Part 1: Course Logistics and Introduction

Section 1: Opening Remarks and Course Language Okay. Thank you. Even without a microphone, without this microphone, is it okay?

Since I'm recording, I don't know the effect of this Bluetooth microphone on the amplification device. If, however, at a certain point, my voice is going to decrease for physiological reasons, I'm going to show you and I'm going to put the microphone on.

For vice-versa, I'll give it a couple of minutes because I don't know if all of your colleagues are already here. You are perhaps less than the total number, someone is missing. Do you know if someone is not here?

Anyway.

Hello everyone, I'm Michele Giugliano. The G is redundant. You will learn this because I'm obsessed with the fact that people write it correctly. Also because on Google, otherwise, there are 25,000 results if you want to have contact or search for me.

The intention would be to do the course in English. I'm not Shakespeare, you'll hear it. It's an opportunity, if most of you agree, to offer something more. Most of the courses in bioengineering and in general, in master's degrees, in *laureate*, in Italy, are in English. You can acquire passive English. I don't ask… if you have questions or comments, you can do it in English. You can do it in English. For an exam, idem. I will give you more details in a few minutes. You can do it in English. In English, all the world is in English. You can be exposed for free, not to a native speaker, *non a un madrelingua*, but to someone who learned it and chewed it up on the road, *a qualcuno che l'ha imparato e l'ha masticato sulla strada*, and you simply get more practice. You have the recordings of the lectures, maybe I will even provide the audio myself, I can provide you with the transcript, so the speech-to-text. And then you can, if at a certain point my pronunciation changes from London Cockney to Edinburgh, becomes Scottish, maybe you can give me a sign and tell me you didn't understand. We have to be together every week from now until mid-December, it will be tough, with this slog of four hours of non-stop lectures. So you can absolutely interrupt me and say, "I didn't understand, can you explain it again?"

If you agree, I'll switch to English. A technical English is not so different. Not only does the world revolve and function in English—the scientific literature is in English, the textbooks are in English—you have a quantity today in your pocket, you have all the knowledge, the PDFs of any article, you don't have to go to a library to make photocopies, and it's all in English. You have a huge amount of tutorials, of videos on YouTube, besides videos of kittens and other various nonsense, but it's all in English. Bombard yourselves, if you can, with English. It's an extra professional skill. You can have it, or not, and you could be better than your colleagues who don't have it. And it's not rocket science.

Okay, I switched to English.

So welcome. I am the coordinator of this course and I'm teaching you this module, which is called "Electrophysiological Signals." In general, please come

to me if you have problems with any other module, particularly at the end when you have to take all the exams for each module. If you have any problems or if you have any advice or criticism on how we can improve the entire course, please speak up.

Section 2: Attendance, Communication, and Course Materials So I'm going to track attendance. Please download, if you don't have it already, the app. I don't do it for controlling you; I do it for my own sake to understand whether it's true, like in the past few years, that after the first couple of classes people got scared and they stopped. No, I'm joking, they did not stop attending, but I would love to see whether also in terms of timing, in terms of schedule, this is doable for you. So at every class, I will give a different code. Today it is **9PSTK**. You put it in the app and basically, it's tracking your attendance. And I think if you send it to a colleague of yours who is not here, it will not work. Please. No problem, I don't care. It's just roughly for statistics. The same thing happened last year, so for attendance tracking, I don't care. For me, it's just roughly the case.

What I do care about, but not for controlling you, just to offer you a communication channel, is Teams. So if some of you are not yet registered, let me know. If you can join in a moment… I'll give you… I'll change the slide in 30 seconds. There is a QR code for you to join the Teams, and in principle, via Teams, we can chat. You all should constitute some sort of mini-community. You should help each other if you have questions about the content of the class and in general the content of the course. You're more than welcome to post your questions and try to help each other, but in general, you can reach me via instant messaging. So… And those who are not yet registered officially might have troubles. So if you do not have a unimore.it email address, please contact me, send me an email with your, say, Gmail account, and I will add you manually in the meantime.

So this is the QR code to join Teams. I think most of you, if not all, were on Teams. And you can do it in two ways. One is by the QR code; it should bring you directly to the Teams of the course, of this module. Well, sorry, no, it's of the entire course, but it's probably only me using it. And then otherwise, if you have already Teams installed, you can join the room, join the chat, the group, the team with that code. All of this, you have on the overhead, which I will post up front before the start of each class. It could be the day before, it could be maybe two days before, it might be five minutes before. So you will have the slides, so don't worry. You don't necessarily have to write down everything, because you are going to have the slides. And the slides are not posted on Teams, although I might say, "Hey, I posted…" We have a website for this module, which is… I'm coming to that in a moment. So if you're not a Unimore student yet, send me an email—remember the extra G, otherwise you don't find me on the internet—and you send me your, say, Gmail account, whatever, your email, because that is needed for me to add you to Teams. Please.

You Google it. You're digital natives, you should be able to search "Michele Giuliano Unimore"—it should be me. Give it a try if you don't. The only thing you have to remember that unfortunately is I am redundant. There is an extra G. So I will show you in a moment.

We have a course website, and we have a GitHub repository. Does any of you know what GitHub is and what a repository on GitHub is? It's a website where people can store files. So if I want to give you something, I put the PDF of my presentations there. Or if I want to share code, I can do it and I do it there. And everybody will, if the repository is free, be able to download the files. In the technical environment, in the technical professional sector, sometimes not only geeks but also people involved in software development, but also nowadays hardware, also in open source, also in open science, it is becoming permanent to have a GitHub account. It's your portfolio. So you're encouraged to create your own GitHub account profile. And if, whatever, if some of the courses at this university are suggesting you or requiring you to do a mini-project, for instance, you can put the code or the electronic schematics or the chemical solution, whatever, as well as your report, if you want to publish it to the world, on the GitHub repository. You can put your GitHub repository, your GitHub username account on your CV. Nowadays people are curious; not only do they find the email of almost anyone, but they can kind of see, "Okay, who is this guy or this lady? What did he or she do?" So having a web presence might be professionally something cool.

I don't remember whether this QR code is pointing to that website, which is posted on GitHub pages, or on this. So github.com is the site where you might want to be curious. It's owned by Microsoft. It has been acquired recently, and it's free for students. With your student email, you can get some sort of free account that allows you to have unlimited public or private repositories. So you can even have your private stuff there. And the website looks like this, so it's a boring website where you have several tabs. One is with just a few lines on aims, what you hope to get from this module. There is a schedule; it's almost every, every, every week on Tuesday. We still don't know the room, the logistics for the other days. I will post it as soon as I have this information. What else is on this website? There is the exact content of all the topics at the end of the semester. You may want to check on this list because this list is what I will have in front of me while asking you questions. But more on the exam later. Now I scared you with this, you say, "Oh my god, you will ask me about the definition of mobility of an ion in solution." I could, but it's a trivial thing, it's a conventional thing, as we will see possibly next week.

Not only the content, and by the way, there is some sort of chapter 0, which I'm not covering. It's about mathematics: differential equations, calculus, basically. So, derivatives, integrals. It's very brief, it's a series of brief videos amounting to, I don't remember whether it's two or three hours. Use this material, it's a refresher if you need it. I'm not a mathematician, I'm an engineer, but sometimes we will use the language of mathematics. I don't know whether you're fond of or familiar with Richard Feynman, Nobel Prize winner, a very famous physicist. He basically said, "Sorry, but nature speaks the language of mathematics." If you want to understand how nature speaks, and since we are talking about biological systems, well, you have to, and it's a natural system, you need to use the language of math. Possibly your background, I will ask you in maybe 10 minutes to tell me in 30 seconds who you are. Maybe your background is all in engineering; you should not be particularly scared about mathematics. There, anyway, you have material to support you. And at any point, even if it's something really stupid, like "Sorry, I don't remember algebra," come to

me and ask questions. I prefer, maybe during class or in the breaks, or later during office hours, that you come to me and maybe I can help you by giving an explanation. And you can maybe catch up and finally remember algebra, instead of carrying over the problem for several weeks or months and having issues with maybe otherwise mathematical parts of the course.

Not only the content, you also have resources. So here it's something where I will put some stuff: PDFs, links to videos, links to papers from the literature. I cannot post PDFs of books, of course, as you know, and I invite you never to look at pirate websites because on these websites, being illegal, you can find almost any book. And if you want to quickly have a look at some book, you should not go on these illegal websites. So this is also a place where I posted some links to tutorials, say about programming in Python, or maybe you are or are not familiar with using computers with a keyboard, with the so-called command-line interface, instead of with a mouse and a graphical user interface. We will not use it, but if you want, there are some links here. And there are also some videos that I will show you today about examples of electrophysiological signals. But the most interesting part are these two links. If you click on either of them, you are brought to the GitHub website where you see it's a sort of file system. Here you have files, here you have notebooks (and I will tell you what notebooks are), and here you have the overheads. And this is the slide that I posted yesterday and then updated a couple of hours ago to add some stuff. Okay, so this is very convenient. You do not need necessarily to reach GitHub; you can reach the website. What else is there? I think that's it. So here, Teams, I'll disable it. Before it was thought to be on Discord, but I will disable this. There is no Discord group anymore.

The Spirit, and here you might see some rambling on why online, regardless of the fact that I'm there also in the same team, but you should be a decent person, you should not harass your colleagues, and my hope is that maybe, depending on how shy or extroverted you are, maybe you can help each other. Maybe someone has a question about, "Well, I did not understand what the guy meant with this mobility of ions in a solution, did you understand it?" "Yes, look, I explained it to myself…"—I'm just thinking of a colleague of yours who might answer—"I explained it to myself as a person, as a body swimming in the sea and finding friction." If you don't talk in real life, maybe try to talk in your digital life. But try to talk, and particularly talk to me if possible. So this is the website. And the website is also reachable from Teams, which has a crappy interface, but at the top, there is a tab that I added where if you go on that tab, you go to the website. And the mathematical preliminaries and the handouts, so the slides, as well as the notebooks, are posted there.

Section 3: Practical Work and Prerequisites Notebooks are, for those of you who know what this is, but I invite you to have a look at a YouTube video in a moment, it's a sort of practice involving Python. But this is not a class on scientific computing, so I don't necessarily want you to become proficient in Python. I will show you something and we'll do it with Google Colab, so you don't have to install anything on your computer and you use Google's free cloud computing time. If you want to install something on your laptop and you have troubles because you're not a computer scientist—neither am I—but you can

come to me and say, “Look, I have troubles, I can’t manage.” I’m not an IT guy but I’m happy to help. In other universities, in the room, there would be like two or three people, sort of teaching assistants, who will be there to say, “Let me, I will do it for you, I will help you.” We don’t have that, it’s just me. And don’t be shy, you can hopefully count on me.

So this is about the mathematical refresher. I suppose, I assume you don’t need it, but it covers very basic things like what a mathematical function is, what a plot is, what does it mean if you take a mathematical function and you add something or subtract a quantity—maybe the graph is going to shift vertically, horizontally, etc. And some notable functions like straight lines, exponentials, logarithms, blah blah. Derivatives, they will be very useful, and integrals will also be very useful, both definite and indefinite. As I said, I’m not a mathematician; this is not a course in mathematics. There was something on the blackboard from a previous class that was geometry. Although we are in the Institute of Pure Mathematics, I’m not in love with mathematics to that extent. And then there are other things you might not have seen. Well, you should, being engineers. Those of you who are engineers should be familiar with the Dirac delta function, typically used in linear systems to probe the response to a pulse, and the convolution integral. Again, it’s something that you might have heard about, maybe people call it filtering, but it’s the same thing. And again, differential equations—only one differential equation, what I will call over and over the only, boring, typical, usual differential equation that I would love you to get acquainted with, and it’s this one. Say this is a function of time; it’s this one, maybe plus something. This first-order differential equation, the coefficients are constant, and I don’t ask you whether you remember what the solution is. I only invite you to refresh your knowledge, not mnemonically, but just by understanding it and reading aloud what it is. It says: what is the function f—since differential equations are equations where the unknown is not a number like in an algebraic equation, but it’s a function, so it’s a collection of… well, it’s a rule, it’s a mapping, it’s not only a collection of numbers—what is this function that when you take its derivative you get the function itself? There are also some memes on the internet where there is a function that no matter if you differentiate it, it just stays the same. Only geeks… do you remember which function this is? It’s the only function that is worth… yeah, I like the exponential. Exponential, yeah, indeed. I thought you said logarithm, `ln(x)`. No, it’s the exponential, perfect. So you don’t need it, but we’ll see even during some parts of the course where I’m going to derive some mathematical formulas, I’m not going to assume that you are very skilled or at ease with mathematics, hopefully.

So there is no guarantee… I thought that maybe that stuff over there was a camera, but it’s not a camera, so I brought my own cameras and I’m trying to record with my own microphones. Hopefully, it will work. Sometimes there might be some glitches, so there is no guarantee, but after… so I’m not streaming, I’m recording, and at the end… let me check that I’m recording because otherwise… yeah, I am… otherwise, it will be disastrous. And maybe the day after, a couple of days later, depending on when I can upload the file to YouTube, you will find it on this channel. There will be a playlist with the name of the course. But again, I hope so. Last year, nothing happened. You never know, the computer, the battery, everything can go wrong. Sometimes I might forget to… well, in

this case, it will not happen because I changed the setup, but say I might… you will only see my face and not the slides. You do have the slides, so it's not the end of the world. And maybe you don't see my face, and okay, fine, you can survive not seeing my face.

Section 4: Class Structure, Exams, and My Teaching Philosophy
Okay, practicalities. Classes are in person, slides, and chalk. I hope they are interactive, really, I mean it. I don't give a damn if you ask a stupid question. I don't care. I forget. I really don't care. I will not remember at the exam, saying, "This person…" No, I honestly don't care. I have other things on my mind and I cannot read yours… I cannot read your mind. So I have some experience from many years of teaching, so I can see whether you are completely blanking and you're brain-dead. Right now, maybe a little bit scared, but not yet. So if you don't speak, I cannot help. I cannot try again, being clearer, explaining myself in a different way. So I ask you to be interactive. And of course, it will be boring if I have to talk for four hours straight, although there will be breaks, don't worry. And I will propose there are some alternatives for breaks depending on what you… what you do. So I don't mind stupid questions. And again, if you need help, maybe you're shy to ask in front of your colleagues. It's normal, everybody, to some degree, is shy. You can contact me. And even if you have some problems with whatever… okay, not your personal problems hopefully, but if you have interests or if you are passionate about something or if you realize that you don't know about something else, ask. I'm happy to help. In principle, that's my main job: to transfer knowledge. And so if you want information about some topic or even in general about bioengineering or about neuroscience or about neuroengineering, come to me.

So handouts, I told you, they are made available to you upfront. Videotaping, hopefully. For all of you in the class, if the slides were the only things required to pass the exams, then I would basically be better off staying at home, and you probably as well. So I'm going to add more stuff, and you can stop me. And I'm not, say, super intelligent or super knowledgeable, but I know a little bit and I can tell it to you, and particularly by reacting to your questions, there might be way more stuff than what's on the slides.

So the hands-on, there will be some practical parts just to make your life a little bit more interesting, hopefully. It doesn't need to be… I mean, you can listen to this stuff completely passively. Again, you don't need to install anything on your computer. I would maybe suggest those of you who are really interested to do that, and it's about installing, for instance, Python and some distribution of Jupyter. I don't know whether you know what this is, but otherwise, if you don't and you only want to use a web browser because you only have, say, a tablet and you don't want to bother, you can use a website which is hosted by Google and it's free to some extent. It's called Colab. And of course here I don't have any notebook to show you. It's some sort of web interface where you can use their computers to crunch numbers, to plot functions, to plot data. And you also can mix text, code, which is not around, which I don't see the code here. Okay, here it's code, whatever it is. I'm not particularly familiar or fond of Python, but okay, so this is Python code and this is a plot. So on the same document you have text, explanations, annotations, code, and the result of this

code. This is becoming a common practice in general in science where people are not only giving you the data—so a few years ago they were not even giving you the data. "Here is the paper, study the paper, trust the authors for the results." No, today, hoping to have reproducible science and research, we give you not only the data that we acquired, say in an experiment—say plugging myself into some electrodes in a moment, although it will not work—and then not only give you the data, I give you the code that I use to analyze the data and I put it on the same page, really for dummies. For dummies in the sense that it doesn't require people to install stuff on their machines and hopefully it will possibly be reproducible. A few years ago, there was this image of the black hole that was reconstructed by means of algorithmic means. Probably you all remember this sort of donut, it's a sort of *ciambella*, it's red with a hole in it. And that was the first image of a black hole. They gave the data and they gave what they call notebooks, so this thing, to people together with their publications. This is what the hands-on parts are, and these notebooks are already posted, all of them. If you want to be curious and you want to sneak a peek, they are on the GitHub repository in the folder called "notebooks." And again, for prerequisites about math, coding, and particularly biology, which I suspect you might not be ultra-strong in, ask. There is some material for the next, not necessarily for the next week but in general for the next couple of weeks, that I posted. It is on the GitHub repository and you'll also find the links. It's some reading material that you may or may not read. It's not something that I will ask at the exam, but it's something that you will need if you feel weak in terms of biology, neurobiology in particular. So on these slides, I gave you the link to that YouTube video from a guy, a blond guy if I remember, that in like two or three minutes explains what Google Colab is, what notebooks are. And this was the solution that I… so the alternative… so if you are in between, if you're not completely scared by computers and so you use that, and if you're not a computer geek that you install your own distribution of Python using Anaconda or virtual environments with UV or pip or whatever, then this is intermediate. And it was a standalone application that you could download on your computer to have, on your computer, your machine, the same thing that's over there in the cloud. It was called JupyterLab Desktop, and a few months ago they stopped developing it. So it still works, but I don't know for how long. You may give it a try. When you download it, it downloads Python inside itself. And so you have Python on your computer encapsulated, wrapped inside this software. This is because installing Python may not be entirely trivial, particularly if you have, say, a Mac or if you're on Windows where there are also another series of complications. If you are using Linux then you probably are sleeping now because you're supposed to be more expert about installing stuff.

So the exam for this module is an oral interview, informal. I don't like quizzes, I don't like written tests, because I'm not there to help you. If you go blank because you're getting emotional, you don't remember things, I'm not there to see, "Okay, okay, good, I want to help." And maybe you need just a bit of information just to restart. It happens all the time, it happened to me as well. I hated particularly the closed-answer questionnaires. This is humiliating to me. You have to be given time, 20 minutes, half an hour, that I'm sitting there and I listen to you while you recreate on a blackboard in detail what I do or present to you during the class. And the overall… I will show you the statistics

in a moment, although it's based only on last year, but it's still something that you might be interested to know. So the final score is the score of individual modules, and it's weighted by the credits, by the CFU. So depending on how many credits, the score, the mark that you get in one module, has a bigger weight. *Cum laude* means that you have to get 32 out of 30 in total, although in my module I gave several *cum laude* last year. And we round to the closest integer. So we don't look at the ceiling, the integer that is approximating by excess or by defect by floor. We just use the closest integer. And it requires, you cannot skip any module, and you're supposed to complete the entire thing in 24 months, in two years, which is reasonable. So for the moment, since it's basically the start of the second year, we don't know, there are only two or three people out of 20, if I remember correctly, who did not complete all the modules. They are missing mine or another one, I don't recall.

So statistics from the past. So that's the average mark. So I think that you can lower your anxiety, particularly because I was told that you are quite smart. I don't know, other lecturers had a good impression and I'm curious to interact with you, just to learn from you. So you see I'm not particularly strict and all the people were good. And one out of three got *cum laude* because they were interested, not because I gave it as a gift to them. And overall, across all the modules of this entire big course, you see the final mark was not particularly low. And so this might lower your anxiety.

Exams and exam sessions. I think I will have six sessions of exams in January-February. I did not manage to add them yet on the ugly online platform called ESSE3, which is very crappy, but that's the only thing. And I will ask you to register with great advance, maybe during the month of December you decide when you want to take your exam, my part, just my part. And you see there are 12 slots a day and there are… 14 from 8:00 in the morning until 1:00 p.m., one after the other. It takes the time that it takes. Unless you're really abusing the time, I will stop you. So if we go 40 minutes instead of… so I will try to be fair, so that all people get 20-25 minutes. So I will try to be fair in that respect. I cannot add sessions in June or July yet, but you will see it's plenty of opportunities. And exceptionally, you can contact me and we can schedule one appointment on a day that you want if this is fulfilling some sort of need. I would love to avoid having this as a rule because otherwise, you will say, "Okay, maybe today, maybe tomorrow, what do you do tomorrow? Can I do the exam?" and maybe you don't show up. If you register, if you contact me, then you must show up. I will not fail you if you don't show up, but as I'm going to help you, you help me not blocking time.

Now for the… I know that you finish the previous class at 1 p.m., so you have only one hour for lunch. My preference is to start at 2 p.m. sharp and finish a little bit earlier—well, 50 minutes earlier, but better than nothing. But it's your call. The alternative would be that we can even start at the famous academic quarter of an hour or even at 2:30. Of course, we would then exceed because I need the amount of hours. And the idea is you can think of it, you don't have to… we don't have to decide. Well, today we started around 2:05, and you tell me what's your preference. Maybe make a poll, make a Doodle, do whatever, and let me know.

Breaks. Normally I do, because I'm used to it… I taught for 11 years in Belgium

to students of several curricula, but they were not bioengineers like you are. And they were a bit intimidated and they were going blank after a while. So the style was that I was stopping, like I'm planning to do today, every 45-50 minutes for a 10-minute break. A 10-15 minute break. We will do three of these breaks. Maybe you have a stronger stamina, I don't know. There are alternatives. We could only stop after one hour. Probably I cannot do four hours in a row, but a couple of hours I can do. I can do it, particularly if I get… you will see some topics will get me excited and I hope that I can at least pass this enthusiasm to you, or at least that you learn, "Okay, this topic is cool but I don't care and I don't want to do this in life." Fine. For me, it would be a success to tell you what you might or might not be good at, or might not be passionate about. So that's another possibility, that we only do one break. For the moment we have another 10 minutes to go and then if you want, we'll break. Otherwise, you let me know. When the timer rings you will tell me whether you want me to continue for another, say, 45 minutes or you want to stop.

Part 2: Instructor and Student Introductions

Section 1: About Me, Your Instructor So I'll tell you a little bit about myself. I'm an electronic engineer by training. At the time I studied in Genoa, I started in 1992, which seems like a really long time ago, and there was no bioengineering, there was no biomedical engineering, but there was already some sort of specialization that was biomedical engineering, and this was what I took. I'm very proud that I did not take some classic exams as engineers do, like economics and construction science, because I didn't care, and I wanted to study biology. I wanted to study some topics that I chose. At that moment it was bioelectromagnetism, and there was another one that was bioelectronics. I wanted to do that. That seemed to be the future. And indeed, that's the case. But it's me. That was me. Then I continued with a PhD, a doctorate in Genoa, well in reality it was in Milan, but it was under the supervision of a pioneer of bioengineering and neuroengineering in Italy, the late Massimo Grattarola. And I basically did a PhD in bioengineering and it was basically about computational neuroscience, which is a discipline related to using models and computers to study the brain. And then I left Italy. That was a very good decision. And I regret that I'm back, but that's another story.

So I did my first postdoc, which in the academic trajectory is what you do, you continue doing research. And I did it in an institute of physiology at the Faculty of Medicine in Switzerland, in Bern, at the University of Bern. To joke, I say that I was looking for the best chocolate. In Genoa, although it's not Liguria, there is this Novi Ligure chocolate, which is still my favorite, but I wanted to try other things. So Switzerland was a clear step to take, so I did that. And then after a few years, I moved to the Swiss Federal Institute of Technology, the EPFL in Lausanne, which is a terrific place. I was a group leader and both during my postdoc and my second postdoc I was doing experiments myself, doing electrophysiological experiments. So that's why I'm here to tell you about electrophysiological signals. And I'm particularly interested in cells, not the entire thing. So I will try to show you something, but it will not work, of course, but I'm more intrigued by the mechanism, in the same way that transistors in this device are sort of microscopic units that determine the

computational features, the properties of the device. Then I moved to Belgium, so the other step for chocolate is Liguria, which is Novi Ligure, it's not in Liguria, but anyway, so Switzerland, then Belgium, although Belgian chocolate, I don't know whether you know it, it's a bit complex. It's not plain good or bad, it's with stuff inside. It was not my thing, but I stayed there 11 years, 10-11 years, and I was a professor of neuroscience. And then I came in 2019, just in time for the pandemic and for the lockdown, as a professor of physiology at SISSA, that is like Scuola Superiore di Pisa. It's special, it's only a PhD, research-intensive institution. And in 2024, I joined Modena because they wanted to, and they did, start bioengineering, and as you know, there was no bioengineering before. So I basically… this is my trajectory.

And as I said, I'm intrigued by the mechanisms, particularly of cells and nerve cells that we will have ample time to talk about and to discuss. They are electrical devices, and to me, it blew my mind as a student, more or less… maybe I was one year younger than what you are now, to learn from a professor that not only were they electrical devices, they were sort of generating signals very fast. This is one millisecond, and on the y-axis… it's a picture from an oscilloscope in the old times. You were not able to record the data with an analog-to-digital converter like this one that is now very small and conveniently attached to a USB cable. At that time you were taking a picture on either a paper recorder or an oscilloscope. So very brief, one millisecond, and about 100 millivolts. That's the amplitude of a nerve impulse. And 100 millivolts is 0.1 volts, and it's not so different from what the internal voltages of a microprocessor are. Maybe you're familiar with your power plug which is 5 volts, or you maybe know if you play with electronics that some stuff is powered by 3.6, 3.3 volts, whatever. Modern microprocessors have internal voltages that are even smaller. And there you eat a banana or chocolate and you fire that. You don't need a small nuclear plant like ChatGPT does. So this blew my mind, and the other thing that blew my mind was that it was possible to write equations. And it was, for me, amazing. How could you study biology with mathematics and physics and chemistry? Well, after all, it is physics, chemistry, and mathematics, so it may not be surprising, but to write an equation that maybe can describe the electrical activity of neurons, maybe one neuron or a network of neurons, really inspired me a lot. And so that's why I basically got into this field, with the dream of understanding how the brain works—of course, this is still a dream—and to make some sort of difference or to sort of bring some positive things in, say for patients, for patients with neurological disorders.

And again, I'm intrigued by cells. These cells that you see here have been filled with a dye. They are from the cortex of a human, but no students were sacrificed at that stage. It was from a patient, and they are called pyramidal cells. They are called pyramidal cells because their soma resembles a pyramid, and you see a lot of cables. Nerve cells are cables; they have a lot of wires, and this is impressive. And when you look at this, you will not see exactly this image under a microscope with living tissue. It's remarkable how dense this network is. And if you open an electronic device, maybe you should try at some point to break something and open it, because nowadays the printed circuit boards are very complicated. You will see that there are a lot of cables, a lot of leads, a lot of contacts. And one of the famous, well, the most famous neuroanatomist that studied these cells was at the end of the last century, or sorry, at the

beginning of the last century, around 1901, 1905. He was from Spain. His name was Ramón y Cajal, and he looked at this and he understood and he got it right, that these things are kind of fetching inputs from there, and something that you don't see here, there is another fiber that is bringing signals out, and that's the output. And the guy, just by looking at these images, understood the function, well, understood some features of the function, which is remarkable. But the reason why I thought of telling you about this is because he called them the "butterflies of the mind," of the brain. And indeed, they are beautiful, as opposed, say, to sperm cells that are the example of minimalism. They have nothing, it's just a cell body and a tail to swim. These guys are baroque, they are so complicated and they reminded him of trees. So particularly all of this branching and bifurcations, like trees. Unfortunately, we don't have windows to look outside; there are trees. Next, as you go out, have a look at trees. In that case, trees are bifurcating, are branching, probably to fetch light, to expand the surface to fetch light. Over there, maybe these cells are expanding to fetch inputs. And over there, it's the top where my hair is, so it's called layer one, or the pia mater, which is one of the dura and pia mater. It would be what is immediately below the bone, below the scalp. And what you have here is the white matter, which is kind of in my ventral part. And so somehow this guy is having this sort of fiber which is called a dendrite, from the Greek word for tree, *albero*, *dendros*, and then it's kind of towards the top, it's bifurcating, it's branching to fetch inputs.

What do we want to do? We stop or we continue? For me, it's the same. Just don't be shy, I'm fine whatever you say. Who wants to stop? So let's stop. So we come back in 10 minutes. Thank you.

Section 2: My Research and Student Introductions So, I lead a laboratory, an experimental laboratory, that you're welcome to visit if you're interested, upon appointment in the coming months or even next year, whenever you want. And we do three things, we combine three disciplines. One is experimental neurophysiology. So we want to study *logos*, the physiology, the functioning of nerve cells, or the electrical function of nerve cells, and we do it experimentally. And then we also use mathematical models and physics and computers to make sense of the data that we record, as well as to make new experiments that involve a computer. We do hybrid circuits where some part is a biological neuron, some biological cells, and some part is simulated cells. And I also work at the interface with neurotechnologies, so nanomaterials or non-conventional tools such as optogenetics. Did any of you hear about optogenetics? I will tell you what it is. In a few years, I'm pretty sure they will give a Nobel Prize to these two people, two American scientists. They invented a way to make a protein that normally real neurons do not express—well, they express it in the retina in some cells of the retina—and you shine light with a laser beam… I do have it, this… well, it's not really a laser beam… hello, yeah… so you shine light with a laser beam literally and non-invasively you are able to excite or inhibit the electrical activity of a cell. Imagine that you are suffering from seizures, we will probably discuss it later today, and you have a seizure that means a very sudden, highly synchronized electrical activity in your brain. In principle, I could shine light with a fiber optic and I could switch it off. It's not yet reality but they will get the Nobel Prize. So today we don't use it for therapeutic

reasons, we don't put it in the faulty retina of blind patients to restore… well, somebody does and they were successful in restoring vision, but we use it as a way to probe cells and circuits and networks because we can interrogate by stimulating or inhibiting just in a non-invasive way. Imagine literally I can have a laser beam and I can move it to different positions, so I can excite this neuron here and I can maybe inhibit this neuron there, but I will tell you in due time, I will tell you more.

Now you tell me… okay, first, before asking you to tell me about yourself in 30 seconds, in my free time, when I have free time, I'm a geek and I'm particularly intrigued by radio transmissions. And I… it's now a few years that I'm trying to learn… it's a stupid thing because you never heard of it, but it's all pedagogically thought out, so you will see that it makes sense. One of the reasons is that I'm fascinated because the way neurons encode information is clearly not by a man-made code, but they do it in a way that seems to depend on the frequency of this nerve impulse. Now I have no idea whether in the cars, say of your parents or your own car, you still have something that is called a radio. When I was younger and even today I have a radio and the radio had two bands, so it's not only podcasts and not only Bluetooth connection to a device, but you could literally hear radio transmissions from a radio DJ or whatever. And it's FM instead of AM. Have you ever heard of this? So AM, maybe those engineers among you who studied electrical communication, they might even have studied the nitty-gritty of it. So with amplitude modulation, the carrier, which I won't tell you what it is, is modulated by the information content by changing its amplitude. Now for a nerve impulse, it seems this is not the case. All the nerve impulses have this 100 millivolt, 0.1 volt amplitude, but what changes is the frequency, like in FM. And in fact, you know that AM is sort of noisy, sloppy. I don't know whether you ever… if you have a radio, try to put it on AM and you will hear a lot of noise. Instead FM, when you are on the right frequency, the quality of the sound is very nice. So encoding information in frequency is more robust in terms of signal-to-noise ratio. And maybe this is not a surprise that my foot is controlled by some neurons in my motor cortex by frequency modulation. And instead in my retina, where the distances are very, very tiny, very small, neurons also communicate by amplitude modulation. It's not really the nerve impulses and other things, but this is very interesting. That's why I'm a geek, because I take things from work home and vice versa. Maybe it can be of interest.

Each of you, I would love to hear for 10 seconds who you are, what is your background. Some of you already told me that they are not engineers and I'm fine, actually I'm very happy that there is diversity. I hope that the engineers among you will help those who are biologists or physiologists and vice versa, the physiologists could help the engineers who might be ignorant in other things. And why bioengineering? That would be very interesting. And if you don't know why, that's fine. I did not know for a while what to do in life, for many years, even during university, and so that's fine. And if you want, what do you do in your free time, if you do sports or whatever. So please, let's start like this, otherwise you will… you will have a start. I don't care, even if you can do it in Italian, in English, whatever, feel free.

Thank you. I can't. Thank you. Chocolate? No, just kidding, just kidding.

Thank you. Thank you. Thank you. Thank you. Yeah. Thank you. Thank you. Thank you. Thank you. Thank you. Thank you. Okay, thank you. Thanks everyone, I really appreciate it, you're a tough crowd. So I hope to work well with you and that you can find interesting ideas in what I tell you.

Part 3: Study Materials and Course Structure

Section 1: Recommended Reading Okay, so as study material, there is one book that covers most of the topics. Of course, it does it in a very structured way, not necessarily in the way that I will follow, but it's the first one. And I think I ordered… well I think, I'm pretty sure I ordered at least two copies last year and they are available in the libraries. Do not attempt to find it on PDF on illegal websites, but sometimes some of the books are good to have, and so that one is a nice book. It is authored by a series of researchers, particularly one from Norway. And of course, I forgot the name of… Okay, it will come to me. So he's a pioneer, the last author of that book. Gaute Einevoll is a pioneer on some of the techniques that we will also discuss later on, the extracellular signals in the brain.

There are other books that you can look at. So these are classic books for… and again, I wrote chapters, so it doesn't… I don't mean from chapter 1 to chapter 20, and some of the books are really, really… it's a sort of Bible. So this is the Bible of cell biology. It's really a tiny big book and it has a lot of details. Same as Kandel, Schwartz, and it has now other authors, possibly it's not 2012, but it's *Principles of Neuroscience*. It's again a Bible and it's only for references. This is a light introduction to neuroscience and the first four chapters might be useful for those of you who are not necessarily that familiar with the biological aspects. And *From Neuron to Brain* by Nicholls et al., 1996, is about… it's a two-volume book and it's about biophysics. Again, not the entire book would be required, but you will find the content of the classes over there.

There are a few things, a few readings that are available on GitHub as well as linked on the website as PDFs. So one is just a few pages… for those of you, probably you don't use it anymore and it's not a thing anymore. When I was a student in high school, there was a company called Bignami and they were making very tiny booklets for Latin or history or philosophy, whatever. So this is a sort of Bignami. So it's very condensed and it's an immensely incomplete summary, but in two pages, it tells you what a neuron is, what a synapse is, what a receptor is. You might want to just have it, to use it, and I think it's just maybe two or four pages, if I remember correctly. This is more blah blah, it's not even aimed at students, it's for the general public on brain facts, and it's an introduction on the… a little bit on the working of the brain, particularly the early parts that are not dealing with behavior. Of course, you can read it, you can read all of it, but for the topics of the course, only the first couple of chapters are relevant. And then there is also the entire PDF, because it was put in the public domain by one of the Nobel Prize winners of several years ago, I think it was in the 60s, Hubel. Have you ever heard of Hubel and Wiesel? I will show you later what they did. So this guy wrote a very tiny book, very high level, very, say, science communication, so it's not a technical book, called *Eye, Brain, and Vision*, and it tells about the part of the brain that we understand

the most. And for me, as a student, it was a very nice primer, a very nice introduction to the neurobiological concepts that came up over and over again. So you might find it relevant. You don't have to study it. I'm not going to ask about material from these things. They are readings for you. You should be selfish. You should study for yourself, not for the exam. And as you could see, the exam at the end is not going to be impossible to pass. You should now try to study for your own professional life, not as a strategy to pass the exam. There is no point if you study just to pass the exam; in a matter of a couple of weeks after, you will forget everything. If not, if you study for yourself, maybe you will learn and remember things for your lifetime.

Section 2: Practical Data and Demonstrations So, what I will also do, because the demo that I would like to attempt now in the next 10 minutes will not work for a series of reasons. The last one is that I had the bad idea to rehearse it last night. And as those who have experience with electronics, they know the term "magic smoke." "Magic smoke" is what happens at some point when something burns and you see smoke coming up out of electronic circuits. And so I don't think it will work. And there are other reasons that I will tell you why it will not work. But you have data on the GitHub pages, as well as you have a notebook that I will invite you to play with. "Play" means you load it into Python, you plot it, and maybe then it's up to you. You can do the Fast Fourier Transform to get rhythms in these traces, although they are crappy traces. It's something that I recorded last year. And another thing that you could say, being an electrocardiogram, there are peaks. It was my electrocardiogram from last year. Maybe you could find a way to count how many peaks there are, but we will see that later. So, you even have a YouTube tutorial that will sort of illustrate this notebook. This is not compulsory. I encourage you to do it. Again, if you don't do it, I don't care, but it has been done just to pique your curiosity. But let me skip this for the moment. Where is the demo? I want to do the demo now. But okay, first I have to talk about frogs, otherwise I cannot say whether I am like a frog or not. Okay, so maybe we do, say in 10-15 minutes we'll break, and then we'll do the demo later on.

The demo has a little bit of a component of risk, that's why I'm not asking any volunteer to be plugged in, because in principle, and maybe I will ask for your help in the future, everything could be working with a battery, because I could disconnect the power supply of the computer, and the computer now is standalone. But the computer, unfortunately, because of the video projector, is attached to this HDMI cable, and that one is a source of potential risk, because the ground of this cable, the reference electrode of this cable could be floating, so I could electrocute myself. I hope it doesn't happen, but I don't want to electrocute you. It would be one bioengineer less, so it could be less competition, but it will not be fair to do this at the university. And this may not electrocute me, but it will create a lot of noise. You will see how bad noise is. So, I'll talk about signals that I'm going to show you, maybe, or maybe not. And I'll talk about noise.

I ask you, what is a signal? What is a biological signal? You have the slide, so you can read what the slide says. Do you have any suggestions? Some of you have been already exposed to signals. I don't remember who, but you told me

that you did data and signals. So you should be familiar with this definition, a rigorous definition of signals that maybe you will see again, you have seen it in the last couple of days, from my colleagues. But I would love to set the topic on what is a signal. What do you think is a signal? What could that be? Thank you. Perfect. And this series of data points, maybe they could be in time, assuming that my heart beats, and at some point now and half a second later, and half a second later, I might record a different kind of distribution of electrical potential. Or it might be a set of numbers that are changing in space like an image, like an fMRI—well, fMRI would be in time as well—let's say like an MRI scan. There will be an image, it's a collection of… it's a bi-dimensional collection of numbers. In every coordinate (x,y) you will have one number, for instance, like this image. So that's correct, thank you. So it's a physical variable, it's an observable perhaps related to a biological system that is subject to some sort of variation. Otherwise, it would be boring, it would be just a value, it would be 26. 26 what? 26 millivolts maybe, or let's say minus 70 millivolts that will come over and over. It will be boring if it's always minus 70 millivolts. If it changes over time, that might be interesting. Maybe you're alive or maybe you're dead. At least I can tell whether you're dead or alive. So variations in time or in space. And it can be represented analytically by some formula, a mathematical function, but it does not need to be. The story of introducing or reminding you of the concept of the function is because a function is a really interesting mapping for mathematics that says from this value of the input, the independent variable, you have only one number in a scalar function. So at this moment in time, the temperature here is probably 22 degrees, only one. And so the independent variables could be one or more; if it's the plane of an image it could be x and y, if it's time it's one. And if the function is not scalar but it's a vector function, maybe at this moment in time I'm getting the oxygen concentration, the temperature, and the heartbeats. So it might be multiple, might have multiple dimensions. Something that… all concepts… this has been discussed in mathematics 1000 years ago, so they were useful even before inventing any device to measure these signals.

These are known functions, mathematical analytical functions like the exponential or a sinusoid. By the way, if time is measured in seconds, what is the frequency of the sinusoid? You know? Or the period? I wrote it in this way because for many years at the university people told me about sinusoids, sine and cosine. Okay, but if I want the sine of 20 Hertz, how do I write it? So T is in seconds and you probably know that inside this function, things have to be without physical dimensions. Well okay, yeah, pi is in radians but it's not really a physical dimension. So here inside, a physical unit cannot survive. It cannot be time. I don't know what the sine of 23 seconds is. It's like an exponential of three apples—it doesn't make sense. I don't know. So this 0.1 is the value of the frequency, it's 0.1 Hertz, which is 100… so it's one… so it's 10 milliseconds… so sorry, 100 milliseconds. Anyway, this collection of data points might also be collected in a file on a disk or on paper or on an oscilloscope. It does not require to be described analytically by a mathematical function. And so these are some examples of scalar functions, like electromyography, that maybe is the only thing that will work. I will try to see whether I can get electrical activity in this muscle. Music, so a voice recorded, now it only has one channel, it's not right or left, it's not stereo, so at every moment in time I'm basically

sending via Bluetooth just one number that is the intensity of the sound. A black and white photo is another scalar example of a signal because you have two independent variables, but for each x and y you have one value that is the brightness, the gray level. If it's black and white, it is only one level. And there are other examples. So if it's a vector function, it might be some sort of observable of a fly that is moving around, and at every moment in time, I can measure the instantaneous velocity. And the instantaneous velocity might be a vector, talking about some direction, some intensity, and some *verso*. So, some direction and intensity. So, it's vectors, you know, that might be described by three numbers in a Cartesian system. So, for every moment in time, I have three numbers. That's the meaning of a vector, sorry, a vector signal.

Section 3: The Historical Roots of Electrophysiology Now for the specific case of electrophysiological signals, it's very interesting and intriguing. I don't know whether you have watched this silly little talk that I gave a few years ago at a science festival. We owe it to… so the entire field of electronic engineering, of electricity, of electromagnetism is actually due to people who embarked on measuring electrical phenomena in biological systems, which I find surprising. And probably you know that these two guys, two Italians, were considered the fathers, the pioneers, and particularly Galvani was the one that just by chance found that there must have been some sort of "animal electricity," because when he touched the nerve of a resected leg of a frog with a metal, then the frog's muscle contracted. So it seems to be… well, it must be some electricity. Now Galvani, unfortunately, got it wrong in one interpretation because he thought that it's a fluid, it's the animal fluid. It's not the same phenomenon that Alessandro Volta, they were more or less contemporaries, was studying in a different lab. He said, "No, this has nothing to do with metaphysics or with some animal principle." It's the exact same electricity that this guy, Volta, got by, inspired by Galvani, placing different metals together. It's the same electricity. And so, but they were anyway giants, despite this rivalry and despite the… yeah, despite that Galvani got it wrong. And he actually died in bad conditions, depressed and convinced that he was, he had been a failure. He was not. So this is a stupid movie to show that by sort of closing the circuit and applying some sort of electricity or metal, the leg of the frog was indeed contracting. And these are the starting observations. So spasms, so mechanical contractions, muscle contractions—today I should maybe call it cellular excitability, but we will see later what this is—is characterizing biological systems, particularly muscles and not only muscle tissue but also cardiac tissue, pancreatic, some pancreatic cells, nerve cells of course. They're all cells that would react if you would be given some electricity. And particularly the story was originally from different metals that were in touch with the… and so creating some sort of difference of electrostatic potential or electric field. I will review during next week's class some elementary concepts of electromagnetism such as electrostatic potentials, electric fields, Coulomb forces, so don't worry too much about that physics. And the interesting thing is that passing a current through the leg was creating a visible reaction, so much so that they would be measuring the speed of propagation. And as if you watch this video, this talk of mine, it basically… they invented neurophysiology. So it's not only them, it's people following, and they were in a… you know, historically, in a situation where people were still convinced that

there was some sort of metaphysical principle in living organisms. And so the brain had this "animal electricity," "animal principle," "animal fluid" that was not knowable. So it could not be understood or measured, it was metaphysical, so it could not be measured. And people said, particularly people like Helmholtz or others, they were convinced that it was understandable by the laws of physics and chemistry, and they proved it. And they started a field that bioengineering is somehow building on today.

So I will stop and then we'll start later. So the question is that, I'll tell you what I will show you and then… so it will be… the question is that I will also have some electrical signals and it's just to entertain you and to make this four-hour class a little bit less heavy. And do you know Murphy's Law? So, of course, as I said, something that could go wrong, did go wrong. This is Murphy's Law. And apparently, I read yesterday that the guy was serious in the sense that he was basically, it was not only just a joke, he was actually saying, in engineering, it's guaranteed that things will fail. So, do organize so that things are not failing. In this particular case, it fails or it will fail, first of all, because I had a last-minute burnout. And secondly, in this environment, the noise is too large. I don't have a Faraday cage that would shield the noise, particularly this damn 50 Hertz noise, which is related to the power, to the alternating current that is in the power outlet. So also here. But even if I disconnect the laptop, it still will capture this very slow, oscillating electromagnetic interference, which is at 50 Hz. And we will see it immediately. And then there is also the ground loop that will prevent me from getting data. And I will use an electronic amplifier. I think that you are more than competent to understand that I need to amplify signals that are very, very tiny. I need to make them much stronger. It will not work if I plug my fingers in the input of this analog-to-digital converter. So the analog-to-digital converter, maybe you've heard, it will acquire analog signals. "Analog" because they are changing over time; they are signals that are changing in analogy to the physical quantities of the real world. And they will basically convert them to digital signals. Not only will they digitize them, but they will also sample them. I think that you might be all familiar with Nyquist. Do you know Nyquist? So anyway, he's one guy who made it very strict that in order to make signals usable on a computer, you cannot… with continuity, with temporal continuity… you need to observe it like with a stroboscopic flashing light at precise times, at precise moments in time. And the period is called the sampling period. And this guy here is sampling at… I will not tell you because it's one of the questions that I would like you to see… so a few hundred or a few thousand times per second. And I need an amplifier. But let me stop, otherwise I'll lose your attention. Let's break for 10 minutes and let's continue later. Thank you.

Yes? How are you? And this is for Teams but I think that you already have it or not? No, because in the university I have a lot of difficulty to start. Can't be yet, not yet, not yet, not yet. Okay. I thought I would ask if there was also a code. I said no, but it was just… Okay, thank you. Nothing. Correct, because we are going from here at 8:00. And in the middle of Mirandola? - But you have to write it if you can present it? - If you want to write it, then you can write it. - Yes, yes, absolutely. I don't know where it is, but it's in the center of the… and there should be a few halls. - No, with pleasure. If you are there, then it's… - If you have the fortune… - Okay. Maybe you can tell… Because in

the center of the community there is not one bar, in a bar there is only a coffee machine and then… if you have a microphone it still works. Thank you. Thank you. Thank you. Thank you.

Section 4: Live EMG Demonstration So I'm a little bit disappointed because there is no noise. It's Murphy's Law in the opposite sense because it's working much better than at home. And there is no 50 Hertz noise. I wanted to show you this… no, well, it is noisy, but it's not the noise that I'm used to. So I will show you what… so I will tell you later the details of what I'm doing. And in the video recording, I'm using the webcam to show that I connected two electrodes to this part of my arm and the third electrode, which is the reference electrode—and I will tell you what this measurement is—I connected here below my… just a little bit below my left shoulder. And there is no noise. So I might attempt also the ECG, the electrocardiogram, also called EKG, but this one is called EMG. And so I am a frog in the sense that the electrodes are amplifying… I think the amplification is 100 times, and I'll tell you how in a moment. It is amplifying signals related to muscles. We know nothing about muscles, we will not study muscles per se, but you probably know that you don't have one big muscle, you have many fibers, and each fiber has its own electrical activity. So it's conceivable that if I do a voluntary contraction of the muscle… and by the way, I'm not able with this setup to look at nerve inputs. I could if I had a needle going in my skin. And in Zurich, when I told you that I attended a few courses during my PhD, there was one very charismatic professor that was putting a needle inside his own muscle, but I'm not that brave. And so what is now recording is something that is probably… I don't know what that pulse was. It could be an electrical artifact. I want to show you what happens if I simply try to contract my fist. And your criticism could be, "Wait, but if you move the electrodes, so if you apply movement, then you have artifacts." So you have to really… in order to convince you that what we are measuring is maybe related to the electrical activity of the living tissue, I have to try to see whether I can stay very still. And the difference will be striking. You will see when I contract, you will see that the variance will increase. It's the crucial moment, come on. Now take your time, I'm joking. But it is the crucial time. So all of these are artifacts probably because I'm moving and it's probably the wires, even the wires. But look what happens when I contract my fist. So I'm not moving, I'm just… those of you who are maybe more expert than I am would probably call this an isometric contraction, so I'm not moving any… correct me if I'm wrong, maybe it's not isometric. And as I keep it contracted, you actually see this increased variance and it seems that there are several oscillations. I just release and it goes away. I contract and I release. I contract and I release. I'm very proud that this works. I would say it took hours to do that. That's it. I wanted only to show that I can maybe zoom in, changing the scale, the vertical scale… that it's not going smaller than 20 millivolts per division, but what I can change is the time base, so how much time is per division by those squares. I can go much faster on a time base that is faster. And here you will actually see that the signal seems to have primitives that are not arbitrarily fast. It's not white noise, it's not arbitrarily going up and down. It seems to have some sort of dynamics of signals that are going up and down within a few tens of milliseconds. Each square is 50 milliseconds. Thank you.

[NOTE: A section of the lecture was not recorded here due to microphone failure. The transcript resumes below.]

Part 4: Future Directions: Computation and Bio-Hybrid Systems

Section 1: The Bionic Dream and Sensory Prosthetics …will be… you have to read the lips but okay, today is blah blah, you have seen it and I will try to find a way, presumably leaving the audio from the laptop and not having this one, so this is better and the audio will be a little bit lower quality but will not stop. I apologize, I charged it this morning but probably they cannot withstand three hours or four hours of class.

Okay, so… so even myself, I was particularly drawn to bioengineering or to the study of the brain for the same reason. In the 80s when I was a kid, there were ugly TV series like this one that was called *The Bionic Man*. It was an Air Force pilot that had a severe accident and because of the injuries, but he was so precious, the Secret Service said, "We can rebuild him." And they were actually replacing his visual cortex with an artificial eye, the arm that was amputated with a robotic arm that gave him super… like a fantastic force, the guy was… and particularly what was interesting is that they had an artificial eye connected to an artificial cortex. And okay, the legs, the limbs were trivial, okay, fine, you have robotic limbs, but it's okay. And then the guy was able to run at superhuman speeds. The funny thing is that they did the spin-off series, *The Bionic Woman*. Okay, this guy's title was *The Six Million Dollar Man*. It's ridiculous, particularly with today's exchange rate. The spin-off was *The Bionic Woman*, and they also had a mini-series on the bionic dog. It was fun.

So the story of being able to… we'll see later about motor prosthetics, but sensory prosthetics is another central point, and people over the past 50 years have made incredible progress. I don't remember, I think in November on Friday in Mirandola, we will host one guy working in Austria, in… I forgot the name of the city, it's not far from the border in Alto Adige. Okay, doesn't matter, and he works in a company that is doing cochlear prosthetics, cochlear implants, and making it possible for deaf patients to hear again. And here it seems to be a relatively easy problem because the cochlea is shaped like a *chiocciola*, from the animal, and it is related to the anatomy of one part of the auditory system, the periphery of the auditory system. And it is special because nature evolved it in a way that if you are able to unroll it—it's all wrapped on itself—but if you are able to make it a line, you have some sensory cells that have mechanical cilia, and these cilia are long or short depending on if you are in one spot of the cochlea or another. And you can probably guess that the longer or the shorter you are, you will change the resonance frequency if these things are sort of in touch with mechanical vibrations due to auditory sounds. So we have the tympanum and we have some liquid system and ultimately we have mechanical vibrations from the air related to these cilia. And depending on their length, they are able to perform as filters. They would not resonate… so the very short ones will not start to oscillate for my pitch of voice, but they would if, say, somebody would start speaking with a very high pitch, and they would because in that case, the frequency of the mechanical oscillation will be higher. You probably know that… okay, you don't anymore… you're not fond

of house music, it's a different generation, so I don't know what you do when… when you don't go to the disco, but there it's very low frequencies, very low bass sounds, and in order to resonate, being an existing relationship between frequency and wavelength, you need long oscillating rods or cilia to detect and to oscillate, so to filter out this signal. So here you have what is called a tonotopic organization. So it's "topic" meaning there is some place, a placement that is mapping tones, which is very surprising. You have, say, violins here and the bass drums of house music or whatever you hear today at the other extreme. So it's conceivable that if you have not just one electrode like in DBS, deep brain stimulation, but if you have an array of electrodes in a way, because you may want to stimulate different spots, maybe you can process my voice, decompose it by Fourier transform into fundamental frequencies and stimulate different electrodes electrically depending on where they are. And of course, since the cochlea is not straight, you may design your electrode array in a way that is flexible and it's also kind of resembling the anatomical structure. So and it will be roughly being able to replace the nerve cells, the sensory neurons that are no longer working. So you don't have this… well, they might still be there, but they don't work anymore. And if you stimulate here, the underlying neuronal circuitry would still be expecting to get activated, because they would expect these guys physiologically to get activated when the frequencies are bass sounds, instead of the other ones from the other extreme, violins.

And the result is surprising, and there have been so many advances in signal processing to make it possible to learn or to adapt the way that sounds are processed in order to be converted into a frequency domain stimulation. And as you can imagine, now I'm making it as if we know how to electrically stimulate nerve cells. Maybe this is a sort of very tiny electroshock, like it is the electroshock here. I'm just exaggerating, it's not an electroshock, they're very, very tiny currents, but it is as if I'm blasting a very loud sound at you, expecting that you will answer a question. You are neurons, the question would be answered by emitting, say, some sort of encoded signal by having an action potential like this or like that, and I shout, and you probably are responding something, but first of all, I'm not able to speak to one cell at a time. I shout, all of you hear me, and maybe I traumatize you. You are shouting back, you are startled, and you are shouting back. So we are far from being able to talk to neurons. The story that I mentioned before, using light and optogenetics, is a more elegant thing, because not only can you shout, you can excite, but you can also silence, something that you cannot do with electrical stimulation.

The same concept has been explored for retinal prosthetics, where instead of having just a monodimensional array, you have a bidimensional array, like a fakir's bed. Have you ever seen an image of a fakir? *Un fachiro, un letto da fachiro.* In India, there were people, if you Google it, you will see people sitting on a bed of nails. So a bed, not with a mattress, but with a lot of individual nails. It's a comparison that I do very often. And it's sort of similar to what, in this case… this is not to be implanted in the eye of a patient, but in this case, it's an array… take a look and pass it around… it's a bidimensional array of microelectrodes. It's so tiny, made with CMOS technology, that you can't see it with the naked eye. And the green stuff that you see around is a printed circuit board, and it has a lot of contacts, and you can individually activate different electrodes in the retina. So here, a surgical operation is inserting this array of

different electrodes on the floor of the retina. And if you have a camera mounted on glasses, you could maybe transfer by inductive coupling images processed on the camera to electrical pulses in the retina. And the guy is, of course, smiling because it was a promotional video from the company. But I think, if I'm not mistaken, this is the guy, or it's a different guy. This is the guy being able to see the moon again.

In the cochlea, I told you about tonotopy, so a tonotopic organization. In the retina, you have a retinotopic organization. So, points that are nearby in the world, just because of the optical properties of the lens, are projected… the light is projected to a nearby point in the plane of the retina. And so, if you stimulate nearby cells in the retina, technically they are called ganglion cells, then you are presumably eliciting some sensation, what are called phosphenes, of sensory stimulation. A blind person will actually see two small lights, two pixels, lighting up, and they will also be close by in the perception of the patient. So the concept is very simple and it's basically the same as a cochlear prosthetic device. Here, you stimulate depending on an image. Maybe the image was pixelated. And so if you stimulate different pixels, for instance, there is a light there that I see, and then there is darkness here, or vice versa. Here I see that it is bright, and here it is darker. I can stimulate a little bit stronger on the left, and then on the right, I stimulate less to give the same transition between bright and dark.

Something very interesting is that this company decided at some point, for reasons that are related to the market, which is another very interesting component that I hope can be discussed again on Friday, one of the Fridays of these colloquia in bioengineering at the end of December, middle of December. The last one will be one person that was responsible for these implants in Italy, and basically, they decided that it was not worth it. And to me, that is amazing. So, of course, I'm not motivated by and I'm not working in a professional, non-academic context, but to think that you, from one day to the next, you decide, "Look, the specific disease that these guys were suffering, Retinitis Pigmentosa, affects… it's a genetic disorder, it's a degenerative disorder related to a genetic condition, is only affecting a small number of people. So, there is no business." So, I don't know who was to make money. In reality, you could get… problems for making money. Because you would restore vision in blind people, which is amazing. I mean, isn't it the dream of everyone? Or you would be able to make a sort of artificial arm for an amputee patient. But then at least this was the situation about 10-15 years ago and Professor Silvestro Micera, I hope he will answer this question. We ran a project, a European project, and in this project, there was a big company called Otto Bock. It's a multinational but has its headquarters in Germany, and we were actually having them on board in this consortium of universities and partners. And they told me very clearly, "Fine, you can do whatever,"—at least 10 years ago, I don't know now if things are different—"you can do whatever robotic arm that you want, you can make a fantastic electrode-neuron or nerve cell or axon coupling as advanced as you want. I'm not going to sell it. I'm not going to sell it for you because health insurance in different countries will not pay for the robotic arms." The only thing that they would cover—again maybe this is different, it was like this 15 years ago—what they would cover is passive prosthetic things made of silicon that maybe have the possibility for a patient to, just with the other hand, contract it so that… so not something like in the *Star Wars* movie that would become in-

dependent, so you could control it by thought. And this was a very big surprise here. They decided that they didn't have a market, and this article says that the company withdrew, and there are still people with this device implanted. And it is like when a company, say, if Apple would go bankrupt, we would have computers and things for maybe a few more years, and then there would be no spare parts and no new computers. What they did, they did not go bankrupt; I think that they are focusing on a different approach that is more rewarding financially. That is, I'm not targeting only blind people because of that specific disorder of the retina, that can be Retinitis Pigmentosa, but also maculopathy, degenerative maculopathy. It's a very specific thing in the periphery, and maybe that's the reason why it worked nicely, because it's so simple. In the periphery, it works nicely. If you go higher in a hierarchy, it might be more complicated. I might be able, perhaps, to understand how to interface some sort of amplifier to the computer because I'm connecting via USB. It's a stupid comparison. I might have a hard time opening my computer and just operating on the motherboard. It would be a mess. It's so complicated. I don't have the schematics.

And here the idea is that they want to stimulate in the visual cortex. So even people that have, maybe they were born without eyes, they have a visual cortex. Okay, it will be more complicated, but they could receive this stimulator. So they would have glasses, and the glasses with the camera would transmit information directly to the visual cortex. How and where to stimulate in the cortex? Good luck. And particularly, so technology in terms of hardware, can be very advanced. Here you see another company… so this is, sorry, it's another company… it's a, if I remember correctly, it's an Illinois technology university. It's so small and this array of electrodes is even long, so they are not just like the one that you see, planar, that I'm circulating. These are able to go intracortically, they go inside the tissue, they don't stay in what is called subdural, so below the dura mater. That's a way to possibly stimulate different spots in the visual cortex electrically and obtain some sort of images, counting on the fact that you have retinotopy not only in the retina but you have it also in the visual cortex. The problem is that we don't know how to stimulate, what is the code. And another reason is that you may not want to electrically stimulate in a tissue that is excitable because if you stimulate a little bit too much, people could have a seizure, an epileptic seizure. And they, and patients had epileptic seizures during the clinical trial. Maybe it's easy because you basically stop stimulating, but the brain is a highly excitable tissue, so if you stimulate a little bit too much, then you have the entire cortex that goes nuts. So there are pros and cons.

Another thing that I invite you to think of is that if you have a camera in the frame of your glasses, and you're blind, okay, so you're blind, anything would work. You can no longer count on saccadic movement. Saccades are the rapid movements that we can do with our eyes, and you don't. Well, you could do that, but you don't have a functioning retina. So what you have to do is you have to move your head. And your brain is not used to moving the head while keeping the eyes straight. The eyes, particularly in a blind patient, would be constantly moving. And there are physiological mechanisms that are compensating for the fact that even if my eyes are moving, my perception is of a stable room. And even if I can move my head, there is a corollary compensation mechanism.

Thank you. Thanks a lot. So this is also something that is worth devoting some thought to, because it's not trivial at all.

Section 2: Motor Prosthetics and Brain-Computer Interfaces Let me move to motor prosthetics. Do you know this guy? Do you like him? I don't like him particularly, for many, many reasons. One thing is that he founded a company, Neuralink, and he started saying, this was several years ago, starting saying, "Neuroscience is over, we only need bioengineers." Okay, that would be good for you, but neuroscience is not solved. We don't know exactly how to make sense of electrophysiological signals. We know a little bit. And Neuralink made very big statements, promising, "We treat all diseases. Not only this, everybody will have, like in science fiction movies, *The Matrix* or *Johnny Mnemonic* and others, people will have a connect… well not necessarily a connector in the back of their head, but they will be able to interact with computers just with their brain, just by Bluetooth." And I don't know whether this is going to be the case, whether, yeah, you could maybe make sense of signals in my motor cortex if I'm a tetraplegic patient, and you can maybe decode if I had the arm, whether I would move it to the right or to the left. Maybe this you can decode, you can make sense of it, but forget about the complex multiple degrees of freedom. I don't remember how many degrees of freedom my hand has. It's probably about 20, 25, of that order of magnitude. And yeah, it's very complicated and we don't understand the motor system so well to say… "Of course, the guy is doing some… so I avoid that… so of course, this is encoding, we can decode his or her thoughts." Bold statement. And they also claimed that they were the first to have, last year, the first human implant. No, it's 20 years that people have done that in humans, even wirelessly. True, Musk, together with Apple, together with Samsung, they have access to amazing technology that would make it possible to do these things. This is just at the level of university consortia that don't have access to clean rooms where microchips are fabricated. And you know how small mobile phones have become. They have state-of-the-art hardware, but they don't yet have state-of-the-art neuroscience, because neuroscience is tough and it cannot necessarily be solved by injecting billions of euros or dollars.

So this is there, it was January last year. The problem is that by looking at specific parts of the brain in the motor cortex, a tetraplegic, paralyzed patient would have been able to use a cursor on a screen. By having electrodes connected to Bluetooth hardware, these are the electrodes that are implanted. So they are soft and they are not implanted by, like in some neurosurgical operations for deep brain stimulation, but they are implanted by a surgeon robot. I hope that it comes… It doesn't come. It's not this video. And of course, they call this telepathy, the first device. And I'm pissed off because this is clearly creating false illusions in patients. Sometimes, it doesn't happen so often, I have people writing via email saying, I've seen… so laypeople, so private citizens, writing to me and saying, "I've seen that you published this paper, could I bring you my mom that is suffering from Alzheimer's or from dementia?" No, I have no way to do it. Maybe what I do will make sense in 10, 20, 50 years. Instead, this guy is calling it telepathy, saying we cure all diseases.

So, I think it's this one. And in a moment, you will see that these electrodes are implanted. This is very cool, implanted by a robot surgeon that is able to

avoid hitting the blood vessels and thereby avoiding causing a hemorrhage. This is very cool and it's done by infrared illumination. Blood will actually… fine, it's wireless so you actually don't see it in these animals, and you can record and stream via Bluetooth tens or hundreds of channels, and each dot is a nerve impulse detected in the sensory area of the animals. Here it's in the motor areas of the animal, and what you actually see… what you actually see… why? I need one of these. So what you actually see is there are markers. They could be stickers placed on a specific place of the limbs or by, say, image classification algorithms, if you want deep learning algorithms, but it doesn't matter, you can process a video, extracting at every single moment where the location of specific points is. And the algorithms are so smart that they could follow across frames in the video where my, say, my knee is in different frames. So if you do that you could plot the angle or the position as a function of time. And here the animal is not paralyzed; the animal is walking on this treadmill. And at the same time, you're recording in the motor cortex in the area that you know is responsible for controlling locomotion, and particularly the articulation of all those degrees of freedom. So you have the ground truth and you have the signal that you know contains that information. And so you build a neural classifier by learning, by training, and you compare it and you say, "Look how cool it is." It is cool that you can predict with high accuracy—so the continuous line is the actual position and the gray, the lighter line is the decoded signal from the brain. It is fantastic. But do this for a patient that is completely paralyzed. You cannot ask, "Okay, can you do a few hours of video walking because I need to… or moving your hand because I need to train my algorithm." And you know that machine learning is successful because it is now fed with huge, large datasets. If you have no dataset at all, it might be more complicated. Let me run it again.

So here you could decode a paralyzed patient. In this case, it's a motor action of the limbs of the animal. It's not particularly relevant or crucial, but it's a fantastic proof of concept. And in a moment you will probably see not only reading, but writing. When you see those bulbs, those cells, lighting up, it's because one particular electrode has been electrically activated. And one thing that you notice is that we are very far, even with Elon Musk, we are very far from the dream of playing the piano keyboard. Imagine that you hear, I don't know, Beethoven's Symphony No. 9, and you say, "Okay, fine, now I would like to replicate it and I have a piano keyboard." Maybe you will never be able to replicate it exactly unless you are a very good musician, but at least you would like to press the piano buttons, the neurons, one at a time. You don't want to just go there with your elbow and press five or six keys of the piano keyboard. Here, you stimulate and you have a bunch of cells that are lighting up. It doesn't matter why they are bright. And this is, again, it's only a demonstration. So all these retinal prosthetics or cochlear prosthetics, sensory prosthetics, they are far from saying, "Oh, look, there is a small fly there and it's now changing direction, I know how…" We are very far even from the point of just the means of electrically eliciting a nerve impulse when we want. And this is the comparison with a keyboard, with a piano keyboard.

And at the same time, again repeating an experiment that was done 20 years earlier, here is the monkey. The motor cortex is again fed into an artificial… to a machine learning algorithm, and it's playing Pong. Pong from the 1970s, I

remember it, it was the first video game. And the animal is not having a joystick; it's a small tube where it gets the reward. And so if you're a patient that is paralyzed… Wait a second, let me show you this. This was in 2012. If you're a tetraplegic patient and you're paralyzed, it might be worth experimenting on animals. And this lady is controlling this two or three degrees of freedom with a connector. So it was 2012, it was not Bluetooth, it was with a wire. I'm just showing you all this because maybe I perceive that some of you were correctly, understandably annoyed by the animal experiments, yet this is the goal, it's not the animal per se.

So, motor prosthetics is not only a cursor on the screen but also this robotic arm. This is massive, it's just because it's convenient to use something, this robotic arm that is already available, it's a commercial platform. And I think here, 2008, so many years ago, a different concept would make a patient, a tetraplegic patient… now this patient unfortunately died for other reasons, he didn't die because of this implant, he died because he did not survive heart complications, I think. And he was using computers almost 20 years ago by his thoughts. He was replying to emails, and here it's simply a different concept. You have an array of microelectrodes, this is like a bed of nails, each can be controlled individually and you're listening to the electrical pulses. It was a question but I think I answered it already. If you could read thoughts, what would you do? And I think in all your introductions, there was the idea of using it for therapeutic applications, and particularly in the case of tetraplegic patients, basically restoring some form of movement. Again, if you can read the thoughts but you have AI or you have some classification algorithm, you could maybe hope to gain insight even if you don't know the code. Maybe you could train with a patient saying over and over: "Please do whatever you want, but think of, if you had your arm, moving it, say, north or south." And at the same time, I record the electrical activity. There are huge problems. When do I start recording? When does it make sense? Maybe I can ask the patient, "When I play a sound, you start doing it." But one thing is to see the monkey there. The monkey was just getting the reward and he was no longer acting on a joystick that was used before for training. But for the tetraplegic patient that is not moving, it's a big deal to infer what the neural control is because we don't understand thoughts. We understand why just a tiny bit of an area of the motor cortex is getting activated a little bit, and we think that we are capturing the movement, move to the right or move to the left. In reality, probably the motor cortex is maybe even planning the trajectory. Motor actions are complex. It's not just "I move right or move left." I control all degrees of freedom to get my actuator in one point. Only to overwhelm you with a situation of complexity.

Swiss and Belgian chocolate in the supermarket, we have the code. We don't have the code for the spike train that was measured a few years ago in our lab. We don't know what it means. It's a rate code, does it matter? Is it the frequency of this nerve impulse that tells about the information? It's also this, but it's also the exact timing of the nerve impulse. And how? We don't know. It depends on where. In your spinal cord, you have neurons that if you have one kilogram, they will start firing at one frequency. And if I add another kilogram, two kilograms, the number of spikes per second—spikes, I call them spikes because they look like needles, action potentials, nerve impulses—the frequency would double. So it would correlate with the weight. But this is in

the periphery. Very, very evolutionarily simple compared to motor planning in a primate. By the way, I'm standing as an inverted pendulum, I'm still gesturing and I'm not falling on the ground. It needs to be more complicated. So we don't have the Morse code, the Rosetta Stone, to make the comparison, what is what. This, we don't have. And yet, I think it's in this video here, I'll emphasize that the monkey… so this is from a pioneer from Duke University many years ago in 2008. I think it's a combination of these two videos. The guy is on YouTube and he has many, many talks and it was particularly impressive because he said, he described the surprise that the monkey at some point… here it's controlling the cursor on the screen with the joystick. At some point, the researchers basically swap and they say, "Okay, now we've trained the machine learning algorithm and basically we'll disconnect the joystick." And as the monkey continued to perform the task, what was interesting is that at some point—and the guy has a very strong Brazilian accent that I won't try to imitate—but he says, "We realized that we were making history because the monkey realized that he was no longer touching the joystick." And again, this is amazing. So the monkey learned to use a tool just by thought, realizing that it was no longer the hand, it was something else in the motor cortex that was controlling it. I show it to you because it's from more than 20 years ago and yeah, somehow we did learn a lot about this.

Section 3: The Limits of Current Computing and Neuromorphic Engineering Before closing, maybe I will give you at the next week… we'll finish a little bit. There is another very crucial perspective that is not therapeutic, it is more related to a novel paradigm for computation. You probably learned that the so-called… what is the name… Moore's Law of doubling the number of transistors on a chip is predictable, so every year or every five years, I don't remember, there is progress in integration and therefore miniaturization and therefore power consumption. Now Moore's Law is no longer… it's starting to fail because transistors are getting so small that we are hitting quantum mechanical effects. They don't work anymore because they are maybe one or two atoms and they are no longer behaving like classic physics. So at some point, forget about having iPhone 19, iPhone 20, iPhone 21. At some point, it's not going to work. We have to end up with what we have. So computer scientists would immediately rise up, maybe some of you as computer scientists would tell me, and that's why now you have architectures with multiple cores, because we are anticipating this. Fine. Multiple cores implies that you need to become proficient in parallel programming. And again, good luck, because it's a very hard field to make, to transform any algorithm to make it parallel. There is still no recipe, and it may be that some things could be made parallel but other things may not.

But the real problem, and I'll show you this… this is from a movie a few years ago, I don't even remember what the movie was. They built robots that were sentient, that were intelligent, and the guy was learning and was scared. Interesting, it was in a nice context of a, say, degraded area of the city, and the guy started bonding with criminals. It's fun. He looked for imitation from cartoons of the 80s or started painting. Yeah, so it was fun. So this is still a dream of building artificial systems. The problem is that we don't yet have an artificial system with the same performance as the human brain. For some aspects,

we are even beyond. For image recognition, we are definitely beyond human capabilities. The culprit, however, is the huge amount of power that you need to not only train but to run this device. I get by with three watt-hours from eating something, and you are probably starving now because four hours with me are heavy. For ChatGPT, you have on the order of two gigawatts per hour, and it's very intensive. There was a paper, an article in the news just reinforcing this story of unsustainable development. So the cloud, I'm sorry for the computer scientists in the room if they consider that to be… it cannot be the solution, it's not sustainable, even if you have nuclear plants that you are producing, because you need maybe 20 years to build one. And resources on the planet are starting now to already be less than what we need to operate our own civilization. So it's simply unsustainable.

There is another problem: the more you integrate electronics on a chip, the more heat dissipation is required. And in fact, you can only run a data center that is very large, maybe as large as this room, and to cool it down, maybe, you probably know Microsoft and many other providers are doing this, they are kept underwater, just in the water, for having a heat sink, just to dissipate heat in a better way. I don't… I can… my brain and your brains are happy, there are blood vessels that are definitely dissipating heat.

There is a sort of direction where bioengineering is going, which basically is skipping the application on humans, and it's basically taking the mimicry that has been very successful in nature. Planes, although they have not been directly inspired by birds, I'm pretty sure they were motivated because people wanted to fly. So here, it's a little bit more similar, so it's way more accurate. There is a field of engineering called neuromorphic, in which the same artificial electronic devices, or maybe even quantum devices, or even spintronic devices, are used or engineered with principles that are exactly the ones of how signals in the brain operate. So much so that they are able to mimic. In the early 2000s, there was one paper that appeared in *Science* entitled "A silicon neuron." It was one electronic circuit made of CMOS that was behaving sort of indistinguishably—although this is a very big statement—from a biological neuron. And if some of you are fond of playing with Legos, it's conceivable that if my brain is a collection of simple elements assembled in a complex way, maybe I can build in electronics neuromorphic circuits, which are a complicated assembly of elementary systems that are behaving and consuming very little power, like the brain. For those of you who are fond of or competent in or have reminiscences of electronics, digital electronics, ChatGPT, von Neumann architectures, use transistors as switches. Switches, and they switch between zero and one, and you probably know that there is a huge power load when you switch. Neuromorphic engineers use transistors as analog devices in a regime that is called weak inversion, and another way to call it, to identify how they operate, is called sub-threshold. So they use them as analog devices, for instance, to compute exponentials in hardware. You don't need to write a program; you have one transistor that is per se behaving as an exponential. Similarly, in a nerve cell, we will see it, one protein that is called an ion channel, it's a protein that is interleaved into the membrane of a cell, that one is a component, it's a protein, and it's performing as an exponential function. So this one-to-one mapping is inspiring a lot. And this is one paper that was published a few days ago, or maybe today or yesterday, where a neuromorphic system involving not a von Neumann architecture, not

deep learning, not backpropagation, not large language models, attention, etc., but involving spikes, is able to perform similarly to the visual system. I'll omit what this predictive coding and what this is about.

Section 4: The Biological Inspiration for Artificial Intelligence So, before closing in five minutes, I would like to emphasize two things you may not be aware of, but a large fraction of, at least at the beginning, how artificial neural networks started, and in general artificial intelligence, but more specifically machine learning, has been inspired by biology. The famous perceptron was invented by psychologists that were aware of how neurons were performing, that they were sort of all-or-none units and they were maybe emitting an action potential if their inputs were reaching some sort of threshold. Now from that point onwards, things diverged, because even the promise of building a circuit… this was John Hopfield many years ago, he got the Nobel Prize last year, probably you should know who he is. I mean, he's very important. He is the person in the 80s responsible for the end of the winter of AI. Marvin Minsky and others in the 60s killed AI, saying a perceptron cannot solve the XOR problem, so it's crap. Today we know that it's not entirely crap, but also because we have a lot of data and we have a lot of computational power. And Hopfield was, however, expecting or anticipating that we would have been building devices, physical devices, even materials that would perform like brain circuits. In reality, we end up having PyTorch, which is a very… it's a Python library and it's used, or yeah, I think it's the one of reference. So we end up having software, which again, it's not a very good idea because of power consumption. So the possibility of continuing to look at biology to extract principles is very, very timely and very relevant, because still, my brain is performing a variety of tasks and I don't need to see all of the internet in order to start speaking in a language. Admittedly, they are useful, but it's not… the holy grail is not going to be the current artificial neural networks.

I won't tell you about Neo, the character in *The Matrix* that is learning Kung Fu, because we are uploading knowledge. Maybe, that would be fantastic, but we are not yet there. The guy is able to have his cortex reprogrammed. We're not there. The guy knows Kung Fu. And unfortunately, we are not there. So, Elon Musk, sorry, but yeah, it's a very nice dream. Maybe what you did, that for sure, you advanced the hardware part, the technological part, in such a way that we can maybe now, if you are able also to release it to researchers, to experimental labs, maybe we can tweak it, maybe we can use it. Because so far, the best that you could do is moving a cursor like people have been doing for over 20 years.

Section 5: The Frontier of Organoids and Biological Computing I think I'll stop here. I only wanted to mention this. Some of you mentioned the possibility of having stem cells or biotechnology, some of you. Something that we are also starting to do in the lab, and it was in the news yesterday or three days ago on the BBC… So, some of you will know that it's possible from skin cells, also from embryonic cells, to take cells and differentiate them into nerve cells, to build what are called induced pluripotent stem cells, and then differentiate them into whatever you want. And it's possible to do it and build mini-brains, although it's wrong, we should not call them mini-brains, although

they do, they are called organoids. There is a company, if you go on this website, FinalSpark, they have an interesting business model. They claim, which is also my view and also the dream of some projects that we started in the lab, that in 50 years we will not only have to charge our phone, we will also have to give it a drop of sugar, because inherently there will be some biological component, a hybrid component that would absorb much, much less power and would have maybe some neurons, some biological neuronal network processing information and working at the same time. If you go here you will actually see live signals that are very similar to what we got in the lab.

Thank you for your attention, see you next week.

Part 1: Course Preliminaries and Announcements

Introduction and Technical Difficulties Ok, I'm sorry that last week part of the lecture was not recorded. Those little microphones have a limited battery and they left me stranded. If you see it during the break with the microphone, please give me a signal because I will at least try to save [the recording]. I have a second ambient microphone, which is not great, but since it was not offered by the university, I have to buy it at my own expense. To be sure, there will be a bit of an echo, but you are here, so it shouldn't be a problem for you.

Okay, so today we continue and we finish this introductory content that I'm quite eager to present to you because in this way, concerning brain signals, you have at least an exposure to what kind of signals you can get.

Attendance Tracking So that is the code for the tracking of your attendance. I emphasize it again: I don't really care. It's just for me for statistical purposes, to see or to disprove the hypothesis that for the first couple of classes you are brave and attending. As soon as I start writing some equations—maybe we start already today—you get scared and you don't show up to class. So please disprove me. It's not the QR code. The QR codes, I should remove them. The QR code is to download the app. It's the numeric code, alphabetical, **DC8R9**, that you have to add. So, if you have troubles during the break, I will put it on the slide again.

Special Announcement: Guest Lecture An announcement. On Friday, we are very lucky, because we have quite a superstar coming to give a class for the entire morning. The program, as you know, is in Mirandola. This is part of the Colloquia in Bioengineering, which is actually a course that I give for the second-year students, or for your colleagues who started last year. And just because he's the one world expert—you can google him and you will see that he's very, very famous. He's a professor at EPFL, not only in Italy, at Scuola Superiore Sant'Anna. He's doing neuroprosthetics, particularly in amputees, restoring not only motor function but also sensation. He has a number of publications in very important journals: *Science*, *Nature*, and so on and so forth. The only catch is that it's in Mirandola. I don't know about the shuttle bus. Your colleagues from the second year told me that it was maybe for 25 seats. And they are less; there are probably 15 people, 16 people. They were fewer last Friday. So you could try to sneak in, and you could try to be on

the bus and to try to go to Mirandola, or you try to travel to Mirandola on your own. Maybe you could ask the university to get refunded. There is the other logistic problem: the room that they showed me that will be next for next Friday has 25 seats. So if you all decide to come, we will not fit. Maybe we can have extra seats, but I would say I hope that I can invite him again next year, but you never know, particularly with very important people. They have a very busy schedule, so there is no guarantee. Yeah, please. No.

Part 2: The Scale of Neural Interfacing

Section 1: Neurons and Electrodes: A Matter of Scale There are still worse infrastructures. I have brought up the talk about the brain; you want to talk to neurons. And I ask myself, I ask for you, if you know how big is "big." So particularly, what is the rough size of a neuron, say the body, the cell body? Do you have any idea? How big is it? Nanometers? So, one nanometer… it's a little bit bigger. It's one order, three orders of magnitude larger. It's on the order of maybe 1 micrometer, 10 micrometers. The soma, the cell soma is around, I will show you in a moment, a micrograph is around 10 micrometers in diameter. And the very fine processes, the branches that branch out, the dendrites or the axons, they can be much, much tighter; they can be a fraction of a micrometer. But nanometer, no. But anyway, this brings up very interesting observations. Maybe you heard about the field of nanotechnologies and nanomaterials. It means that today we do have materials and possible devices that are smaller, a thousand times smaller than living cells. So in principle, you could think of answering the second question about "what about electrodes?" So in this case, I'm particularly referring to the deep brain stimulation electrodes, which are massive, as I tried to tell you. But I think in 10 years, electrodes, even those of Elon Musk's Neuralink, will become nanometer-sized. We can already build things that are nanometer-sized. One example, I don't know whether you've heard of it, is called carbon nanotubes, which are very tiny, and also silicon nanotubes and other materials, but carbon nanotubes are particularly interesting because I work with them, trying precisely to interface them with neurons. If you're smaller, like a Trojan horse, *Cavallo di Troia*, you could maybe think of putting something in the "stomach" of the neuron and maybe you're not injuring the neuron. And we will see why the injuring, the stabbing, this idea that I had that with an electrode you may puncture a neuron, will become maybe more delicate if you just have a very tiny needle that the neuron will not even know is there.

Now, any idea about these deep brain stimulation electrodes? Last week, I showed you the guy with Parkinson's that has an immense improvement in his motor symptoms. It's purely symptomatic, of course. And I told you that that technology is directly taken from heart pacemakers, with small modifications. So what do you think if these neurons are 10 micrometers in diameter? So, on the order of micrometers, what do you think these electrodes should be? Micrometers, but in reality, do you know how big they are? Just for you to think that people undergoing surgery, neurosurgery, to have these things inserted, they don't have a nanoscopic thing, but they have big stuff. Do you have any idea?

I'll show you, I think in the next slide. This is a sort of cartoonish reconstruction

of a piece of cortex. It's only one type of cell that is depicted; it's the one that I like most. They are the pyramidal cells, and they are pyramid-shaped because their cell soma (somata, if it's plural) is shaped like a pyramid, and then you have this very long apical dendrite. It's called apical. From Greek, "apo" means "far." Right now we are going into the wintertime and the planet is getting closer to the sun, in the perihelion. But in summer we go to the aphelion. Anyway, so it's called "a privativa." And here you actually see the basal dendrites. And the axon here is not depicted, it's not reconstructed. This is a real example of a cell stained with a color technique, with a dye technique, invented by Camillo Golgi. And it's from the visual cortex of a cat. And here you see, purely by geometrical comparison, the very tip of a tungsten electrode. So if this thing is around maybe, again, tens of micrometers, then the tip here of this—this is an experimental tungsten electrode; it's a needle, it's an antenna—in research labs, it's probably maybe one micrometer or less. But see, in a moment, coming from there, you will see a huge deep brain stimulation electrode if it comes… come on… Yes. So it's immense. And imagine the big damage that it's causing. And the electrode is not like here, the very tip, that has not been insulated. There was here a USB-C cable that I wanted to play with. So you probably see that here it's all insulated with the exception of the tip. The tip, I'm just making it simple, the tip is metal. But here, so here I can get electrical signals; here no because there is insulation. So here is the same; here you don't, it's insulated by some plastic or Teflon. Here you have just this huge ring that is metal. It's typically platinum, and this is the insulation. There are maybe more than one active electrode. So you see, it's huge, and it is a massive damage to the structure of the brain where it is inserted.

Section 2: Challenges in Neuroengineering So the challenges of the future, some of you will face, and they're still open in neuroengineering, are the fact that with these big electrodes, if you want to talk to the neurons, you're not selective. It would be like me shouting at you, instead of wanting to give a message only to one of your colleagues. Not because of privacy, but because I want him or her to receive and to listen to my voice, not the entire class. Otherwise, if I scream "fire, fire!" all of you will get panicked and will leave the room. Instead, I want only maybe one of you to leave the room, just making stupid examples. It's one of many stupid examples that I do, but it maybe gives you the analogy of why the stimulation is non-selective. And by the way, I cannot talk to a neuron on the basis of its genetic identity. If you are excitatory or inhibitory, it doesn't matter, provided that you are in the range of action of this extracellular voltage stimulation, or current stimulation, electrical current stimulation, you will be reached by the stimulus. Instead, I would love to talk only to those of you who are excitatory neurons. I can't today. Not with electrical stimulation. I can do it with optogenetics, but we will talk about that another time. With recording, if you want to listen, they are discontinuous and again non-selective. It would be as if during the break I start to listen to you, whether you are bored, whether you are hating my classes or not. I cannot, I can hardly distinguish your voices and I will hear a huge summation of all the voices. For diagnostics, for the EEG, electroencephalography, maybe I can live with that, but I'm more interested in understanding the information content of individual cells, and today I cannot do that.

And then there is an important thing, which is the fact that any tissue, any biological tissue, after you put in an implant, you might have a sort of inflammation and therefore tissue rejection of the foreign body. So the electrode starts to be immediately recognized as a foreigner and, as you know, particularly the brain has a very peculiar immune system as opposed to the rest of the body. The brain is encapsulated in the blood-brain barrier, which is easy to remember because it's BBB, blood-brain barrier, and it has its own immune system where you have cells that are called glial cells, *cellule della glia*. They immediately engulf whatever foreign object or bacteria or whatever, because it's so severe that if an infection occurs in the brain, you're not surviving. So evolution made it in a way that is so exquisitely reactive and so tight. So there is no rule. It's like in a cloud where you cannot enter, not even if you're invited. Well, maybe the blood-brain barrier can be fooled, but that's another story. Drugs, neuroactive compounds, are smaller than the pores of the blood-brain barrier, and they can enter.

And then there are issues with the information content. I have no code, I have no translation, no dictionary, so I don't know how to decode the signal. And something that is still not particularly developed, but it's a topic of bioengineering and neuroengineering in general, is the fact that you can also match experiments with mathematical model computer simulations. There is an entire course in the second optional course, only for those of you with a neuro-curriculum that I'm teaching. I started teaching it on Friday afternoon in the fantastic venue of Mirandola, just because your colleagues are already there.

Part 3: Modern Approaches and Modeling

Section 1: Advanced Electrode Technologies So the alternative, these are experimental directions that I would like to tell you about in one minute, just because I work on these solutions. Instead of having a massive electrode, maybe you could use an array of nanoscopic or, say, microscopic needles. So these are microwires, and these are even silicon nanowires that are even smaller. Here you see a comparison; this is an electron microscopy picture, scanning electron microscopy. You see that these things are so tiny, so the aspect ratio, which is the ratio between one size and the other of the geometry, is very high, meaning that they're very tall and very, very small, very tiny. They could actually penetrate maybe the membrane. So in this case, you don't see those that are vertical and they are where this neuron is sitting on. And in general, over the past maybe 10 years or so, some of these concepts have been used. So not only you have a piece of metal like this one and you keep it as an antenna, maybe you try to get closer to the speaker, you get closer to the neuron, but you want maybe to bring… this is inappropriate to say… So if the deity is not coming to the mountain, maybe the mountain comes to the deity. And so the idea is to have needles or protruding, three-dimensional electrodes, and you try to get closer to the neurons. There is one particular research that we play with in which, because neurons are so reactive, and so if you are not friendly with them, they will try to reject you. What we thought is that if we were to have some sort of structure that was decorated—so these are gold protruding three-dimensional microelectrodes that could be connected in the substrate—so if you decorate, if you attach to them, by some surface chemistry technique,

some protein that makes the neuron think that that's food, it's not gold, it's not a metal electrode (in this case it's gold because chemistry is easier), maybe the neuron can be brought to embrace, to phagocytose. Technically it's called pinocytosis, but all cells are eating; it's called phagocytosis or endocytosis. But of course, if the neuron tries to engulf or embrace or phagocytose, the electrode will not be able to be broken by it. So it's not a particle or bacteria that the cell can, or food that the cell is trying to encapsulate, and then internally it cannot break it. But in this engulfment, the proximity between the electrode and the cell will be exquisite, will be very small, and if you pass a small current, you are guaranteed that you are talking only to one guy, because it's that one that is hugging you, not all of them. And it kind of works. So here you actually see, it's a work that was, it's a European project actually that I coordinated and it featured many collaborators from many countries. And I'll make a long story short. So here, indeed, at least in vitro, that means that we did not do it—well, we did not succeed at doing it in vivo in animals—cells, rat cortical neurons, seem to embrace these protruding things. This is the lateral section of one of these mushroom-like shape electrodes. And what you actually see here on the top of these electrodes, these are carbon nanocubes, for reasons that I'm not telling you.

Section 2: Mathematical Modeling in Neuroscience So another thing that is very important is about the modeling part. So not only improving the technology, microfabrication, nanofabrication or nanotechnology, but also looking at mathematical descriptions. And we are going to use, to exploit, even in this course, mathematical descriptions to understand things. And maybe you are aware of this, maybe not. So this is just for purely historical reason. The equations that in 1952, two physiologists—so not engineers, not mathematicians, not physicists, two physiologists—wrote, it's a differential equation that describes, if you solve it, if you have a computer solving it, how the nerve impulse looks like as a function of time. So it goes up very rapidly, and then it goes down, and then it recovers, and then it goes to around minus 70 millivolts. And these two physiologists were so cool, they got a Nobel Prize in the 60s. They were so cool that they formulated an electrical equivalent circuit model. Of course, neurons are not circuits. They are not capacitors. They are not resistors. But you can have, by analogy, that the biophysics of cells is behaving with the same equations as the physics of electrical circuits. And this is very powerful, for instance, inspiring a field, neuromorphic engineering, where people are building electrical circuits that are mimicking neurons. Both are electrical devices, so why not have them in hardware?

This is one example of a big 10-year project that was concluded recently, a couple of years ago. It's called the Human Brain Project, in which individual models, mathematical models, with all this morphology, can be incorporated in a supercomputer, in a high-performance computing system, and all these differential equations associated to these hundreds of thousands of virtual neurons can be solved, and the communication between neurons can be studied. Here, it's a pretty movie where the color is encoding for the electrical potential. If it's red, it means that the membrane potential is positive, say plus 20 millivolts. If it's blue, it means that the membrane potential was minus 70 millivolts. Of course, people did more interesting things than this three-dimensional fly-

through, which is purely aesthetic. To some extent, it's not particularly useful, because the only thing that you can see here is that maybe you get red, and then you get bluish, and then, okay, here it's too short to see. So maybe it's some sort of global… because the entire network was in a kind of synchronization, global oscillations, just to tell you that people are using that.

Out of curiosity, how many of you know what a supercomputer is? Any suggestion? Can you tell the others? Can you tell what particularly is fancy or not fancy about a supercomputer? "Ma perché hai un microprocessore bello carrozzato?" (But why do you have a nicely beefed-up microprocessor?) Things faster. So the only way, and I told you maybe last week that we are already doing it on, even on mobile phones, we are doing a sort of parallel computing with multiple cores, and a supercomputer is a collection of nodes, literally computers attached with Ethernet cables in a network. And so if you run a simulation, that's your responsibility, you can distribute the job. So you can distribute the program across many computers. So, and how would you run a simulation of a piece of brain on a supercomputer, having said that it's a collection of hundreds of thousands of computers, of cores or processors? Any idea, what would you exploit in this parallelism of the electronic hardware? How could you make it work? In principle what people have been doing is having one neuron per processor. To mention how difficult or how computationally intensive it is to simulate only one neuron. So if the brain is a collection of things and they are communicating, maybe you could do the same if you have a collection of processors and the processors are communicating with an internet local area network cable. Just for you to be curious.

Part 4: Electrophysiological Signals: From Single Neurons to Networks

Section 1: The Visual System: Hubel and Wiesel's Discoveries Now, I would like to give you some examples of brain signals that have this electrophysiological component. And I'll mention, I will start with the work in the 60s of two researchers. They were both, I think, medical doctors. And the guy here is the author of the book that I suggested to you, that is in the readings, in the GitHub repository. I strongly recommend you to read it. It's very light. Probably it will take a couple of weeks if you read a little bit of it. But it's a tiny book. Of course, as a PDF, you don't have any indication of how big it is. So David Hubel and Torsten Wiesel—this guy is today 102 years old, 101 years old, I was surprised. I am happy. They both got the Nobel Prize in 1981 for their discovery on the visual system. And they made experiments looking at the electrical activity, at electrophysiological signals of neurons in a cat, anesthetized, and they were recording from the occipital part of the cat's cortex. It would be as if, but I'm not suggesting it, someone would stick an electrode into your brain, into your occipital cortex, while you're staring at this screen. They didn't have a video projector, but they had a system where they could, you'll see in a moment, project stimuli, light stimuli, say for instance a small white dot, and they could change the position of the dot, they could change the diameter of the dot, etc. Maybe they could show a bar, we will see, while the animal, anesthetized with its eyes open, was staring at them. And of course, one objection is clearly the animal is anesthetized, so you're not guar-

anteed that the brain is actually processing information in the same way, and this is a well-taken objection. On the other hand, sensory systems are normally not necessarily affected by anesthesia. What matters with anesthesia is that your cortex usually is like when you dream, particularly during slow-wave sleep, when you are in the second phase of sleep; you are basically disconnected from the sensory system. There is a station in between that is called the thalamus, and the thalamus is like a gate, and the gate is closed while you sleep. Possibly because if the phone rings, you are not necessarily reacting. The cortex is not… maybe the cortex can still dream, but it's disconnected from sensory systems, with the exception of one sensory system that is very ancient, which is olfaction. The olfactory bulb bypasses the thalamus. It's going directly into the cortex.

So I will show you some recording. Why the animals… and what they did, they had an amplifier, and they connected the amplifier to a loudspeaker. So if you have a very fast transient, you probably know that if sounds are made of frequencies, and Fourier proved this, and if the changes over time are very abrupt, it means that in the frequency domain you have a very broad distribution of energy. If it's broad, it means that it's also broad for high frequency, in the frequency domain for high frequencies, that means you will hear it. And you hear it as a sort of "tak-tak-tak-tak-tak." During the weekend I was playing radio and I was trying to make contact with Morse code. My radio has a decoder. Just to give you an example in which in this case you have small sounds that are short, "dit," and longer sounds that are longer and they are "dah," and you will hear it. And the guy in a moment is giving some numbers and gives his name. He says "operator is…" So here is different, because we don't know the codes that neurons are using. And we don't have two symbols. For Morse code you have brief and long, it's digital. You also have the pause, but that's another story. For a neuron, you only have when the neuron is firing. And the interesting thing is, okay, when does it fire? It could be that it fires not once, it fires a given number of times, and so maybe the frequency of firing is making sense.

Now you will hear about one neuron that is very close to the tungsten electrode, so we only hear its voice. We are sort of confident that we only hear from one neuron. And the animal is staring at the screen. So you will be staring at the same screen. And in this case the recording has been done in the retina, in the outer layer of the retina, which is called the ganglion cell layer. I'm here and I'll ask you to pay attention, to try to do, although you can't because you don't have the Rosetta Stone, the dictionary, like I tried to do during the weekend, tried by ear to decode the message. So here the question for you is just be aware, be concentrated, just to try to understand when this neuron is responding. And the neuron is responding with a sound. You will hear—it's why they call it neuronal firing, *sparo*—you will hear it in a moment. Second is, not only when or what is the neuron responding to—maybe it responds when it's dark, maybe it responds when it's light, maybe it responds with a particular shape. Maybe some of you heard about the story of the "Jennifer Aniston cell." A few years ago, there was a paper showing that a patient, a human patient, awake, with an electrode, had one cell that was only showing, only firing, only responding, when the patient was shown pictures of Jennifer Aniston. It was not always the same picture. It could be Jennifer Aniston at the beach, Jennifer Aniston drinking coffee, Jennifer Aniston in the early morning. So it was not the same

picture. And that cell was not in the visual cortex; it was more in the associative areas, say, in the frontal areas. Listen. Here is different. But the question would remain: Jennifer Aniston would not be shown, but what features of the stimulus are exciting the neuron? And so, if you are very advanced, you could start thinking, assuming, and I tell you that these neurons are performing some sort of information processing, some computation, what kind of computation is it? So here it is. No. I want to use that loudspeaker. This is me, and it's not a mirror. *coughing* I'm going to go.

Do you want to see it again? Or do you have some ideas? Go ahead. "Naturalmente." (Naturally.) In fact, that's why they were trying maybe to have an elongated stimulus instead of a focused stimulus. Maybe they tried to change the size of the stimulus. They tried to make it dark in the center and light around. And if you were careful, you could hear that the neuron was firing sometimes differently, sometimes not differently. Go ahead. No, the retina, ganglion cells. And in a way, being in the retina means that it's already a major complexity in terms of information processing. It's not like a webcam. It's not a pixel of the webcam, for sure, because you could see that it was doing something strange. It was not responding to… So apparently there was one area that was indicated by, so it was written, highlighted on the paper, on the paper screen. I cannot write on this screen, otherwise they will kill me. So that one is called the receptive field. And people, so they actually tried to search for it, tried to see what is the area of the world, in the sensory part of the world, where if you have a stimulus there, the cell is responding. Because if the cell is around here or here, it's not responding selectively to that stimulus.

Let me show you it again with some commentary. It seems that it needs to be there, in the circle. But it seems that it's also responding when the light is much larger than the small circle. At least now, with this, it seems to be the case. I'll throw some random ideas because it's very, very interesting. And they could do, of course they did some quantitative analysis, but it was just because they could hear and they could see these correlations that they understood what these neurons were doing. And then of course it's about almost 70 years of research trying to understand how this is done.

One thing that you notice is that the neurons are also responding spontaneously when there is no light. They are not completely silent. Normally, so they have a spontaneous activity. Let me very briefly… Sorry… Ah… Spoiler… So even when the stimulus is not around, the cell is still having some spontaneous activity. Maybe it could surprise you, maybe not. After all, we are 37 degrees Celsius temperature animals. So there is some noise, and even the systems that we are building in electronics, they are noisy. So it's not a surprise that if you don't do anything, still the system is responding. But it's not responding strongly. Something that maybe those of you who are maybe musicians will hear is that when the light is on, you may hear some sort of "zzzzz" and then the sound frequency is decreasing. It's as if you hear this. So there is some sort of what is called adaptation, some sort of slowing down. And this is interesting because it's in all evolutionary sensory systems. You also have it in your skin. You have sensory receptors in your skin. And if somebody would grab you from the back right now, you would startle. You would be scared. You would have a reaction. There would be a reflex arc, *un arco riflesso*. It would be perfectly normal.

But of course, now you are probably wearing your shirt or whatever, t-shirt or whatever, on your shoulders for many hours. And you're not completely aware of it, but you still have it on your skin. It's because your sensory cells, in that case they are mechanoreceptors, here they are not light receptors because these guys are not really, they're not light-sensitive per se. It's the last layer of a chain of processing, but these guys are also losing interest. If the light is there for too long, at the beginning they respond, but then it relaxes. Because maybe evolutionary what matters is if a tiger enters the room. So it's the change. When the world changes, then I have to take notice. If the world is stationary, it's boring. But that's another story. And there are things that you might hear. So when the light is off, still you hear spontaneous activity. But look what happens now. So it seems that if you have light all around, but in the middle, in the same receptive field, you have darkness, then things are a little bit different. Could you understand? Could you not? Could you hear it? What was the difference? No, the retard that you feel is because when they are on the stimulus there is a rebound and the cell starts to fire. I will show you why it is crucial. You have to feel what you do immediately before the stimulus is given. No way. Could you hear it? It might have shut off. So if you have dark in the center and light around, it stops. And if you have one particular orientation or another one, it's the same. So any more ideas? Any other things that you noticed? You heard it on this slide, you have all the comments that we could make.

So, one is that there exists a space in the sensory world, in the world, that is called a receptive field. It's a field that, if the stimulus is there, the cell is responding to. So, you could probably imagine that other cells, so other neurons sitting nearby, would respond to a different receptive field. There is no orientation selectivity, suggesting that there might be other cells, maybe not in the retina, maybe there are other cells that would have some preference, some preference for a particular orientation. Here it's bright and here it's dark. So maybe it matters if you have these contrast differences. And the cell is firing nerve impulses, even spontaneously, but at a low rate. And another thing that was interesting is it's not doing it as a pacemaker, but it's doing it like a sort of Geiger counter, like in radioactive Geiger counters. It seems to be random. It's a random process. And just opening and closing a parenthesis about this randomness: Even Mandelbrot, the mathematician who studied these Mandelbrot sets, these beautiful things, fractals that you might have heard of, he was also studying neurons because of their stochastic properties, the fact that they are irregular and they are kind of unpredictable. And technically, the best model is a Poisson point process, for those of you who have vague reminiscences of stochastic processes in probability theory.

When the light is in the center, it fires, but when the light is around and in the center at the same time, there is a little bit of suppression. Sorry, no, no, sorry, sorry. This means that if you have the black spot in the center, so if the light is in the surround… So, we have a distinction between surround and center. It's a sort of ring, and then the center can be… so there can be light in the surround and darkness in the center, or vice versa. And if it's vice versa, it responds. If it's instead darkness in the center and light around, then you have suppression. So, yeah, the cell is firing normally spontaneously, but when darkness is in the center, it actually is also even suppressed, even below the spontaneous rate. And the cell fires if the light is everywhere. And something that I told you, it's

not… if I should depict it graphically, you will see that there is some sort of fatigue. So the rate, number of pulses per second, number of sounds per second, is decreasing in time. Please. Of course, of course, absolutely. The image that you see of me and of your colleagues is the result of a population code. Each cell is like this. It seems that cells are highly specialized, but cells are connected in a network. And the connectivity gives rise to these emerging properties. This one, it's only isolated, and we could understand that this one was specifically caring about that receptive field, and it was what is called a center-surround cell. And in this particular case, this one is center-on, surround-off. There are other cells in the retina, and the ganglion cells that are the opposite. They will be center-off, surround-on. So they would fire with a different logic.

And in the story of on and off or this center-surround, the fact that you have contrast, darkness around, darkness in the center, or if you have darkness in the center this one was suppressing, instead the other type with darkness in the center would be instead more excited, is the basis of one typical operation of image processing that maybe none of you today with this generative AI models, with Sora, with Gemini, whatever model for generating images, you probably never played with Paint software or Photoshop. But maybe you did, I don't know. Okay, something that is also important is that switching off the light causes a rebound. So when the darkness was in the center, the cell was silent, all was silent. But then when the light was removed, when everything was removed, there was some sort of rebound. So before there was some spontaneous activity, and also later there was spontaneous activity, but during the presentation of the darkness, there was silence. And then here there was a little bit more, like a rebound, like when a drug addict is not consuming his or her drug, then, when you remove this inhibition, when you disinhibit, it has a rebound. It has some sort of abstinence crisis. Here, in engineering terms, you can see that this is a sort of high-pass frequency filter. So, it actually doesn't want to respond to some steady input. It wants to take the derivative, and you probably know that the derivative of a step… Maybe you don't like the step. Assume that the light is switched on with some ramp. You know that the derivative here is zero because here it's constant in time. Here it's also zero because it's constant in time. The only time when the derivative is non-zero is here, when there is a peak. So these cells are doing derivatives. And of course, when you are switching off, so here you also have some sort of derivative. So these cells are still a high-pass filter and they respond to the change, to the transitions. And other cells are showing the opposite.

About computation, did anybody play with Photoshop? Or with any image processing things? Did you ever apply filters? There are filters to extract things. So there is one thing, if you can, if you have any software that could do some elementary image processing, try to play with it. If you apply a spatial derivative filter, which is enhancing whenever there is a contrast difference, whenever there is a light and dark, you extract the edges. So if you have a picture of my face, you will actually process it with this. Maybe I can try to do it, but I don't have Photoshop on my computer, but I will try. You end up with a picture, with a new picture, in which whenever there are areas that are not having contrast changes, they are dark, because in space the derivative is a derivative of something that is uniform, like here the derivative of a constant in time is zero. The derivative of an area that is roughly uniform is zero. But what matters are the edges. And

indeed, you would extract the edges. So the retina, it's an oversimplification, is an edge detector. Here you don't actually see it because with the edges, you would actually say, "No, look, I would like to have an understanding if the edges are vertical, are horizontal," because if they are vertical, I could probably say, "Oh, this guy has a big nose and it's vertical. It's in the middle of the face, so that must be a face." And by the way, this seems to be the way we recognize faces, but not in the retina. So for me to enter the room, for me it's very important to see vertical transitions, bright and dark. And something that is interesting is that retinal prosthetics, this artificial device that was implanted in the eye, since it was stimulating ganglion cells electrically, probably was getting for free some spatial processing, so these edge detectors. And so it's a contrast detector. It detects edges. Because in the end, you could probably… it's boring to see, with the exception of when I write, it's boring to see or to devote energy or information, even metabolic energy, to the world, to represent the world in your brain when everything is uniform, when it's not interesting. You have the impression that the brain is filling in, so you actually see that there is a wall that is white, but no cell is responding to the white. The cells, you have cells that are responding to the transitions, which makes sense because you have very limited resources. If you have to build a system with a few hundreds of thousands of detectors, maybe you want to optimize and not have something that is pixel-by-pixel related to the intensity; you want a way to extract some information that is relevant for behavior. I'll stop here by asking, and we'll start later, how if you could, even if you know nothing about how the retina is wired, but imagine that you have at least one layer that is photoreceptors, like a camera, like a CCD camera, pixel and pixel and pixel, how would you build the circuit for a ganglion cell to receive this information and to relay it downstream, to extract edges? How would you do that? Knowing that in the center there is a positive response, it's a sort of excitatory response, and in the surround you have some other field, and if you have light in the surround but not in the center, you are inhibiting the cell. We'll stop here for 10 minutes. Thank you. Thank you. Thank you.

Section 2: The Neural Circuitry of Center-Surround Fields Okay, let me restart. Let me again ask you the same question about how we build it. How we build a system that is center-on, surround-off or vice versa. So, let me say that I have this… This would be the photoreceptors. Receptors of the retina. So here, maybe you can imagine that there are pixels. And here you have this neuron that is the one that you were recording its electrical activity from. And there is an area of the world on the screen that was projected through the lens inside the retina. So you could say the receptive field on the screen was actually mapped onto an area that was probably circular, because the lens is a linear system, and so unless there is aberration… Okay, it's not an ideal lens, so there will be some non-ideality, but on a first approximation, a circle in the world would be a circle projected in my retina. So you will have different pixels, different sensory photoreceptors. By the way, they are called cones and rods. *Coni e bastoncelli*. I believe that in elementary school, in primary school, you heard abundantly about that. I don't care about the… of the eye. I don't care. What is cool is that the cones, if I remember correctly, those are for the colors, are specialized in red, green, and blue, which is the typical… from the medical

bonus that you regulate. Again, if you're playing with Photoshop, this is what you play with. But that's another story.

So how would you make it in a way that… so this would be the surround, and again here you have a lot of other cells, and these cells are, they have axons, they have output wires, okay, and they are like all the neurons, they are very eager to connect, because being in isolation is a crack for people, cells, societies, countries, etc. And so, how would you wire, what would be the wiring diagram of this system, which I anticipate is not correct, but it doesn't matter. Just for you to do the exercise. So what you have is the possibility to distinguish between synapses that are excitatory and synapses that are inhibitory. An excitatory synapse would be a synapse that, if it's activated, will excite the target. For the moment, I will not tell you about… Maybe Zoli might have started telling you about biochemical signals and might have already told you about neurotransmitters, excitatory and inhibitory. Did he do so? Yes. So you know already about the story. So why? No. So glutamate and GABA, gamma-aminobutyric acid, are two of the main central nervous system neurotransmitters. One is excitatory and one is inhibitory. So you have that some synapse could, you could choose it. So you could have excitatory or inhibitory at your will. This may not be entirely correct, but that's what we'll talk about later. And this is your target. So this is your post-synaptic cell, post-synaptic neuron. And this is the ganglion cell that we recorded from. Hubel and Wiesel recorded from it. So this is… It's in the ganglion layer. It's a ganglion cell. And this is, for instance, surround-on, center-off. No. Surround-on, surround-off, center-on. That doesn't matter. So just if you tell me that here you want to have light, when you have light here, and these guys are activated, I want this guy to be excited. How do I do it? How do I connect? Can maybe some of you come to do that or you tell me how should I wire? So here I have axons. Give me some ideas. What's the difference? What's the difference in terms of cables at this level of understanding that I'm trying to pass to you? You have processors and you have connections. Connections are, I'm inclined to think they are feed-forward, so they are not recurrent. You don't have bidirectional connectivity, and they are feed-forward. So it's like the perceptron, although maybe you know or you don't what the perceptron is. You have some sort of algebra, that you have excitatory, which is making the post-synaptic cell go up, and inhibitory to go down. Down in a supposedly excitatory, excitation state. You want to favor or you want to depress. If I have an inhibitory synapse and I'm projecting to one of you, if I'm excited because you shine light on me, but the synapse is inhibitory, when I'm excited, I will secrete GABA and my post-synaptic target will be depressed, will be silent.

So what I'm trying maybe to inspire you to do is to see whether maybe you would connect the center with excitatory synapses. And the surround, if the surround is off, I made a mess here, okay, where the surround is off, so if I have light only in the surround, like the one that we have seen, if the light is here, these cells of the retina are excited, but this guy is silent. So I was hoping that maybe you could have said, "Okay, so this cell is going to be excited." It's a photoreceptor when light is invading it, but the axon is projecting with an inhibitory synapse. So all here are inhibitory synapses from the surrounding, and from the center you have the plus. You can try to think of this conceptual model, and it works. At least it works explaining some of the observations, not

all. And in fact, we know now that this is not how things work in the retina. But it's interesting because just by a simple algebra, you could have that some projections are like on a car, are accelerating, others are like brakes, and they are stopping the same neuron. The post-synaptic neuron, you can think of something that is making the summation. You could think of a capacitor, and it would not be a wrong assumption, that is summating inputs. The capacitor, as you know, it's an integrator in time, it's integrating the signals, and it's keeping memory, right? In the charge, in the plates.

And so, in order to get this, this is the same thing, but not with sound, but with traces. If the light is in the center, you see, before there was spontaneous activity, then here, this is when the light was switched on, the frequency increases, and here, if you squint your eyes a little bit, you see that here, the number of small pulses is with higher density. So there, I don't bump on the table, but I bump here. So it's… So here it's higher spikes or pulses per second, and then it's relaxing. Here it's… okay, here when you switch off, it goes back to spontaneous activity, which has even lower activity. Here, when the activity, when the light is in the surround, you see here it's truly silent. Well, not entirely, there was one random pulse emitted, but it's really suppressed. So it means that when the light is in the surrounding, you probably have to invoke some inhibition, some inhibitory mechanism to brake the same post-synaptic cell. So the connections, the wiring, might determine the function. And here is if you have light on the entire thing, what happens is that you have both at the same time, you have activation of both the excitatory and the inhibitory synapses. And the fact that here you have more spikes means that, okay, these synapses from the center are either more numerous or they are more effective, they are with a stronger coefficient. So yes, true, you are doing simultaneously the averaging, the arithmetic combination of both the excitatory and inhibitory inputs. Excitatory, I imagine, like positive numbers. Inhibitory, there will be negative numbers. Okay, here, the excitatory numbers are winning. Fine. Not particularly shocking. What was important for me is to push you at least once to try to think about these circuits.

Section 3: Higher-Order Processing in the Visual Cortex Now, I'll show you what happens if you record in the visual cortex. So, a couple of stations, of relay stations… I'm skipping the thalamus. In the thalamus there is a region that is called the LGN, doesn't matter if you know it or not. In the Hubel book, *Eye, Brain, and Vision*, *Occhio, Cervello, Visione*, it's indicated. So you have all these things explained in a very nice way. Here we are skipping the thalamus. We are directly recording in the primary visual cortex. Because as you can imagine, there is a hierarchy of areas in the cortex for vision. Like in deep networks. By the way, they were directly inspired by the visual system. So here is a cortical cell. And again, the question is the same. What is the feature the neuron is responding to? Where and what is it? And again, what are the specific features that are making the neuron fire or maybe be inhibited? And what computation? Is it the same computation? No? No? No. No. Okay. It will get a little bit better in a moment in the video. It's from the 80s or earlier. So it's actually putting some plus or some… So even here, you have a receptive field, but it's not circular. You have the same thing that when the light is on, you have a lot of spikes and then there is adaptation. And it seems

that if you excite a sound in the periphery, it's sort of silenced. Okay, it's not writing minus, but this is like the surround-off of a moment ago. There it was a ring, here there is a direction preference, also for the off-response. It's a little bit more difficult to hear because you have less spontaneous activity here. It doesn't work. You need to have the exact orientation, otherwise you excite both, you illuminate both the on and off centers. So it needs to be 45 degrees. If it's like this, the response is much less. So there is a tuning preference. The quality of the stimulus has to be tuned to the correct angle. If it's everywhere, it means the "off" part. And the fact that here you hear the rebound is because you are in the off part. Now you are both in the on and off part. So the on wins.

So this is called simple cells. There is one orientation. This cell was specifically responding to that, and this is another sort of silly cartoon for a wiring diagram having different ganglion cells, so these are different ganglion cells, each with its own receptive fields, they are particularly aligned on the same line. So, because these four cells have receptive fields that are aligned, if they all project with an excitatory or inhibitory synaptic connection to this cortical cell, then you will get this preferential direction-selective cell.

This is another example, which is more interesting. It's still in the visual cortex. Let me know if you understand what this is doing. What is this doing before the guy writes something that will spoil it? Could you see it? Only seems to be direction-selective… Ok, I spoiled it. Not only is it orientation-selective, but it's also direction-selective. It's only when the stimulus goes from right to left that you have the response. In the other direction, it doesn't work. It only works if you have something moving in one direction. You can try to think whether you can do a sort of circuit, a functional circuit like this. You will see that it's not trivial because what is happening here is that you have time. You have movement. So it's not that straightforward. You could try. It could be an interesting mental experiment. So in this case it's both orientation and direction-selective. Which is very cool.

Something that I mentioned last time, I wanted to clarify, I don't know whether… So I mentioned retinotopy as well as tonotopy. Tonotopy was in the auditory processing areas of the brain as well as in the cochlea. That's why cochlear implants are doable, are not easy, but they are doable. Retinotopy is the fact that points nearby in the world are projecting to points nearby in the retina, and they are, I tell you now, projecting to points nearby in the visual cortex. So in principle, if you want to restore vision in a blind patient, you could think of having a camera, and if there are two pixels of the world nearby, you could stimulate two points in the cortex that are nearby in the cortex. There is a small caveat which is interesting enough. We are over-representing what is in the exact center. If you keep your eyes straight, it's called the fovea. So it's not like a CCD camera. This one is 4K. It means that it has 4K, 4000 pixels, or 4 million pixels, it should be 4 million pixels, and they are uniformly distributed in the plane of the sensor. There is no area in which there are more. If you had it, you would have a hard time displaying the images because you don't have more pixels and your screen would need to accordingly have more pixels in the central part. We do that because when we read or when we have to do something very carefully, we need maximal spatial resolution. And so the only way is to have the largest number of photoreceptors. But in the periphery, you

can try, I cannot read. I cannot read these letters if it's, not only because I wear glasses, but I don't. I see that there is a bottle, and that's maybe okay for if a tiger enters the room while I'm looking at you. But if I want to read, I have to bring it into the fovea where I have the maximum resolution. In the cortex you have this sort of deformation which I'm not telling you about here. Many, many years ago they did an experiment with a macaque monkey staring at a screen while being anesthetized. So the gaze was fixed, there were no saccades, there were no movements, it was fixed. And immediately after they could process the cortex, they sacrificed the animal, and immediately after they processed the tissue and they could see where the areas of the visual cortex were most metabolically active. So they could basically have some sort of imprinting of the image, at least radioactively, to tell what's in the cortex, where the neurons were that were most active. And this, I admit that it's not a technique, or it would deserve more explanation, but it's not worth it, it's not crucial. There is some sort of deformation.

Section 4: The Brain's GPS: Place Cells and Grid Cells I'll show you another example of electrophysiological signals. These are more higher-order, they are not related to sensory systems, and they resulted also in a Nobel Prize a few years ago, it's about 10 years ago. John O'Keefe is a UK researcher who first studied the hippocampus and found something very cool that I'll show you in a moment. And then two Norwegian researchers that were, before they were a couple, Moser and Moser, who studied similar experiments in the entorhinal cortex. And they were, as you can see, this is not about vision, it's about behavior and cognition and navigation. And it was worth a Nobel Prize. But again, I ask you to pay attention. What you will see is not an animal. We will not be recording with electrodes attached to a loudspeaker. You will actually see the top view of a small arena in which a rat is moving. And the animal has one electrode implanted in the hippocampus. So the animal is not anesthetized. He is awake and behaving. And whenever there is, in this recording, a nerve impulse, just because I'm recording the video, I actually put where the head of the animal is in that moment, I put a small red dot. So if the cell, if I'm here and the cell is firing, then I put a red dot so that it will stay in the plane of the image, it will stay that when the animal was here, the cell was active. It will make sense in a moment. So pay attention. I'm asking you whether you can understand when or what this neuron is paying attention to, and what computation it might be useful for. Computation meaning maybe what kind of behavior, what kind of something that maybe you do, maybe not every time, every day, that you probably are doing.

So this is the top view. This is a big rat. And you see that the red dots are at that moment in time when there was a pulse recorded by the electrodes implanted in the hippocampus. And the experimenter is giving some treats, some food, so that the animal is exploring an otherwise boring arena. Typically after a while, animals are not exploring; they are getting bored. And there is one specific receptive field, if you want, that this hippocampal neuron is responding to. What is it? It's relatively easy. Any idea? No. I'd say I have no time, maybe I can replay the video, but it's not so complicated. Say now even when the hand is not there, ok, it's difficult because it's accelerated, but even if there is no one in the room the cell would perform the same way. It's only that the

rat may not be willing to move around. So it's only when the animal moves and explores the arena that something in the hippocampus happens. You know that you can even answer, even propose, I don't care, even if it's a crazy idea, I'm not saying, I'm not judging and remembering, "Ah, this guy was at the exam…" I don't care. I would love to invite you to think, to be critical, to formulate hypotheses, to try to be curious, to feed your curiosity. "Potrebbe avere ragione, ma non è." (You could be right, but you're not.) "E qui, purtroppo, la mano è disgraziata perché arriva sempre dalla stessa parte." (And here, unfortunately, the hand is a nuisance because it always comes from the same side.) Because you are thinking about the visual system, but it is not.

So these cells are called place cells. And the Nobel Prize was given and in the news—you were probably not even born, or you were born ten years ago, you were younger—and they said, "Found the Nobel Prize because…" because people explained the GPS that is inside the brain. GPS is the global positioning system that you use with your mobile phones maybe to find whatever pizza or ice cream place, *gelateria, antica gelateria*, whatever, where you navigate. So your hippocampus is specifically helping you to navigate because it allows me to orient myself, and there is one hippocampal brain cell in my brain now that is firing and it was not firing when I was there. When I'm here another cell is firing. So at every moment I know where I am. It doesn't come for free. I need a few minutes to explore the surrounding because I need to create, to integrate information about visual, maybe other sensory modalities saying over there there is a lot of noise and maybe there is a very nice aroma of chocolate or perfume, here there is instead a bad smell. All the information integrated gives rise to these place cells and it's surprising, it's fantastic that you have neurons encoding space. The… okay I'm not saying the Euclidean space but it's… it's amazing. And to make some sort of circuits like this for place cells is a much more, it's a harder endeavor. If you have 20 minutes, there is a nice TED talk from a few years ago by Matt Wilson, who is a researcher at MIT, that is also telling you that when you sleep, you are replaying the sequence of activation of place cells. While the animal, or even myself, is creating, is moving, is navigating in the environment, different place cells are activated in sequence, and when I'm asleep, I'm replaying the sequence. I'm replaying the sequence of activation, because this is, maybe it's something that I have to consolidate. I have to sleep so that memories are consolidated. Spatial memories. I need to go there, because there might be some chocolate, or there was a nice smell, or a perfume or whatever, and I might need to concatenate, correlate all the place cells that finally led me to find here the chocolates, and I was really rewarded. I have to remember the street. If you use a mobile phone, you are probably not having this. It's very cool because when you are still, when the rat is still, or when it's in non-REM sleep, you have this replay of memories. It was particularly interesting, I don't remember whether it is in this talk, is a collaborator of Matt Wilson that I worked with, I collaborated with when I was in Belgium. The animal, still awake, not sleeping, was replaying the sequence and people could see the sequence of cells replaying, possibly because the animal had to plan ahead where it wanted to go. Extremely surprising because it deepens the understanding of the mechanistic functioning of the brain.

I'll show you another one that is in the entorhinal cortex. Same exercise. It's a little bit more complicated, but it's still related to space. And I wonder whether

you see it. And this is particularly interesting. It is still for navigation, but you will see that it's interesting. Here it's a mouse. It's not a rat. And it's a smaller arena. And it's the same drill. I don't remember whether now there is food. There is no food. The wire that you see is because the electrodes are connected. And here you see again one small dot that is added to the image when and if the animal was there, there's a spot, and the recording electrode was detecting a nerve impulse. So clearly it's not one preferential spot, like a corner or like the front of the board here, of the desk. And after a few… this was… it's truly remarkable. It's a Nobel Prize and they got the Nobel Prize. It's amazing. For those of you who have some interest in science, or mathematics, or geometry, here it's surprising. I'm not, of course, it's not my field of research, but it's surprising that in the brain of an animal, you have some element of Euclidean geometry. Do you see what kind of, sort of, graphical, geometrical shape is resulting? So what I'm trying to delineate here is that it seems that these blocks of activity are not random, it seems to be at regular spots, and I could maybe build either hexagons or triangles. And maybe some of you, particularly if you had to renovate your kitchen or maybe your house, you know that in mathematics there is this idea that in order to cover a surface with a given geometry, I might have, depending on the geometry, if it's a triangle, if it's a hexagon, if it's a pentagon, I might have an easier or more difficult life to tessellate, *tassellazione* of a surface. And the triangle or the hexagons are the primitives that are able to tessellate, so to subdivide in domains, in regular domains, any surface, any flat surface, of course. That's why I kept saying a couple of times Euclidean space.

So these cells are called grid cells, for obvious reasons. And the grid, this is one cell. One cell has this tessellation, and another cell will have a tessellation that is might be rotated or scaled or translated, offset. It's what is sort of giving the underlying substrate for place cells. So it's only because you have these that you can get place cells. And again, you could, if you have grid cells, of course it will not be the retina, it will be some sort of space-effective domain, you could try to see how grid cells project to place cells in this sort of diagram, this sort of wiring diagram.

Section 5: Network Activity *in vitro* Something that we will also see is that electrophysiological signals can also be recorded from isolated portions of the brain. In this case, these are recordings done a few years ago in our lab, in which you don't have a rat, you don't have a mouse, you don't have a monkey. You have a bunch of cells that have been removed and digested enzymatically. They've been cultured. You probably know about cell culture. You've heard about cell culture. And these cells are plated on a chip, basically, like the one that I showed you last week. And these small things that you see here, small dots here, are the cell bodies. You don't see the dendrites and the axons because they are very, very highly packed. They are very tightly… the concentration of cells is very high. And because this is a microchip, these are metal electrodes. It's as if the chair where you are sitting is made of metal, like the example of an otherwise isolated antenna, an isolated conductor, and only here you have a metallization exposed, exposed to the electrolyte, or exposed to the bottom of some cells, like your bottoms. It's all very elegant, and when the cell is firing an action potential, it's not me with an electrode trying to pinch or to get closer to

the cell; here the cell is sitting on my electrode. And for instance, in this case, the cell sitting on top of this electrode, or maybe nearby, was, again, producing over a time scale of maybe a fraction of a second, producing a series of nerve impulses. Something that might be capturing your eye is that this impulse, it seems to be a negative deflection. Negative deflection makes me interested because it's a… Let's say some of them seem to be smaller in amplitude. So here, maybe, it's like having a microphone in the room. Let me actually see if this guy is still recording. Yeah. So it's like having a microphone and having two people talking simultaneously, but one guy is a little bit further apart. So there is attenuation; there might be some attenuation like sounds, or like charge, or like electrical potential. You know, there is some sort of attenuation with distance. One over the distance, one over the square of the distance. We are going to see it and discuss it or refresh it later today. But it's interesting that if I have one neuron here, and I have one electrode, so let me connect this electrode to an amplifier. Just because I want to impress you that I'm an electronic engineer, so I remember how to make amplifiers. That's all. We don't need electronics for the moment. It's interesting to see that this electrode is recording a signal that is like a negative deflection. So this is zero. It's probably zero microvolts. And this is probably minus 100 microvolts. So it's very, very tiny. You need a big amplifier to analyze it.

In solution, although we are going to discuss it, in solution you have, as you probably know, you have ions. And these ions can be positively and negatively charged. In the electrode, if this is made, say, this electrode is made of platinum and this cable here is made of copper, Cu is copper, right? Okay, so here you probably know that the charge carriers are electrons. Fine, okay, but it's interesting that here ions, say for instance you might have sodium ions and chloride ions, or you might have calcium. Okay, calcium will have double valency, but you might have positive and negative ions. Here the antenna is recording a signal that is negative. Why would it be negative? Well, why should this part of space be negative? Think of it. We are going to slowly approach this apparent mystery. It must be something about the distribution of charge inside and outside in general, and during when the neuron is excited and it emits a pulse. It's very interesting because it's the same language that you might have been seeing while studying semiconductors. In semiconductors, you have two charge carriers. One is the electron, and the other one, you know what it is? Have you ever studied silicon? *Lacune*. Holes. Yeah. So, it's not so different. You have positively and negatively charged carriers, and the analogies are many, so many, that even the pH, the acidity of a solution, that means if you have some proton, some H+, also because water is dissociated, so if you have H+, if you measure this, it's a measure of pH. There is some sort of equivalent in the semiconductor world. So the analogies… so at the beginning of last hour, because of the suggestion by your colleague, I mentioned that in terms of spatial scale, nanomaterials, nanotechnologies are even smaller, if not comparable, to biological processes. Well, it turns out we have even the electronics that is maybe speaking some language that we are used to.

I'm showing you this because even in this very simplified circuit, you have some sort of emerging behavior. Different spots here, different dots are from different electrodes. Here, here, and here we have 60 of these electrodes indicating when in time there is one of these peaks at that electrode. So, electrode number 1,

number 2, number 3, number 60. And time is in this direction, and what you actually see here that seems to look like fireworks, is the top view of an 8x8 array of microelectrodes, similar to the one that you held in your hands last week. And when there is a small explosion, it's because there is a small signal, like this. And you see that just by eye, looking here, there are situations like now where the activity is irregular, asynchronous, and epochs in which the activity is almost synchronous everywhere. Oh, and there it seems as if it's not tired. There is not a lot of spontaneous activity now, then it started again. And here in this other representation, this synchronized activity is represented by stripes. What I found interesting is at this point, you probably noticed that immediately after, there is fatigue. It's as if things get tired, which is what I mentioned before, knocking and saying, if I ask this population to fire a lot of action potentials, a lot of nerve impulses, after a while they will get exhausted. So they might need a few milliseconds to recover, which is… this is one stupid example, but I hope it will stick. It's disgusting. If you have these animals, the llama, you know that llamas spit. If you ask the llama to spit repeatedly, say 20 spits per second, after a while the llama will not have any more saliva in its mouth, so it will not be able to spit. But if you give it time, it will replenish its reserves of saliva. Maybe here is the same thing, and citing Professor Zoli's classes, maybe in the synapse that maybe he has shown or maybe not, the vesicles containing neurotransmitters, maybe they got depleted. So all the neurotransmitter was released and they got empty. So even if you ask, "Okay, release, release,"… All the other comparisons with urinary functions and other things will not be very elegant, so I'm sticking with a llama spitting, although it's a stupid comparison.

So, the fact that you have a network in a dish is hinting at the fact that you could study, in a reduced preparation, signals and how the signals are organized when you have a circuit, which is very interesting, very convenient, besides what I mentioned last week, that you could maybe build some sort of biological computer by having neurons in a dish, not anymore in the brain of a living animal.

Section 6: Macroscopic Signals: Electroencephalography (EEG) Instead of this microscopic recording with this microelectrode array, probably you're familiar with a more mesoscopic, macroscopic recording of the electrical activity, which I wanted to do, but in class I wanted to demonstrate but it's not really possible because of the noise. Later, I'll tell you why last time I had a lot of noise, but that's for another time. And this is called the electroencephalogram, and it's due to the observation from Hans Berger, that was from the last century, or the 19th century… well, the 20th century, in which by having an electrode, first he did it in a dog, in dogs, he had an electrode on the scalp, so very far from the actual brain. So not only you have hair, skin, bone, and then below you have membranes like the dura mater, the pia mater, so you have a lot of stuff before you start having neurons. But what he could observe is, this is one of the original traces, that you have electrical signals that are changing over time. So you probably are familiar with the EEG as a way to reveal rhythms. And what does it mean? Why? Okay, yeah, I get it that compared to what I did before or what Hubel and Wiesel did, they had one microphone and the microphone was in the mouth of one neuron, it was so close. And this is similar, more similar to somebody sitting outside the room. Okay, now you're very quiet

and I'm grateful for this. But if we were all talking and chatting, some people, somebody from the outside of the room, will simply hear some rumble. They would not be able to distinguish individual voices, they will actually hear the summated effect. Maybe even attenuated because of the distance. Like neurons are attenuated because the electrode is a surface skin electrode. And because of a lot of noise. So, yeah, it's a very sloppy, very poorly characterized signal. But it might be worth understanding or trying to understand a little bit because this is what in diagnostics people use, for instance, to predict whether you have some disorder of excitability like epilepsy or whether you have some sleep disorder or whether you have some sort of connectivity disorder. Schizophrenia, autism, would maybe reveal their signature on EEG. So for understanding first principles, forget it, it's too broad. It's actually, I want to speak with each of you to understand what you're saying, but if you want to simply classify as healthy or diseased, go for it, because people accumulated more than 100 years of experience for understanding whether this is normal or this is pathological.

So this is electroencephalography and we will sort of slightly go around, I'll tell you what it is, and it's for what I consider to be more like a classification. So you put the electrodes, hopefully you put the electrodes always in the same position, because hopefully you want maybe to try to compare different patients, different experiments, different measurements from different doctors, different people, different hospitals, different countries. So you may want to have a convention, and it's purely a convention, there is nothing to understand. In fact, I'm not going to tell you about this convention. And it's what you end up with, what people did, was simply observing. Okay, so the guy is sleeping. Oh, the guy is keeping his or her eyes open. Or no, the person is with eyes closed. Or the person or the animal, the rat, or whatever, is running or is staying still. And they could see that in different brain states, which I don't know if they are really brain states, they are conditions that are easy to classify, to recognize behaviorally, because if I think about my childhood now, how can you really be sure that I'm thinking of my child? If I'm snoring and sleeping, maybe you can say, "Okay, the guy is sleeping." So you characterize, you classify, it's purely a categorization. There is no understanding, but you can annotate rhythms based on their frequency. So if it's slow, like… particularly today I was watching a YouTube video where beta and alpha apparently are enhanced by practitioners of mindfulness. If you sit and you focus on your breath, your frequency content or your waves in the cortex of the beta band or alpha band, that means in the frequency component of part of your spectrum of your EEG, beta and alpha are higher than when you are instead doing nothing, you are scrolling Instagram or TikTok. Instead, if you are engaged in sleep, then your activity slows down. It's sort of getting so-called theta rhythm, or even deep sleep with delta and even slower. And people now start a little bit to understand, okay, but at the level of individual nodes. So, one node, what does it do when it sleeps? Does it also oscillate four times per second? Does it fire one pulse once a second? And the answer is yes. But it's very peculiar because all the nodes in the cortex are roughly doing the same stuff. So somebody sitting outside, if we were all clapping our hands with a rhythm, they would probably be able to get at least the rhythm, if we are all synchronized. If we are… probably you have experience, when you go to a theater and you clap, you're not synchronized with your peers, unless the singer or whoever is asking you to do so, is imposing some pacemaking.

And if there is randomness, from the outside, you will actually hear no rhythm, noise. If instead everybody is synchronized, then outside, so the EEG amplitude will record some sort of oscillation. So, yes, but they also need to be synchronous, like in vitro those stripes that I showed you in a dish, in a culture dish.

Part 5: Pathological Brain Activity: Epilepsy

Section 1: The Penfield Homunculus and Seizures Have you ever heard of the Penfield homunculus? Before the break, I would like to know whether you know what the sensory or motor homunculus is. Some of you are nodding. Just because in a moment I will discuss with you what happens when an EEG will actually see this, when the rhythms of the brain, so the signals that you can detect by EEG, they go completely crazy. They get synchronized, and when they get synchronized… Ah, I spoiled it. Okay. When they get synchronized, they get synchronized across in space. So they're actually invading the entire cortex. And the question is, this cortical homunculus, okay, I spoiled it, is where in the sensory area of the cortex—I think here is sensory and here is motor. Yeah. So, in different areas of the cortex, you have different spots, different surfaces of the cortex, devoted to different body parts. Both in terms of sensation, so tickling, or in terms of movement. And one question that I have for you before the break is: Do you know why hands are deformed? Or even the lips, both for the sensory and the motor, and the motor homunculus, which is a sort of graphical representation of what this mapping is. And Penfield is a neurosurgeon that was the first to, during surgery, neurosurgery, start stimulating the sensory cortex of an awake patient, and the patient was saying, "Yeah, you're touching me on my hand." "I know, I'm just electrically stimulating one part of your somatosensory cortex." Or, probably in an anesthetized patient, saying to the patient, "Why are you twitching your finger?" "I don't know, it's not me, it's not my voluntary control." "Yeah, don't worry, it's the neurosurgeon that is stimulating electrically with a small electrical pulse in the exact same area where your finger is mapped." So we have this map, and like for retinotopy, it's some sort of deformation. I would love if you could think of why hands are over-represented and also lips and tongue. 30 seconds, you have any idea? It's about evolution. Why? Why? Why? Sensory feedback, and we also have to have a very… not only piano players, but also us. We need to, in order to be dexterous, to be skilled with hands, with movements, we need to have big hardware. And the lips, I think, not for language, but because of food. So you want to know if it's very hot or if it's a scorpion. It's an interesting thing. I have to read if it's about… I don't think it's about… because in primates, non-speaking primates, you have the same. You have the same deformation. But it's definitely related to survival. So we'll stop for 10 minutes. And I'll change the microphone battery. Thank you. Which is what? But thank you. "computer vision e in generale in image processing esistono dei filtri, se avete fatto un corso di soluzione di reti neurali la parola 'convoluzione' vuol dire applicare un filtro lineare all'immagine. Quello che vi ho detto è per fare il detector di edge è applicare un filtro che sia una derivata spaziale in una certa direzione e nella direzione opposta e questo è un kernel. Questo kernel non lo so perché sono ignorante se se lo fanno lo fanno questo kernel, questo kernel è un filtro spaziale che è esattamente il circuito che avete implementato e molto probabilmente è

il finale della…" (In computer vision and in general in image processing there are filters. If you have done a course on neural networks, the word 'convolution' means applying a linear filter to the image. What I told you to do to make an edge detector is to apply a filter that is a spatial derivative in a certain direction and in the opposite direction, and this is a kernel. I don't know, because I'm ignorant, if they do it, they do this kernel. This kernel is a spatial filter that is exactly the circuit that you have implemented and very probably it's the final part of the…) Thank you. I think this. And you can finish every 45 or… Okay, so other two blends of topics or contexts that I think might be relevant when you study electrophysiological signals, particularly pathological signals, particularly something that, as I said, electroencephalography would reveal as a pathological signal: ultra-synchronized transients known as epilepsy. And I will show you a couple of videos without omitting the so-called graphic part, the part that will be maybe disturbing to some of you. But I wanted to just evoke the story of the homunculus because maybe you already know that different spots of your sensory cortex or motor cortex are encoding for different areas in the world. Well, the world would be the skin or the motor action. So also in this case, by the way, you can talk about receptive fields. The receptive field would be an area on my skin that would be, if stimulated in some way, mechanically, thermally, with pain, then I would have neurons in the corresponding cortex activated by it. But what I wanted only to refresh is that you know that you have some parts that would be, if you have electrical activity here, maybe stimulated by an electrode or maybe by some crazy wave of synchronous activity that gets generated and it spreads pathologically out over the whole cortex, maybe invading here would give the impression that something is touching your leg, even if you don't have anything. And similarly, if you have activation, again, by an electrode, by somebody like Penfield stimulating like a neurosurgeon, or by a wave of synchronous pathological activity in your face, in the motor part of your face, maybe you will contract both, you will extend both muscles, and you will start having some motor actions that are clearly highly unphysiological. And from the behavioral point of view, would not be normal, would not be conventional, would not be something that you're used to. Because normally, say for some motor actions, you're contracting only one muscle and not both. You would contract the extensor or the contractor, but not both at the same time. Okay, you answered: "And if a wave of abnormal synchronous electrical activity…" further you understand that we could experience sensory sensations and even motor actions. And okay, the story about how it could propagate from one spot to the next… I kept drawing some axons and synapses and another neuron and a neuron with another axon, so it may not be completely impossible to understand that if the connection is for doing some computation, it is so intricate, and maybe just by chance as a side effect, it's a system that can be very easily recruited for propagating pathological electrical activity. This is a nice video that you probably have been, I hope you have been seeing some variations on, in which if you have a set of metronomes and you are having some way to couple them, not by, in this case, by electrical synapses, by connections, but some sort of reading that is propagating vibrations and effectively coupling them, after a while, the metronomes get synchronized. So if you have a system that is highly densely connected and something goes wrong, it could explode, because all the elements could get synchronized, unless you remove some sort of medium for connectivity, for the spread of activity, for connectivity. Anti-

epileptic drugs either slow down the pacemaker, they make all the neurons fire a little bit less, or they try to interrupt connections. And clearly, I hope your objection will be: "Hey, wait a minute, the connections are there for me to talk, to understand, to reason, to love, to dream, so if you interfere chemically, maybe I will have side effects." And this is indeed the case. So maybe in a near future, there are already many clinical trials, you will not have anymore a pill that is indiscriminately shutting down all the neurons. You will have a small brain pacemaker that when a sudden abrupt rise of synchrony is occurring, will try to desynchronize, like in this case it would be… could be somebody, at some point, shaking this, so that the metronomes that got synchronized would not be anymore synchronous.

Section 2: Epilepsy as a Disorder of Hypersynchrony So I'm talking about these abnormal rhythms and synchronization. They are relevant and typically very commonly described and measured by EEG, by electroencephalography. And it's interesting for me to just to mention, if you're not already familiar, epilepsy is a collection of many disorders that result in this sudden synchronized activity. So the seizure, *convulsione epilettica*, is just a symptom and not always can be treated by drugs. So one out of three people are drug-resistant, unfortunately. And this involves seizures, and I will show you a couple of types of seizures, which are sudden and excessive electrical discharges of the central nervous system neurons. And obviously because of the homunculus, of the Penfield homunculus, and not only that, because where is my sense of religion? Where is my sense? Where is my awareness? Where are my breath centers? It's not only in the cortex, but for the cortex and the homunculus it's easier to find. You have unexpected changes in behavior, in motor functions, in sensation and in consciousness. Whatever it is to be aware, to be conscious, to be able to receive information and react appropriately must be related to electrical activity. Unless you believe in the soul, but that's a different story. I don't. So if you have electrical activity that goes crazy, you might affect, not necessarily, but you could also affect consciousness. And it's quite frequent, 1% of the population. There is also a set of epilepsies that appear in the pediatric age, and they tend to disappear. So I'm not asking if, but it could be that some of you, as a kid, could have had one seizure. And because of traumatic injury, you could even experience, I hope not, but in the future, all of us could experience a seizure. Even in Alzheimer's, in dementia, there are seizures. There is some sort of excitability disorder. So a disorder of the electrical state of neurons.

So these seizures are typical events that last a few minutes, and if they don't persist too long, they do not cause persistent damage. For the moment, we would not understand why there should be persistent damage. You could maybe, by some analogy with electronics, you would say, if my computer would be electrocuted, so there would be some sudden electrical activity in all the parts of my laptop, probably it will be fried. Some component will be broken, mechanically or electrically. It will be burned. You don't have heat in the brain. You have glutamate, which is the excitatory neurotransmitter, which nobody really knows why this is the case, it's also cytotoxic. So it's toxic to the cell. Cytology is the study of the cell. So cytotoxic is something that would kill the cell. So if you have a lot of glutamate release, cells start to undergo cell death, apoptosis. And despite in the past, epilepsy was associated with stupidity, to witchcraft.

People were burning, so people were burned alive because they were thought to be witches or wizards, just because of epilepsy, and there is no relationship to intelligence or to violence. There is still a societal stigma that if you, I hope that maybe you know somebody suffering from epilepsy, yeah, they're fine. And there are two kinds. I will show you an example of petit mal and grand mal. The petit mal, they are also called absence seizures. Well, this one I will not show you the video. And you will see in a moment why they are called absence, depending if it's French or not. Instead, the grand mal are more graphic to see and in both cases consciousness is compromised. There are other seizures that instead are not involving… consciousness is not compromised. This is a typical EEG plot, different electrodes placed as I told you conventionally in different places of the skull, they record very large, so large amplitude and also fast oscillations, which is interesting that they get large, while before they were not large. And the comparison, it was a stupid comparison of us clapping our hands, synchronously or not synchronously, and somebody from outside the door, doing some sort of average of the sounds, would actually hear very prominently the bang of us clapping if we are synchronous, and not really large, in that case it would be auditory sound pressure, very strong when we are not synchronous. It's the same phenomenon, and I have another cartoon to show to you, where here we don't have… we have asynchronous activity. Millions are oscillating, they are firing, but they are firing in a scrambled way. So there is no summation, no big build-up. If instead they do, and they do everything… There is probably cell number two that started going crazy before, and the others are maybe following. How would you know that? Maybe some signal processing technique, like cross-correlation. If you ever played with signal processing, it would maybe give you, it's a linear technique of course, it would give you some hints about how delayed one signal is to the other one. Cross-correlation means that you quantify similarity, and it could be similarity but not at the exact same moment, so isochronous, but with some phase shift. And indeed, you could reveal that, say, electrode number two is earlier, the signal from electrode number two starts earlier. But okay, just to give you a glimpse that what you studied, or at least what some of you studied in your previous years, is very useful. By the way, even trigonometry, but we'll see that another time.

So I'll show you now a focal, which means it's not generalized, it's not invading the entire cortex, it's only focal. And this young kid, you probably can guess where he has the focus of the epilepsy. The guy is without… he's contracting, moving the mouth, so you probably can tell me which part of the cortex in the Penfield homunculus this focus is located. At least you can tell me whether it's the motor or sensory cortex. This should be easy. Motor. So it's a motor symptom. And obviously it's likely to be related. It's around here. Not that necessarily it's always easy to say, "Ah, yeah, I see." And therefore you do it with EEG, showing where it is located. And in this case it doesn't spread everywhere. It's… you know, it's very easy to understand something, something that went wrong with the population of neurons that started to oscillate at the same frequency as the motor symptoms. And something interesting is that it's not in this case, it's not spreading, although there are connections. For instance, there are connections between the motor and sensory areas. It's not spreading, it's not invading, luckily for this guy, it's not invading the entire cortex. So in this case consciousness is not affected, it's unilateral, it's localized. Of course,

it's unilateral, it's only in the left part of the face, in the motor areas, so in the cortex you have a representation of all motor and sensory areas of the body. And it's intermittent, and the fact that it's intermittent has something to do with neurons, and we will see later on when we discuss excitability. Why would a nerve cell be inherently starting to oscillate? Why is it an oscillator? And so this is clearly here, I'm not talking about one neuron, it's probably going to be millions of neurons nearby sitting here and being faulty for some reason. Maybe they got too hyper-connected. Maybe there is some fault in the genetics and when the neurons are firing, maybe some positive ions are accumulating too much and they are not able to diffuse away, like ink in a glass of water that would diffuse away. Maybe there is something wrong with, technically it's called ionic buffering, that means removing ions that are in excess, and this movement in the population starts to go crazy.

I'll show you now, it's quite… it's a little bit dramatic, but not as dramatic as a tonic-clonic seizure that I'm not showing to you, but if you're curious, have a look on YouTube. It's not… the person is not in pain during the seizure. Of course it looks to you as very unnatural. So it's not a surprise that people thought, "Oh, it's the devil that is possessing this person." But the consciousness of the person is gone. So clearly, if it's your brother, your boyfriend, it has an emotional impact, but the person is not suffering. So you could look at those videos on YouTube where people are showing them to say, "I'm not possessed by the devil, I'm not more violent, I'm suffering from a disorder of excitability." Here, it's typically the absence seizure. They are generalized, so consciousness is gone. It's happening several times a day, so they can be very disruptive for kids that are learning at school. They typically go away with puberty, so some sort of reorganization of the cortex and the thalamus is happening with age. This is known, so we know that the development of the nervous system in humans is continuing during adolescence, and I think up to 18, 20 years of age. And if you record the EEG, it's not this very strong, high-amplitude, ultra-synchronized, crazy EEG; it has a very peculiar peak that is called a spike, although it's not a spike like in an action potential in a nerve impulse, it's visually a peak and a wave. So it's a small needle and then a wave. Needle-wave, spike-wave, spike-wave at a very specific frequency, 3 hertz. When a neurologist, an expert in reading EEG, looks at this, they immediately say, "The thalamus is involved and this is an absence seizure." The thalamus, as I said, is the station that is in between, that is connecting sensory information and relaying it to the cortex. So you could probably understand that maybe the connections are not only in one direction; there is feedback, like everywhere in the brain, there is almost always feedback. So you have basically a station that is, just by some pathological state… If some focal epilepsy starts here, it can be recruited and it can spread the activity everywhere. Okay, the point is not relevant. It's only that the father is calling him again and again. In a moment it starts and he's blanking. He's not there anymore. Here it starts. And he does not have any memory, any recollection of what's happening. For him, time was moved forward by a few seconds. Now it's back. Imagine if you're at school, and now it starts again. Even with your accent… "did she send me the whole thing when I was absent?" The twitching of the eyes is also another interesting thing. It's gone. So this is quite dramatic and some of you told me about your interest in consciousness. Well, it seems that the thalamus and the fact that the cortex is

not completely synchronized and the thalamus is remaining as a relay station for sensory information and it's not going crazy like in this case, seems to be relevant. So here consciousness is gone and it's like a switch: on-off, on-off. So I don't know what consciousness is, nobody knows it. Only a little bit we start understanding it, and the route of looking at pathologies like coma, locked-in syndrome or anesthesia or some forms of epilepsy are relevant for objective information, also with the idea of treating, for therapy, of course, patients.

So this is an example of a generalized seizure. Again, it starts in one specific area. I really don't care about the positioning of the electrodes. As I said, this is a convention. You can learn it, and maybe after a couple of weeks you will forget it. Knowing that it's a convention might give you some hints that we don't really know. We know that if we do this always in the same way, we can compare it to the literature and to books, which is precious. So an experienced neurologist would say that this was a sort of event that looked funny, and this is the start of the seizure. What happens normally, and you will probably understand why, after having been confronted with the fact that you have the Penfield homunculus somewhere, and also that the brain is responsible for cognition, thoughts, emotions, memory, dreams, hopes, etc., smell, etc., it's all in the electrical activity of the cells. You can probably understand that at this stage, a few tenths of seconds, a few seconds before the loss of consciousness, patients are reporting the so-called aura. And typically they start responding, saying, "Okay, something is wrong. I start smelling something that is funny, that it's not real, it's not authentic, it's not real." It's clearly not real. Let's say there is nothing in the world that smells. And there are signals that are normally not compatible with actual sensory things because the world smells differently. If my olfactory cortex is activating synchronously, it starts to get activated in a synchronous way, maybe this does not correlate to any chocolate, any Belgian chocolate, but not even any food. Or I will start to feel, to have visual hallucinations. Again, we know that in the visual cortex you have cells that start to be activated if you have, in the world, light stimuli with orientation. So there is no surprise that maybe sometimes you can see a geometrical pattern. There is a nice book by… Ah, damn. He's a very famous neurologist. He died a few years ago. He was the author of *Awakenings*. It was a famous movie with Robin Williams. And he was reporting about hallucinations. In his case, because he was blind, but in general about geometrical shapes. Can you help me? Do you know the guy? No, you should read him. He's a very famous… I think he won the Pulitzer Prize for the book. So he was a scientist, a medical doctor, but he was also a very talented writer. Oliver Sacks. Oliver Sacks. Thank you. Thanks. Please believe me, he's a fantastic guy. Very humble, and he died recently. So, no surprise that you could see during the aura this, and report geometrical patterns. Now we know that you have cells that are encoding for orientations, etc.

At some point you have the tonic stage in which the motor cortex is invaded by… so consciousness is gone because you are, anyway, all the cortex is doing the same thing. And it's not a big surprise that people are reported, if you look at the movies, at the videos on YouTube, people obviously fall on the ground, this is the risk of injury, particularly if they are driving a car or flying a plane, these are the risks. Not necessarily the seizure per se, unless it happens very frequently. So there is a co-contraction of all the muscles and it starts

progressively and clearly it doesn't look real, it doesn't look normal, and of course it's not normal. And something that was always capturing my attention is the so-called epileptic cry. If all muscles are contracted, it's also the muscles of the chest for respiration. So if I have air in my lungs, it gets extruded and if my epiglottis is closed, I will have some sort of sound. And of course, people around will get impressed, but technically it's just the muscles that are all contracting, because the entire motor cortex is invaded by this synchronized activity. And then after this tonic phase, it starts the clonic phase, so some sort of rhythm that is activating muscles, all the actuators, so extensors and contractors, all the pairs of muscles that are allowing movements, start to oscillate. And so the patient is typically at the beginning after a tonic phase, has a clonic phase. Many videos of dogs, epileptic dogs, are on the internet, unfortunately. And you would be surprised that epilepsy or neurological disorders in animals are in general advancing, even psychiatric disorders, depression, schizophrenia, are advancing our knowledge on the same diseases in humans. And as a side effect, treatments for the animals are getting better and better. This was sort of inspiring. And here we have, again, that maybe it starts with a focus, maybe it involves the thalamus as a way to relay pathological activity everywhere. And so this is sort of… so it can start, okay, I should have said, it could start in the thalamus and spread, or it can start somewhere and involve the thalamus and spread everywhere. This is probably the future in which either a subdural, sub, below the dura, the dura mater, or intracortically… so, ah, sorry, we're done. Or DBS, this is a DBS array of microelectrodes, this is a surface subdural, they could be maybe wireless, but wireless is not, okay, it's striking, it's cool, but it's not particularly mind-blowing. The mind-blowing thing would be that if you have a brain pacemaker that is able to interfere, maybe you can disrupt synchronization. With a big electrical pulse, you would scramble things. It would be as if you start making a mess, you start talking. You're too polite, too gentle and too silent, so it doesn't happen. If I start shouting, maybe you will get, for a moment, you will get shocked by my reaction. So if there are neurons, maybe I can shock them and they respond and later on they will be tired. For a few milliseconds, I showed you, real neurons are getting tired, they are adapting, so maybe I can desynchronize a population of neurons going crazy by giving them a sort of reset signal. And there are some of these devices in clinical trials, they are not yet on the market.

Part 6: The Biophysical Origins of Neural Signals

Section 1: The Origin of the EEG Signal Now, what is the origin of EEG? We are getting a little bit closer during the course, particularly during the second half of the course, but roughly I can tell you that the EEG, already now at the level of this cartoon, is related to the activity of one kind of cell, those that are my favorites, the pyramidal cells. Rita Levi-Montalcini, in her memories, was saying that on the microscope, looking at different brain sections, so, resected brain slices of maybe humans or chickens or rats, whatever, she was impressed by looking at all of them within the cortex. And this is actually quite impressive. No matter how many times you do the experiments, you do the preparation, the cortex looks always the same with all the pyramidal cells aligned like this. So, this is where the hairs are. And this is where the white matter is in the ventral

part. Here is the dorsal part: the hair, the bone, the dura mater, and the pia mater. So they are all aligned in this way. She actually said that they resembled pilgrims walking along lines during a pilgrimage. Okay, it stuck in my brain and I wanted to tell you. And it's because these guys are aligned, they are aligned as electrical dipoles. Maybe you're familiar with magnetic dipoles, north and south. Sort of the same thing, although it's not magnetic forces or magnetic phenomena here. Maybe under some circumstances that we will see, the soma will get charged, say, positively. I'm talking about outside. And the dendrites will be charged negatively. And so this separation of charge will act as a dipole, and because they are all aligned in the same way, all the electric fields, vector fields, will summate. And if you have an electrode here, you will sense it.

If you are like me, you probably got extremely disappointed, saying, "Okay, yes, these cells represent the majority of the cells in the cortex. But the brain is not only the cortex. What about the thalamus? What about the basal ganglia? What about the base of the encephalon? The cerebellum. Who cares about the cortex?" Yes, it's probably evolutionarily the most interesting part. It's probably why we are human, because of our cortex… But come on, the EEG is only about one spot of the brain? Not only the surface, only 80% of cells, all the cells that have this geometry. What about all the other cells? There are many other cells that are also excitatory, and there are many inhibitory cells, which are here, and they are all making a very dense network. They don't count, they don't play any role for the EEG, because their geometry is not aligned, and their electric fields cancel out. Which to me is depressing, because it says, "Okay, so EEG may give you some information, but it's not the golden standard." On the other hand, this is the only thing that we have. Unless you go invasive, but ethically, unless you need to have some sort of implant, you cannot look at the brain invasively. Functional magnetic resonance has a very sloppy temporal resolution, and spatially, you don't really see the electrical activity; you see the blood oxygenation level, which is indirect. It's metabolic, it's delayed, and it's averaged, and it may have nothing to do with the neurons. So we will try to understand not the entire EEG, but at least how the so-called extracellular fields can be studied, given the morphology of a cell, and what happens for the cell to have here positively charged ions outside and far apart from the apical dendrite, negatively charged ions. This is where we can start understanding, and we will call this local electrical phenomenon, we will call it local electric field, or local field electrical potential, local field potential, LFP, instead of EEG which clearly involves a mess of the entire block of cortex that is below it. You probably know that the cortex, particularly the human cortex, is not flat. It's constantly a sequence of gyri and sulci. It's not flat. The cortex of a rabbit is flat. The cortex of a human, probably because we had such a large growing surface extended, has to be packed and folded. Like a piece of paper, I do it for the sake of science, like a piece of paper in order to have the same surface but occupying a smaller volume. Whoever will forgive me.

So this is a cartoon that I promised for only this cell. Okay, I'm basically neglecting all the other interesting interneurons, inhibitory neurons and other excitatory neurons that are not shaped like this for dipoles. And here you probably understand why roughly the same activity… So this is very stylized. You don't see any pulse, like I told you the neurons are emitting those pulses. But if you allow this poetic license, then here you have that this activity is very differ-

ent from neuron number one to neuron number two to neuron number three. So if you do the arithmetic average, the sum, you have the EEG on a first approximation, and you see that maybe you have, as a result, a small amplitude, high frequency signal. Small amplitude because it's never one transient… okay, here this is one big transient, but the other big transient for the other cell starts not in the same spot, it starts here, and this one has it here, and this one has it here. So they're not synchronous, so they don't sum up. So, amplitudes are small and they are at high frequency just purely as a result of this arithmetic summation. Not because the individual cells were firing or were oscillating at high frequency. Simply because of phase differences. And when they are synchronized, you actually see it and you have large amplitude, slow frequencies. These words of "large amplitude, slow EEG" or "desynchronized EEG, small amplitudes, fast frequencies" are what neurologists talk about all the time, because this is sort of their stereotypical language that they use qualitatively to refer to maybe some state, or learning state, that we don't understand. We understand partly why this happens and how it can be treated, if they're really synchronous or not synchronous.

Section 2: The Need for a Deeper Understanding I'm done with this long introduction, calling for bioengineering and neuroengineering to actually develop an understanding. So it's not that we want to, for the sake of science, understand the origin of the EEG and understand what happens or why, in this case it was a microscopic EEG, why you get a negative signal and then you get maybe a small positive bump. Why? I want to do that because if I understand slightly what happens, maybe I can design that brain pacemaker to interfere with epilepsy better, or I can have some brain-machine interface based on EEG, like most of the brain-computer interfaces nowadays, for people that do not have any invasive implant, work better. Today they are not suboptimal. However, it's a mess to understand, and it's not just the algebraic sum. So it's more than this, and it's very complicated because these cells are electrical devices. So the local field potential, that is what we are going to investigate a little bit. EEG and even the magnetic counterpart, MEG, are involved, and they are still not completely understood. This is a computer simulation of one guy and we are going to use… So this is from one of the authors of the book that I recommended for this course. And right now in the simulation he is activating only the soma. And the neuron here becomes red and the EEG, the simulated EEG or local field potential, shows a positive deflection. Let me see again. So, when you only have inputs arriving in the basal dendrites, then the local field potential starts to be positive, depending on which depth your electrode is at. If the inputs are arriving in the periphery, in the apical dendrites, you actually see it's the exact opposite. And if the input arrives everywhere, in a moment, for the simulation, the cells will start firing and communicating and getting bright, whatever. You almost record nothing. So, the naive expectation that on the surface, with this simulated output, whenever you see a positive bump, it means that you have one or the other activation, is wrong. It depends on the activation, what caused the activation, and where the synaptic inputs impinged and activated the compartments. Now it doesn't make sense, I only want to show it so that we can understand a little bit better. And the way to understand it involves understanding, first of all, that neurons are not points;

they are not systems that are concentrated in one point, although the point-neuron approximation is useful and we will use it. So neurons are distributed in space, like maybe a small cable and a ball, which is the soma. I'm particularly using the word "cable" because I'm a geek and I told you about my interest for Morse code. In the late 19th century, Lord Kelvin, the same guy famous for thermodynamics, wrote a mathematical equation for submarine electrical cables, bringing telegraphic signals, Morse code signals, under the ocean, because they were difficult to design or difficult to understand the variations of signals that are propagating through such long cables. Believe it or not, the exact same equation, the cable equation, will be used in this course to try to make sense of this local field potential. And it's surprising that it's the same equation as for an electrical cable. In this case, it's a biological cable. So the key point is, it's not a point, it's distributed in space, and it matters where and how the electrical activity of these cells is behaving. Right now it doesn't make sense.

Section 3: Biophysical and Mathematical Preliminaries I'm going now to start with light preliminaries. So we'll finish in five minutes and then we have the last hour. It's going to be light. I'm going to refresh some concepts from electrostatics and biophysics. Do you have questions so far? I invite you once more, if you have any questions, to come to me, to contact me. I don't care about repeating things over and over for you or giving you additional explanations of material. If you don't speak, I cannot deal with it. So my suggestion would be to start studying about these topics throughout the course. You don't necessarily need to do the exam in the first session in January, but do it. Because you have me engaged and active. Not that I go away in the second semester, I'm still here in Modena. But maybe we can refer and count on these, maybe it will work or will not, on the Teams chat that you could use as a forum to ask questions. And maybe some of your community will say, "I found this analogy useful." The one of the llama spitting was disgusting and was not very useful. But these other analogies helped me understand it. So please, count on me.

So let me switch to preliminaries and then we'll break. And for this part the slides are already in the repository; I posted them this morning. You could refer if you want to these two chapters in this book, particularly *Cellular Biophysics* and *Foundations of Cellular Neurophysiology*. I don't know whether all these books are… at least I know that this should be in the library, this one should also be in the library. If not, let me know and I'll ask them to buy it. And for no reason, look at… well, you could even consider buying some of these books. The idea is that they are supporting your studying and understanding. It's not necessarily that you will see here verbatim, word by word, what I'm saying. I try to make your life even simpler, even taking it and refreshing something that you probably already studied extensively in high school or during the first couple of years of your bachelor, at least the first year of bachelor in physics. And there are these four concepts. The first one is purely a convention, and I would like to… it's a nomenclature thing, and I would like to make, because, sorry, explaining it will give me the possibility to clarify some concepts. So, physicists normally always talk about the density of particles in space. Chemists talk about concentrations of a solute in a solvent. It's the same thing, it's a matter of jargon, and I will demonstrate it to you. Second, I would like to, since we have to talk about electrical potential and charges, I have maybe to

refresh basic electrostatics. So it's not Maxwell's equations, eh? Don't worry. It's maybe Coulomb's force and what is the potential of a force? What is the electrostatic potential? Third is something that you may or may not have heard of. It's mobility. I need to describe the ions that we will start looking at. I need to understand and to give a number to possibly measure if a fat ion swimming in a solution in the aqueous medium will encounter more resistance than a slim ion. I would like to give a number. This number I call it mobility, which is not particularly original. So the mobility of a particle in a fluid, in an aqueous solution. And fourth, again, this is another definition in the sense that it's me defining what I would love to describe. It's a flux or flow of particles in a fluid. Particularly because I want to understand whether maybe ions are going inside or outside or they go away. How do they work? How does it work? Because so far, particularly for electromagnetism, I know about forces. Okay, you have a particle, and maybe you are in an electric field, I know what force it is. But I want to understand how these things move. And to do that, it's very convenient to talk about flux. Flux is the biochemist's word for current. Those are going to be electrical currents. Well, strictly speaking, it's a current density, because it's per unit of area, per unit of cross-section. I imagine a river where you have water flowing, but this is nothing else than current. So electrical current, ionic current, this is resistance, electrical resistance, but I call it mobility, and all the rest are definitions or fundamental laws of nature.

And I'm going to use mathematics, like it or not. Okay, Feynman was even saying that it allows us to reason. So it's not only a language that is very precise, but per se allows us to make inferences, allows us to make discoveries. And it's true, if I can write something in mathematical terms, maybe I can then shut down my brain, shut down my understanding, and simply crunch equations, and I will derive consequences. And of course, then I go back to the experiment to see whether it works. Of course, I'm not claiming that you will have to remove your intuition. All my efforts, today and in the coming weeks, is to try to tickle both your mathematical preferences as well as your physical intuition. I hope that you will roughly feel that you understand in your stomach, and you can replicate it with a pen and paper on the blackboard with chalk. And okay, so this is a sentence that I already told you. Nature speaks the language of math, and it's beautiful. It's a beautiful language, so maybe we can try to use the same language. This guy was a Nobel Prize winner for quantum mechanics, was a very prolific author of books, of science dissemination books. Maybe you know this guy; he was playing bongos and was also very fond of opening locks or *casseforti e lucchetti*. I mean, these people are a bit original. But he was really a genius. I think he was the guy who is considered to have invented nanotechnologies, because in the 80s he gave some remarks in one famous seminar talk that he gave at Caltech where he was working, talking about the opportunities of nanotechnologies, when technology starts to build devices that are small, nanoscale small. So I will stop now for 10 minutes and in a few slides I'll use the symbol "remember" to tell you "this you have to remember." And I'll just only give you this so that you are scared. So I will ask you informally to remember or to learn for the first time the plot, the graph of three functions, because, say four functions. One over r, which is what people call a hyperbolic function. Okay, it's one over r. This one is the famous exponential transient. Okay, so there is one minus… Okay. And this is

the natural logarithm. And this is a linear term, it's a straight line. So you should be able, roughly, it's not that I'll ask you, "Here's millimeter paper," no, but roughly you should be able to plot these functions. Now that you're scared, we'll take a break for 10 minutes. I hope that you're not scared, but if you're scared, please come to me for mathematics. Thanks. Thank you. "ho caricato il giorno stesso però" (but I uploaded it the same day). I will be able to find the video that I have told you that I will find the description. What I've done is to make the description with Whisper and then put it into the… Thank you. Thank you. *pain* Thank you. Thank you. Thank you.

I'm sure that inadvertently, the other time I started at 14:00 and today it was the same. In theory, I would end at 18:45, so I have to put an eye on it for a little less than 50 minutes. If not, it doesn't make sense. If not, I'll start first and finish at 18:00. But it won't happen. Ok, so I understand that you are tired and I hope that the… I hope to make it… I'm interrupting often, I'm just giving you videos and questions to make it a little bit more lively. Please speak if you are too bored or if you feel from now on that the style of the class is getting too hard. Not today, in general. So I know that you are tired, but these are very simple functions. And so I'm not asking you to do the so-called study of the function, say 1 over r. The limit as a function of r. And I'm not asking you to take the limit for r that goes to plus infinity, or r that goes to minus infinity, or to calculate the derivative or the second derivative. These are functions that just roughly by eye you should be able to do. So this is not a course in advanced calculus, it's not a course in mathematics. So it's a rough understanding of these functions. So any idea about who is somebody willing to help me plotting f(r) = 1 / r? You will have it on the slides, but if you want to challenge yourself, let me know what should I sketch. Okay, okay. So your colleague was making a sort of gentle curve, and indeed this is what I have in my head. Okay, maybe for negative r, but okay, negative maybe we will not care, because we are thinking of r as maybe a distance and the distance is only positive, but it is indeed the case. And again, you see, r is in the denominator, so when r gets very large, 1/r is getting squashed, it goes to 0. Yeah, okay, a mathematician would say it goes to 0+. Fine, okay, it will go to 0 from this part of the plane. And for r that goes to 0 from the right, it goes to plus infinity, because 1/r is something that grows over and over. So I'm not a mathematician myself, but roughly knowing that you have this profile is very convenient and useful.

The other one is only present for the charge or discharge of capacitors. So it's not because I'm in love with exponentials. They are nice, but I'm not… I'm in love with other things, not necessarily exponentials. So, and the way I would, it's f(r), what is it? It's f(r) = 1 - e^(- r). The way that I still teach it to myself is looking at the exponent, assuming that alpha is a number, it's given, it's a positive number. You know that if you want to make me happy, the overall exponent of the exponential needs to be negative. Because if it's positive, you probably know that the exponential is exploding. And in biology, biophysics, I hope that nothing is exploding, particularly because I'm invoking the fact that the property that all physical systems should be dissipative. They should basically dissipate energy, so it's unlikely that things will go to plus infinity. Okay? So, if that f(r) is the value of the membrane potential across, so inside with respect to outside of a neuron, I don't expect it to explode, to go to plus

infinity. It's starting to go to the other extreme where the exponential is… So, for r that goes to plus infinity, e to the minus… it goes to e to the minus infinity, so it goes to zero. So, you want to make me happy, check the exponentials, and this is also going to be relevant for differential equations. So, in that case, I know that the exponential, for r going in this direction… at some point, I will not have the exponential anymore. And as engineers do, a friend of mine made it very clear, saying engineering is not math, it is the science of approximation. So I would like to approximate things, and here I can see that it's the sum of two things. So even the graph, the plot, will be the superposition of two things. One that is always there, and the other one that at some point is not there anymore. So if at some point, yeah, ideally it's going to be at plus infinity, but in reality it's going to be on the order of 1/alpha, that is setting the scale of this equation. So here I only remain with 1. Because if r is so large, this can be neglected. If you want, you can do things properly, but yeah, I can see two pieces. And other things that I normally do, I test what happens when I put the argument of the exponent to zero. I do it because it's something that I always remember, what is the exponential of zero, that you all know is one. So if it's one, one minus one is zero. So, yeah, I should look for the concavity, but it's an exponential. So I know that it doesn't change. So the only thing that changes with r is this term here. And I know that it's something, it's an arch, that is either exploding or attenuating, going to dissipating, going to zero. So it's likely only going like this.

The logarithm, I think you know it or you don't. Logarithm of r. I'm on purpose using r instead of x, instead of t. So it may be that it annoys you a little bit, but it's purely just a variable name. So, fine, call it the way you want. It's purely a label. What is important is that I understand what is the independent variable, that is r. And you probably know that the logarithm, at least for real values, is not defined when the argument is negative. So the logarithm is the value that you have to give to the base, so it's an exponent that you have to apply to the base, that is e, in order to get the argument. It's the inverse operation of an exponential, and for an argument that is negative, it's not defined. You know, it's only one thing that you have to remember. And the graph of the logarithm is crossing this axis in one specific point. That is easy to remember. Do you know when, in other words, I'm actually searching for the value that makes… So where the logarithm is zero. So what is… One of my questions. So its value is one, am I right? So why it does not work… so I wanted to say what is the exponent that I have to give to the base e in order to have 0? It doesn't work, e to the 1 is 2.7 blah blah. Where is the problem? I have to think about it. But the plot is correct. I have to think about it. Again, it's not a course in math, and I will try to solve my own puzzle another time. And here for the straight line, you have one thing that is very important, is the angular coefficient, m. It tells you about the slope of the line, and then you have another value, p, that tells you where it crosses the axis. I don't remember it by heart. I simply say, "Okay, what happens if r is 0?" So r=0 means this axis here. So when r is 0, this term disappears. f needs to be p. So this is the value where the straight line is crossing the vertical axis. So these are crucial that you understand, that you remember that you are able to handle and to master roughly for the plots, for the graphs of these functions.

And another thing is the derivatives. So we're not talking about, well, we are

also talking about integrals, but these are the derivatives that I would love you to refresh and remember. You know that the derivative of a constant, any constant, is zero, and 1/x is another one that, yeah, you could derive it from the incremental ratio, but do you remember what 1/x is? It's important because it comes in Coulomb's force, in the gravitational force you always have 1/r or 1/r^2, so this is somehow a polynomial with a negative exponent that is very important. No, I'm not asking for the integral, I'm asking for the derivative. So, in principle, you should write it as x to the power of -1 and then apply the rule. But it's, or vice versa, it's the ratio, say numerator and denominator, and you say it's the derivative of the numerator, which is 0, times the denominator, minus the derivative of the denominator multiplied by the numerator. So, it's 1, and because of that minus, and then you have here that you get x squared. So it's -1/x^2. You have time to refresh it. The derivative of the log is indeed 1/x, particularly if it's only if the log is a natural log. And then there is also this property that is very important, which is to say that the derivative is a linear operator. So if you have the derivative of a sum or of a subtraction, of a difference, what is the derivative? Yeah, and would it be the same? If here I would put some coefficients, 25 and -30, how would it change? Linear, ok, you know everything. So it's linear. And the other thing that we are going to use is the derivative of a composite function, for instance in this case the derivative of the log of c(x). It's a chain rule. I don't remember how it is called in Italian. *La regola delle funzioni composte*, but I don't remember if it's called the chain rule, it seems not. *C'erano i carabinieri un tempo, c'era la regola dei carabinieri, ma era un'altra cosa.* (There were the carabinieri once, there was the carabinieri rule, but that was something else.) Do you remember how this is… what you have to do here when you have a composite function? You take the derivative of the external one, and then you multiply it times the derivative of the argument. So in this case, it will be 1 over c(x) multiplied by the derivative of c. Let me know if it… Okay, I could have had them appearing slowly, slowly. Okay, and this is the only thing that we are going to use. Even written like this. The derivative of the log of C, that would be the concentration. And it's very important, because this also gives rise to some relationship with Einstein, which he is remembered for. It's about diffusion. It comes from that simple trick.

And I think the other thing is the concept of integrals. When you have the limits of integration, you invoke… So you know that integration is the inverse operation of the derivative, and the indefinite integral is basically… You could also call it an anti-derivative. And so, when you have the limits of integration, by the fundamental theorem of calculus, you actually have the primitive, and you calculate the primitive at one limit, minus the primitive calculated at the other limit. Does it ring any bell? And now, because this one is a log, and again, we are going to see exactly this integral later on, probably not today. Let me put it back, I'll keep speaking up until 7, and you may not like it. So here we are going to have exactly the log, and it's a difference of logs, and you know that because of the properties of logarithms, if you have a difference of logarithms with the same base, the result is the logarithm, yeah, yeah, it's the log of the ratio. So this is going to be omnipresent. So this you will get acquainted with. And another thing that we may… so this is not crucial because we are only using… I want to give you an intuition of the electric field in terms of the gravitational field and I want to use the Taylor expansion up to the first

order. So a polynomial expansion up to the first order where I know that any function, any continuous and differentiable function, I can write it as the value around one point, so this is a number, this is like 27. So around 27, if h is very small I can approximate the function as the value of the function at that point plus basically a straight line, and this is the angular coefficient which is given by the derivative calculated at that point. It's the slope of the tangent to the function at that point. But this is not really crucial. We're not going to be… There is another way, another point in the next, probably next week, where we are looking at, maybe two weeks where we are using Taylor expansions, but maybe I'm not doing it. I'm only telling you that if you want, you can try.

And first of all, the differential equation, I tried to introduce this sign already last week. This is the only differential equation that I would love you to remember or to learn to solve. And again, you have this online refreshing video that, although it's my voice, it's a little bit monotonic and it's a bit boring, but I refresh different techniques, particularly when the equation is non-homogeneous. So when you do add some term here, and this term, say, for instance, is a constant. So if I read it aloud, this is a differential equation, meaning that the solution is not a number, so it's not an algebraic equation. The solution is a function, and it's a function such that when I take its derivative, I get the function itself. Okay, multiplied by some term, which is a constant, which is a number, a parameter. Okay, plus b, that's a little bit more involved. You need to be able to calculate the so-called particular integral. Or you do some heuristics. And the heuristic is that clearly the solution of the homogeneous equation associated with this ordinary differential equation is an exponential. Actually, it's a family of solutions. It's an infinite family of solutions. So this k is the one that you will fix by the initial condition. And here you claim that because here the right side, so this forcing term, depending if you're an engineer, you see this immediately as a linear system. So if this is the input to this equation, if this has the plus of being constant, then the solution must be of the same type, must be some constant. Now here I gave already the solution, but I should have called it big K. Something to identify, particularly by replacing this big K here and saying, "Okay, the derivative of a constant is zero minus A times big K plus B equals to zero." I can determine what K, big K, is. And we will not do anything more than this. You know that if B is instead a trigonometric function, then the solution is also of the kind of trigonometric functions. Actually, I think this is more general, and people were calling them *cisoidi*, *le funzioni cisoidali*. It means that you have trigonometric functions also pre-multiplied by an exponential, but we will not see that. And I remind you that if this b should be an arbitrary function, then the only way out is the convolution integral, which is maybe something that I… No, I did not, and I will probably only mention it once in the forthcoming classes. It's a filtering operator in the end, so this thing, this particular thing, as written like this, it's a low-pass filter. And, yeah, you need to remember that these exponentials are going everywhere, and that's why 1 - e^(- r), or times x, or t… And you see that you will always see me being very worried when writing a differential equation, that here, the same state variable, F, appears here with a minus sign. If it's not a minus sign, then I get stressed because it's not a dissipative system and it can… it's exploding, and yada, yada. So I hope that with these stupid things, something will stick or will be refreshed in your brain.

Another thing that I will use is the orders of magnitude, particularly this giga, mega, kilo, so it's 1 billion, so 10 to the plus 9, sorry, 10 to the 9, 10 to the 6, 10 to the 3, and again, I will basically use milli, micro, nano, pico, and I remember, because milli is 3, I always go like this, I don't remember, so if you tell me pico, okay, maybe now I know that it's minus 12, but I do, so milli is 3, micro is 6, nano is 9 and pico is what follows nano. I cannot remember things by heart. There are of course exceptions because they are not in the MKS system, like the decimeter or centimeter. Fine, I remember that it's 10^-1 or 10^-2. This will become interesting because I will constantly push you to check your damn physical units. You have one way to check whether you are correct if you can check and test the measuring units. If you are summing at some point apples and pears, something is wrong. And if at some point in a differential equation like this, you have that B does not have the units of something, say that instead of being x it's time, it's a differential equation with time being the independent variable, B needs to be something per unit of time, because on the left-hand side you have something divided by time. And so the units here need to agree. So you will see me obsessing with these things, which is just an easy way to spot possible problems. Something that I remember that maybe is familiar for those of you who like beers and who've been to Germany, where you get in these beer gardens, where you have, particularly in summer, it's fantastic, they actually bring you a glass that is one liter of beer. And I cannot drink, although German beer is good, I was used to Belgian beers, and with Belgian beers you don't drink one liter. Only to say that you have something that you can keep in your hand that is one liter. Because this conversion… that is… if all… both liters and cubic decimeters are all volume indicators, they are all measuring units for volumes, it's only that liters are something that is more familiar to chemists, that basically had to deal with liquids, and they got used to this unit. Engineers and physicists, maybe they got used to something that was measured with a measuring tape, and they use meters and multiples and submultiples. But, yeah, they are the same. And I kept forgetting, but it's one cubic decimeter because it's something that you hold, 10 centimeters cubed, you can hold it in your hand, and it's beer in Germany. I don't know whether this will help or not.

Section 4: The Resting Membrane Potential So why are we bothering with this concentration and density, mobility, electrical potential, and what was the other one, the flow, the flux? Because we have some investigative task for the couple of weeks that await us, that are the next two weeks. And it's, why if I take any cell, let me draw a cell, a neuron that gives me comfort. So I have one cell, and it's in any cell of your body, unless you are dead, and I will tell you why you could be dead, you have that conventionally, I'm referring, I'm taking the convention that is referring the electrical potential inside with respect to the outside. So outside is what I call conventionally, because potentials are defined with respect to a constant, an arbitrary constant, and I will refresh why there is this constant, why it is arbitrary, that I call it zero. So here it's zero millivolts. Inside your cell, all the cells of your body, there is an electrical potential that is non-zero, and it's negative. And for neurons, it's around minus 70 millivolts. There are different cell types in which you have minus 80 millivolts, minus 20 millivolts, and minus 70 millivolts is quite a lot compared to 3.3 volts of electronics or even smaller values of electrical potentials

in microcircuits, microchips, is even a fraction. So it's a lot. Why is it minus 70 millivolts? I want to understand why it's negative and why it is minus 70. The reason why it's there is because you are not at thermodynamic equilibrium. Lucky for you and lucky for me, we are not yet dead. And being dead means that there is nothing, no more, at least maybe transiently there is, but when you are really dead, there is no difference between you and the environment. There is no energy spent to oppose entropy. You are as disordered as the environment. What there is in the environment is inside yourself. And if you are a cell, basically the membrane is rupturing, or at some point, things start to flow in and out, and you have identical things inside and outside. You are nature, you are completely embracing nature, you are dead. But if you are not dead, if you are eating bananas and chocolate, etc., you have disorder, sorry, you oppose disorder, you create order by spending energy, and you create some sort, apparently, of unequal ionic distribution inside and outside the cell. That's the whole story, but I would like to understand why. The reason is not that I'm obsessed, it is because if we understand why, we can make sense of why not, and why sometimes this electrical potential might change over time. If I know the mechanism, I can understand if things are changing over time, whether the mechanisms could change over time as well.

This is one example that I showed you. I don't know if I anticipated that I would have shown some neurons. So this is an experiment that we did many years ago. Just a cell from the cortex of a rat that we explanted the brain and we cut the brain in slices very quickly so that the tissue was not dying. It was actually still alive. And we put it under the stage of a microscope that we used. This is called a transmission microscope because light is going through, it's transmitted through the sample. And we are using infrared light because infrared light increases contrast. After all, cells, you know, are water, made of water, and they are surrounded by water, so there is no contrast. If you see them with a normal microscope, you see nothing because they are transparent, made of water, so there is no contrast. You can get contrast by some technique that is called differential interference contrast microscopy, which I'm not telling you about, that gives this beautiful picture like craters on the moon. But these are sort of three-dimensional renderings of the somas of cells. So you see that each cell has a body and each cell in this case has a pipette implanted into its stomach. So here, what we did was a little bit more ambitious than what was technically impossible to do in vivo. Instead of Hubel and Wiesel that had this tungsten electrode, very tiny, nearby an electrode, so like a microphone was here listening to the extracellular signal, here we did more. We got a pipette. This is the sort of top view of a conical glass pipette that has been sectioned by light, just because the microscope is creating some sort of light section. So that's why it looks like a triangle, and it's empty inside, and I will tell you why it's empty. And we push it almost inside the cell, because I wanted to see, to measure the electrical potential inside. I'm not happy with outside. Outside is boring. Okay, the only thing I get is a very tiny signal of a few, say, 100 microvolts, but this is just me speaking and somebody from outside of the class listening. I want to hear the whole story. I want to enter into the stomach of this cell. And if you do, you measure it on a voltmeter, you measure a negative minus 70 mV. And people call it with some strange nomenclature compared to engineers; they call this polarization. They say that the cells were polarized in the sense that they

were not zero, so there was a polarization.

And sometimes this polarization goes away because these cells, particularly these pyramidal cells, in a time that is so fantastically small, and I'm fascinated by that because in a matter of a millisecond, or hundreds of microseconds, you have that this electrical potential can change very rapidly, incredibly rapidly for being a biological system. The world is able to commute and to open and close or to switch like a transistor so fast, and it's made of proteins, blobs, gel, ions, and the ions are moving slowly. It's not like electrons that are moving… okay, they are not moving fast, it's the electric field that moves fast in a wire. Let's say you don't have transistors that are operating at the gigahertz range. Here you have proteins. You have fatty acid molecules. What is changing so fast? And fast is fascinating to me because this is the time scale of thought. I'm thinking right now with action potentials changing rapidly over the time scales of around one millisecond. So the time of an action potential, this is called an action potential, as opposed to a resting potential, for obvious reasons. One is at rest, one is doing something, we don't know what, but it's doing something, is a fraction of a millisecond. Let's say half a millisecond. And it goes up to +30 millivolts. So minus 70 to plus 30 is 100 millivolts, it's 0.1 volts, which is remarkable. One… okay, now I had one banana but I also had a dish of pasta today. But still, it's not chat GPT and it's not my computer that is powered by an 80-volt power supply. It's with a few watts I'm able to sustain myself. So understanding why the hell you have -70 mV… if I can understand as a next step why the membrane potential can be changing in time, and it changes over time in cells that are called excitable. Neurons, some pancreatic cells, they are secreting hormones such as insulin, so the pancreatic beta cells. Muscles, of course, myocytes and cardiomyocytes. These are all cells in which the electrical potential changes, and apparently it's a trick that nature evolved to convert electrical signals into either information in the world of the brain, or motor actions, or mechanical contractions, so changes in reality, changes in elasticity. And because electricity is so fast and so easy to transmit, that was the way you could command and release, say having chemical release, so having chemical messaging and electrical messaging and mechanical messaging. So that's why there's this investigative work that I have to involve you with.

So it's essential, and if you are dead, you don't have this. This could be a question. You could probably say, "Okay, I could understand why this is -70 millivolts." Maybe your first guess would be that inside there are many more negatively charged particles than outside. It's a legitimate hypothesis. If you think carefully, you could also reach the opposite conclusion that you have a lot of positively charged ions outside and very few positively charged ions inside. And I would say you're close with both but it's more complicated than that. And the fact that in half a millisecond the membrane potential can change so rapidly leaves you with a question: how could it be? I mean these ions are fat, they have a mobility and this mobility is not so… they're not supersonic. They still have to swim, and if you are a swimmer or if you like swimming, you know that in solution you have friction, and this friction is quite remarkable. It's increasing the faster you go. So if you double the speed, the friction doubles. So it's Stokes' law. We will see it probably next week. So it's not clear how you could get something that is reacting electrically so fast with ions that are so sloppy. You can think, if you want, maybe you already

know how this happens. But first I'll start with an omnipresent description that is unfortunately adding a little bit of a degree of complexity that I don't like. And this comes because you have physicists as well as chemists dealing with this electrolyte. An electrolyte is a solution in which you have charged particles. And, I mean, both were studying these systems. Chemists, they studied electrochemistry. And physicists studied electromagnetism. And they used, unfortunately, different conventions and different words. So, you have some solute, which is aqueous. Maybe it can be water, and we have a solvent, which is what is dissolved in this aqueous solution. And you can basically refer to the amount of this stuff, these balls, these particles, in two ways. They are equivalent. You could count per unit of volume. So you could literally take a small volume. Maybe you want to take it very small so that you can move this volume here and here and here. You count how many molecules are there. And you express this as a density, something that you know already. So the density is the number per unit of volume. So you take the number and you divide by the volume. And you divide by the volume because you don't want your choice to be affected by that specific volume that you chose. So you normalize by the volume. And chemists did the same, but they were actually not using counts, because they were already aware that in solution you don't have, like in the solar system that you have, how many planets are there? 10, 15, whatever. So a few numbers. In solution you have billions and billions of particles, so it's not practical to count them, and they are used to using a different measuring unit, which is very similar to when you say half a dozen, *mezza dozzina*, or three dozen. So, okay, what is special about the number 12? Okay, instead of saying 6 or 24 or 36, I just make it shorter and I say half a dozen or two dozens or three dozen. It's the same thing, or you could say centuries, four years. You could say one century instead of saying 100 years. It's purely a matter of laziness, maybe, or of convenience. So chemists were using moles. Have you ever heard about moles, molar concentration? And so these guys were counting in numbers, these guys were counting in dozens, and they were also using liters instead of volumes measured in, say, cubic centimeters or cubic decimeters. They were using liters because they were handling liquids. And moles per liter is called molarity. And it's indicated by a big M. Instead, the mole is sometimes indicated by "mol," written m-o-l. So it's not the animal. So this is a mole, but it's a different mole. And it's a purely conventional thing. It's the number of atoms in 12 grams of an isotope of carbon. So, okay, and how many were there? It's the Avogadro number. So it's 6.022 times 10 to the 23 molecules. This is a pure convention. So this could have been in another… no, another universe, in another timeline where it was not Avogadro or other chemists, whatever. So if it was the Middle East, like it was highly developed in terms of mathematics, and particularly mathematics, I'm thinking of Persian mathematicians, we could have ended up with something else. But, okay, conventionally we are stuck with this Avogadro number. This is the reference. So they are used to counting in 6 times 10 to the 23 multiples, because molecules are so many.

I'll conclude here and we'll close it. So if I want to go from one to the other, the only thing I have to be careful about is that I have to remember how a liter is converted into volume, and how one mole is converted into a number of molecules. So these two numbers. So if I have a solution that is concentrated one millimolar, it means that I have one millimole per liter. So 10 to the minus

3 moles divided by a liter. Literally I express it with, okay, 1 cubic decimeter that I can write it as 1,000 cubic centimeters. And then, okay, I'll divide this and I can express it as 6 times 10 to the 17 ions per cubic centimeter. So here, there is nothing to understand. The only thing is that going from one world to the other, you have a conversion factor. That's all. For next time, although you do have it on the slides, the solution, try to see whether it's a very trivial thing that I do as an exercise anyway. So, okay, this is, apart from the way you measure the volume, be careful, so it's the Avogadro number, but the volume has to be carefully converted. I'll ask you to tell me how many ions would be in a spherical cell filled with 150 millimolar of potassium. Just try. It's the only thing that you have to remember is maybe the volume of a sphere, which fine, you can look it up on Google, on a book, but it's not difficult per se. Maybe you don't remember it anymore because you didn't use it. And another thing that is interesting, you could, if you want, you can ask, what if all these ions were not uniformly distributed in the volume but they were instead distributed just around the membrane, which is actually the case, because the solution is a conductor. What would be the surface density? So can you go from a volumetric density to a surface density? And we can see, well we do have it on the slides, but otherwise we'll see it next week together. Thank you, and as usual, if you have questions, please come to me, otherwise I will not be able to help.

Okay. Okay.

This is the third test of audio because the other time, despite the ambient microphone, the audio was not a great deal. I hope it *did* help you, but in this way, you should feel better here and I'm for sure able to provide this signal to the registration. Let's see how it is.

Announcements Three announcements. The first one is my personal curiosity. Has anyone read the material given during the first lesson on GitHub? The readings? The *Bignami* of Neurobiology, 2-3 pages. The book of Hubel, "Occhio, Cervello, Visione". Why do you think? Ok, ok.

Potete iniziare a guardare quel materiale. Non è, diciamo, deletario, è fatto per potervi dare degli elementi utili. Se invece sapete già tutto di neurobiologia e di elettrofisiologia cellulare a un livello divulgativo, eccetera, ignorate il mio commento.

The second comment, the second comment, the second comment is *legato* to my deficiencies. The other time I was *impantanato* to remember the definition of logarithm. The problem is that I don't remember the things I have to remember. The only thing I remember is that the logarithm is the inverse. *Se vi ricordate il mio*, I crashed because the mnemonic phrase didn't come to me: the logarithm is the quantity that I must put as the exponent of a certain base to get the argument of the logarithm. And here I am going to be because the logarithm of 1 is 0, and it is the only point in which this $\log(y)$ is remembered because it is the only point in which it is traversing the *asse*, intersecting the axes. And the other thing I have remembered is that for the argument that tends to 0 from the left, the logarithm becomes negative, becomes negative, becomes negative.

So the only thing I should remember is that the inverse is the inverse of the exponential. So if I apply it to the natural logarithm, which means logarithm

in base e, this means that here remains x. You can also think that this is a rule that makes it a logarithm and becomes a logarithm in base e, e^1, or, or, is the inverse. *E quindi qui c'è scritto che questa roba qua è l'esponente da dare alla base per avere l'argomento.* If the argument is 0, what is the exponent that I have to give to e in order to have 0? I am going to write it again. It is the contrary. What is the exponent that I have to give to e in order to have 1? $y = 1$, e^0, $e^0 = 1$.

Anyway, third *annuncio*, interesting or less. During the first lesson, I told you that with that small amplification of electronics, I was able to measure an electromagnetic signal in a very banal, very *rosso* way, and I was not sure… I have a card of aluminum for food and I have all the circuit, of which I had no time to create something like that. So my operational amplifier with its various things, and its various electrical, was a different one. Two electrical and ground I applied to my body, and here there was the exit from the reference. I made a kind of *carte* and I've covered this circuit in the card. Do you know why I had this idea of getting into the card? If you've ever heard of it, I would like to make two words.

Ok, *e vi dice qualcosa se io dico "gabbia di Faraday"?*

The Faraday Cage It can be demonstrated, but we will not do it, maybe you have seen it in some course of electromagnetism, that in reality, dependent on the frequency of the electromagnetic radiation of an arbitrary electromagnetic field, at a certain frequency, it is possible to shield what is inside. And in some way, it has to do with Gauss's theorem; in some way, it has to do with the fact that in a conductor, the charges are arranged, at least in a static, quasi-static way, in a static, quasi-static regime, they are distributed on the surface, not inside. And therefore, it is used to shield electrophysiological recordings.

And if you come to the laboratory, I'll show you that we have, apart from anti-vibration tables that are optical tables—they are tables to avoid vibrations, but that is not a big problem—but above the table, we have mounted a Faraday cage above each table, which costs a *botto*. I am from Genoa and I am sensitive to these aspects because aluminum costs *l'ira di Dio*. In effect, in some cases, instead of an aluminum plate whose thickness is not the *stagnola*, which is even thinner, I believe the paper is about 0.2 mm of *spessore*, in the case of aluminum, more than 1 mm or 2 mm of *spessore*, it is very *pesante*, it is extremely tight and therefore it is not particularly practical. So people use literally some of the *reti da pollaio*, that will be smaller than the wavelength of the electromagnetic field that you would want to cut off.

I will show you that the interference based on the cell phone is of the order of the cm, is of the order of the centimeter, so I have a fraction of a centimeter if the frequency is of hundreds of megahertz. For this to do it, I don't know, I remember the speed of light and I can, given the frequency, find the *lunghezza d'onda*. This is the same goal.

However, the key that I have not done at home is that this *scatola*, this and this is a point of… On the other side, there is a cable and I can attach it to the oscilloscope or to the *aggeggio* that was a digital-analog converter, digitized, that I would attach via USB to my computer. What I've done is I've *pinzato*

this alligator, which is called alligator because it looks like a little alligator. I've *pinzato* the wire, because I wanted to contact the zero ground, but I've *pinzato* the *schermo*. I've *pinzato* a piece of paper. This has probably put all the same level of potential, the entire surface of the external surface was not *more*, in other words, *"appesa"*, as it says in terms of technicality, *"appeso"*, not "floating", and therefore I could have been able to be exactly the same potential as I made my measure.

It may be or may not be of interest.

If there are problems—now, none of you has contacted me, we haven't been doing anything particularly math, we're going to start a little more—I'm at your disposal. Don't expect necessarily the day before the exam, in which maybe I won't have much time to get into it. So if you can, if you want, if you need, you know where to find me.

Let me switch to English. And so, two questions for you.

Review: Membrane Potential and Avogadro's Number Do you maybe remember that last time—this is very key, it's a fundamental property—last time I said that all the cells, unless you're dead, unless you are at the thermodynamical equilibrium with your environment (so you're not any more distinct from it, you're completely whole with nature, and you're not Buddhist, so it's for really important things). Every cell has a difference of electrostatic potential inside with respect to the outside. Do you remember this?

And do you remember whether this difference of potential is negative or positive, assuming the convention that is inside with a reference that is outside the cell?

Okay, so you even remember the number. So I don't want… So this is really, really key. So it's negative. And for neurons, it's roughly around between -60, -70 [mV]. Other cells might have different numbers, but it's still always negative. I'm not aware of any cell that has this difference of potential that is positive.

It's very important because it's a way employed by living matter, by biology. Evolution found a way to make, through possible pores… I'm indicating the door because I'm thinking that I'm making in my mind the stupid analogy that this is the inside of a cell, and if I want to traffic molecules or stuff or food or whatever, coffee, whatever you eat and drink, maybe it's good to have not only doors, but force fields that are maybe working to move stuff from inside, from outside to inside, or vice versa.

Of course, you might have other mechanisms, devices like ionic pumps, and we will see at least qualitatively (maybe Professor Zoli might tell you about them), that are spending energy. And it's… I'm imagining outside a club there is one big muscle guy that is against maybe the electrochemical gradient and is actually taking in or pushing out stuff from inside the room. But that is consuming energy. Instead, if already *per se* you have some sort of electric field, say you have wind because we keep the windows open and just for free you get food in, this is easy, easier, and it doesn't cost energy.

The second thing that I would like to ask you is whether you remember the Avogadro number that we briefly mentioned. It's purely conventional, so there

is nothing deep there. And I remind you this is the number of particles or molecules in one mole of matter. So this is in a way the convention. I call 'mole' a stuff that contains, say, an amount of sodium ions or about, say, whatever, water molecules, about chocolate beads, whatever, that have as many elements as the number of Avogadro. Do you at least remember the order of magnitude? 23, okay, 10^{23}. That's it. It's just this rough intuition that I would like to push you to develop. Not necessarily to remember things by heart; it will be gone. But at least a little bit, just a couple of things, it might be worth investigating.

Review: Concentration vs. Density Problem Did you have troubles attempting at this problem? I assume that the only thing that you had to do in order to answer the question, "if I give you the concentration, can you go back to the density or to the absolute number of ions?" is to remember the Avogadro number, to be careful, and to remember that chemists are friends. They simply had different units and they use litres instead of cubic centimetres, for instance, for defining their reference volumes. And so millimolar is referred to one litre.

And the other thing is maybe the volume of a sphere that you all remember being… Just to be always sure, if it's a volume, it's likely to be measured in cubic meters, cubic centimeters, whatever. Liters is also a measure of a volume. So there must be some r^3 or there must… in terms of units. So, okay, $4/3\pi r^3$, it's been, for me, imprinted, I don't know, in one, I think it was the elementary school, and it was such a very bad experience that I got it imprinted. But you can check that makes sense that this is a volume, and so indeed, the radius of this small cell, spherical cell, is micrometre, so it will be cubic micrometre. In this context, it's stupid, because of course, this is the context so I can convert it to…

I did not convert. I simply made the multiplication $4/3 \times \pi$ (3.14 blah blah) and then the other thing is that I went to this answer remembering the conversion factor and this glass of beer that you get in Germany that is one liter and is one cubic decimeter, and you can convert it to cubic centimeters if you want.

So it's a huge, huge number of ions. So it makes sense that maybe you want to call this concentration 150 instead of millimoles, so it seems to be relatively small. It's OK. It's actually quite large concentration for being inside the cell. Normally you don't get such a high concentration. And instead of talking about 18, 19 orders of magnitude, numerous molecules or particles.

If you didn't try, I mean you have all the steps. And even for the second question that is: assume that all these ions are distributed in the surface. So they don't stay in the room, in the bulk, but they stay close to the interface with the outside, because maybe the inside is conductive and maybe it's like when you go to the cinema and you don't want to stay… Yeah, this is a stupid comparison. You go to a cinema, you don't want to stay close to your friends because they are not friends, they are enemies, so you try to maximize the relative distance. So if you go to the cinema and you are an ion, a sodium ion, you are going to be repelled from all the others and you will repel the others. So they don't want to stay together. They will be trying to maximize the distance, the relative distance. So they will be accumulating at the surface, at the inner side of the membrane.

And it might be worth just roughly understanding what is the order of magnitude of ions when they are concentrated there. Try. It's stupid. It's very silly. But it may reactivate, unless you have it already engaged, it's a mental mechanism of simply writing and doing algebra and being careful not to make mistakes.

So after this first concept of purely conventional equivalence between concentration and density, we are going to work, you will see today, with these two concepts interchangeably. Mostly we will be talking about concentrations because it's easy to refer to an actual experiment.

Electrostatic Forces: A Review I would like to talk about, to refresh, because you certainly are an expert, about the electrostatic forces, particularly the **force of Coulomb**, a French scientist who first described it, and the associated things. So you have a **force**, you have a **field**, and you have a **potential**. It's not that every time that you have a force, then you have a potential. You probably vaguely remember that's what I'm referring to. And I would like just to refresh this, telling you again about the gravitational force and the gravitational force field and the gravitational potential, because the gravitational potential is something that you have, particularly if you go with a bike, you are very comfortable with.

Analogy: Gravity So again, let me first talk about gravity. I don't know why I got this meme. It does random gravity checks. So this guy is Newton, and in terms of gravity compared to electrostatics, it's a little bit easy because you only have one type of mass. I'm not a physicist, so I'm ignoring antiprotons, antiparticles, so I have no idea whether the antiparticles are… they presumably are only antiparticles in terms of electric charge, not in terms of mass. I know about dark energy, but again, I'm ignorant in physics, so I'm only sticking to this classic description that Newton, one giant in the field, had to cope with and intuitively got it. That is, you have mass and the mass is attracting always.

How much? It depends on how the two masses in this case are far apart and how big they are, how concentrated, how dense they are. This is very silly, but I'm just using it just to remind you that forces require vectors. That means in one given point if you want to express quantitatively a force, you have to give me, say, in a Cartesian system, Cartesian axis system, you have to give me the three components. Because I have to identify an arrow. That means a direction and *verso e una direzione*. I don't know how *verso* is in English. So a direction and where the vector, the force, would be pointing to.

In this case, vectors are easy because the direction is the one that is going through both centers of each of those planets or those particles, those masses. And the *verso*, so the direction where these forces are pointing to, is also easy because it's only attractive. So this should be easy, and you probably know that this is a very famous expression for the force, which reconciled planetary motion and motions on Earth.

So, famous apples that are falling from the tree is governed by the same thing that makes planets going around the Sun or orbiting around each other, described by Kepler's laws, but it seems to be as a different thing. The Kepler's

laws were okay, they were phenomenological. Also, this is phenomenological because we don't understand gravity, at least I don't think that we understand gravity in a very fundamental way. So this is describing in a unified way. And you know that the force, you remember, is proportional to how big are the masses, according to the product, and inversely depending on the square of the distance. And there is the famous gravitational constant that I don't remember, I don't indicate it.

So I indicate like this the intensity, the norm, the modulus, the amplitude, the intensity of the force, because the direction and the *verso*, the direction and the way they are pointing to, are just mentioned verbally. No, the direction is the line that is connecting the two centers of masses.

And sometimes, like particularly say when you have really, really big masses in astrophysics, maybe you don't want to deal with a property that depends on both. If you have two masses, you want to say, I want to have something that is a property of space. This is a very deep concept in physics, and it's when you switch from force to **field**. So, yes, you put a mass in one spot, and then the space starts to have, depending on where… there is a symmetry, of course, like this shading that is a little bit grayer in the center and lighter in the extreme. It's a property of the space. How much? If I place a test particle, a test mass here, then I will experience a force directed in the direction to connect in the minimal direction, in the minimal distance direction, connecting my test mass to the center of the bigger masses, but it could be even a small mass, it doesn't matter, and the direction is attractive. So the sign is attractive.

When you do that, you want to basically, assuming that the mass here is the big M and say is the mass of our planet, you want to normalize, you want to have some description that is no longer measured in Newtons. This would be the measuring unit of this thing, which is going to be Newton. It's not going to be Newton anymore, it's going to be Newton per kilogram because I don't want to have something that depends on my test. I want to use any test, so I want to go to this description that I indicate with E. And you see there is no small m. But I can go if you give me the field, I can get the force and vice versa.

Does this sound completely unfamiliar to you? Probably not. Okay, so you're not too scared. Let me remind you. Okay, so Newton per kilogram.

So the other ingredient that I would like to mention, to refresh you, is that some force fields, they are **conservative**. That means that the work done to move one particle from one spot to another one depends only on the initial and the final position, not on the trajectory. You know it well if you are biking because you love to stay at some… Okay, I'm spoiling something that comes later. So those fields that are conserving mechanical energy are called conservative. And I'm just giving you as a fact. I'm not proving it. I don't even remember how to prove it, although I knew once many years ago.

You can go, you can get rid of the complexity of vector fields. The fact that in principle at every point in space you have three components. And so it might be a little bit complicated to handle and to manipulate and to make calculations on vector fields. So if there is one scalar field, one numerical quantity, that's fine. Okay, in every point might be different, but it gives me just one number,

not three numbers, one number that is called **potential**. Then I can maybe use it more conveniently.

The point is, okay, how do I have, is it possible? For this conservative force field it's possible. There is a function, it's a function of the point. Okay, it might change also in time, but it doesn't matter. That is such that if I take its derivative, or more correctly, the **gradient**, so the derivative with respect to space, and of course in 3D it depends, okay, so which of the three directions you want to take the derivative? You want to assess how these things are changing in space. So you have to tell me where. And you maybe remember that in vector analysis, you have the gradient. You don't have the ∇ operator. You have this thing here. And you don't have necessarily the derivative. Well, in a case of a spherical symmetry, yes, you have. And we will actually stick only to spherical symmetry.

And you also remember that this is basically, since some derivative differentiation operation is involved, I could define V, well, I cannot define, I can spot V, but also V plus any constant, 25 or -52, would work equally well. Provided that this constant is constant in space, when I take the gradient, or the derivative with respect to r, it's the same. So that's why we say that this potential is defined apart from a constant. You should all be familiar with these things.

And particularly, I remind you that we conventionally, I stress it in a moment a little bit more, we call zero gravitational potential the level of the sea, purely by convention. We claim that at the level of the sea, the gravitational potential is zero. But yeah, okay, you could have made any other choices. So in other words, you add or subtract something so that the position r where that's potentially zero, sorry, where, no… So at that position, say at the level of the *sea*, these V and the constant are, say, cancelling and you get what you want, that is conventionally you call it zero. So through tweaking of that constant, you can say that at this point the potential is zero and it's an arbitrary choice, there is nothing magic. And mathematically is written there. You go from a scalar field to a vector field because basically you have a gradient.

The other catch is that for this definition we consider **minus**. So the potential is the function such that when you take **minus** the gradient you get the field, the gravitational field. And so for instance, without particularly being a genius, if you consider this mathematical expression and you remember one of the few derivatives that I asked you to remember, $1/x$, okay since here the independent variable is r, if you take the derivative of $1/r$, you probably know that is $-1/r^2$. Of course you can derive it properly that you have to take the numerator, you multiply by the derivative of the denominator, etc. But say it's one of the few derivatives that you could learn, you could remember by heart. And indeed if you define $-Gm/r$, when you take the gradient, you take the derivative with respect to r, and you change the sign, you go back to the expression of the field that is Gm/r^2. So r^2 is always there in the field and in the force. In the potential it goes like $1/r$, which may or might not be deep as a concept but it's a consequence of this.

I don't know whether it's now or later I have a very silly meme where you will see that one person is maybe overwhelmed by working with vectors but with scalars it's a different story, it's easier. I have also a demo to show you. And

okay here you get back what I just said.

So something that we can do, we can just to motivate something that you might learn or remember mnemonically, that masses (like your bike) move in the gravitational field of the planet from points with **higher** gravitational potential to points with **lower** gravitational potential, from higher altitude to lower altitude. It's the same thing because the force field is attracting and is attracting everything to the center of the planet and that's why the potential is… as more when you… is negative and when you increase the distance the potential is getting smaller in absolute terms. And in fact, if you think, okay, so there is here the big mass, here is the distance. If I plot the function $V(r)$, it's minus. So the minus is making it flip that hyperbola from the first quadrant to the fourth quadrant. And so intuitively you would say if I have a test mass and I put it here, well it will be attracted. So technically it's moving along this potential profile, indeed going from points where in absolute terms you have higher potential to points that are lower potential.

I'm making this as a premise, although maybe it's irrelevant and trivial for you, because in the electrostatic case it gets a little bit trickier, and I wanted to refresh it. There it's trickier because you can have different kind of properties of the particles. They can be charged positively or negatively.

Something that I'm not telling you is that if you consider that expression and you take the Taylor first order… I don't know whether you have been taught like this ever, I was not, but if you take the Taylor expansion to the first order and you simply are considering that you move around some certain distance from the center of the mass, here I'm precisely thinking of our planet, and you are around this value, so you're approximating locally this function just with a straight line, around this R_0, and you call h the quantity that you want to investigate. It's $R_0 + h$. h is your new variable. You basically recover, because of the sign, this is just a number, and it's where the story of the level of the sea… you recover the intuition that the more h, the more the height, the larger is the gravitational potential. Play. Just try to do it by yourself, and it may connect few synapses in your brain.

The Coulomb Electrostatic Force But I would like to talk about the **Coulomb electrostatic force** and I wonder whether, if you consider these two fundamental forces, you ever thought which is stronger? Well, you have it in the slides, but if you knew already which one is stronger. Is Coulomb better than Newton, stronger than Newton? Okay, so the counter-example would be that I have to find a mass so large but weakly… with very, very small charge. Okay, let me consider not an arbitrary case, but just the case of chemical or even biological molecules.

The fact that Coulomb is extraordinarily larger, I think it's 38, 40 orders of magnitude, I don't remember by heart, is the reason why life could develop. It's because chemical macromolecules or even chemical molecules could stay together. Because if it was only due to gravitation, it would be so weak that basically it's as if it's not there. And molecules or ions or macromolecules would simply fly away. A DNA molecule would never be able to exist in a universe where you don't have such a huge predominance of the Coulomb electrostatic

interactions.

And do you know why there is attenuation for both, for the gravitation as well as the electrostatic forces, 1 over the square *root*, sorry, the **square of the distance**? You could try to search on the internet for this explanation. It's something related to the geometry of space. I'm not daring saying space-time, it's space. And particularly it's clear when you think of electromagnetic radiation in the visible. So light is electromagnetism, and light, the attenuation goes like $1/r^2$. Because if you imagine that you have a star in the center and it's emitting some certain density of light, after a given distance, this sphere of light is becoming considerably larger and larger and larger. For some conservation of energy, you would expect that everything is conserved, it's not being removed or deleted. But your eye is very tiny, so it's not integrating the entire volumetric, actually it's called *angolo solido*, it's not integrating in 3D, it's just picking up a little bit, a fraction of this. And the further apart you are, the more what you capture is $1/r^2$. It's very interesting and there are many even light talks or light papers and things on this $1/r^2$.

So it's 39 **orders of magnitude stronger**. That's the French Coulomb, instead Newton was British. Near the St. Pancras Station in London, if you go there, there is a statue of Newton. And again, this is, if you consider elementary particles, they are charged. And addressing the correct criticism by your colleague, I could say that the mass is probably not as represented as charge. Charge is… so you could have a very tiny particle in terms of very light, but extremely charged in comparative terms. But it was a good objection.

So for electrostatic fields, you have two… so I'm considering the static or quasi-static case. So I'm considering a regime in which you don't have the generation of a magnetic field that is concatenated to the electric field because this is changing over time. If it changes over time, there will always be, but the intensity and the wavelength can be neglected. So I'm considering the case where things are not moving. And I remind you that you have two classes, two families. You have one property that the particles, if they are charged, they are say blue and they are red. So they can be of one class or they can be of the other class. Charged positively or charged negatively. It's purely conventional that we call something positive and something negative.

What is important to remember is that if you have the same quality, then the force is **repulsive**. See the sodium ions going to the cinema but not liking each other and so being repelled and causing repulsion from each other. If they are of different species… the opposites attract, even there was a song by Paula Abdul many years ago in the 80s. And so if they are of a different quality, they **attract**.

And it's the same thing. It goes depending on with some sort of constant, and here it has been unpacked because it's relevant in a moment to talk about it. But it depends on the product of these two qualities or quantities that are called the charge. So it's an elementary property. I'm not entering into the details what is the charge. And apologies here, I should have not called it r_0. r_0 is the specific distance in these two examples over the independent variable or the name of this axis that is called generically r. So it goes like $1/r^2$ like for gravitation and it depends on the product. But here the product can be negative, so the sign

of the force can be indeed… can be repulsive, not only attractive.

And again, same story, if I have say a positively charged particle and I put it here, assuming that my test particle is always positive just purely by convention, I would like to have something to tell me that if I place something… to express that if I *press*… if I push or place the test particle in different… test charge in different spots around this, I am basically measuring a property of the space, not of the two specific charges. And again I go from the Coulomb force to the **force field**. And you see that there is only one Q, the Q the charge of this one, not the other one. And again it's not Newton, it's Newton per… before it was Newton per kilogram, here it's Newton per Coulomb. And by reworking the unit you can also express this as Volt per meter.

I have to think, I don't remember why… oh sorry… yeah sorry, I'm… yeah, my brain was interpreting V as voltage. I'm tired, I did not sleep, and this *Olt* I thought it was dt and I thought, no why? Why should be dt? The time should not be there. No, it's **Volt per meter**. I remember this because if you go on the news and you look for safety limits of electromagnetic or electrostatic force fields near the antennas of Radio Maria in Rome, you actually have a number that is expressed in Volt/meter. If it's too large, Volt/meter, then it's dangerous for cells. Obviously it's fascinating to understand, okay, but why it's dangerous? What exactly is the interaction with the biological hardware?

Permittivity What else? Something important that is different from the electrostatic case, sorry, from the gravitational case is that you have this quantity here apart from the 4π that again has geometrical reasons, but… and about spheres, but I'm not talking about that. You have one value of a constant that is called **permittivity of the vacuum** (ϵ_0) and another one that is adimensional and is called the **relative permittivity of the medium** (ϵ_r). And here there is another lesson. So this one ϵ_0 is the permittivity. Permittivity means that it gives permission. So the larger it is (because it's at the denominator) the smaller is the force.

And this is key for having chemical reactions and life being developed in **water**, in aqueous media, instead of in the vacuum. Because the other value, so this is ϵ_0 is expressed in Farad per meter, but the other relative permittivity is without dimension. One is the permittivity of the vacuum. If you look at what is the permittivity of water, it's almost two orders of magnitude larger. And being at a denominator it means that you have a sodium chloride molecule and you put it in water, then you… because the force that was much more intense in the vacuum, you put it in water, or even in dry air is not too far, okay it's around 1. But as soon as you put it in water, the two ions constituting the two molecules… constituting particles constituting the molecules, sodium and chloride, may fly apart, fly away, just because you have 1/100. So it's 100 times less intense this electrostatic force.

And what it's interesting is that for phospholipids, now I'm throwing it like this without any justification, you have a value that is in between. Cells are made, particularly the cell membrane is made of lipids, and I'm particularly fascinated, we will briefly discuss it later, that the first time people started thinking of that cells, it was in the 19th century, that cells might have a membrane. And the

membrane is a crucial point because it makes inside different than the outside. It makes a distinction between the environment and what is not the environment, what is *me* and what is *not me*, is not necessarily so obvious concept. And people could not observe with microscopes, because it's so thin, so small, could not make inference or guess about the thickness. And they did it purely by… the first time they did it by electrical methods, precisely because it was possible to *know* the relative permittivity of individual components of the lipids.

And to me it's fascinating that you don't see something, compare it to a capacitor, and we will see later why the membrane is acting as a capacitor, you can guess the distance between the two plates of the capacitor, so the thickness of the membrane, by an electrical measure. It's not the first time. There is another… for understanding how these pores, these doors that make the inside of the cell connected to the outside of the cells… the first time people could not see them and they could only measure it electrically. So it's a primate of electricity and electrical methods compared to optical methods, to optics and microscopy.

Quantization of Charge The other things that I would like to remind you, because we will use it often, is that the electron, so the charge in general is **quantized**. You cannot have an arbitrary value of charge, it comes in multiples of an elementary value that is 1.6×10^{-19} Coulomb. And for instance in the case of this sodium chloride that is dissociating in water, you probably know better than I do this is reversible. And indeed when I write Na^+ and Cl^-, what I mean is that this guy has a positive charge and because there is only one plus and I'm thinking of the valency etc., I'm specifically referring to this value. And when there is a minus, so this one is negatively charged with the opposite sign so that both are somehow canceling so that the molecule is electroneutral. This you should be roughly familiar with all these things.

So this is the story of vector fields. In every point even in 2D it might be quite daunting. So this guy is depressed. Maybe you don't know who this guy is but is famous in my generation, well even in the previous generation. If you have scalar quantities it is much better.

Electrostatic Potential and Superposition So even in this case, the electrostatic force fields are conserving mechanical energy and therefore you have one function of the point such that if I take minus and I take the gradient, I can describe the electrostatic field. So in other words, given the electrostatic field, it always exists a potential function. And this potential function is again, is defined apart from a constant. Instead, if here it was, instead of V it was $V + 25$ millivolt, okay, when you take the gradients the constant will go away because the derivative of a constant is zero. So you get the same electric field regardless of these reference. And the reference of these aluminum foil that I wrapped my circuit around… so it's not anymore the level of the sea where I say, so the height is zero and so the potential I call it zero. Here is just an arbitrary point where I call the potential zero. And normally is, it is considered in what is called the electrophysiological convention, not a very original name, zero for me is outside the cell where I have some sort of reference electrode. But we will be more on this.

So the space or the expression of this potential… before for the gravitational

was easier because it was G and here it's just a number, there is nothing to understand. This is one number that it's known… goes like the charge divided by the distance. So it's $1/r$ for the exact same reason.

Let me probably stop… let me see what is next. Yeah, okay, this is… this is important. One minute and I'm done.

So something that is very cool, but I'm not… I have no time to really to go more in details, is that Maxwell equations are linear in the sense that the **superposition of the effects** holds. If you want to understand what is the electrostatic potential, so it's a property of the space, in this point, P, I choose that point… When you have this collection of small, large, positive, negative charges, it's enough that you consider the superposition of the situation that you would have if you had only one charge at a time.

If you had only this big one that is red, you would have… you have to calculate the distance, and you will have, okay, this pre-multiplication factor is just a number of stone, you will have only Q_1 divided by the distance between this Q_1 charge to the point P. Now you make disappearing the red large charge, you make the appearing only the blue small that is considerably further apart and you would have in that case, you would have Q_2 divided by the distance.

So if you want to understand or express what happens if you have in one point, say inside the cell, you have -70 millivolt with respect to the outside where it's zero… you may say, okay, well if you… if I know where the charges are located, I can basically consider a sort of superposition of the effects, which is some sort of weighted sum of the charges. Clearly, okay, one big charge, if it's a big positive charge, is going to contribute positively to the potential over there. But if it's very far apart, $1/r$ will attenuate that contribution.

So with this you could maybe start thinking, okay, maybe if I see -70 millivolt, if this holds true, maybe it means that I have many negatively charged particles inside the cell. And that would be perfectly legitimate, but it's not entirely correct.

If you have questions now in the break… let's stop and wait for 10 minutes.

Let me restart. You… I will point you to it in a moment, where with a so-called web app, that means it's an application that is only requiring a web browser, you can go and say, let me not take it, say, I can take a lot of negative charge, in this case it's 1 minus… -1 nanocoulomb, -1 nanocoulomb, it's a very small charge. And I can also have a sensor, so this is the test particle, and you see that these are the lines of the force field and it's relatively complicated. I'm having fun, you see? Saturday night, what do you do? I'm having fun playing with charges. And yeah, you can play with it, but see what happens if you are only looking at a scalar quantity. So there is no more arrows. And what remains is some sort of shading that reminds maybe the concentration of, say, smoke. If I should smoke here, there will be a very high density of smoke, of particle here. There will be maybe diffusing, there will be less intense concentration of smoke, less intense value of the electrostatic potential in one spot far that it goes like $1/r$ and it goes far.

And if I have different distribution of charges… so it's not… This sensor is a voltmeter. So in this… so you see at some distance from this distribution of

charge, the electrostatic potential that I… that I'm detecting here in the points identified by the cross, I see minus four… so it seems to be negative. If I get maybe close to the… to the positive charge, they get positive, but otherwise is… is negative. So maybe doesn't get… doesn't get -70, but this might say… You could play. After a few minutes you… you will be fed up. This could give you some sort of intuitive understanding of why, first of all, is easier to work with potentials and what does it mean this superposition of the effect, when I remind you the task is to understand why there is this damn -70 millivolt.

Potential, Charge Movement, and Current Something else that I wanted to… in addition that I wanted to talk about is the exact same story saying that if you have positive charge, so you have one big charge that is positive, a **positive charge** would move from point with **higher potential** to point with **lower potential**. I think I have here… if I have a battery… I hope that you, maybe I said it, I didn't, well it's difficult but I hope that you, well don't do it because it's dangerous, but I hope that maybe in your childhood you had… you licked batteries, particularly the 9 Volt batteries. But anyway, so one pole of this battery is 1.5 Volts with respect to the other, it's the negative, but you could… you could say that the difference of potential from here to here is 1.5. So it means that in this point you have 1.5 Volts. And so if you have a positive charge, because of the potential generated by this big blue charge, it would move along… will be repelled and it will be move along these potential profile, indeed moving from points of high voltage to points of low voltage.

I tell you this because for many years there was the misunderstanding of… centuries? No, years… there was the understanding and still it's the same convention, that electrical currents are… in conductors, in metallic conductors are… is carried by positive charge. And indeed you know that if you have here… you have a circuit, the current goes from the plus to the minus. Well, electrons are negatively charged. And so actually it's the **negative charges** are moving from points with **lower** electrostatic potential to points with **higher** electrostatic potential.

I wanted once in your life to revisit this important concept, to know that it's wrong, it's a different convention, but it kind of fits whatever you do with the story of repulsion or attraction. So if you are in the world of Coulomb, of force field, you only have to remember the sign of the two particles, but if you're in the world of potentials, it's easier. And you only have to remember, like in the gravitational field, you are by bike and the bike naturally goes from points of high potential to points of low potential. And for electrostatic is the same, unless you are a negative charge, which is the exact opposite. And this is indeed what the real electrical currents are carried along.

So here I'm just, it's already since last week that I'm stimulating and saying, okay, -70 millivolt, what do you think? I told you that now we know or we know again the superposition of the effects and so if you have -70, well, it could be that you have a lot of negative charge. Would not be shameful to reason like this, but it can also mean the exact opposite, as we will see in a moment. And again, I hope that you started thinking. I would love if you could keep these *critics* in your brain alive.

Biophysical Primitives: Mobility and Friction But first I need other elements, other primitives in biophysics, and one is called **mobility**. It's purely a description, is a conventional description as you will see, of how tough is for one particle to swim in solution, to move in in an aqueous medium, if it is a sodium or *chlorine* ion and if maybe one ion molecularly is fat and another ion is thin, slim.

And I start with the… with the parachute. Do you know why these guys are not dying? Probably you would say, well, it's because of the parachute. Do you know what the parachute is doing? So you probably know that in proximity to the… of the… of the surface of our planet you have an acceleration of 9.8 square meters per second or meters per second square. So every second you accelerate, you change your velocity by roughly 10 meters per second. So why is not dying *to see*, crushing himself or herself in the ground?

Which is by the way, is the same reason why when it rains, the drops of rain are not damaging your scalp, killing you on an instant. They should. The reason why they don't is because they have, well there is not really the parachute, but because they are… the drops of water, of rain and the parachuters are moving in a dense medium that is air. And air is causing **friction**, viscous friction.

So you have in the general case… you don't have raindrops and you don't have parachuters… but you have a particle that is moving in an *aqua* solution under some external force. I don't care whether it's gravity, whether it's electrostatic potential, sorry, electrostatic forces, I don't care. And I only have to invoke or remember one guy, **Stokes**, who studied friction. And actually it is very interesting because he studied how the geometry of a particle in a solution is affecting this friction. But for the sake of simplicity here we don't care, we just basically say that the friction that is familiar to those of you who are maybe swimming in the sea is increasing, is proportional to the velocity, is not constant. The faster you go the more the deceleration you experience. And it goes in the opposite direction of course from your motion because it is again, it's a friction, it *tries* to oppose.

So let me invoke Newton's second law of the dynamics, $F = m \times a$. Force equals mass times acceleration. Of course acceleration I express this as a first time derivative of the velocity. And… And again you will see why I'm obsessed by that always boring usual differential equation because it's always the same linear first order constant coefficient equation that comes over and over, at least in this course, not in nature but at least in this course.

So here I can write the overall sum of all forces. I'm thinking of a monodimensional case otherwise I will be making a necessary complexity. So I'm considering that there is some sort of direction of motion that I call x. x is a coordinate, so I can have some velocity. I can even define an acceleration if you want. And I can define a position. And I can go from one to the other by differentiating, by derivatives. And along this monodimensional coordinate, which is describing the system in all its complexity, I have two forces that are the sum, so F equals mass times acceleration, M… is the total force, and the friction is opposing. So if I take this axis along the direction of the motion, say along the direction where I call positive the force that is positive in this direction, then the friction force needs to have a minus. And this λ is a constant, is a positive constant

and is the… basically is the… is a factor that is describing how big is the viscous friction. And you see here this is given, it's proportional to the velocity. The faster you go… the slower you are… so… so the slower is the… the faster you go the stronger is *these* friction force.

I remind you that these are forces and *these* are the world of velocities, positions, and acceleration. So they are slightly different beasts and the difference is because here you have a derivative. Again, it's the always usual boring differential equation and if I write it down, so you actually see here, friction I replace $-\lambda v$.

I told you the other time that if you want to make me happy you have to ensure that if you present me with a differential equation that the state variable in the left hand side, in this case, has a minus sign. Because in my brain I have always the automatic reaction saying, okay, yeah, the solution, at least the solution of the homogeneous equation associated, is an exponential. I have to put the exponential to something and if this something has a negative sign, I'm happy because the system is dissipative and this friction is dissipating energy. It's actually dissipating energy in an irreversible way as heat. So things should not explode. I should not expect the velocity to exponentially increase, *vice versa*. They should be exponentially *decay*. So make me happy and always keep an eye on what you have from the other hand side of this equation because this is what you're going to put in the exponent. True, there is this constant here m, but m is positive so okay, I could divide both sides of this equation by m and here will be $-\lambda/m$. Okay, still it will remain positive. So the minus, I'm grateful for this minus which is the Stokes law.

And of course this is not an homogeneous equation, so in principle I'm hand-waving, but you allow me to assume that this non-homogeneous term is constant in time. It's given and it's a constant. It's not an arbitrary function of time. And it could be as constant as gravitation or as constant as the Coulomb force that a big charge is exerting on one charged particle that is entering in solution and moving along this direction. It doesn't change in time. I don't care whether it changes in space. Here it's a differential equation with time. If that's the case, I can apply those heuristics. And so if the force in terms is constant, then likely the input term is also constant. And I can identify which constant it is by direct substitution.

Anyway, to make a long story short, I don't even care about the initial condition that would allow me to select one among the infinite solutions of this differential equation. Because you see here, it's when I'm very happy. So this term is going to vanish. And the reason why this is vanishing is because friction has the property of making particles approaching the so-called **limit velocity**. So, parachuters, because of friction of the air and the same for water raindrops, are not constantly accelerating over… At some point they stabilize. I spoke to people throwing themselves from an airplane, just skydiving, I think it's called, even without, before opening the parachute. The first few seconds are ugly, because you feel it in your stomach, the acceleration when you're falling, but at some point, then you don't accelerate anymore. And in principle, it's indistinguishable from a reference frame that is not moving.

And this second is given by this constant, which is the inverse of the time constant of this equation. It tells when the transient is gone, is over. So this

friend of mine doing skydiving, after a while, his velocity became constant. Okay, it might be unpleasant, because particularly your visual scene, the visual field is expanding, so you're clearly, if you don't open the parachute, you're dying. You're not constantly accelerating and your stomach is not telling you that. And… and the reason why this can be done, we can neglect it, is because the mass and the… so this ratio between λ and m is very, very, very large because the mass is very, very small. And if I have this that is very, very large it means that one divided by this quantity, that is the time constant, is very small. So in a matter of picoseconds or faster, an ion in solution will reach this limit velocity.

And therefore I can write this relationship which is the limit velocity, so the **velocity is basically proportional to the force field**. Newton would be upset and would say, "beware, I think this is… it goes to the time before Newton." People… forces were directly translating into movement, and Newton said, "No, force is equivalent to an acceleration, not to a velocity." Unless you're in a viscous medium. If you are in a viscous medium, then if you double the force instantaneously, well, it's not really instantaneously because you have still some latency, but say, very rapidly, you're changing the velocity. This is crucial because we are swimmers. We stay in solution.

And people, instead of calling λ the Stokes coefficient, Stokes parameter, Stokes coefficient, they call u **mobility**, $1/\lambda$. So this is my definition of mobility and it tells that the velocity is proportional to the force field. And you will see in a moment why this is relevant.

I warn you that in the literature, there is, in books, you have also the tables with the numbers, with numerical numbers of people that calculated the mobility, and how they could calculate it, well, they simply… they had to calculate the velocity. They knew the force, they calculated the velocity, and they basically had the mobility. But they had… they worked with the charged particles. And in our case, this is what is called **absolute mobility**. So it's a relationship between the force and the Stokes parameter. And it's measured in meters per second Newton.

If you have the charged particles, in the book you call it… and they are basically referred to not absolute, but **electrical mobility**. And instead of being a force, it's a force field. And so somewhere compared to the force you divide it by a charge. So here in between there is the charge, or if you want really to *spaccare il capello*, if you want to be very picky, you have to put the total charge, so that is the elementary charge multiplied by the valency of that particularly ionic species. And the valency is here in the absolute term is the… the modulus, because I don't care whether it's positive or negative in the settings that I'm used. It's enough that it's making the particle moving. Only just to tell you that if it's there it is measured as meters per Coulomb per second… meter per *cool* divided by seconds per Newton. So just be aware that in books you may find other numbers. It's the same thing apart from this conversion factor.

But anyway, we always stay here and anyway we don't look at numerical numbers. So you can go from one to the other apart from the elementary charge or, yeah, the total charge if you… if you're talking about say calcium ions that in solution they dissociate and they have $Z = 2$. So the valency of a calcium ions is 2, they have 2 positive charge, elementary charge.

These are some number and you see that the potassium and sodium and chlorine they are more or less of the same order of magnitude. Some ion has an easier time to swim than others. And you might see some other numbers but they are different units so that's why you don't see exactly the same. It's 10^{-8} and here it's 10^{11} so you would say it's crazy. No, it's because here somewhere you have 1.6×10^{-19} Coulomb at the denominator. But okay, that's it.

This is however wrong, but we don't care, we stick with this approximation because as you know ions in solutions are not swimming like this naked. They don't like to be naked. They are immediately neutralized by water dipoles that are constituting what is called the **hydration sheet**, hydration layer. So sometimes you might have ions that are very slim but they have a very big hydration sheet around them. And otherwise ions that are fat but they have maybe… they are not necessarily more fat than one slim ions with the hydration sheet.

Flux of Particles The last conventional things that I need for the following is the **flux of particles**. So I'm thinking of some sort of external fields or forces. I don't know what forces they might be. I anticipate it's not gravity because gravity is, with all due respect to Newton, is very weak and it's not really playing any role. I'm considering mobility, so I'm considering how friction basically, how good is one molecule, particle. Look, particles could even be not electrically charged, and we will see together. So it's not that if you're in solution you need to be electrically charged. There are other phenomena that happens if you're not electrically charged. And then the other thing is some sort of way to describe, to measure, to understand quantitatively how under a certain force, with a given mobility, I give rise to a flow, to a flux of matter.

But I need to be precise because there might be several ways to define flux of particles. How would you define it? What I'm thinking is, I'm thinking of a river. And the river is flowing, and it can be a small river or a large river, and the velocity of the particles can be really large, even if it's small or if it's big. So there are two kind of things in this view. I wonder whether you could suggest me what they are. One needs to be if they are small or large, so some sort of section or cross-section of these flux, so space, surface. And of course there is another quantity which tells me how fast things go. So time is also an important thing.

Any idea? What would you call it? Flux. You would say it's an amount of water… well maybe for electrical currents you know that currents are measured in Amperes. And Coulomb per second. So this, apart from the formal definition, is because people thought, well it's a flux but it's in a wire, maybe in a conductor… well I don't care. It's not a density, it's just an absolute flow and is basically in a given amount of time I move a certain amount of charge, one Coulomb. If I move one Coulomb at every second and I really imagine one big charge, blue charge, that is one Coulomb and is moving, moving to the left in one second, so it's not particularly fast, then I will have one Ampere, which is quite a big current.

Here, I told you I'm interested in if the section or the cross section is large or small. So I'm interested in **densities**. So again, let me see if my method would

match yours. So I call it J. J might resonate with a current density, but in this case, the particles may or may not be charged. So the units are not necessarily, we will see what the units are with this definition, are not Amperes, because there is no Coulomb, at least not necessarily.

And here is the first time that I can work with both, with either densities or concentrations. And I know how to go from one to the other, so they're not scaring me anymore. And this flux, just for simplicity, I'm considering one-dimensional movement. There is some velocity and there is some external force. But one way is this one. And here you see why physicists would be happy with densities and the chemist would say, "No, let's use mole."

So it's a **number of...** Pick your field, pick your part of the game field. So if you want to be a physicist, say number of particles moving through the surface, the cross-section of the surface. Maybe I would like to make that the surface of passage is not really having an impact. I would like to normalize. So if it's a big 10 square meters, or if it's a very small blood capillary, I don't care. I would like the flux to be a density measure, a specific measure, not an absolute measure. So it's a number of particles moving through a surface, a unitary surface, say one square meter or one square micrometer, in the unit's time interval, exactly like for the current.

And if you like more chemists and you like the moles, it's basically the number of moles moving again, the same thing through the unitary surface in a unitary time interval.

Let me try to graphically unwrap it and you tell me whether I'm… well, it's a crappy sketch. Here again my direction of movement, now I put it horizontally because it's… I don't know, I go in the lexicographic order from left to right. And although here I was thinking of something falling in a jar made of liquid… but… so with the jar with a bottle made of liquid is because maybe gravity would make this particle slowing down and reaching a limit velocity. Here I don't care. So I'm assuming that the force is along this direction and it's pushing the particles from left to right. And here I conventionally identify a reference surface that I call ΔS, delta because I'm perverse, at some point maybe it will go to zero or I will simply normalize it, I will divide by it. And I have a watch and I wait one Δt. Nothing is infinitesimal so far. They are not infinitesimal measures yet.

But I basically say if things are moving, it means that they do, in a unit of time, they move of some length, some meters per second. So in one second or in alpha second, things will be moved. If I take two pictures, one now and one after one Δt, I can probably identify a displacement of all these particles that were, at the time of the first picture, they were all stuck in this coordinate, at this x coordinate, that they were basically in this frame. I'm really imagining, I'm imagining it like many particles of really a fluid or smoke, so there are many. So if I slice it in that plane I will have many, not all of them because of course they are dispersed in a volume as concentrations or as density, but if I slice it, there will be few of these particles at that position.

So after Δx, basically those particles, I assume that they all move with the same speed and there is only one direction that they can move, so… well then I draw another rectangle, another area, that it tells me where they are now, those that

where Δt ago they were in the first… in the first surface. Maybe you see where I'm going because now that I have Δx and Δt I'm tempted to say, yeah, okay, but $\Delta x/\Delta t$ is a velocity. Well, it's a uniform velocity, otherwise I would need to take the limit. But even in that case I'm fine, I know… no… calculus, so in that case I could take the limit. So if the velocity is not, should velocity not be constant in time, I could still handle this situation.

But here I have that $\Delta x/\Delta t$ is basically the velocity. And another interesting thing is that basically here Δx and ΔS identify a **volume**. And this volume is the volume that these particles basically they identified, they, whatever, in the Δt. And I know how many particles are in a given volume. If you tell me the volume, since I know the concentration or the density, I can tell you what is the absolute number of particles in that volume. And this is what I'm going to do.

So the definition here is number of ions that cross the first surface, blah, blah, normalized by the, so per unit of area and per unit of time. So I have to divide by the area and divide by the time interval. But I know how many ions are here in this box. Because it's enough that I have the density and I multiply by the volume. If I like to work with densities… if I like to work with concentration, I do the same. But there will be an Avogadro number in between.

And you see that if I do this… I think I have it here for concentration for those who are like me, more fond of moles. So here you will ask, I want to work in moles because I know that sodium is 150 millimolar concentrated there. And what happens if I apply some electric field? And so they will move, and I want to use the fact that I have 150 millimoles per liter. So here you see that I can simplify ΔS, and I remain with C or ρ, whatever it is, the density or the concentration, times the velocity, which is simple, which is okay.

The Nernst-Planck Equation (Thorell Formula) Let me do another step invoking mobility, because with mobility, I can go from velocity to force. So here, with this definition, which is called the formula of Thorel, is a guy particularly, say, for the molar fluxes, not for the particle fluxes. Look, we have to commit, but we know how to go from one world to the other.

The flux is given by the **mobility** multiplied by the **concentration** multiplied by the **force**. $J = u \cdot C \cdot F$

And it's not so impossible, so strange, because if the mobility of this particle is very, very small because the particle is a very poor swimmer, it's very big, and Stokes is slowing him or her down a lot, then the flux will be small. If you don't have enough molecules, if the concentration is small, the flux will be small. It cannot be larger if you don't have stuff there. And finally, this is clearly, it makes more sense, the stronger the force, the stronger is the flux. This is also making sense. If I blow air, the stronger is the force that I put with my lungs, the stronger will be, the more intense will be the flux with this definition. So, and the fact that here is a linear, it's a proportional dependency, is something that I like. It's sufficiently simple.

So this is called the Thorel formula, and again, depending on your choice, if you're talking about moles or particles just be careful, connect your brain for a moment and then disconnect it and play or use units. So when you write

units like moles divided by square meters divided by second… will be the unit of this flux. And you see it's a density because in the denominator you have a square meters which is the cross section. Again I don't want to work yet with the current because I want to work at some point with densities. I don't know the sections like I don't know how many particles I have. I'm happier to talk about concentrations.

So here is if you want to go from the flux to the current density. So flux is agnostic in terms of electric… electric charge. But if you want to do that it's enough if you want to go for… this is **current density**. The current density means Coulomb per second, fine, but it's per unit of area, per unit of cross area. And so you see that is per square meters, Ampere per square meters. And if I want to do that, nothing is easier. You simply have to multiply by the charge.

Again with this stupid example that I'm imagining some molecules of smoke and this… and there is some wind that is pushing them in one direction. If they are all charged, I have to multiply… if I have the flux, in order to get the current density, I have to multiply by the total charge. And in this case, I have to multiply by the charge of an element. If the element is a particle, I have to multiply by the elementary charge. Maybe the valency here. Maybe here I should write also z. But if I have moles, then I have to multiply by the charge of a mole. And if a mole is made of 6 point something, 10^{23} particles, then okay, each particle will be charged Q, and I do the Avogadro number times Q, which is incidentally called **Faraday's constant (F)**, which is the amount of Coulomb per mole.

Nothing to understand here. It's simply that if you want to go to the world of electrical phenomena, electrical currents, you need to give this label to the particles. And depending on your choice, if you are in the… if you're in the… in the physicist quarter and you like densities, multiply by the elementary charge. But if you are in the moles, you have to multiply by the Faraday constant. And we will love to stay with concentration simply because it's so easy to prepare solutions and work with concentration.

So remember it will be at some point, although for a very brief moment of time, but at least now you know that the world of electronics is… could be reconciled with the world of particles swimming in a solution… ions. Because after all, ions are charge carriers. So maybe they can bring currents if they move in solutions. And that's why my neurons are firing and they generate electrical phenomena, because it's the same thing. But they're not electrons.

And actually it's even more interesting because in the… you know that electrons and even holes are in semiconductors or only metals are the only charge carriers. In electrolytes, in biological solutions, you have a multitude of charge carriers. You have magnesium, you have chloride, you have sodium, you have potassium, you have *chlorine*, you have many other ions that if they are in solution they might be positively or negatively charged just purely because of the dissociation due to water, to the properties of the permittivity of the water. So it's the same thing. And here basically we are reconsidering, we are approaching the elementary bit. We already kind of revisited the potential and now the current. So basically Kirchhoff is our friend now because we… we know that Kirchhoff laws of current and voltages… okay here are the same… currents… okay current

densities… and voltages.

Two Types of Fluxes: Drift and Diffusion And now the interesting bits. What kind of fluxes can occur in solution? Because after all, okay, fine, this can be an arbitrary function that is very elegant mathematically but… but okay, what could it be? There can be only two types that we are going to consider. One is if the particles are electrically charged and there are electric fields, electric force fields, they are basically Coulomb electrostatic interactions. And the other kind is **diffusion**.

So that's why here there is a waterfall, because the waterfall is a **drift flux**. There is gravitation that is pulling the particles. Okay, here the particles are not electrically charged but they have a mass so it's a similar thing and they are pushed in this direction. Here you have smoke and the smoke is diffusing. There is a lot of smoke here so immediately the concentration of smoke here will be high. But after a while, just purely by thermal agitation, and by bumping in air molecules or water molecules, the ink, I'm thinking of an ink in a glass of water, or the molecules of the smoke in air, will basically diffuse. And if you wait long enough, they will be everywhere equally diffused. Unless they might interact. But normally, smoke particles, they do not interact among themselves.

If they are ions and they are unequally distributed, maybe they do both. They could diffuse, but as they diffuse purely by, again, thermal agitation, they are too much concentrated here, so randomly they would go in all random directions, but the net flux would be that they would diffuse away from the point they were concentrated. But they are the ions that were going to the cinema, so they were, for instance, if they are all with the same charge, they will start to repel. So there will be some sort of combination between diffusion and drift. And if you have not just one, but you have maybe two or three or four or n types of charge carriers, things may start being interesting because they will be, each will diffuse, but they will interact with each other by electrostatic forces.

To make a long story short, we cannot easily understand how these two things combined would give rise to this -70 millivolt because over there there is the… the answer to our problem. And so we are now using this… this framework. We know how to define fluxes. This is… I told you already the diffusive and drift fluxes.

1. Drift Flux Let me consider the **drift flux**. Somehow it's easier mathematically. So I'm considering, of course, charged particles, otherwise there is no point. Coulomb will not give me any satisfaction. Coulomb only holds when the particles have this physical property that we call charge. But allow me to assume that they are uniformly distributed. So they are everywhere the same, so there is no diffusion. They might stay in solution at 37 degrees, but overall, statistically, they will not move. What they do, they will repel and attract each other.

But let me make it easier. I'm thinking that there is an external force, and this external force, I told you, that can be related to charges by the knowledge of the electric fields. And if… since I don't like working with vectors, instead of E I work with minus the gradient of the potential. So the external force acting

on a certain charge with a valency that can be 2 in case of calcium… so is the amount of charge times the electric field. Or the amount of charge with the minus… the gradient of the potential. This is the specific force field that I would like to obsess now.

So here if you like working in the… in the world of densities, and so here is Q is the elementary charge of a proton and z will be 2 say for calcium, will be 1 for sodium, will be 1 again for potassium because they dissociate and they have unitary valency. If you like, like I do, working in the domain of concentrations, nothing easier. So this is a force, still it's Newton, but what I have to address is that I have to refer the force field or minus the… and I wonder why there is no minus here… why there is no minus? Okay, later… Avogadro number times the charge of the individual particles. So this is z times the Faraday constant. And this is going to be multiplied by the gradient of the potential. Minus. Minus. And here there is the minus. So here is no minus. So I'm losing minuses, but then they reappear magically for some magical property. Apologies, I will amend the slide tonight. And I will re-upload it correctly.

So now that I have the force field, and okay, depending on which is your favorite team, I can deal with it. Let me plug it in this expression of the flux. Nothing easier. You have the mobility. You have the concentration (because I'm working in this framework). And here is the force. Of course, it's the force related to the concentration world. So it's the, let me see here. So it's $-z$… minus, and then $N_A \times Q$ is the Faraday constant. So this is the total charge. So this is Coulomb. And this is because the Coulomb… the electrical force, is a conservative force.

So, okay, it's basically done. Don't worry about the story. I think that you will not be tempted to, you will not have the urge to consider Faraday constant as Coulomb per mole. If you do, we can talk about that, but I think that that will be okay.

So this has the same unit of a flux. That means moles per square meters per second. It's not a current density. It's just the flow of particles. If you want to make them into a current density, you have additionally to multiply by zF, but we don't do it for the moment.

Why am I doing this? Because this flux is the flux of matter. Fine, the matter is electrically charged, and these charged particles are moving because there is an electrostatic force. Fine, but this is measuring a flux of stuff. So it's like the flux of a river. So, so, passively and, sorry, positively and negatively charged, they experience a force, and this force here I basically put it in relationship in a monodimensional case with the electrostatic potential.

What I'm trying to go… so where I'm trying to go is the following. To give you a slight bit of intuition. I have a cell and I have this electrostatic potential V that I told you is negative with respect to the reference electrode. So I have somewhere a potential that I measured. It's not something that I imposed. I measure a potential. So I would love to translate, to relate electrical phenomena that are being characterized by potential to fluxes. Because intuitively I'm thinking that maybe there is something related to charges inside and outside and these charges, as opposed to what I did on the demo before, they are not glued in place. These are glued in place. See what happens if I put a positive charge, the negative is not… is… It should be kissing the negative. Instead, they are not

attractive. They are glued on the table. If instead I put these, it became now proverbial. So the sodium very close to another *peer* in the cinema, they're not repelling each other. These are, just for the sake of simplicity, these are *stick*, they're glued on a table.

But in solution, everything moves. So I hope that maybe if things are moving, I can describe fluxes. And if I can describe fluxes, I can maybe sort of understand if there is any relationship between fluxes and potential. Maybe, even ambitiously, some relationship which is self-consistent. There is no external force. It's just the thing that is reorganizing, redistributing, because things are… maybe initially they're not equal inside and outside, and if they're not equal they will start giving rise to fluxes, and the fluxes are going to create an equal distribution, there will be repulsion or attraction, and at some point, -70 millivolts will come up. This is my plan. It's not entirely correct, but it's almost what we are going to do. It's not exactly how things will work.

2. Diffusive Flux Let me show you this animation and then we break for 10 minutes.

If you don't have any electrostatic charge, any electrical charge, so they are not charged particles, they are in aqueous solutions and they are unequally distributed. Because otherwise if you kill me also for the distribution, so they are not interesting, they are not spicy. They are not charged. And everywhere is the same. Well, they don't do anything interesting.

Here you know that if they are in solution and they are not charged, they **diffuse**. Diffusion is omnipresent. So the ink in water, the smoke. And this happens because you know that there is thermal agitation, so non-zero kinetic energy of water dipoles. They are constantly agitated and they bump into each other. And they do this because they are not at 0 degree Kelvin and so there is thermal agitation. And by doing this they transfer kinetic energy to the… to the particles, to the calcium ions for instance. And they do this by random isotropic collisions. Isotropic means that there is no preferred direction. So it's the same. So a dipole would bump me from the bottom or from the top or from the left to the right, it's gonna be the same. I will randomly make a step.

Probably you heard in this context the analogy with a drunk sailor… *sailor man* that is drunk and it can be… just because it's drunk he can make a step on the right or a step on the left. If it is a mono-dimensional *sailor… sailor man*, it will go randomly from one direction or the other. Here I'm basically thinking of the underlining things and this very fantastic… I will at some point I will make it nicer. This is a stupid cartoon that I made, not really implementing the diffusion equation. The diffusion equation is something that I would like to derive. The only thing that I… so here it's what it's called the Monte Carlo simulation where I'm basically considering the microscopic scale and microscopic phenomena. And here the microscopic phenomena is that they are only bumping and making random movements. In other words, I don't know whether you have ever heard of this, this is the so-called **Langevin equation**. So the actual stochastic bumps are translated into a force field that is just a random number generator in a computer.

If they start all concentrated in one spot, purely by this random, and believe

me I did it, there is no direction that is preferred, you have that they were all in one spot at time zero and now they tend to be distributing everywhere in the *same* in space. This is a box so there are boundary conditions so you see that particles are not allowed to escape. And this red and blue lines are basically histograms of the horizontal and vertical coordinates. If at the time zero there were two Dirac deltas because they were all concentrated in one spot, at t that goes to infinity they should go flat. So this is the concentration somehow, and the concentration should be the same everywhere. You see they did not stop moving but statistically their overall population did not change. The only difference is that they are boundary conditions so you have these two spikes at the *other* boundaries because yeah, I cannot… I make a random… if I'm here I'm only… I can only make a random step on the left because if I make a random step on the right, I hit the wall. So there it's a restricted diffusion, so you have this anomalies compared to what the diffusion equation would predict. But here there is no diffusion equation.

But the question is, after the break, can I make the flux? Can I understand how do I have to write the flux? Because here it's a bit complicated, I leave you to think of it. What is the force field in a diffusive environment. There is no… well there is a force because things started *to* move around, but what is the force field? Is it conservative?

Let's stop for 10 minutes.

Okay. Okay, let me restart.

So this slide is a bit dense and there is one concept that I cannot make it… I cannot make it easier for you and so it's a bit disappointing for me because I wanted to show you the steps as I derived the drift flux. And in the case of the diffusion, as this is not a course in thermodynamics, I cannot come with a… because this involves statistical mechanics and thermodynamics, I cannot easily tell you what is the force field and what is the potential that is associated to the force field in the context of a diffusion.

So the elements that I clearly intuitively are there is that temperature has to play a role. So the warmer the bath, the faster is the… So the higher is the kinetic energy. So the story of the kinetic energy, which is clearly well represented in a statistical mechanical representation of thermal phenomena, is there. And the other concept that is there is the concentration. So this is something related to the concentration. If things are *unequally* distributed, then there is some sort of net force, even though the elementary forces are random and isotropic.

So this is sort of given. It follows from other principles that when you have temperature that is larger than zero, so you have non-zero kinetic energy of the water molecules, and kinetic energy is exchanged, it is as if you have an external force field which depends on the temperature. k is the Boltzmann constant. And then there is this funny gradient of the logarithm of the concentration. So the two elements are there. The temperature is there and the concentration is there. And okay, the temperature, I'm happy that is here, that is proportional, so I double the temperature this force seems to double so that, okay, it could be intuitive. What it's not intuitive at all is why this has to be this potential… so minus the gradient of this function, $\log(C)$, with respect to space. But this is the potential of the force field, how this is came to be. And of course it's also

more complicated to state, oh okay, so a potential exists, so the equivalent force field is conservative, but I don't venture in that concept.

So this is the exact same case for the world of densities. If you are in the world of concentrations, this is a force that has to be scaled to an Avogadro number of particles, so this Boltzmann constant has to be pre-multiplied by the Avogadro number. And you end up having this $N_A \times k$ which is called R which is also called **gas constant**… is given the final expression.

So the flux was: mobility × concentration × the external force. $J = u \cdot C \cdot F$ So the external force here is $-RT$, and then is the gradient of the logarithm of the concentration. With respect to r or x or whatever you want to call it. So just this… just this unidirectional, just monodimensional case. Let's call this coordinate x.

Something that you might be tempted to do is that here this seems to be a composite function and the derivative of the logarithm of a concentration. So this concentration we don't know what it is. It's a given or maybe becomes self-consistent but that will be later. So here you will be tempted to simplify this term. Would you tell me how? How can I rewrite this? *Analisi 1.*

Okay. $1/C$, dC/dx. Indeed. And you will see that C is simplifying, is canceling. And I hope that you have seen this expression because this is the law of diffusion. I think it's in the next slide, but I want to just, I cannot resist simplifying this. So you have that diffusion is proportional to the gradient, which is exactly what diffusion is. Okay, yeah, I'm spoiling even myself.

Fick's Law of Diffusion So if I do that, you see there is no longer concentration here multiplying because it has been simplified. And apart from these quantities that are constant, and maybe I call them big D, I call it just… it's a number. Then I have that: $J = -D \cdot (dC/dx)$ So the minus comes from the idea of the… of the potential. And you know this as the **Fick's law**. I think it's Fick's law of diffusion.

If you have a profile of concentration that is like this, so moving in this direction in this coordinate you have a lot of stuff, so it's a pile of sand. Okay, if you move from left to right you encounter the pile of sand, so the more you move to the… to the… to the right the more stuff you find, the more concentrated it is. Well, you know that the pile, if it should fall or if it's not a pile it's maybe smoke or ink or whatever, will tend to diffuse from points where they have higher concentration to points that have lower concentration. To me this is a pile of sand that when it relaxes it ejects sand in this direction. It doesn't eject because that would mean spending energy to become more concentrated. So if I have nothing and then at some point I have all the ink in a glass of water, the ink will diffuse in the opposite, so this minus direction of where the concentration is increasing. The derivative of the concentration with respect to space is where the concentration is increasing. Minus is where it's decreasing. This is the law of the diffusion that you probably have seen many times, I hope in the past. It's called the Fick's law of diffusion.

And the fact that people could link this microscopic thermodynamical constant, R, T, particularly T, the temperature, and the mobility to this **diffusion co-**

efficient D, which is exactly what people have been measuring for decades and centuries for quantifying diffusion of particles, the diffusion of that ionic species or that molecule or hemoglobin, so whatever… oxygen… D has been related, and this is due to **Einstein**. So Einstein worked out, as you probably know, the link between the microscopic mechanism, the microscopic view of Brownian motion, the thermal agitation, to its macroscopic description, which is here. Here you don't have anymore the single molecule. Here you have the single molecule, the particle… okay here you have a mole of particles. Here you don't, you don't have anymore the microscopic, you don't have the kinetic energy. So it's very powerful synthesis in a way and it's exactly what we want, what we need, because now we have diffusion and drift. Sometimes we are going to simplify that, some other times it's easier to work with the… with this full expression. It can be ugly but it's not particularly difficult now that I guide you and I gave you perhaps intuition to what this thing is. It was as expected, it had to depend on the concentration.

So you have drift and diffusion and the question now will be what do I do with this? What do I do with this knowledge?

I think we are… I don't remember this quiz or questions or whatever. Okay, so this is exactly the point. What happens if you have both? Particles that are charged and they are non-uniformly concentrated. They will both drift and diffuse. The point is that it's not trivial and it's very difficult unless maybe you could think of running a computer simulation to understand how… there will be a Monte Carlo simulation where you track individual things, how they would tend to diffuse because they are too packed, but if they are diffusing they are getting further away from their opposite charge friend and they will be kind of being attracted. But if they are attracted too much, then they will start igniting diffusion. So there will be this sort of ping-pong, maybe some sort of equilibrium, balance between these two things.

So balance would mean what? That this is exactly equals to that? Or maybe… maybe it's a thermodynamical equilibrium that means that everything is dead. So there is no flux. This is zero, this is zero. Maybe the sum is going to be zero. We're going to see that this is the way to understand why this is -70 millivolts. The whole story is if they are free to move, then it's not what your intuition might have guided you in at the beginning or… or last week to say, "-70 millivolt, well superposition of the effect, it's because I have a lot of negatively charged particles inside." Which incidentally is in part, it's true, it's not wrong, but it's not the reason why you have this electrostatic potential. And by the way, it's not… otherwise would not be explainable why this electrostatic potential can even change abruptly in excitable cells.

Something that, of course, has happened is that in the video, the last couple of hours there was no projection of the slides, but at least the slides you have them. Now I think I solved it. I have now understood where a bug is, but at least you have blackboard material and my voice.

Neuroelectronics: The Real Thing So let's continue with this chapter that is not any more preliminaries. Now it's the real thing, it's **neuroelectronics**. And I hope that now it makes sense why is electronics. It's about current,

current densities, fluxes, electrostatic potentials, distributions. Not too far from what maybe some of you might have studied in the case of semiconductors, diodes, transistors, is similar, it's not so different.

So, again, as a supporting means to your study and understanding, these are the references that might be relevant for this part. Particularly both in this book and in this book, it's just one or two chapters. It's not the entire book. Instead, here you have way more stuff, more chapters are related to diffusion, drift, to the emergence of electrostatic potential across a biological membrane and how this is affected by membrane properties. And this is also another relatively general introduction on the cellular biophysics, at least the first couple of chapters.

So this is a list. It's what we are going… I don't know whether we are finishing today, most likely we are continuing next week. Let me for a moment focus on the first three things. So I would like to make it even more explicit that we are focusing on wet systems, so aqueous solutions that have electrochemical properties. So they have electrolytes, so they have ions and cations, which is just another convention to say if these are positively charged *or* negatively charged, unless I'm swapping them. They are particles that are charged and they are ionic species and they are born, they come from the dissociation of molecules in water. And again I will tell you how we do measure the electrostatic potential by electrodes, particularly with one electrode interface which is silver chloride, silver chlorinated interface.

And again I will again emphasize that our task is this tackling this -70 mV under rest, resting. What is rest? It's rest but I'm still alive, so maybe it's not thermodynamical equilibrium. And at rest I do have -70 mV. For excitable cells, sometimes in a matter of less than a millisecond this electrostatic potential jumps 100 mV. So something here, or maybe not, or maybe something has to change rapidly over a time scale of a millisecond, or maybe not. And then I will go with other things. So I will refresh, I will tell again the superposition of the effects, I will tell that the anchor charges are fine for intuition but they are not what happens, and again drift and diffuse and I will tell you what, why, how do we use it.

Okay, first three parts.

So I'm thinking now of a world where you have aqueous solution, so you have a solvent and the solute is made of molecules that are charged. They might even be not charged. And they are, yeah, these are the ions that we are probably going to comment mostly. And they dissociate because salt, because of solvation, is this reversible reaction that happens because in water electrostatic forces are weaker. And obviously, this is an important thing. Remember that **electroneutrality** always holds, globally. Locally, it might be violated. But globally, if I put one gram of sodium chloride in one liter of water, it's going to be electrically neutral. But it will be dissociated, so in some spot I can observe electrostatic potentials that are non-zero.

This is for the tenth time. In metals you have some charge carriers, in solution you have a multiplicity of charge carriers. And there is no particular novelty to tell you that people started observing many, many years ago, 50 years ago or more, the comparison between transistors and electrolytes, even proteins and semiconductors. However we are going a little bit more in details, more in depth,

and we are going basically to study electrochemical system with electrodes. And just for… For the nomenclature, it depends on the book that you look. You have anodes and cathodes, particularly when you have, maybe not only you measure, this is supposed to be some sort of gauge, just to measure what is the difference of potential between these two points of the solution. Or maybe you want to apply an electrical potential with a battery, an electromotive force, and you make, let's say, the positive or negative electrodes are going to accumulate the opposite sign particles to that electrode. So this is the framework that we are considering.

Electrodes: The Silver/Silver-Chloride (Ag/AgCl) Interface And about electrodes, I have immediately to tell you that like what I *tried* to stick on my chest and on my arm during the first class, we cannot use metal, a piece of metal. Well, we could, but it's different. Because electrons are not going to jump into the solution and ions are not going to be entering in the crystalline *reticulum* of a metal and will be kind of fighting and kind of finding their way in the metal wire. This is not going to be happening.

What people did, and this is the most conventional and the easiest kind of electrode to be used and to be understood, although as you can see is the most fragile, and it's definitely not the one that Elon Musk is placing, it's not the way Elon Musk is placing electrodes permanently in the brain of patients, it's called **silver chloride junction**. And it's based on this dissociation reaction. If you take silver and you put it in a solution that is very rich of *chlorine* ions, *Chlorine* and silver can bond together, can bind together, and in the process they release an electron. Because charge has to be conserved, and this electron, if by chance you have an electric wire that is closing a circuit, maybe to another electrode, through a meter or through an amplifier, so you close the circuit, this electron could or can give rise to a measurable electrical current. So an ionic current, these *chlorine* ions getting closed and binding, give rise to an ion.

So this is the perfect interface to exchange. It's like when you go to a foreign country and you have different currency, you want to pay with Euros and they only accept Swedish Crowns. Well, okay, this is making the conversion and so it's transparent to you. It's so transparent that this is an **ohmic interface** in the sense that there is proportionality between the difference of potential and the current that flows in.

Also, the opposite takes place. So again, charge is conserved if you have a block of silver that is bound mechanically or because you kept it in solution for many hours, or because it's produced by an industrial process that is called *sinterization*, that is attaching *chlorine* to the porous structure of silver, you make it porous. If you pass a current, like the battery that I was attaching a moment before in the previous slide, if you pass a current, then you may create a dissociation of *chlorine* ions that were attached to the silver metal, and they are expelled, and they are now free to swim in solution.

So it works as an interface, as a bidirectional interface, and that's why placed on my skin you could get signals, and if you attach electrodes to my brain and you apply some sort of electric current, you can excite my cells because you can create some fluxes, some current densities in terms of fluxes of ions. Although

you start only with electrons. So you can measure and you can inject, read, write. And this read and write is linear in the sense that it is linear, it's an ohmic relationship. So if you apply with respect to the solution you apply a certain electric potential, electrostatic potential, the current will be proportional to how intense is the electrostatic potential.

Again please remember this the comparison with the gravitational potential. If I have two *reservoirs* of liquid, I don't know whether this helps or confuse you, if you have two *reservoirs* made full of liquid, full of whatever… wine or petrol or water whatever… and you are making their height, you create a difference of electrostatic potential. The electrostatic potential is basically referred to the height. So if they are not at the same height but one is higher than the other, then you will create the condition for a current, for a flux to flow from one container to the other. Here is the same concept and it's a ohmic relationship. Of course there is resistance because there is non-ideal, not perfect, it's not a superconductor, it's not a perfect match, one electron, well it is one electron, one *chlorine* but the… clearly resistance in the wire because it's not an ideal wire and there is a resistance… Stokes law… in the solution.

If you have sodium hypochlorite… so if you have *candeggina* at your place and you have a piece of metal wire… well don't do that because you will… well it's not ruined but it's a… if you have a silver chain or silver ring if you put it in *candeggina* it will be immediately covered by a silver chlorinated layer. And this is how we build our electrodes in the lab. There are other ways that is by electro-deposition. So we apply a specific current, so it's here, and we make the *chlorine* attached to the… to silver. So we subtract current and we get as a result silver chlorinated electrodes.

Pipettes and Patch-Clamp The other element I mentioned many times… the **pipettes**. This is a close view of one machine that we use in the lab to build these pipettes. So we start from borosilicate glass that we buy and they're very expensive and they are very tiny. It's a 1 millimeter or *half a* millimeter diameter made of glass, borosilicate glass particularly. And if you ever seen *of*… being to Venice and the islands around Venice, you know that you can do by heat, you can melt the glass. So if you have this tube of glass… they got a Nobel Prize for this idea… and you put it in one part where there is a filament, I don't think that you see here, but there should be here a sort of ring, and this ring is attached to a battery so when current is flowing in, because the ring has some resistance, is going to heat up a lot. If you go with this capillary inside this ring in the center it will melt.

And if you mount all of this in a system that cost about $12,000$ Euros… it's not very cheap… in a system that has some wires, this is another sort of pulley that is pulling the two extremities of, in this case it's an horizontal puller, it's called pipette puller with a lot of originality. So if you *hit* in the center and you start to pull, the glass is melting and getting softer and getting thinner. At some point where you pull with more force and you end up having from each side this melted and very, very, very tiny capillary. The other one remained from the other side of the system. Initially it was just a cylinder inserted in the middle with this ring, it was heated, pulled, heated, pulled in a certain cycle and at the end you end up having with a tip that can be smaller than a micrometer or

even smaller.

And you're familiar with this because you see this in in-vitro fertilization of oocytes, of human oocytes, where people are injecting genetic material taken from men in the oocytes by some sort of pipette. This is obviously for different reasons. And what we use this, we put here inside a very, very tiny silver wire, silver chlorinated wire, silver that stayed in the *candeggina* for overnight and we fill the pipette with a solution that is very rich in *chlorine*. So at the tip of this pipette we have a perfect interface. So we have here… this is silver, this is silver chloride and so this is connected to an amplifier, to a system, to some sort of a battery or whatever, and at the very tip here we can basically count of *chlorine* ions to be our messengers. And if you have some sort of electrostatic phenomena in the bath, in the solution, in the stomach of a cell, I can really be bringing some sort of detector very, very close, and I will be guaranteed to be able to transduce this difference of potential, this current, into a world that I can manipulate with electrodes.

And again, when you do that, you put it under, okay, in this case you need a special contrast technique. I think I show you already this. What you do, you push it into the stomach of a cell, inside. Here there are many pipettes. There are six pipettes and six neurons. And what you see is the experimenter that is changing the focus. And when I say -70 millivolt I'm referring to what the tip of the pipette is detecting with respect to another electrode which is typically called pigtail because it's a… it's a piece of wire, it's… we don't need another pipette. And this piece of wire is just to expand its surface… keeping the surface… sorry, expand its exposed surface keeping the volume small… we wrap it around and it looks like the tail of a pig. Although pigs are… well I don't think they are so curly but still.

And so when I say -70 mV it means that the difference of potential inside minus outside is -70, is negative, because outside by convention I say that this is 0 millivolt. I attach this to the ground of my amplifier so I say that is… that is zero. And so that's why I'm basically saying inside the electrostatic potential is -70. Now in the… today and next week I will keep indicating *in* and *out*, but at some point I will maybe drop *out*, because for me *out* means out of the cell and means zero. So here it is *in* with respect to *out*. This is supposed to be the pigtail electrode. And this is the silver chlorinated with a very trivial, very crappy drawing, a very crappy scratch.

So I would like to understand from these first principles, from drift and diffusion, the existence of this difference of potential. And basically somehow I told you that this is not only theoretical, people are measuring it, we do it all the time, by this sort of electrodes and techniques.

The Cell Membrane as a Capacitor And the first things that we might have to encounter is a brief discussion of what the membrane is. Because I understand that I can pinch with a pipette because nowadays I have microscopy techniques that allows me to roughly see the boundaries of the cell. I will not be able to see how thick is the membrane, but I'm able to see whether I'm pinching or not, like for oocytes. And so it might be interesting to see whether this is ringing any bell. That is something that we already know.

And the interesting thing is that these endeavours towards this −70 mV will ultimately be resolved in terms of the permeability of fluxes and ionic permeabilities. Permeabilities are relatives of mobilities and they are related to the fact that the doors are small, they're large, they're closed, they're open. So basically it's only in terms of this selective ionic permeability and ionic flows that I can understand this neuroelectronic world.

In the process, we are going to end up having a fully **electrical equivalent model** of a membrane and of the cell and of the doors and etc. Which is very useful because not only we can say that we understand better a system, an electrical system that we are maybe familiar with. I mentioned in the past hours, Kirchhoff laws of current and voltages. So all this set of things of linear network theory, electrical network theory, that is very easy, very conventional. I might review it for some of you who might be completely empty. But say you have something that it's not obviously the physical system, it's a model. And having models is essential, like it is for a civil engineer, wants to build a bridge. You don't try to build a bridge and see what happens. You have a model, you have equations, you try to combine it, and then you make predictions and you build your system on the basis of these guidance. And you will see how naturally this electrical equivalent model will come.

And this is basically where we are going. So we are interested in excitability. What is, in all the things that I said before, what makes it possible for the membrane potential to change so rapidly, and apparently with some meaning. So not only the resting potential, again, the potential at rest, rest without any stimulus maybe, rest because you are resting, you are not consuming power maybe, but you're not dead, you're at rest. And here there are electrical potentials that are maybe conveying some action. And that's why they are called **action potentials**. They are also called **spikes** because they look like needles, like spikes, like spiky… *punte*, *spilli*. And they are faster. So this is– I show you already many times this particular recording. Let's go on.

We measure this −70 millivolts across the cell. So inside with respect to the outside. And I hope that you had a happy childhood and you played at least once in your life with oil in water. I don't know whether you had a dish or you had a glass. You probably know that oil in water does interesting thing. It basically creates some sort of spheres or circles and it's not mixing because the molecules of oil are **hydrophobic**. It always takes me a couple of milliseconds to remember what is hydrophobic and hydrophilic. Hydrophobic, I imagine, is particles of oil. They are phobic, they have a phobia for water, but it's because of this phobia that they are creating these phases. And that's why, thanks to this, that life evolved. Again, you needed to create some boundary from what is outside and what is inside. And this is the mechanism.

Of course, the membrane of a cell, of any cell, nerve cells or even pancreatic cells, muscle cells, skin cells, blood cells, white cells, is not only composed of lipids, of oily molecules. There are many, many other things interleaved into the membrane, but the properties that I'm referring to are basically mostly made of **phospholipids**. And these phospholipids, I'm just spending one word, you have only to vaguely remember that they have a **head** and they have **tails**, and the head is **hydrophilic**. Instead the tails are **hydrophobic**. And just purely by this electrostatic interaction with water dipoles, I don't have any more wa-

ter dipoles around, these things thrown into the water, they **self-assemble** in membranes, which is to me mind-blowing. So you engineer something according to the rules of the game, which is physics and electrostatics, you put the ingredients there and out of the blue the lowest energy configuration is the one that creates a compartment inside divided from the outside.

Those using hydrating creams probably know that there are two kinds of stable configuration that lipids could get in water. One is what they are called *micelle*, **micelles**, where all the tails are pointing in and all the heads, hydrophilic heads, are pointing out. So they are basically shielding what they, so the tails really don't like to be in contact with water, so they try to really to escape. And they are, sometimes they are used in cosmetic products, they are very common to see this. And another very common for hydration creams is **liposomes**, also for drug delivery. So I'm just making it trivial, assuming that you never heard of liposomes, in which you have that there is another configuration in which it forms, a **double layer** is formed. So the tails are facing each other and there is an internal compartment that is formed as well. And this is the most interesting part because this double layer, phospholipid double layer, is stable. And this is what generally, just on a first approximation, constitutes a plasmatic membrane.

And the story that I told you about this very tiny, so it's a nanometer thick layer of the membrane, of this double layer, has been measured electrically. Because people started thinking, okay, there must be something, there must be a membrane. Well, this was the membrane hypothesis. And outside, I have a lot of ions. Ions are charged carriers. And then inside, I have a lot of charged carriers. So I have two compartments that are basically **conductors**. Not ideal conductors, but conductors. And in between, I have maybe something that does not allow water or other things to flow in. You see, it's very packed. It's like an army, like all soldiers. Okay, the army is a bit unhappy, comparison analogies, like a crowd, and they do not allow anything. And water cannot enter because, energetically, you will need a lot of energy to push a water dipole in, which opens an interesting reflection. If it takes a lot of energy to move something from inside to the outside, maybe something went wrong. This is not an ideal thing because I'm a cell, I need to eat. You also need to take coffee or whatever you can find from the machines here or whatever. You have to go in and out. You have to exchange even information. And if it's so difficult to enter and exit, maybe you need to have some, you have to devote some specific structures, *ad hoc* structures like doors, because going through the walls might require a bit of energy. You could do that if they push you, but it's going to be really, really costing.

But let me go back to this story that there is some conductor here, there is another conductor here, and this seems to be an **insulator**. Does it ring any bell? You have two, yeah, **capacitors**. I know that you are all experts in biophysics, but I hope that those of you who may not would maybe get this analogy. Of course, it's not a capacitor. It's definitely not a parallel planar plate capacitor. It's a biological membrane, but it behaves like, at least geometrically and also functionally, behaves like a capacitor.

So if I have, again, if I know how the electrostatic potential, particularly here, across these two plates is related to the charge, and I know that this is the basic definition of capacitance, $C = Q/V$, I know that maybe I can do, I'm in

the game. So I know that for parallel plate capacitor that at some point… now this is a mathematical model. It's not the real thing, but it's useful and it's actually even beyond the good analogy. It works so nicely that if you take the formula that was derived in physics one whatever of the capacitance… like the ratio between the difference of charge or the change of charge maybe over time, let's say the difference of charge, the different distribution of charge and the difference of potential or the variation of the potential in time, then you can do this and you can understand maybe why you have -70 millivolts from inside with respect to the outside. Maybe you can guess what is the charge difference inside and outside if you know the value of the capacitance.

And the value of the capacitance is known from first principle to depend on the area (A). The larger the plates of this capacitor, the bigger is the capacitance, and the *closer* closely spaced are these plates (distance d), the higher is the capacitance. And you see here that there is the… is the permittivity. So it's the $\epsilon_0 \times \epsilon_r$. And I told you that for phospholipid *belayers* this is around seven.

So if you could, and this is how people did, if you could engineer just maybe not the real cell but if you could take this phospholipid and you can maybe put it say on water… you can assemble something that you know how large is the *surfaces* and you know the permittivity of the lipids. And you can measure electrically the capacitance. You can measure the capacitance injecting some current and seeing how the voltage is changing. So I'm thinking of the constitutive equation of a capacitor, which is $I = C \cdot (dV/dt)$. And I'm tempted to say that if I integrate both sides, I can get some sort of relationship between the voltage and the integral of the current. So if I can maybe inject some current, I can measure the voltage and I can infer the capacitance. This is one way. It's not for instance, it's not the way we do it today for measuring the capacitance of a real cell. We do it in a different way, but people did it and… and they got that it was around in the order of a few nanometers. And for me, this is brilliant.

So, one, you could calculate from first principle, okay, now you know the thickness and the permittivity, and so you can get the capacitance, or vice versa, like they did. You don't know the thickness, but you measure the capacitance. And so this is the formula that you would use. And if you are interested in the capacitance per area unit, so the so-called **specific capacitance**, I'm attached to this because I don't know how big a cell would be. It could be a small sphere or it could be a bigger sphere. I would love to have some value that is a specific physical quantity. So specific means per unit of area. And the number that you can burn into your brain, although in humans, in human cell, people found at least one paper found slightly different value that was half of this value… so it's $1\ \mu\mathbf{F/cm}^2$ (one microFarad per square centimeter).

So you see it's per square centimeter. So the total capacitance is… will be known if you specify the area, otherwise it remains this specific quantity. And here you see I only use the nanometer… 6 nanometer… 10^{-9} because it's milli-micro-nano… so it's three… and 10^{-9}. And here I think it's 8.85×10^{-12} Farad per meter, this is ϵ_0, and 7 is the relative permittivity. And you get roughly for free that you get 1 microfarad which is what you measured experimentally.

And something that you could do is you could get this 70 millivolt interpreting this ΔV and ΔQ as the delta… as a difference in space. So with the two… so

not necessarily in time but in… okay that's another story. And here you will see that if you plug the value of 1 microfarad per square centimeters you can see that a membrane that is 70 millivolts charged is actually storing this amount of current per unit of area. This is kind of interesting *per se*, it doesn't answer still my question saying, but why you have -70 millivolts. Because here you have, this is given. You could also reason the opposite way. You say if this is a difference in electrical charge around the membrane, then you will have -70 millivolt. Okay, but why? Why did you end up with this value of the charge? Nonetheless this knowledge that is basically taken from elementary physics or from electrical engineering allows you to make prediction, to make calculations, to make… to *deeper* your understanding of a biological system… of any cell by the way, not only *your*… of neurons. OK, in humans, people found a smaller value.

The Membrane as an RC Circuit And you might remember that people are fond of studying **RC circuits**, where you not only have a capacitor, but you have a capacitor in parallel with a resistor, which is, by the way– So this is an analogous to having your bathtub or your sink where you wash your face in the morning with a hole. So you have a certain height, and the height is equivalent to the potential that you have on the capacitor. And you have some dissipative system that here is represented by a leak that is contributing to removing the liquid. But anyway, to make a long story short, you probably know that this electrical circuit, for reasons that now I don't want to bother you with… in order to do that you have to derive the equation that describes V as a function of all the other quantities, and you will find the usual boring differential equation, but you will find that the exponent that makes me happy, there is $e^{-t/(R\times C)}$. So the RC of an RC circuit is the **time constant (τ)**.

And so today we measure the capacitance by analyzing the time constant, the decay time constant of a real cell. Now it makes no sense because you would say, "Yeah, okay, but here there is a membrane. Where is the leak?" Okay, maybe you intuitively could say, okay so it's not only the membrane, there is… there are some resistors. And there are some resistors and so even if we don't know necessarily the value of the resistor, in one measurement we can see how fast or slow the system is relaxing and we get… we measure the capacitance. But in the old days they did it in a… in a different way because they did not have any… they did not play necessarily with intact cells or they did not play with cells like neurons. They do have resistors.

Let me skip this and let me, before breaking, let me just remember what I wanted to tell you. Okay, this I told you already. And so with this, I wanted to show you two graphs… This is the demo, I did it already, so you can go, you have the link if you want to play once. But I wanted to show you what I did having fixed, glue, anchored charges in free space, at least in a *B-dimensional* settings.

So, I tried to answer this question, and what I did is I myself randomly, so it's a *B-dimensional* system. I have one part of the plane that I call outside, I have one part that I call inside, and I dispersed here inside and outside positively or negatively charged ions. So it's me clicking and I did that, I did this. So negative, you see there are many negative inside and there are very fewer negative outside.

And I put a little bit of, I sprinkled a little bit of positively charged ions. I know I should do it. This sucks. Graphically is not very appealing but I didn't have time and today one could think of doing these things with a… with a Python library that is called Manim. If you are fond of YouTube channel 3Blue1Brown, whatever. So he's actually using this library for making beautiful animation.

Okay I'm not so skilled but I wanted to do the following: if I have a certain distribution of charge then for every point I could measure all the distances and I can calculate the potential in that point. And here is what I did. Clearly you have… you have a sort of thermal map. You have in every point you have a color with a gradient scale that tells if the potential is positive or negative. Now forget about the unit. I… I only care about the negative or positive. Here I actually put random number for distances. I put random numbers for the charge. I did not expect to actually get -80 or -70. I just wanted to see if… now the question is, so you see the potential is negative inside and it's positive outside. But what I'm measuring with the pipette is a **difference**. So I put the pipette here and I put the other reference electrode here and I take the difference. Here you cannot see, so let me convert this into… Okay, here you are seeing it. So from this point to this point, the difference is -42. Okay, so the point is it's not the absolute values, it's the difference that should go to -70 millivolts.

And if you do it in 3D, maybe it's easier. Inside you have this negative potential and outside they are positive. So this height should be -70 millivolts negative. So here should be lower than here. So when you take the difference you have something that is already minus, minus something that is positive so it remains minus. So here you would be… you would be tempted to conclude, okay, that's the explanation. That's why you have -70 millivolts. You have a lot of negative charges inside.

Let me show what happens and then we break. Sorry that I'm abusing a few more minutes and we stop early. I will not do another 50 minutes later.

If I put a lot of positively charged ions only outside, very little inside… so this is the dual case. Intuitively I like to think -70 it's negative. In reality is the difference between inside and outside. And this difference would still give rise to negative numbers if I have not only a lot of negative inside and a lot of positive outside, but also if I have a lot of positive outside and little positive inside. So the difference, the height, will still be negative. So you would have the same explanation for an unequal distribution of positive ions. So you will be at square one. Why the hell you have -70 millivolts?

The problem is **anchored charges** and I hope that I did not spoil but in the past two hours I gave you some elements where we are heading, otherwise it would be too boring even if with some culmination in a moment, well probably next week.

So we break for 10 minutes and we continue until 5:45.

Okay, so there are two key points. The first is that ions are not anchored, they are not stuck, they are free to swim, free to diffuse and to drift, to experience drift forces due to their own electric fields. And the other element is that there is a very thin insulating membrane across which we measure the electrostatic potential, the **transmembrane potential**. Transmembrane because it's across

the membrane, inside with respect to the outside.

And what I wrote here is again the concept of capacitors. Capacitors are, the formal definition I think is that you have a set of coupled conductors where the distribution of charge and the distribution of electrostatic potentials are linked, are linked by a property that is called capacitance. And if you can think of this relationship and you divide both terms by Δt, so you're thinking of this change as a change over time, maybe you will recognize that here $\Delta Q/\Delta t$ is a charge divided by time, is a **current**. So somehow the two elements, the two worlds, the two concepts, currents, current densities… Okay, fluxes, but say this is basically currents and capacitance, capacitors that are able to integrate currents in order to generate the potential are the ingredients that we are, as electronic engineers or whoever we are familiar with. If not, I tell you these are very powerful concepts. And it took centuries to get to this relatively simple and elementary picture. Of course it's not over because it's not so trivial now to combine everything and to say, "Okay, so tell me how V comes up." So maybe I tell you, apologies that this is capacitance, maybe I call it with this symbol, is that these ones are concentrations. These are concentrations.

So, summary of the facts. 1. Everything is isopotential, but locally you can lose the electroneutrality. 2. You have multiple ionic species. 3. These ionic species, although we don't really understand how, but they must be unequally distributed inside and outside, otherwise we would not have, we would have no electrostatic potential. 4. And the membrane *belayer* is impermeable to water and ions, so it is acting as a capacitor, as an integrator of current. 5. And okay, this membrane potential that we measure, again, remember it's a difference of potential inside with respect to the outside, and the outside we consider zero. 6. And again if the cell is not dead, and I'm repeating over and over again so there is no thermodynamical equilibrium… and you will hate me in a moment because in a moment I'm going to take this as an hypothesis at least temporarily… but say if I'm not dead it's -70 millivolts. 7. And in excitable cells things are even more interesting, more spicy. This changes, it goes -70 to $+30$ and back.

Ionic Concentrations So first I start with telling you what people did in terms of quantifying the ionic concentrations inside and outside cells, starting from the most famous experimental preparation that is the **giant axon of the squid**. So there is a squid which is this invertebrate that you may have eaten, maybe it's a small one. So it's a giant axon… this guy here, if you dissect it, has a very big thing that is a cell that you can possibly put an electrode inside and you can measure things inside and outside. It's not the axon of the *giant* squid. The giant squid is a marine monster, probably it exists, it's very rare, but this has not been done on marine monsters. It has been done on squid, normal, but they have a big axon.

And this is the situation expressed in millimolar: * **Potassium ($\mathbf{K^+}$)** is concentrated a lot **inside** (400 mM), and not that much outside (20 mM). * **Sodium ($\mathbf{Na^+}$)** is the **opposite**. Sodium, you have a lot of sodium **outside** (440 mM) and a little bit of sodium inside (50 mM). * **Chloride ($\mathbf{Cl^-}$)** is like sodium, you have a lot of *chlorine* **outside** (560 mM) and a little bit inside (52 mM). * And basically **Calcium ($\mathbf{Ca^{2+}}$)** is very important because basically calcium is outside, there is a little bit (10 mM), but **inside** basically it's almost **empty**

(10^{-4} mM). You heard probably at Nauseam in other courses that calcium intracellularly is considered a second messenger, is one of the most important actors in biochemical cascades. Well, it is because inside it's basically empty, so as soon as you start having some free calcium ions inside, the cell knows that it means something and it has mechanism to detect that.

Now I tell you something that I hope you will remember for the rest of your days, how to mnemonically remember these, because this is extremely important. In Italy it works nicely. Abroad I had to mention that I was Italian, that for lunch I was preparing pasta, which is the only thing of just a few things that I can cook. And in the water of pasta after, so before, well that's another thing, before or after water boils, I was putting sodium chloride. So, *sale da cucina*. Of course, it's not the only salt, but this is what I have at home and I put it in my boiling pan when I prepare pasta. So, if I taste this, it's salty, okay? It's trivial.

Now the disgusting part starts. In order to remember that outside you have a lot of sodium, so you have a lot of sodium chloride, allow me this, also it *also* for chloride. Imagine that you go… this is disgusting, but I could not find a better alternative. You will maybe propose me. So you go do, you do sport, you sweat a lot, and you lick your own sweat. It's salty, right? So closed… so outside the cell, presumably in the intercellular space. So this is inside and this is outside. In my perverse mind, the sweat is what is outside. So I lick my sweat, I lick and I taste it salty. I'm licking my *exacellular* medium, which is not wrong, of course. What is wrong is that by no means the other salts will taste differently to my tongue. But I hope that you will remember. So if I think, okay, I taste my sweat, it's salty, so it means that **outside I have a lot of sodium**. I have a lot of sodium and inside I have a little bit.

Potassium is the opposite. And you will tell me, have you ever tasted potassium chloride? No. I think it's not even… I don't know whether it's *eatable* if I take potassium chloride. I don't know. So potassium chloride is the opposite. You have very little potassium outside and a lot of potassium inside. Chloride, just because of the same story, goes like the sodium. So you lick, it tastes salty and that tastes salty because it's outside in the extracellular space you have a lot of ions.

It's important and we will see and it's intriguing that you were thinking, wait, I thought that you had a lot of negatively charged ions inside, but no, it seems that you have mostly positively charged ions, but you cannot really conclude what is what, because this guy here is 400 millimolar, it's a lot, and it's inside, instead sodium is 440 millimolar outside. And we said that we should not take for granted the story of anchored, glued charges. So I don't think that we can conclude anything about this. But this is key for the next point.

The Role of Selective Permeability The next point, so the spoiler, at the end, we are going to understand the electrical potential across the cell membrane because, okay, there is an heterogeneous distribution of charges. This we have understood. I think I repeat it too many times. And this, however, it's not enough. What matters is the fact that the **membrane is not permeable to all ionic species in the same way**. It's as if some of you would not be able to come out from the door, and the door would be only making those that are

particularly slim or whatever… would be selective. So the permeability across the membrane is partial. And despite the fact that maybe you have, you see the most beautiful person from the other side, so you're attracted because you're blue or red. Okay, that's maybe not a good comparison. So you are attracted because of a drift or because of diffusion, yet you are not going to cross it because the membrane is not permeable.

And things are getting complicated because we are not talking about only one ionic species. There are multiple. The second point is that we are not talking about the thermodynamic equilibrium because you're not dead, but I will start with the dead cells. And because of these distinct concentrations. So the fact that you have a lot of sodium outside, it's a lot of sodium outside because if I lick my skin, it's salty. So it means outside the cells in the intercellular space, you have a lot of sodium and a lot of potassium inside instead is what makes these -70 millivolts. So it's a collective thing.

This has been, for me, very complicated to appreciate intuitively. I can see why maybe if the charges are not anchored, if they are all concentrated unequally, say, there will be some diffusion, but then after diffusion, diffusing I will get far from my partner and then I will be attracted or repelled. So this I can feel. So there can be drift, diffusion, ping-pong, this I get it. What I don't get is, okay, but what does it matter? What does it mean that if you have a membrane that is semipermeable, then across the membrane you will have a difference of potential. So an unequal distribution say of positive and negatively charged.

So here I did the same cartoon that helped me. Not this one but something similar helped me. So again this is… it's purely a Monte Carlo simulation in the sense that I fake and I put the equation of motions so it's like a Langevin dynamics. There is no gravity. What I did here I only… if I remember correctly I only put… so I put friction of course, I put the random collisions so that I could get diffusion, and if I'm not mistaken I'm only taking care of attraction and not of repulsion. But definitely this is not what a proper molecular dynamical simulation would take into account. So if two particles are getting very, very close $1/r^2$ will go to infinity. I don't have this and at some point I clip so that two particles can… can be one after the other. There are methods from physics where physicists already know how to do this system. But it was already computationally tough, although there are not many particles, to create a stupid movie like this.

So the difference is that we start with the same situation, that you have a lot of positively and negatively charged particles from one side of the membrane, and from the other side of the membrane you have almost nothing. In one case, which is this one, the membrane is as if it's not there. It's permeable. There is no selection. Here, black can cross, red will not. Red will stay on the left. And the numbers that you see below, if I'm not mistaken, are the number of black particles. We will see in a moment. So I launch the simulation and you will see what happens. And you can make a bet what happens after a sufficiently long amount of time what will be the distribution of the red or the black, whether they will be equally distributed or not.

You can see that here the red are crossing, here the red cannot cross. So it seems that because they stay here, they stay behind, the black will say, oh wait,

you didn't come with me, then I'm attracted. I cannot, you don't come, you don't move the cloud, oh, sorry. I don't move the entire cloud, I don't move the entire cloud of things at once. Here it's as if the center of mass of this smoke cloud, the black and the red are basically diffusing and they are bringing each other. Here this cannot happen because the membrane is semipermeable. And this is the entire story.

So in a couple of seconds, or maybe 10 seconds or 20 seconds… this number here is the number of frames, the number of step of the simulation. It's something for me to show me that things were moving. Here, you end up having, I think it's the red. No, sorry, it cannot be the red, because otherwise it would be zero. So the black here will be 50/50, because there is no selection. Here will not be 50/50. And you can already appreciate it. OK, it's fluctuating because it is a stochastic simulation. It's a numerical simulation of a system that is, say, it's at the equilibrium, but in a statistical sense. But here you actually see that there is a so-called breaking symmetry, instead on the left. So the fact that the membrane is opposing the passage from one ionic species is enough to create an unequal distribution, a dynamical unequal distribution. So something has to do with membranes and something has to do with permeability, but I have no idea what permeability is. There I'm talking about fluxes in the open space, so there is no permeability. Okay, maybe I could imagine that the mobility, I could make the mobility at some point in some, although it's a property of the molecule, but I could maybe say at some point the mobility goes to zero. But it's not very elegant because it seems that I have to tweak something. In other words, electrically, I'm talking about that here, there is no resistance. So the current can flow in both directions. Here there is only… some ionic species have a non-infinite resistance. So here the resistance is zero, here for some ionic species selectively is infinite. So there you cannot change. Of course if it's not infinite but it's very large, still you would have this breaking of the symmetry.

Ion Channels, Pumps, and the Electrical Model Okay. So the doors that I anticipated to you are physical doors, physical pores, and these pores are called **ionic channels**. I don't know whether in the module of Professor Zoli you have heard of something like this. Are proteins… they are called surface proteins just because they are expressed at the surface of the membrane. So you actually see here the double layer, the phospholipid double layer, *b-layer* whatever. And these proteins are not inside the cell, they're not outside, they stay in between. They stay in a part… in… in a domain that is called lipophilic, so it's philia for lipids. So… So they love being with lipids. And these proteins might have a different distribution of charge, of electrical charge, so that they are stable there. So you cannot pull them away unless using a big force. What they can do, they can slide horizontally on the surface of the membrane so much that this cartoon, this conceptual model, was called for many years, I think it's still called **fluid mosaic**. So the membrane is a collection of particles and structures, lipids, and it is as if they swim on the surface of the sea like kids when they have these inflatable things, *braccioli* or *salvagente*. Of course if you want to go down or up you have to spend a lot of energy and so naturally this does not happen.

But if these proteins are creating some sort of space in their inside due to their

three-dimensional conformation, and if you are an ion, you can move freely or not freely inside the pore, then this is like a passage of ions across the membrane with a bridge, with an easy path that does not require a huge amount of energy like the lipid *belayer* would require this strain. And in fact our colleagues went through a pore and not through the walls.

So these are pores and it turns out that you have specific pores only permeable to sodium ions, they are called **sodium channels**, and you have pores that are only selective to potassium *channels*, that are called potassium ions, they are called **potassium channels**. You have *chlorine* ion channels, you have some ion channels that are permeable to all ionic species, and you have some other mechanism of transport, which they are not passive transport like these two cases, where you basically, if you have the energy to do that, or simply by purely diffusion, by purely thermal agitation, can go in and out without problem.

Of course if you have a -70 millivolt difference of potential across the membrane it means that you have an electric field, then it might be easy or difficult depending if you are positively or negatively charged ion species. Say for sodium ions will be very easy because ion is concentrated a lot outside (because I lick and I feel tasty and my *swept* is salty) and so it's positive. Inside is more negative than the outside. So I can move along the electrochemical gradient, the electrical gradient. Well it's also electrochemical because I have a lot of sodium outside and a little bit inside. So by diffusion as well as by drift I will be able, if I'm a sodium ion, I will be able to enter. If I'm inside will be more difficult because I will move against both the diffusion and the drift.

But this is still passive. There are active mechanisms, like I cited a couple of hours ago, called **ionic pumps**. And these ionic pumps, I imagine them like some sort of mechanism. And they have been described mechanistically like some sort of rotating things or machinery, molecular machinery, that are able to exchange against the electrochemical gradient. So for instance, sodium I told you it's very easy to flow in, but very difficult to get out. But if you have ATP, so if you eat your bananas and chocolate and you have adenosine 3-phosphate molecules that can be used as a currency for converting, so they are used for storage of energy, and you can spend this energy, you can activate from one cycle this ionic pump, and you can extrude three sodium ions, and you pull in two potassium ions. Remember that you have a lot of sodium ions outside and a lot of potassium ions inside. So this truly requires a lot of energy. It's not passive.

So this is important because these ionic pores or ionic channels, the pumps we will not probably consider. We will not discuss it in details anymore. But the ionic pumps are to me resembling a metal wire or a **resistor**. If I'm an ion, I can go along. There is a capacitor here, but this capacitor must be some sort of parallel between the capacitor and there are these resistors. Because if I'm an ion, I can, yes, I can impinge in the membrane here and I will hit the insulator. Okay, if I'm approaching here and I'm a positive ion, just by electrostatic induction, I will attract my opposite from the other side. But this is going to be only a so-called displacement current. But if I just move a little bit on the right in parallel to this capacitance, I have a resistance because ions can flow and this will be a transport current where the current is physically,

explicitly moving. It's not just an indirect effect because I'm getting close to the membrane and because of my charisma, I'm attracting somebody from the other side. No.

Yes, there will be some movement but this is not a transport current. A transport current I need to have pores.

So this is another model, and it's some sort of physical wire. Of course, the channel is very tight. It maybe will make only one sodium, one potassium ions at a time, so it will be tough for the ions to pass. It's kind of tight, so friction will be large, or at some molecular scale will be not easy to have two or three sodium ions going simultaneously. But it still matches perfectly the definitions of the **Ohm's law** that says that you have some relationship between a difference of potential and the transport current that is proportional one to the other by a resistance.

So this is just to set ideas for what an equivalent mathematical model would be. So this is what is called an **electrical equivalent model**.

So if I'm a sodium or whatever, potassium, so here it's different, so this is a potassium ion. This is inside, this is the outside. Potassium is highly concentrated inside and very little concentrated outside. In fact, if I *lick*, I *lick* the sodium, not the potassium. I know, this is the fault of that comparison. And *cross* the membrane through this resistance. No, sorry, this is, sorry. Inside the cell and outside the cell, ions can move freely. Maybe they encounter some friction because they have a mobility and this mobility is not infinite. So they do encounter friction due to the viscous property of the medium. And if one ion wants to go from inside to the outside, if there are the conditions to do *so*, it can do it provided there is a bridge.

The conditions would be that you have an electrochemical gradient. You have a lot of potassium inside, so diffusive. In terms of diffusion, you will diffuse. This is the **Fick's law**. The movement, the diffusion will happen from points where there is high concentration to points where it's lower concentration, minus the derivative of the concentration with respect to space. But if you can do that, well, here I need the pore. Otherwise I will hit the insulator, this capacitor.

So the capacitor is there and it's a sort of property per unit of area. So this is clear, it's fine, but they are not only capacitors, they are resistors. Some of them are resistors. And if you allow me to make a first approximation of many, many approximations that I will do and that I did already so far, this resistance is much larger because here it's a very tiny space. So although we call the Stokes law… we only call it λ, we did not open and say, okay, what is in the geometry of the hole… the *map*… the particle moving… you can probably understand that here moving inside here it's different than moving freely inside and outside. So by comparing this resistance that is placed in parallel to this series of resistance that are… that are placed here inside in series and outside in series, maybe I can approximate that this is like a shortcut. So it's basically negligible. It means that if I have an electrolyte, in solution, the conductivity of the electrolyte is such that, OK, the conductivity is not infinite, but it's as if it's a piece of copper without resistance, with very low resistance, both inside and outside.

So by the way, now you get the **RC circuit**. So the suggestion that I told you that you could measure the properties of the membrane by looking at capacitive transients, now you get it for free. Because you do have resistance and capacitors as I *introduced* the pores.

Okay, there is the membrane potential and this seems to be it. And the question is now, are we done? So basically you have a series of parallel of capacitors and resistors. Maybe you have one resistor per ionic species, but I don't see how you get -70 millivolts. I can maybe say or see that if you put here charge and you have -70 millivolts, I know that after a while because of these resistors you will not have any more -70 mV. You will decay to zero. You will have leak. So this is not clearly enough.

And it's not enough because this is not the perfect model of the pore. It's not the perfect model because yes it's acting as a resistor, but inside here and outside you have two solutions made of different ionic species. It's like Alessandro Volta in his battery. He had two environments with different solutions. And they are joined together. So when they are joined together, there is a battery. There is an effect of an endogenous generation of electric potential.

But this is blah, blah. Let me, probably next week, but let me at least tell you what will be the plan to understand it.

The Path to the Nernst Potential So ions are flowing across the permeable membrane, and these two compartments are made of unequal distribution of concentrations. And we only, only, where is it? Yeah, I only simply tell you the strategy and we do it next time.

What we are going to derive is the **Nernst potential**, which is at the **equilibrium**. So now I am really assuming in the next three minutes that we are dead. And the rest we'll see.

So if you have diffusion and you have drift, one thing that you can try to do is you can say in general one molecule or one mole could experience simultaneously drift and diffusion. So what you might have to do is understanding what is the total flux. The total flux is $J_{diffusion} + J_{drift}$.

Let's think that we only have one ionic species. We only have one ionic species. We will not do it. I simply tell you what the plan is. One ionic species. The membrane is permeable to this ionic species. And what else do I need? I need that I consider that it's over. I'm dead. And basically we are at the **thermodynamical equilibrium**.

If we are at the thermodynamical equilibrium, it means that nothing is moving. Nothing is moving. So the **total flux is going to be zero**.

So next week, what I will try to do is to say that everywhere across the membrane, the membrane is… I want to keep the membrane because I want to have two points where I will try to attribute a membrane potential, and the potential is there, so it only awaits to be manipulated, *massaged* in some way. I anticipated we will have to integrate it.

And the assumption that is clearly wrong, although we will see later on that is useful, is follow what Nernst, one of the greatest biochemists and biophysicists

who existed, what he assumed. He said, okay, in general you have both terms, but it's zero. So zero means not that the drift is compensating for the diffusion, it's that one is the opposite of the other, because it's the *total* flux that is zero.

$$J_{total} = J_{drift} + J_{diffusion} = 0 \implies J_{drift} = -J_{diffusion}$$

This is maybe a little bit, it's not counterintuitive, because for me I see it, the total flux is the superposition of both. If you have an ionic species that is charged and *unequally* distributed, I both drift and diffuse. I cannot do only one. And if I say, look, it's dead, so there is no net flux, that means that this is zero. I cannot assume, it would be wrong to assume that I only do diffusion or I only do drift. No, it's their combination that is zero.

And you can, next week, we are going to, if you want, you may try to do it by yourself. If you assume that one is equal *to* the opposite of the other, you end up with two terms with an equation that is, it is a differential equation, but it's by no means boring and not trivial and not usual. You have this stuff, so this is cancelling out because it's there. So that's why I wanted to keep it like that. Okay, so mobility and concentration are done. Although concentration keeps existing here. But it's interesting that the mobility goes out of the way. So it doesn't matter if it's a big, fat ion or a small, slim ion. Apparently it doesn't for this picture. In the more realistic picture, it does.

And then you have… So this is a number, this is also a number. You have this term and *these* terms that are basically one minus the other. What do you do with this? We'll see you next time. The spoiler is we may try to cancel the derivative, integrating both sides. If you're brave, try, and I'm happy to answer questions.

Okay.

Introduction and Announcements

Alright, a bit too much… now there's volume. I'll try to speak more softly now, maybe lowering the microphone a bit. Good morning. A service question: do you guys use Teams, or do you just hate it?

Because every now and then, last week, I threw some information in there, like the fact that you have a limited window during December to book your spot… slot… during the six exam sessions between January and February. But also articles, or news, or links that might be of interest to you.

I think I did this two weeks ago regarding a topic a colleague of yours mentioned to me, that of research on consciousness, specifically the electrophysiological correlate of consciousness. And I posted videos, articles, and books—links to books. I did the same a couple of days ago with that ***Nature Communication*** article, which had its news picked up by this other site, ***IEEE Spectrum***.

Take a look, it might be interesting. I don't care if you 'like' it, but it's to know if you are alive and if you are potentially engaged, potentially interested.

Quiz: Ionic Distributions In particular, there was a stupid quiz that I am re-posting for you, because only two of you responded. And it's this one.

In the meantime, while you register for the attendance tracking, this is the question. It is: what are the ionic distributions inside and outside? You remember the disgusting, but I hope, lifetime memory-effective hack that I suggested to you. So you have these four possibilities. You should tell me which one is the correct one. And of course, I'm talking about a biological membrane, and so 'in' and 'out' is around the inside and outside the membrane, the plasmatic membrane of a living cell. Which do you think it is?

So, how many of you think that is the first one? So, you have more sodium outside than inside and you have more potassium outside than inside. Who thinks the second is correct? The third one. Fourth one. There is maybe a fifth possibility. I think those of you who raised your hands for the second one, indeed, you're right. Sodium and potassium, their concentration is one the opposite of the other.

And it will be very clear to you why the swap is relevant. You will remember without problems. The fact that you have a lot more sodium outside is sort of easy to remember with this disgusting thing that I suggested to you. Well, you could think… I think I spotted in my slides, I forgot, there is a gentle kitten, like a cat that is licking its paws instead of me licking the extracellular solution. But anyway, it's salty, you have a lot of sodium outside and a little bit inside.

The Resting Membrane Potential

So, let's go back to what happens. Our task is to understand why this damn **-70mV** is the so-called *resting membrane potential*. Let's call it equilibrium, but I'm not talking about thermodynamic equilibrium; I'm talking about the *resting condition*. And this resting condition is meaningful when you are talking about excitable cells, where the membrane potential can change. But anyway, in all cells, even those that are not able to rapidly change their membrane potential, you have a negative electrostatic potential inside with respect to the outside.

Diffusion and Drift To understand it, we have to start from here. And I already have, over the last two classes, I think I tried to push, particularly last week, I tried to push this idea: charges are not fixed; they are free to move. And there is drift and diffusion. And once they drift, they are somehow triggering diffusion, and vice versa: as the diffusion is happening, then you start having electric fields.

So, this is the natural tendency of everything to diffuse and to concentrate equally everywhere in space. And this is the fact that the particles are electrically charged. And so, if they move, they might sense Coulomb forces, and they are effectively moving in an electric field.

So here we are talking about the formation of an electrical potential in space across a membrane. The membrane is semipermeable. And the starting point… actually, you can see it in two ways… but the starting point is that you have an unequal concentration of ions, and therefore, an electrical potential is generated "for free." This is the principle of batteries, of chemical batteries.

So, you have an unequal distribution. You have to spend energy to make things different inside and outside. And for free, or not for free, but in exchange for

this energy that you provided… (that's why you eat bananas and chocolate, etc., just to establish and to maintain over time this electrostatic potential through the unequal concentration)… then you have these electrical phenomena.

We start first with this, which is wrong. You will see why it's wrong; I already mentioned it: which is the fact that we are invoking the condition of equilibrium that I told you, [is] only when you're dead. Let me take it anyway.

Mathematical Preliminaries: Integrals and Taylor And the elements that I… the mathematical preliminary that I need to refresh or to remind you is this simple definite integral of the function $1/x$, which is very famous. And it is… the integral of $1/x$ is the logarithm of x ($\ln(x)$), whose derivative is the integrand. And because it's a definite integral, it has the extremes. And so the fundamental theorem of integral calculus says that you take the primitive, you calculate it at the upper extreme minus the primitive calculated at the lower… *lower interval element.*

So in the case of the logarithm, you end up having the difference of logarithms, and log is a nice function that the difference of logs is the log of a ratio. The sum of the logs is the log of the product. And this is what… just to vaguely remember… I told you already that this is what… I forgot to bring you the ruler, the slide rule. In the old times, engineers did not even have digital calculators, and they were making products and divisions by simply adding things, adding measures. So they were adding or subtracting quantities, provided that the numbers, the lengths, were not encoded linearly: they were encoded as logarithms, and they could do ratios and multiplications for free.

And the other things that I will hint at and leave as an exercise to you is the Taylor expansions into a series of polynomials that are just arrested to the first order. So I will be soon, maybe in a couple of hours, giving you the task, and you should do it by yourself without troubles: how to approximate one function around one value where the function is calculated, is known. And you know that is the value of that function plus… it's a straight line, it's a tangent line… it's the new independent variable and the slope, so that the first derivative is calculated in that point.

The Nernst Equation So, that's Mr. Nernst, another giant, who followed these simple calculations. The hypothesis is that there is equilibrium, and we are for simplicity considering just a single ionic species. I don't care whether it's sodium, potassium, but just one: calcium, magnesium, just one. And it is at equilibrium.

Equilibrium means that the total flux (J) is equal to zero. I will give you later a couple of more elements to get this intuitively, because indeed, you could maybe think that the right thing to do is to say diffusion must be equal to drift. This is not going to capture the equilibrium. That's another condition. This is something else. It's not… this would be a balance. Instead, I want to investigate what follows from the idea "everything is at equilibrium."

So the total flux is zero. And if the total flux is zero, it means that one is minus the other. Because the total flux is the sum of the two fluxes. And it's the sum… you will see in a moment… because of conservation of mass. That's why

you have this summation. But it should be straightforward, even intuitively, to understand that fluxes would be composed linearly, additively, by the two terms.

So let me take this hypothesis and let me consider just one unidimensional, monodimensional case in which I have a semipermeable membrane, but I use it just for subdividing space, basically in three domains, but I will not bother you with what happens within the membrane. It has a thickness that is not infinitesimal. It's finite, although small.

And that sum is zero, meaning one is minus the other. I have the mathematical expression of each of them. We have derived them, most of them… not for the diffusion. It's only partial, because I told you that I cannot easily explain to you the shape… the analytical shape of the potential for that force field.

And I have it here. This is the diffusion flux… sorry, the *drift*… no, diffusion flux… and this is the drift flux. And you see that you are tempted, and we will do it, you are tempted to simplify those elements that are common and they are of course non-zero. So the mobility (u) and the concentrations (C) are in general non-zero. And something already striking occurs: that in the expression that you remain… So that's the hypothesis, and this is… we logically derive all the steps… is that the mobility disappears. As if the mobility plays no role, which is a bit strange. But in the case of equilibrium, yes, this is the case. It doesn't matter if you are a fat or a slim ion moving in an aqueous solution with all these dipoles of water, so the hydration shell. It doesn't matter, just the equilibrium conditions will be the same.

And you see here, I don't know the concentration (C), and I don't know the potential (V). So it's not properly a differential equation that I can solve. But at least, because you will see that there is a derivative here (dC/dx) and a derivative here (dV/dx), maybe if that equation holds true, it will also hold true by linearity, the same equation after applying the sign of integral to both terms, to both sides of the equation.

And I take this integral across the integration range, the integration interval, which is spanning, describing the membrane. You see x_{in} and x_{out}. So I removed $u \cdot C$, and I didn't do anything else. I hope that I did not forget the minus. Yes.

So, it's easy, because here these are… perfect. So the argument of these integrals are perfect differentials. So it's already the derivative, and the integral and the derivative are canceling out… you will not be… but you're not mathematicians, although we are in the building of mathematics, so we have to be careful. You cannot simplify this. This is not really a ratio (dC/dx). It's a Leibniz notation for derivatives, but you're not allowed to do that.

And again, for the fundamental theorem of integral calculus, RT is taken out. It's just a constant. Likewise Z and F, they are not changing within the membrane. And what remains is the integral of a derivative, so the primitive is $\ln(C)$, and here the primitive is V. I calculate each of them in each extreme, and I subtract.

So this one is $\ln(C_{out}) - \ln(C_{in})$, and I end up having this expression.

$$\Delta V = V_{in} - V_{out} = -\frac{RT}{ZF} \ln\left(\frac{C_{in}}{C_{out}}\right) = \frac{RT}{ZF} \ln\left(\frac{C_{out}}{C_{in}}\right)$$

So I wonder, maybe some of you attempted during last week this calculation. It's not complicated. There is no other things to hypothesize. Because it's a difference of logs, you can calculate… you can rewrite it as the ratio of the concentrations.

And that's why it's absolutely fundamental that the only thing that you should remember of the logarithm… say, of the function logarithm of x, is that it crosses the axis, the horizontal axis, at 1. And for the argument that is larger than 1, it's positive. And for the argument that is lower than 1, it's negative.

I'm doing my best, but sometimes it's tough… and you should talk in case you're scared.

Now, this is really important because the ratio C_{out}/C_{in} is this thing that I'm starting to insist on. I don't care whether you remember 400 millimolars outside, 50 millimolars… What matters is that you remember what is larger, if 'in' or 'out'.

Applying the Nernst Equation to Ions So, if we are instantiating this equation, assuming that this $[C_{out}/C_{in}]$ is known and this $[\Delta V]$ is not known, for **sodium (Na)**: sodium outside is more concentrated than inside, so this quantity $[C_{out}/C_{in}]$ is positive. So we are here [argument > 1], so the logarithm is positive.

RT, whatever… so T is in degrees Kelvin… states at room temperature… Z is 1. So, I can tell you that this thing here, RT/ZF, that you could also write it equivalently as kT/Zq if you are coming from the family of physicists instead of chemists… it's the same. You can see over in the past class elements for going from one side to the next. Basically, R and F, the Faraday constant and the *Riemann*… gas constant, are close relatives.

So, this quantity for $Z = 1$ (the valency of sodium, that is only 1, the valency is 1, has only 1 elementary charge, positive), this quantity at room temperature is around **26 millivolts (mV)**.

So, first of all, you should burn into your brain: this is millivolts. Of course, the logarithm of something is dimensionless. And it makes sense that it's log of something that is dimensionless. So this quantity $[C_{out}/C_{in}]$ has no dimension. It's a ratio of two [concentrations] millimolar. And so the units are canceling, it is no longer a quantity, a physical quantity with physical measures. So that's okay. It's a guarantee that we are on the right track.

And so, yeah, this is millivolt. Indeed, this difference of potential inside with respect to the outside is in millivolt.

I don't know if any of you has a calculator or even on your mobile phone. Sometimes you have, if you tilt it in the other direction, you get the scientific calculator where you can calculate logarithms. And if I recall correctly, 400

divided by 50… we could do it simplified… No, I cannot do it because this is not a log in base 10, so I cannot be just doing logarithms without a calculator.

If some of you have a log… as a calculator and does for me logarithm of 400/50, for sure you should get a positive number because the numerator is larger than the denominator. And we are going to see, multiplying this quantity by 26 millivolts, we will see what is the electrical potential that you will have across the membrane if you have an unequal concentration, like you do, of sodium ions.

I can tell you that it is around **50 millivolts**. So I think this quantity [ln(400/50)] is roughly 2 point something or around that. What was that? Okay.

So in the… 26, 25… Okay, it's not -70 millivolt. So I'm disappointed. But it's very intriguing because in this way we have some sort of hint, the first step to understand why you have electrical phenomena, particularly the arising of an electrostatic potential for unequal distribution of charges, which is not trivial.

This is not the only story, because first of all, you don't have a single ionic species, and you don't have equilibrium. But equilibrium… let me discuss it in a moment.

The Nernst Potential as a Battery So, equipped with this knowledge, what you basically have in front of you is a mathematical model. You have a membrane, you have a distribution of ions, and you basically have a **battery**, like the Daniell cell or Alessandro Volta's pile. And this is also called… so it's the Nernst equation, and it's called the Nernst potential (or equilibrium potential) for obvious reasons. And it is specifically for that ion.

And now I will give you the numbers for sodium, for potassium, for chlorine, for calcium. The only thing you need to know is the amount of the concentration inside and outside the membrane. And, of course, roughly, and I hope that you will remember for the rest of your day… The only thing that you will be able to say, even without remembering or without knowing the numbers, is whether this equilibrium potential is positive or negative for different ionic species.

Just because I started stressing you with this: for **potassium ions (K)**, which I told you are the opposite… so, sodium you lick and you taste salty outside because there is a lot of sodium outside and little inside. For potassium it's the opposite. So you have a lot of potassium inside and little potassium outside.

So that ratio, C_{out}/C_{in}, instead of being larger than 1, for potassium is **lower than 1**. Lower than 1 means [logarithm] negative. It doesn't go… The argument is still positive. It's just the ratio that is lower than 1. It is still a positive quantity, so we are not violating any… mathematical… so we're not exceeding the domain of this function. And we are in this part [argument < 1] where the logarithm is negative. It goes very rapidly into negative. So you would probably think that I'm very hopeful that maybe -70 millivolt comes from potassium. Of course, it cannot come from sodium.

And okay, here is what I already said. 300 degrees [Kelvin], which is roughly 20 degrees, 25 degrees [Celsius]. So it's… you know that the degrees in Kelvin… well, it's like zero degrees [Celsius] when you are at -273 Celsius. And so T is

in Kelvin. So this one is in degrees Kelvin. But anyway, 26, 25 millivolts is the things to remember when the denominator Z is 1. For **calcium (Ca^2)**, it will be smaller. It would be 13 millivolts because you would have a number two below. Magnesium would be the same, but we will not talk about magnesium.

So some of you have the calculator. You could try, but otherwise I will give you… But the only thing you see, the potassium is the opposite than the sodium. So potassium, for sure, this equilibrium potential will be **negative**. Sodium will be **positive**. **Chlorine (Cl)** is a little bit strange because Z is **-1**. So you have a minus. So it should be, say, 50 millivolts, or maybe it's going to be 60 millivolts. But in reality, because the C_{out}/C_{in} is larger than 1, but there is this minus of Z, the valency, because it's negatively charged… So this $[\ln(C_{out}/C_{in})]$ will be positive, but due to $Z = -1$ the potential will be **negative**. And this one [for Calcium] will be positive because you see outside you have really a lot. So it's probably going to be very large. Maybe not so large because the logarithm is not growing… yeah, it's growing and it goes to infinity, but it's not growing so fast.

Values of Nernst Potentials So here are the numbers that you can get, just once in your life to make this calculation with a logarithm, which, however, should be cross-checked by your intuition, by this graph of the logarithm.

And so here it is: * **Potassium (K): -77 mV.** Damn, it's not -70. * **Sodium (Na): +56 mV.** * **Chlorine (Cl): -68 mV.** Close, but not the right one. * **Calcium (Ca^2): $>$ +100 mV.** (more than 100 millivolts)

And all these batteries, depending if the potential is positive or negative… just purely by conventions, I swapped them, the symbol, the electrical symbol, just to convince you that one is positive inside with respect to the outside, and sometimes is negative. That means it would be positive in the other direction. But it doesn't matter. It's only for those of you not necessarily familiar with this purely conventional symbolic… So the symbol of electrical component, just to tell you what it is.

So I'm just… I hope I'm not exceeding. You will tell me if I'm too theatrical. What I wanted to convey is that it cannot be -70 millivolts. So the resting membrane potential cannot be, or should not be, or in principle should not be, the result of only one ionic species if inside and outside you have many ions.

And so, here, this the Nernst equation is very powerful, but it leaves us with a question, which is: how do I combine them? How do I… so do I average them? Is the arithmetic average? Is some sort of other way to combine them? Because I don't know how to do that, but I would like to consider one cell in which I have sodium, potassium, chloride, and calcium in and out. And I would like to know, okay, why if I take two electrodes, one in and one [out], why do I measure -70 millivolts?

So this is a key. That's why I'm presenting it to you, despite the fact that the hypotheses are, I underline it, the main hypothesis is that everything is at equilibrium.

A moment ago, I made a mistake. Instead of calling them equilibrium potentials, I call them **reversal potentials**. Possibly in one hour you will see why it's a legitimate name, why these are also called reversal potential for one ionic species. For the moment it's not clear. Reversing what? Reversing maybe some flux, but under which conditions, since we are thinking of equilibrium or resting conditions? So it's not clear, but it will be clear.

So, it must be a combination. But how?

The Electrodiffusion Equation First I would like to very briefly tell you why the story… because some of you could be annoyed by the fact that very quickly I end up saying: "if it's equilibrium, then I put the total flux to zero and one is minus the other." Some of you will accept it, but I'm maybe speaking to those who are… no, they don't believe, they want to see.

So I will make a step backwards and say: let me tell you about the **electrodiffusion equation**.

So, in general, I'm not talking about any steady state or any equilibrium. And the only thing that I'm invoking is the **conservation of mass**. Mass is not destroyed nor created. And if I have an… *accurate*… aqueous solution, I can basically… allow me to consider for simplicity just a monodimensional case. And I have one point. In one point, I might have the fluxes (J), I might have the concentrations (C), I might have the electrostatic potentials (V), if any. I can characterize… So all these quantities that I mentioned are depending on space (x). Maybe they depend also on time (t). But they will depend definitely on space.

And like I did for the flux, let me take again one surface that right now is not infinitesimal. Maybe it's small. I call it ΔS, it's the surface. It's a cross-section surface. And I take, in the other case, I was thinking of the definition of flux, and I was measuring the time Δt, and because the velocity… this… *neural*… formula, linking the velocities to the forces, and then the fluxes to the forces and to the velocity… here I'm just thinking of Δx.

So in this Δx, I span a certain volume between x and $x + \Delta x$, and there I'm claiming that in this box, things will not be destroyed or concentrated. And there is nothing that is like… for, say, for cubes made of some elastic material, there is no way to blow the volume or to… So I'm thinking of some sort of elasticity that I can feed more stuff inside, some sort of inductor or capacitor, equivalent capacitor in this word. No, it's just the portions of the volume. **What comes in must come out.**

This is the reason I'm, again, invoking Lavoisier with this story of "nothing is created, nothing is destroyed." In a Δt, again, Δx, and here I can calculate, express how many particles are at a certain time, $t+\Delta t$. It's the concentration… let me do it for one ionic species… the concentration (C) multiplied by the volume ($Vol = \Delta S \cdot \Delta x$). If the volume is expressed in liters, so if the concentration is expressed in millimolar, I have to be careful that the volume that I'm multiplying is expressed in liters, but nothing wrong with that.

So this amount of particles at time $t + \Delta t$ must be equal to what they were a moment before, at time t, **plus** those who entered the door **minus** the others

that came out from the door.

$$C(t+\Delta t)\cdot\Delta S\cdot\Delta x = C(t)\cdot\Delta S\cdot\Delta x + J(x)\cdot\Delta S\cdot\Delta t - J(x+\Delta x)\cdot\Delta S\cdot\Delta t$$

This is simple because it's unidimensional, so by convention I take as positive the fluxes, here generic fluxes, when they are pointing in the same direction of x. That's why here I put it plus and here I put it minus. You see it's at t, both at t, which is the previous step, but here is at x, so at the entrance, and here is $x+\Delta x$, which is at the other end.

If you do the same thing for a three-dimensional case or even bidimensional case, of course you have to take into consideration a small cube and you know that you can have influx and outflux from all the possible sides. Here it's simple, it's monodimensional. You enter and you exit.

So that's it basically. This is translating that sentence: "nothing is created, nothing is destroyed." So that means whatever I measure now must be whatever it was before, plus and minus the new particles and the particles that I've lost. And I remind you that here I multiply by Δt and ΔS because the flux itself is per unit of time and per cross-section of area.

I rewrote the same equations over there and you see that ΔS can be simplified. This is independent on my choice of cross-section. And what remains is the Δx in the left-hand side and the Δt… well, Δx is… yeah, the Δt on the right-hand side.

So I can divide… I can multiply and divide both sides by Δx as well as by Δt. Or I move Δx from the left to the denominator on the right, or Δt, I move it from the right at the numerator, I put it in the denominator on the left-hand side.

$$\frac{C(t+\Delta t)-C(t)}{\Delta t}\cdot\Delta x = -(J(x+\Delta x)-J(x))$$

$$\frac{C(t+\Delta t)-C(t)}{\Delta t} = -\frac{J(x+\Delta x)-J(x)}{\Delta x}$$

And you know I'm now very much tempted to take the limit for Δt and Δx simultaneously that go to zero, because those quantities are exactly resembling a differential ratio. It's the definition of a derivative.

On the left, you see that it's on time. It's a function of x… x is fine, whatever. But it's a function of t, and t is calculated in $t+\Delta t$ minus whatever it was calculated in t. And then at the denominator, you have the amount of this variation. On the right, you have the same thing, apart from the minus. And the function is a function… considered of the other quantity. It's the first argument, x, calculated in $x+\Delta x$ minus the function calculated in x. And in the denominator, you have Δx.

You take the limit, and you get the partial derivative. You cannot use the symbol of total derivative because these are not total derivatives.

And you get what is called the **electrodiffusion equation** that basically links fluxes and how they change in space, to how the concentration is changing in space and in time.

$$\frac{\partial C}{\partial t} = -\frac{\partial J}{\partial x}$$

It's a partial differential equation and in general it's very difficult to solve. And you can plug the drift and the diffusion in that part $[J]$, because you don't have any other fluxes.

The GHK Equation (Permeability Version) What was historically done—so this equation is what is called the **Goldman-Hodgkin-Katz (GHK) equation**, with conductances for obvious reasons—can be done with the Goldman formulation.

Because (and now you see how), if you allow me the simplification that I consider all currents related to ions with the **same valence** (if the valences are different the expression is not so simple, you can do it numerically but it doesn't come out so simple), I can take these fluxes [from the Goldman equation] and say J_1 (which is sodium) + J_2 (potassium) + J_3 + J_4… all equal to zero.

$$J_{tot} = \sum_k J_k = 0$$

It's certainly not immediate, let's see what happens.

So these are all the terms [of the Goldman equation for each ion k], where I simply put a subscript k where things are different.

$$J_k = -P_k \cdot A \cdot V \frac{C_{out,k} - C_{in,k} e^{AV}}{1 - e^{AV}}$$

They are different in **permeability (P_k)**, certainly not in V_{in} and V_{out} (because as in the other case, again, the channels experience the same V_{in} and V_{out}), and I put the subscript k (or 2 or 1, whatever) on the **concentrations ($C_{out,k}$ and $C_{in,k}$)**, because those depend on whether you are sodium or potassium (inside there is little sodium, outside there is a lot of sodium; if you are potassium it's the reverse, inside there is a lot of potassium and outside there is little potassium).

I can factor out the common term $A \cdot V/(1 - e^{AV})$.

$$J_{tot} = -A \cdot V \frac{1}{1 - e^{AV}} \sum_k P_k (C_{out,k} - C_{in,k} e^{AV}) = 0$$

By default, this term $[A \cdot V]$ and this term $[1 - e^{AV}]$ do not depend on the subscript. And here I get something that is a little annoying, because I have the sum of these binomials: $(P_k C_{out,k} - P_k C_{in,k} \cdot e^{AV})$. The exponential $[e^{AV}]$ I could… *[error in speech, e^{AV} is inside the summation]*… No, I can't. I had to

split the summation. And it's not a bad idea, because I'll show you what we can write now.

$$\sum_k P_k C_{out,k} - \sum_k P_k C_{in,k} e^{AV} = 0$$
$$\sum_k P_k C_{out,k} - e^{AV} \sum_k P_k C_{in,k} = 0$$

So, when I say that the total flux is zero, for the same reason as Kirchhoff (where I am saying again: it is a dynamic equilibrium, not the "thermodynamic death" where the individual fluxes are zero, but… *death*… it is a dynamic equilibrium, the total, their sum of effects is, on average, null), I realize that there are two cases here to make this total flux zero. It means that: 1. Either the intracellular potential (V) is null. It's okay, it's a legitimate case, but it doesn't interest me because experimentally I don't see it as null, I don't see it as zero. I see that there is a potential drop across the membrane, which is what I would like to understand. 2. This in the denominator doesn't count. This term [the numerator] can be zero, being a product of terms, only if the first [$-A \cdot V/(1 - e^{AV})$] or the second [the summation] are 0. The first… okay, [$V = 0$] I'm not interested in the simple case. 3. Or the case that this quantity [the summation] is 0. And it's a little… it intimidates me, because apparently… *it's not a mess*, but it's big, it's complicated, I don't like it.

The sum of terms means that this difference is 0. That is, this sum here [$\sum P_k C_{out,k}$] is equal to this other sum here [$e^{AV} \sum P_k C_{in,k}$].

$$\sum_k P_k C_{out,k} = e^{AV} \sum_k P_k C_{in,k}$$

Apart from this term [e^{AV}]. So I can write it and I can divide both sides by this quantity here [$\sum P_k C_{in,k}$].

$$e^{AV} = \frac{\sum_k P_k C_{out,k}}{\sum_k P_k C_{in,k}}$$

No, sorry. Okay, I move… C_{in}… underneath. And I'm left with a ratio of sums, which will be ugly but it is what it is. And on the right with $e^{AV_{in}}$.

I don't like the exponential. *No problem*, I apply the **logarithm** to both sides, so as to kill the exponential. Here I have the logarithm of the ratio of these two sums, and on the right I have $A \cdot V_{in}$.

$$A \cdot V_{in} = \ln \left(\frac{\sum_k P_k C_{out,k}}{\sum_k P_k C_{in,k}} \right)$$

(Remembering $A = ZF/RT$)

$$V_{\text{rest}} = \frac{RT}{ZF} \ln \left(\frac{\sum_k P_k C_{out,k}}{\sum_k P_k C_{in,k}} \right)$$

(Considering Na , K (Z=1) and Cl (Z=-1))

$$V_{\text{rest}} = \frac{RT}{F} \ln \left(\frac{P_{Na}[Na^+]_{out} + P_K[K^+]_{out} + P_{Cl}[Cl^-]_{in}}{P_{Na}[Na^+]_{in} + P_K[K^+]_{in} + P_{Cl}[Cl^-]_{out}} \right)$$

I find an expression strangely similar to the Nernst potential. Here there are no G's [conductances], here I am seeing the P's [permeabilities]. And I continue to see the concentrations (which I "hid" from you in the ohmic part).

That is: the ohmic part for an engineer is simple and immediate, they are resistances. In total there will be some resistance that wins… some conductance that wins, that makes the membrane potential "lean" towards the battery that has the largest conductance, that wins.

Here it is more complicated because I don't see it, but it looks a lot like the Nernst case, in which here the **average is weighted by the permeabilities** and the concentrations continue to appear.

So it seems that if… so I should rephrase it… here the dependence is even with the logarithm, so it is more sophisticated. Depending on what the permeability of the membrane is to one species or another (compatibly with the concentrations of the species itself), the resting potential leans towards one or the other. Does it lean more towards the sodium reversal potential (which is +50 millivolts) or the potassium reversal potential (which is -80 millivolts)?

The Nobel Prize-Winning Intuition Before finishing I would like (and take a 10-minute break) I would like to simply draw what I just told you.

So, I said that… you have to call this axis V. Here, **+50 millivolts** (approximately) is the sodium reversal potential. So it's E_{Na}, the sodium Nernst potential. Here, **-80 millivolts** (approximately) is the potassium reversal potential (E_K). It's a little more depolarized… it was -68 millivolts, this is the chlorine potential (E_{Cl}), if we really care.

This is because maybe it might occur to some of you now to say: "If this resting potential, which normally is planted here [at -70 mV], were to change rapidly and go, as during an **action potential**, go towards +20 millivolts, and then go towards -80 millivolts, and then return to a resting potential…". As it seems from the traces I showed you, if you have the eye, if you remember something.

It might be strange, or interesting, the fact that it does not exceed this range: +50, -80. Which are exactly, under certain conditions, the maximum and minimum values that a distribution of sodium and potassium ions allow the membrane potential to assume. If there were only those, and if the other permeabilities disappeared (if the gates are closed for potassium and for chlorine, there is only the open gate for sodium), then this is possible.

This intuition is what I will call in the next hour "Nobel Prize-winning intuition," because something like this allowed Hodgkin and Huxley to win the Nobel Prize, explaining not only the resting part, but the action part.

Excitability

Thank you. … the next chapter. The next chapter is that of **excitability**.

The thing that strikes me, or has always struck me (beyond the fact that I am probably 30 years older than you), is that anyway, clarifying the origin and mechanisms of the resting potential, and then of neuronal excitability, is not something that has been known for centuries and centuries. It is relatively recent. Recent as in: in the 1950s. Less than 60-70 years ago, a few years more than 60 years ago.

And what I am telling you today in class was not so obvious, and in a flash, I hope without too much difficulty, you can also understand it both at a quantitative level and at a more intuitive level.

Historical Context People had begun to see that the electrical characteristics of excitable cells, like nerve cells, were not constant over time. It spiked every now and then, in very short times, in fractions of a second, in the millisecond in particular. Several characters, who are indicated here (but I am only interested in showing you the dates), had initially thought that there was a kind of propagation in some parts of the neuron's morphology, in the axon. There was a kind of propagation of an "irritation" or a propagation of ***inward* ionic currents**.

In the end, these people saw that the potential inside with respect to outside (I'll call it V_M from now on, membrane potential; outside is my reference which I consider zero, because I have a reference electrode, I attach it to the amplifier, to the other plug where it marks zero), they had seen that at a certain point the membrane potential became very positive. So it wasn't so strange for them to say: "Boh, there must be *inward* currents," which means incoming, and somehow they brought positive charges inside the membrane.

Parenthetically, now that you know what the balance of ion concentration is inside and out, you could tell me what this ion might be called. What ion is it? [Referring to Na] It's not calcium because it only has one "+", it's not chlorine. For the same reason that there is a lot of it outside, it could be that if something happens, purely… in accordance with a… (the term escapes me)… with an **electrochemical gradient**, given that there is a lot of sodium outside, it can naturally enter where there is less. And if this happens, the potential could, should, increase.

It was not so clear. It took **Bernstein** and others at the beginning of the twentieth century to think that it wasn't necessary… it was better not to think in absolute terms of currents, but of **permeability**, of changes in permeability. Precisely because of what occurred to you: that is, if there is a condition whereby if I open the door, a horde of people comes in (because there are many outside and few inside), maybe the key to everything is to understand permeability.

At the time… it wasn't until the 1970s that people understood that there were channels, pores in the membrane. Hodgkin and Huxley had not imagined that there were pores. They thought there was a kind of transporter, some electrically charged particle (because we will see now why electrically charged) that acted as a kind of bridge. But the fact that there were pores was seen in the '70s and '80s by **Neher and Sakmann**, two German electrophysiologists who also won the Nobel.

Vole… until '40, before the Second World War (where there was an interruption), they were all people in England in a very, very notable laboratory in Cambridge, particularly Hodgkin and Huxley. Now I don't remember if Cole and Curtis were there or at another university, I don't remember now. I don't remember if they were in Australia. Anyway, during the Second World War everything stopped.

And it took several years after this hypothesis by Bernstein for people to measure a change in permeability. And this change in permeability, Hodgkin and Huxley with Katz etc., then correlated it with these incoming currents. And in the end, the mechanistic model, that is the mechanistic explanation that describes how ionic permeabilities can support (and how they must change, what changes and why) to generate or to explain the action potential, came about in the 1950s.

And the interesting thing is that these gentlemen, who won the Nobel Prize ten years later (Hodgkin and Huxley), formulated it with a **mathematical model**. That is, the type of mathematics and circuit equivalents that I told you about, at a certain point becomes a bit complicated to do by hand. As long as it's a matter of invoking equilibrium or resting conditions (for which the total current is zero), this is relatively easy. Understanding it in a dynamic context, where quantities change over time, is a bit more complicated. Just as, for that matter, the analysis of circuits that are not linear circuits (that is, made of linear components: capacitors, resistors, inductors, etc.) is not necessarily trivial. Differential equations emerge. Then in the end, the differential equation is always the same, but in some cases they become non-linear equations, like the one it is.

So the tool of the electronic (or mechanical, as it was in their time) calculator proves fundamental to understanding anything.

Plan for the Excitability Chapter Second episode of this chapter, it's called excitability, which obviously… *crescia…* Excuse me.

Partly following this book, **Sterrat**, and you can also find material in **Abbott & Dayan**, which as I said perhaps in the first lesson and also perhaps last week, contains some chapters, some material (it's not in its entirety), and this Abbott & Dayan is a much more difficult text. And again, these should be support texts for your study.

So the starting point is that there is a concentration, a distribution of charges and ions that is not homogeneous between inside and outside. Here is the kitten, which is much more dignified than the comparison I told you about. I hope that one way or another, either with the kittens or with the sweat, you can remember the composition of the extracellular fluid.

We have seen the electrical equivalents. I would like to try to put everything together, to put into an equivalent circuit (not because I am an engineer, but because Hodgkin and Huxley, who were physiologists, did it) both the **resistive** and the **capacitive** properties. And I put them together because I see that they are all equivalent components that can be described as all experiencing the same extracellular and intracellular potential. If they are in parallel… if the balloon that I made for you, that I drew earlier, the membrane with the individual pores

and the individual currents that somehow insisted in parallel, doesn't already convince you enough, sufficiently enough.

This is the starting point, I'm repeating it. And this again at rest. Thank you, we understood that.

We see that in the continuation… (I should have shown you one thing, later I'll stop to show you the notebook for the Goldman equation)… the usual first-order differential equation with constant coefficients will pop up shortly. In reality, the coefficients will not be constant, but in a separate treatment that we do for simplicity, it will become exactly that: with constant coefficients.

You remember that if you want to make me happy, you must pay attention that the state variable (which obviously in a differential equation is a function) has a **negative coefficient**, because it goes into the exponent and biophysical, biological systems should be dissipative systems. They should not explode, but turn off or at least converge to some quantity. For sure, they are not quantities that explode, at least they don't always explode or at every moment.

So having understanding, memory, knowledge of how to solve this differential equation is essential. And also how to plot a function, how to derive the graph of a function of the type: **constant + exponential**, where the exponential is decreasing ($k \cdot e^{-t/\tau}$).

I told you the other time that I can imagine that the exponential term disappears at a certain point. "At a certain point" means when the state variable… let's say… is sufficiently large in absolute value. The exponential of a very negative quantity is zero. So at a certain point, I can neglect this quantity. This way of looking at solutions and mathematical functions sometimes helps, rather than having a clear functional analysis of how to do limits, derivatives, concavity… because "roughly" one can get more or less the same result.

Specific Objectives So the idea is to combine these capacitive and resistive aspects into what is called the **charge balance equation**, in which I no longer consider the hypothesis of rest, of the resting condition.

1. I'll show you how it is possible to obtain a **lumped-parameter circuit**, even though the membrane is a spatially extended circuit.
2. Then, in fact, I have already bored you during the first lessons where I told you that neurons, described as the "butterflies of the soul" by a great neuroanatomist, are spatially extended objects. They are as spatially extended as the distance between my pyramidal tract and my foot, as a first approximation. So it might be that this thing about lumped-parameter descriptions is not always valid. But for now, we'll consider it. I'll tell you why, what "lumped parameters" means if you haven't done something similar in some course on electromagnetism.
3. And since we are talking about circuit equivalents, I would like to **derive the equivalent circuit model**. Again, the ambition is that by looking at the model, I can infer considerations about what the electrophysiological signal is that I measure experimentally.

4. I will talk about a model (this is the side thing I'll do for you simply) where the usual boring differential equation with constant coefficients emerges.
5. Here I'll show you what is technically called the **Thévenin equivalent circuit**. There was also Norton who was a friend, but I prefer Thévenin. In the case where the cells are not excitable (and now I'll tell you what this has to do with excitability), it allows, in fact, to have an **RC** [Resistor-Capacitor circuit] which perhaps you have already heard of or chewed on.
6. So we will look at how one can see the response to a stimulus. And I can do it with pen and paper, or anyway I'll do it, you have everything on the slides. And to have some insights about the steady state.
7. And then there is an exercise where I tell you **how many ions are exchanged** during an action potential. So how often you should eat bananas and chocolate to support the fact that most of the cells in your brain right now are firing like crazy for several hours, since this morning and even during the night. So, how metabolically costly is it to be excitable?
8. And at some point (I'm not sure if we'll do it today) I'll talk to you about what is the kinetic, effective, phenomenological description of membrane channels, which are not all pores that just sit there and do nothing. They are structures that change very rapidly (the geographical area lends itself to the comparison: they are the Ferraris of the molecular world, of molecular biology) because they are able to change shape extremely rapidly, in a fraction of a millisecond. But I don't want to give spoilers.
9. This description is the same description that many of you already know from chemical reactions. But perhaps no one has shown you that chemical reactions (sodium plus… sodium chloride), this notation with some little number above it that you might have indicated with the constants K_1, K_2, whatever, is actually a differential equation. But perhaps no one told you that. They are called Markovian kinetic schemes, in due course.
10. And I will tell you what they have to do with the complete description, which combines all these things from before, the **model proposed by Hodgkin-Huxley** to mechanistically explain why the action potential occurs.

The Charge Balance Equation So I recall Lavoisier, "nothing is created, nothing is destroyed," and among the other simplifying hypotheses I had made, the main one now was that of considering [the system] at rest, or at steady state. It would have been more correct to say that the quantities did not change over time (-70 [mV] remained constant), so there were no quantities that varied over time.

I remove that one too, and I anticipate that this means I also want to include the **capacitive effects**, which in themselves have a characteristic of temporal integration. The capacitor has a capacitance; it accumulates. It's like the bathtub in your house or the sink: it's an accumulator. So it's a term that has "memory." Memory makes one think of time (not… not yet neurobiological memory, but dynamic memory, of a dynamic system).

And by invoking the conservation of charge, and therefore invoking Lavoisier, I can combine things.

In particular, I say that the total charge balance is zero. Charge is not destroyed or created. In an electrolytic system, in my brain, in a neuron, there is no destruction of charge and there is no creation of new charge.

And the changes in charge (ΔQ) in the case of a neuron's membrane can occur for two reasons (I've already anticipated this and I'm insisting in the hope that it's not too complicated or too technical information):

1. On one hand, there is **displacement** (displacement currents, I_C). I don't open the door; I get close, but I'm very positively charged… actually, negatively… and from the outside, positive charges come to neutralize me, but they hit the dielectric, the insulator. So I know that in the case of a capacitor, this displaced charge (ΔQ_C) depends on the potential difference (ΔV). A potential difference that, for example, I am representing in the temporal context. Again, I take the clock, I let a Δt pass, I know there are… so I express in terms of current ($I_C = \Delta Q_C/\Delta t$) this ratio Δcharge over Δtime. It means: how much charge has changed (and it's a displacement) in the unit of time, Δt.
2. And **transport** (ionic currents, I_{ion}). Instead, this is the current… this is a transport current. It is given by those currents; it has nothing to do with capacitance, but it has to do with those fluxes, or with the current densities, determined by the ionic fluxes of the individual species: sodium, potassium, calcium, chlorine.

So the total charge… *[error in speech, means charge variation]*… is the current times Δt. And in this [capacitive] case, it is given by ΔQ_C. This for the capacitor… time doesn't matter… *[error in speech, $Q = CV$ is the base relation, but current depends on the change of V over time]*. This is the potential difference across the membrane, period. If I know that, if I know how much is across the membrane, I can understand what the ΔQ_C is, the amount of charge displaced. This is the transport charge ($\Delta Q_{ion} = I_{ion} \cdot \Delta t$) which requires remembering what a current is, which is charge over time.

If this total current… *[the sum of charge variations]*… doesn't change, it means that… $\Delta Q_C + \Delta Q_{ion} = 0$

(Remembering $Q_C = C \cdot V$ *and* $\Delta Q_{ion} = (\sum I_k) \cdot \Delta t$*)* $(C \cdot \Delta V) + (\sum I_k) \cdot \Delta t = 0$

…$C \cdot \Delta V$ must be equal to the sum… sorry, $C \cdot \Delta V$ plus this summation of currents times Δt must be equal to 0. So $C \cdot \Delta V$ equals **minus** this quantity $[\sum I_k \cdot \Delta t]$. Before, they were all on the first side; now I have moved the currents to the second side, changing their sign.

And I *screwed* them… I divided both sides by a quantity that is not zero (so I can do it), Δt. Here I got $C \cdot \Delta V/\Delta t$. Here I no longer have the Δt term.

$$C\frac{\Delta V}{\Delta t} = -\sum_k I_k$$

And taking the limit for Δt that goes to zero, in fact, this thing here is the **constitutive equation of a capacitor in parallel with a parallel of resistors**.

But here I didn't have to use the electronic-engineer mindset. Here it's the charge balance. In fact, this equation comes from balancing the charge. Incidentally, it's the same equation that Kirchhoff would propose. And in the end, obviously, the parallels are striking.

The Importance of the Minus Sign (Dissipative Systems) Speaking of making me happy or sad: notice this **minus** sign. These individual currents (I_k), if you remember, were $I_k = G_k \cdot (V - E_k)$.

$$C\frac{dV}{dt} = -\sum_k G_k(V - E_k)$$

The fact that there is a minus sign, regardless of the convention of whether it enters or exits, could help mnemonically to think that when you write the charge balance equation ($C \cdot dV/dt = ...$), the fact that there is a minus (which obviously I never remember, in fact I got it wrong when I said it out loud a moment ago, I didn't consider it)… this minus makes me **invert** this difference.

This difference ($V - E_k$) incidentally also has a name, it's called the ***driving force*** (it's not the electromotive force). It is a forcing term: having a particular intracellular potential, for that ionic species the fact of being more or less distant from the Nernst potential causes a more or less intense current.

Or… the minus becomes something that becomes $G_k \cdot (E_k - V)$.

$$C\frac{dV}{dt} = \sum_k G_k(E_k - V)$$

So I am happy because there is the **minus** V. So don't forget this thing to make me happy: to keep an eye on the fact that what you write or what you see written (what in theory you could numerically validate with a computer simulation) might not explode, it should not explode.

Because this system, despite being an electrical, ionic system, is a **dissipative system**. Ion channels are systems of **passive transport**. It is not necessary to consume ATP *per se* to make the ions work. You consume ATP to make the **ion pumps** work, which are **against** the electrochemical gradient transport mechanisms. So [if] I am a sodium ion, I need an ion pump to get me kicked out; I need energy because outside there is a lot of sodium.

Anyway, please make me happy because here it is the same state variable, V, which appears with a negative *exponent… [error, means coefficient]*. I know that here there are a lot of terms ($G_{sodium}(E_{sodium} - V) + G_{potassium}(E_{potassium} - V)$ ...), there are many… But conceptually, even if there are many, it is likely that this propagated "minus" term can still justify the existence of a dissipative system. Thus, of solutions that have something to do with decreasing exponentials and not explosive ones, that biophysical quantities do not go to infinity.

The Equivalent Circuit (Lumped Parameter Model) So this equation here is nothing more than the **parallel** [combination] of a capacitor and a series of resistors (obviously resistors with batteries also).

This is the circuit diagram of a small piece (it is also called a *patch*) of membrane, in which you see that the phospholipid bilayer is indicated as equivalent to a **capacitor (C_m)**. So if I am an ion, I stop here, I don't go inside because there is a… even energetically it would be impossible, I would need a lot of energy to be pushed inside. Just like in capacitors you need the so-called *breakdown* of the dielectric to have a transport current inside a capacitor. Lightning is an example of dielectric breakdown and obviously you have enormous energy. Here you do not physiologically "puncture" the dielectric of the membrane.

But, if you are an ion, you can (as I did in the little drawing the other time), if the channel lets you pass (because it is the one selective for you) and if the channel is open (it could be like a closed door, maybe), it lets you pass from inside to outside or vice versa.

Here I have also indicated the tip of a **glass electrode**, the one I showed you during the first two lessons, because in theory the tip of this electrode (which you remember contains a silver-chloride wire, which, when appropriately operated by an electronic current, releases or "sucks" a chlorine current)… So here, in theory, I "spit" or "suck," therefore I interact with the intracellular ionic environment. And it is as if this can be characterized by an equivalent circuit equal to an **ideal current generator (I_{ext})**. Whatever the potential drop across the membrane, the current is what I put in. Right now I'm not putting any in because I want to see what the neuron does. If I want to stimulate it, maybe, I inject a few tens or hundreds of picoamperes (in the case of a pipette and in the case of a mammalian neuron it would be a pretty tough current).

But the membrane is extended. And so there are many channels. You see that some channels are of different types, in fact the battery is oriented in the opposite direction. Nothing to understand, it's just for me to remember that some are for sodium (E_{Na}), others are for potassium (E_K), and each has a Nernst potential that is not equal, it even has differences in sign (those for sodium are +50, +56, whatever; potassium is -80, etc., etc.). So they are not all identical. Beyond the fact that they are channels that are selective only for one ionic species, but electrically I don't care if they are selective for one ionic species. But the batteries, the sign and the amplitude, I must take them into account.

So one thing that is done… (which ended like this without suspense, I thought… something I didn't remember would appear)… one might say: "But couldn't I perhaps **simplify** this circuit which now in this configuration has distributed parameters?"

So in theory, or rather, no, it is still lumped parameters, especially because the points here inside and outside are isopotential. There is no resistance that would describe (either inside or outside) a difference in distribution, a different distribution of electrical potential when changing the point. But I could decide to simplify it because they are annoying to manage eventually with pen and paper.

Review of Circuit Theory (Kirchhoff's Laws) So I'll give you some hints of so-called circuit theory, or electrical engineering, in which I don't have a fast approach, I'll just remind you. I don't have an abstract and formal approach that would require invoking graph theory and saying that a circuit is a collection of nodes and arcs… (I don't remember how to say *link* in Italian)… nodes and edges. And for each of these nodes I can associate two electrical quantities. I can do it arbitrarily. I can orient these electrical quantities as I want, so they will have an orientation, an algebraic orientation. I can do whatever I want, as long as I am then consistent.

So I'll make it much simpler than that. I remind you that these quantities are used in the so-called **Kirchhoff's Laws**, they descend from physical principles of electromagnetism: 1. **Kirchhoff's First Law (Current Law, KCL):** From the conservation of charge. Given any node, the algebraic sum ($\sum I$) of all currents entering or exiting (with sign) is **zero**. 2. **Kirchhoff's Second Law (Voltage Law, KVL):** In the case of the circulation of the electric field being zero, thus due to the conservative energy properties of the electric field. The path I take (I go from here to here and then here… a closed path, so a loop) has the sum ($\sum V$) of quantities of these electrical potential magnitudes… the algebraic sum, taken with the signs, with a direction you want (as long as you are consistent), the sum is **zero**.

So one thing that is done in electrical engineering or electronics, one can choose (but it is purely conventional) is the so-called **passive sign convention** (convention of users). In which given two nodes, typically one takes the potential (V) oriented like this (with the arrow pointing up) and the current (I) going down. Simply because in the case of a user, of a passive component, of a resistance, the constitutive equation of a resistance says that if you take the potential oriented like this and the current oriented like this, $V = R \cdot I$. With this convention. Otherwise, they would have different signs. It has to do with the fact that… with the usual story of the height difference, the comparison with gravitational potential: if one has a difference in altitude, one has a flow of charge. The drama is that it was thought that positive charges conducted electric currents. So if you see the last lesson or two lessons ago, the positive charge, when the electric field is oriented in this way, would move downwards, as mass does when there is a difference in altitude (a river goes downstream, it doesn't go up a mountain). But this has remained and Ohm's law works with these assumptions.

It is not fundamental. I am telling you these things, first: to suggest to you, if you don't have them fresh, maybe to review them. If you have never done them, you might be curious, you might be ambitious to say: "But why does it take a genius to have a smattering of circuit theory?" No, there isn't this. This feeling of inferiority was particularly alive when I was in Belgium, where students did not have a technical background and so they said: "Ah no, I am not a physicist, a mathematician, an engineer, I won't do it." Try. You will see that it is simply a matter of algebra, nothing particularly else is needed.

Here I use it only to show you that that parallel of resistors and capacitors (because the capacitor is also a property in theory distributed) can be compacted into one point.

Constitutive Equations of Components So, in theory, I need all the tools of circuit analysis, which I have partly already proposed to you without actually making a formal reminder. These are Kirchhoff's equations. And the *hardcore*, let's say the formal electrical engineering enthusiasts say: "Ah okay, I have a number of equations equal to the number of unknowns and so any circuit I can describe it, provided you give me Kirchhoff's equation and the constitutive equations of the components."

Anyway: * **Current Equation (KCL):** Between each junction (cocycle, node, whatever you want to call it… the important thing is that this closed surface does not intersect the part, for example, of a component like a resistor or a capacitor, it doesn't go inside, because otherwise the hypotheses of electrical engineering no longer apply, one would have to resort to electromagnetism). There are very interesting YouTube videos by Veritasium, who is an exceptional popularizer, who perhaps last year spoke precisely about phenomena of electrical propagation, of the current or the electric field moving in space. Anyway, if you are interested I'll give you the link, he is very good. So, Kirchhoff's equation, Kirchhoff's law for currents: the sum of currents is zero ($\sum I = 0$). Charge is conserved. In the end, current is charge over time ($\Delta Q/\Delta t$), Δt who cares, total ΔQ equal to zero, so it is conserved. * **Loop Equation (KVL):** Or cycles (*loops*). The potential, the algebraic sum for any loop or cycle that starts at one point and *finds*… returns to the same point, involving as many nodes as you want, ensures that the sum is zero ($\sum V = 0$), where they are oriented quantities. This is due to the conservative nature of the electric field. This is a consequence of the circulation (if you are familiar with Maxwell's equations) of the electric field. But anyway, these are reminders eventually to resonate with those of you who have this information.

The equations of the components, in the end: * The **capacitor** and the **resistor** are the only two components we see (apart from perhaps ideal current and voltage generators). * **Resistor:** $V = R \cdot I$ * **Capacitor:** $I = C\frac{dV}{dt}$

They are the only equations that we have, in fact, already chewed on. And keep in mind that they are valid like this if you take these conventions, which is the passive sign convention (if you take the voltage pointing up and the current going in the opposite direction, for reasons I told you about). So V is V up here minus V down here, but typically it means, writing it like this, V is the potential difference between these two points.

In the case of an inductor… we will never see them because they don't exist, or at least inductive phenomena are not appreciable in the case of the biophysics of excitable membranes and neurons, of excitable cells in general.

What we see is, at most, the constitutive equations of a **battery** (or ideal voltage generator) or **ideal current generator**: * **Voltage Generator:** They are very easy. If the voltage generator is V (I repeat, taken like this, V), $V = E$. Where E is assumed to be positive, but even if it's negative, it's the same. $V = E$, then it will be E that plays its part with the sign, if maybe it changes over time it doesn't matter. * **Current Generator:** $I = A$. Where in fact you have to pay a little more *tension* [attention], because for generators another convention applies, which is that of **generators** (active sign convention), in which current and voltage are aligned and go in the same direction.

If you put them in an electrical circuit where you have a voltage generator (in which this is V, in which this is I) and this is a resistor (for example the circuit of an incandescent light bulb), you understand why people used this convention in which from the generator the current has that sign. When is it positive… if it were negative, amen, it would have a minus sign. Anyway, this is a convention that you might need to know, but I repeat, I don't think you will ever use it in the case of… except probably for the description… okay, if you go on to become neuromorphic engineers, obviously, but you would have to chew on circuits *per se*.

Exercises: Series and Parallel Before taking a break. A possible exercise to derive on this basis: 1. Why two **resistors in series** have an equivalent resistance (R_{eq}) that is the sum of the resistances ($R_{eq} = R_1 + R_2$). 2. And instead, a **parallel of resistors** is equivalent to a resistance that has as its resistance a value whose inverse is the sum of the inverses ($1/R_{eq} = 1/R_1 + 1/R_2$).

If they were conductances: 1. The parallel is the sum of the individual conductances ($G_{eq} = G_1 + G_2$). 2. For conductances, the series of conductances would be an equivalent conductance whose value, whose inverse, would be the sum of the inverses ($1/G_{eq} = 1/G_1 + 1/G_2$).

It can be done exactly with the laws I told you about, that is, by putting constitutive equations at each node for the electrical quantities of voltage and current. If you want, I repeat, we can do it together later.

These are Kirchhoff's equations for this node and this node [series circuit], and this is the equation for the only loop that is present from this node to this other one and back. These are the constitutive equations. It is a system of n unknowns in n equations. At a certain point, you will be able to do it by eye, to see that since there is no wire "exiting" here… from the hydraulic point of view, it is as if you had a pipe that has a certain flow rate with liquid here, so whatever the flow of liquid that enters here must be conserved, it must continue to be here and must continue to exit. So yes, $I = I_1$, $I_1 = I_2$. But at a certain point, maybe you should see these things by eye, if you don't already see them by eye. But [I say this] simply because someone among you told me they are not fresh, not particularly comfortable with circuits due to their background. And this is Ohm's law repeated twice.

If you do the same thing here [parallel circuit], you should somehow do the same thing for this circuit on the left and for this circuit on the right. And understand when the two relationships between V and I (so between this V and this I, which is the same as here… between this and this and the large I that is here) become equal. In this case [series] you would see that the only way is that the equivalent R here is $R_1 + R_2$. Here you have to call it in a different way. While here [parallel] you would see that it is a little more annoying, but it's algebra.

Okay, this if you want, again, you can do it on your own and I am available if you have problems. It's simply putting in numbers, because many times these things remain hanging. Suppose you have this type of circuit, which could be the sodium Nernst potential, and two… in fact, in this way here it would not

be biologically realistic, I should change it and make it biologically realistic. Anyway, you have two resistors in parallel.

The first question might be: but what is the voltage across these two resistors? Here, even if one has no rudiments of Kirchhoff and does not invoke Kirchhoff, one can see it: that between this point and this point the electrical potential is that imposed by the generator. So V is known. The thing that is not known (because again, in the hydraulic parallel, in the hydraulic analogy, here whatever comes out is faced with a bifurcation, one part enters here and one part enters there)… so it depends. So there I might need to use Kirchhoff.

It might not be strictly necessary anyway if you think that the current could have a greater or lesser difficulty in going into a path with higher or lower resistance. **The current goes in the direction of lower resistance**. Just as… not bicycles, but also bicycles if they are in free fall in a valley… I'm thinking of gradient descent in machine learning. So a stream goes down, follows the direction along the potential (gravitational in that case), obviously choosing the path that has fewer restrictions. But in the hydraulic case it is not… but maybe yes, even in the hydraulic case it is.

To do this exercise you simply have to write Kirchhoff's equation at this node and the constitutive equations. You would find the current flowing in each of these resistors, or the current flowing here in this parallel of resistors.

It could be interesting because when the membrane is a parallel between the sodium conductance, the potassium conductance, and the chlorine conductance, you inject current… it could be of interest. So you don't have a voltage generator. In fact, there are experimental modalities where one can apply (it's called *voltage clamp*, but I won't talk about it) where one can apply a voltage. In that case, the current you would see is the one that comes from the path of least resistance, or highest conductance, as one intuitively expects.

If you have problems, tell me, I am available. Here was the story of the resistive divider, for those of you who have vague reminiscences of electrical engineering, where indeed, while the current follows the path of least resistance, the voltage divides proportionally with the resistance. But it's not crucial.

Simplification: Lumped Parameters Before I break, I would like to tell you then: what do I do with this equivalent circuit in which I have a lot of capacitors in parallel, with many channels interspersed as well? I will have some tens of millions of sodium channels, one next to the other… maybe they are not all close, but for me everything is isopotential in this membrane, in this potentially spherical, isotropic, and uniform cell.

So I have a lot of capacitors in parallel and a lot of resistors in parallel. Can't I just write a single capacitor that has the **equivalent capacitance (C_{eq})**? * **For capacitors**, it's easy, and for conductances, it's easy too. Because if you imagine the expression for capacitance ($C = \epsilon A/d$) which scaled linearly with the surface area (A) and inversely with the distance between the two plates (d), you might think that this case here [parallel] is as if you were putting (and in fact, you are) capacitors with parallel plates, one in electrical *conduction* [connection] with the other. So it's as if you were expanding the capacitance. And therefore, the **total**

equivalent capacitance is the sum of the capacitances ($C_{eq} = \sum C_i$), because the total surface area is the sum of the surface areas. So in the case of capacitance, it's simple and intuitive, and that's enough for you. But if you want to "split hairs," you should write $I_i = C_i \frac{dV}{dt}$ (constitutive equation) for each of these capacitors, and write, since all the currents must be conserved (their algebraic sum must be 0), you might discover that this is equivalent to writing many times $I_{tot} = I_1 + I_2 + \cdots = C_1 \frac{dV}{dt} + C_2 \frac{dV}{dt} + ...$ And you see that $\frac{dV}{dt}$, since it is shared, can be factored out. So you can also see it algebraically. * Whereas **for conductances** with a battery in series, it's a bit more annoying, but conceptually… if you have not one door, but you have (because someone breaks through for renovation work and makes many openings), it's obvious that as conductance, permeability, the possibility that you can escape from this room in case of a fire increases. It's easier; you are facilitated if you don't just have here and there (you have a bottleneck). If you have more, the current can distribute. Again, the total current is the sum of the currents; the **[equivalent] conductance is the sum of the conductances** ($G_{eq} = \sum G_i$).

One last [way], before I stop, a method I use to remember this is that the **membrane capacitance** (which you must memorize) is **1 µF/cm²** (one microFarad per square centimeter), the specific capacitance. So if I have a membrane that is small or large or very large, given that [the unit of area] is in the denominator, if I want the total, I have to take this thing and multiply it. So the larger the membrane, the more, in fact, I have many of those capacitances, the more the total capacitance will be increased. So: the larger something is, the greater the capacitance (because you have the unit of area underneath).

And the same thing happens for **conductance**. Now I'll say a random number because I don't remember it: 3 µS/cm² (microSiemens per square centimeter). The specific conductance is also per square centimeter. And again, this is easily understandable if you remember the definition of resistance as resistivity (ρ) times length (L) divided by area (A). Conductance is the opposite, so it grows linearly with the area.

So: the larger the surface area I multiply by, the larger the capacitance [and conductance].

So it depends on your tastes: if you are particularly intuitive, visual; or if you are a fan of units of measurement; or if instead (and it's legitimate, I'm halfway) you prefer to have a little machine, a mathematical method where you switch off your brain, activate the math, and get the right answer that tells you about the same system. Even better. You all have the same tools.

Try to do it: to demonstrate that the [equivalent] capacitance is the sum of the capacitances and the [equivalent] conductances are the sum of the conductances. Be careful: because I'm not saying that you sum together conductances of different types. I'm talking about the same conductances, of the individual ion channels [of the same type], summed together. And you have a single branch [per type]: "all the sodium goes there."

As one of your colleagues pointed out to me during the break, in the end, it's quite obvious that the equivalent circuit model of a membrane, which is made up of many individual ion channels, can be grouped by type (otherwise, if they

have a different reversal potential, or NERNST equilibrium potential, *and they mix*… they couldn't be put together, you can verify this in the previous exercise).

The thing I pointed out is that if, in general, even the exercise of making you think about the parallel of capacitors is useful (it may seem trivial here, even if at every interval between two channels, in theory, it's as if one had a capacitor), it becomes useful in the case where there are cells that are spatially distributed. That is to say, where the isopotentiality hypothesis condition does not hold; the potential is not the same at all points inside or at all points outside, simply because it is long, because it is extended. We will see this very precisely, because this spatially extended component allows us to understand the origin of extracellular signals. But before understanding extracellular signals, we must understand intracellular signals.

The Complete Charge Balance Equation So you see here that the capacitive properties are concentrated in a single branch of this circuit. There is the ideal current generator (I_{ext}) which is placed in parallel (the tip of the pipette continues to be here). And similar types, analogous types, related types of conductances or ion channels—selective to sodium (G_{Na}), selective to potassium (G_K), selective in general (this is a term that Hodgkin and Huxley defined as non-selective and which is called *leak* here, G_L, *leakage* is an English term for "loss")—are in fact different branches and are placed in parallel.

So, given this circuit, or vice versa, looking at that charge conservation equation we saw at the beginning of the previous hour, one wrote: $C\frac{dV}{dt} = -(I_1 + I_2 + ...)$

Whatever you want, plus maybe I'll add, I'll call the current I-capital-ext (I_{ext}), I don't know if you can read it, it says I_{ext}, which is the external current due to an experimenter, due to me injecting currents into the neurons because I don't know what to do. Or vice versa, it could be a current due to another neuron "talking," so some process which we will see (and perhaps with Zoli you are already extensively exploring) of neurotransmission, conversation… The situation between electrical signals into chemical ones, and from chemical back into electrical ones.

So you either write these equations by invoking charge conservation (the charge balance equation), or by looking at this circuit you write (as Hodgkin and Huxley did) the differential equation that describes the evolution over time of this potential across the membrane. In the end, across the capacitor. I could say across the sodium conductance, but since they are in parallel… Similar to the case of an RC circuit where it was the capacitance, the capacitor, that somehow had memory. As in this biological case, it is the capacitance that accounts for the dynamics, for the fact that quantities do not change instantaneously but have an inertia.

So whether it is a hydraulic, mechanical, chemical, electrical, or biological system, there is always an inertia. Nothing, even if you are Schumacher, works by changing instantaneously. Nothing in nature has instantaneous transitions, because everything has a certain latency, dynamics, inertia. And this is an electrical inertia, the one due to the charging and discharging of the capacitor.

So you can take that approach [charge balance], or here [circuit analysis], using the constitutive equations of the individual components. But mainly you do **Kirchhoff at this node** [intracellular], writing that the total current… this total current is zero.

$$I_{tot} = 0$$
$$I_C + I_{Na} + I_K + I_L - I_{ext} = 0$$

So 0 is the sum of all these terms: sodium, potassium, in the end this sum here, plus the external current term. Actually **minus** [I_{ext}], because it goes with the opposite sign. So here I wrote it with a plus [in the slide], but precisely because all of these [ionic currents] should be on the first term… this minus [I_{ext}] accounts for the fact that they are oriented in one direction [outward] and the external current is oriented in another [inward]. You see the external current *and it goes* from outside to inside. I assume I am injecting positive charges inside, because I would like to be there with a pipette making the neuron "fire" (that's practically what it's called), firing the neuron, by injecting a current that depolarizes it, that makes it more positive inside with respect to outside. I would like to be the one throwing positive ions in there.

The missing thing is the capacitive term (I_C), which, however, is also a branch like the others. So from the point of view of electrical engineering, I don't have to make an exception. Here it is a current, as it is physically a displacement current, but from an electrical engineering point of view, it is a current for which I have the constitutive equation, which is this one: $I_C = C\frac{dV}{dt}$.

$$C\frac{dV}{dt} + I_{Na} + I_K + I_L = I_{ext}$$

These are the terms for the individual [ionic] conductances: $I_k = G_k(V - E_k)$

And this is the Nernst term which, as you see, despite having been derived under *[unrealistic]* conditions, continues to appear in the mathematical description of what the currents or current densities are.

The charge balance equation thus takes this form:

$$C\frac{dV}{dt} + G_{Na}(V - E_{Na}) + G_K(V - E_K) + G_L(V - E_L) = I_{ext}$$

I repeat, this G_{leak} (G_L) is a term that conventionally includes generic conductances for which there is no selective conductance. In the Hodgkin and Huxley model, it has yet another slightly different meaning, but here if you want you can add branches for calcium, for chlorine, for magnesium, etc., assuming that for each there are specific conductances for which you may have measured or want to measure those quantities.

Analysis of the Model (Passive Case) So, seeing an equation like this, the first thing one should think is: "It's one of those equations… okay, there is a **minus** sign in front of the same state variable (V) that appears here in the first term under the derivative sign. So Michele Giuliano is calm, he doesn't panic. So presumably this thing here doesn't explode."

The first thing you might think is: "But these other terms, which are numerical, are they constant over time or not?" E_{Na}, E_K, E_L [leak]… you can think of it as the equilibrium or Nernst reversal potential of the chloride currents… they are numbers. Hoping that you continue to eat bananas and chocolate, presumably your ion pumps continue to always keep sodium around +56 millivolts, potassium at -80, -90, whatever it was, chlorine at -68.

What remains, obviously, are the **conductances (G)**.

If the conductances were **constant**, I could also… I could factor them out, as I have done now. And you see that I always do the same thing (now obviously I won't do it anymore), where I am in a condition… so I am in a much more general condition than before, because here I have the time-varying phenomena.

$$C\frac{dV}{dt} + V \cdot \sum_k G_k - \sum_k G_k E_k = I_{ext}$$

I could now say: "I'll study the *steady state* conditions, the resting state." How do I do that? I assume that the state variable (V) is time-invariant, is constant over time. But if it is constant over time, it means that the derivative of V with respect to time ($\frac{dV}{dt}$) is zero, because it's a constant, the derivative of a constant is zero.

$$0 + V_{\text{rest}} \cdot \sum_k G_k - \sum_k G_k E_k = I_{ext}$$

So: zero = that whole pizza. *For free or what, I don't know if.*

So, making the hypothesis that a *steady state* exists (I don't know if it exists, but I say: okay, suppose it exists), I put 0 here. I can get V_{rest} by taking this part [$\sum G_k E_k$], moving it to the first side (the minus sign goes away), and then I divide both sides by this sum of conductances [$\sum G_k$].

$$V_{\text{rest}} = \frac{\sum_k G_k E_k + I_{ext}}{\sum_k G_k}$$

I find the equation based on the conductances of *Goldman* [incorrect, GHK or Resting Potential Eq.], Hodgkin and *Axley* [Huxley], which tells me that the resting potential is the weighted sum of the reversal potentials of the individual ionic species.

Note: when you do this, keep an eye on the units. You have, in that case, in the numerator (Conductance × Millivolts), fortunately divided by Conductance (divided by the sum of conductances, which is Conductance). So Conductance and Conductance cancel out and you are left with Millivolts. So it works out.

That $V = ...$ *[formula]*… it can greatly help you to keep an eye on the dimensional analysis.

One thing you might notice from this is that it's not true that V *must* be the weighted average, unless you have turned off your current generator ($I_{ext} = 0$). If you are injecting a current, you might have an extra term. It's not strange, because in the end, an equation… this is an RC circuit. If you inject a constant current over time, at a certain point you charge or discharge the capacitor and you are left with that as the *steady state* value.

Simplification: The RC Model Anyway, what I want to do is imagine that these quantities (G_k) are **constant**, because I want to understand what the dynamics are. Does it really explode? Does it not explode? What's the story?

So they are constant. If they are constant, I can call them: * This $[\sum G_k]$ I'll call G_{tot} (or G capital). * And this term here $[\frac{\sum G_k E_k}{\sum G_k}]$… I'll call E.

This is exactly the resting potential given by the Goldman, Hodgkin, and *Axley* [Huxley] equation (conductance version, not permeability version, not Goldman version, not with Goldman fluxes). And G is the total sum.

Again, what I did was to factor out this quantity that I called G capital.

$$C\frac{dV}{dt} + G \cdot V - G \cdot \left(\frac{\sum G_k E_k}{G}\right) = I_{ext}$$

$$C\frac{dV}{dt} + G \cdot (V - E) = I_{ext}$$

Here obviously it wasn't there, I had to multiply and divide to do this factorization. Since I divided… and before I called it V_{rest} (resting potential), now I call it E (anyway it's a number, suppose -70 millivolts).

I manage to get this equation here, which is very simple, because it's the usual differential, *boring*, annoying, and wretched equation, about which everything is known.

$$C\frac{dV}{dt} = -G \cdot (V - E) + I_{ext}$$

Apart from this $E - V$… *[Rearranging]* … again, I am happy that there is the **minus** V sign. The fact that there is this term, this forcing term (*driving force*), is not particularly complex. You could make a change of variables, perhaps you've been accustomed to it. You could call this $V - E$, you could call it v lowercase. *[The professor inverts the definition in speech, but the sense is to translate the origin]* Because if you think of substituting v lowercase here… it's the same thing… *[confused steps in speech]* Mathematically it's boring, it's trivial, but it means that physically I took the axes and translated them in such a way as to make it, instead of being -70 millivolts here, I took the X-axis and pushed it here, so "0" for me is there. And I get something that is the equation of an **RC circuit**, which perhaps you have seen in various forms.

Response to a Current Step I would like for once (and again, I hope you will also try to solve it by hand) [to analyze the case] when the value of the external current (I_{ext}) is **constant**, or at least **piecewise constant**.

I am imagining a case where I have a cell, I have an electrode that I put in the "belly" of this cell with the usual silver-chloride wire, and in here I inject a current that is first zero. At a certain point, I turn on the current generator, I keep it constant for a while, and then I turn it off.

And what could this cell possibly do if it's similar to a capacitor? Explode… or it doesn't explode, I guarantee you, because thank goodness we saw that the minus sign appears, there are no errors, and the minus sign appears.

So it will respond to the current input like a **passive circuit**. There are no transistors, there is nothing. There is a damn capacitor and a damn resistor. Which means that the charge you put inside accumulates on the capacitor (in this case, it accumulates inside the membrane, you are spitting out many positive ions) and… *negative*… it can't… they accumulate inside. But there are holes, there are ion channels, and so it is clear that there will be a *skin*…

Like the sink: where the faucet of your sink is this current generator (I_{ext}, water current); the sink is the capacitance (C), the basin that holds the quantity; the potential (V) is the height of the liquid in the sink (this can even be done mathematically, it is exactly equivalent); and the *leak* (G), so this loss, this dissipative component, is the hole, because you haven't plugged it. If you plug it, you spite me because it is no longer analogous to this system. Okay, in that case, there is no analogue of the reversal potential (or resting potential, in this case).

So this situation is probably what you see: that is, okay, you turn on the faucet, the hole is not particularly large, the level at a certain point rises, stabilizes, and then rises no more. But you have to keep throwing water in. As soon as you turn off the faucet, the level starts to drop again, and then completely disappears. Here it doesn't disappear. Because of the way this term $[E]$ is written, remember that I moved the axes, so eventually the potential is decreasing exponentially, dissipating energy… whatever… it actually goes towards this resting potential which is E.

Solution of the Differential Equation (RC) Again, I'll do it here too, but it's trivial; conceptually, it's the same thing I did before. Here I can say: "Ah, what a beautiful differential equation." The first thing I do is see what the dynamic equilibrium points are, not from a biophysical point of view. And I say: "Is there a *steady state*?" If there is a *steady state*, it means that the quantities do not change over time. If V does not change over time, the derivative is constant [null]. So what happens when I put 0 here?

$$0 = -G \cdot (V_{ss} - E) + I_{ext}$$

For what value of V do I have this *steady state* (V_{ss})? 1. **Without current ($I_{ext} = 0$):** You can simply… with an algebraic equation discover that in the case of zero external current, you have this case only when $V_{ss} = E$. 2. **With**

current ($I_{ext} = I_0$): And instead, you have an extra current. Okay, V_{ss} will also depend on this current.

$$V_{ss} = E + \frac{I_0}{G}$$

It depends on this current based on Ohm's law. In fact, the membrane capacitance (C) is no longer there; it disappears because it only appears here [in the dV/dt term]. So you can have a neuron or a sink as big as you want, in the end, at *steady state*, the potential should not depend on the capacitance. I have to think, because intuitively I wouldn't say so, but here it's true. Here V depends only on I_{ext} and E [and G]. It depends on the size of the hole (G) and not on the capacitance (C). Capacitance means the size of the sink. This is the point I can't tell you. I think I'll tell you.

So, the usual differential equation.

$$C\frac{dV}{dt} = -G \cdot V + G \cdot E + I_{ext}$$

You can map things if you want, apart from this change of variables that could make your life easier. If not, simply expand this product and you have $-G \cdot V$ (which is equal to this $-A \cdot x$ [generic notation]). Note, it's wrong here, it should be f, this f is not x. So $-G \cdot V$. And then you have a constant forcing term (B), which is $G \cdot E + I_{ext}$. So it's not a homogeneous equation; it's an equation with an extra term. Not just the one from the constant current, but the extra term (unless you change the coordinates). So this term B is due both to this I_{ext} and to $G \cdot E$. But nothing wrong, nothing wrong. Okay, written here.

Capacitor Charging (Current Injection) Okay, the exercise is as follows. I look at this differential equation, and this is the current (I repeat, piecewise constant). It means that I know the analytical solution, in theory, with my eyes closed.

Phase 1: $I_{ext} = 0$ (for $t < 0$) So I simply look at it when the forcing term I_{ext} is constant ($I_{ext} = 0$). If it's constant, I expect it to have been constant for a long time. Okay, typically I should say that the current has been 0 since $-\infty$. But I could say: okay, at a certain point the solution to this differential equation will be the sum of two terms: a decreasing exponential plus a *forcing* [particular] term. And the equilibrium here is what is given when it no longer changes, the potential no longer changes, and when the external current is zero. So here I put zero ($I_{ext} = 0$) and then I say what the equilibrium potential is. I put this [dV/dt] to zero. The only [solution] is when $V = E$. So even if no one provided me with the initial condition (which I just realized I did provide), which tells me what the initialization value of the charge on the capacitor is at this point… I can in theory, knowing that this condition has persisted forever, assume that there was a transient anyway. Once the transient is over, presumably there is an equilibrium. What is this equilibrium? The one that nullifies the second member (because I set the first member to zero under the hypothesis that equilibria exist, that an equilibrium point exists).

Phase 2: $I_{ext} = I_0$ **(for** $0 \leq t < T_{pulse}$**)** As soon as I move I_{ext} from 0 to some value, which I call I_0 here (suppose 100 pA, 50 pA, whatever you want; 50 pA could be a reasonable order of magnitude for a relatively small cell), you obviously have a differential equation where there has been a step. You have to calculate the step response.

To make a long story short: you have the initial condition ($V(0) = E$). You have the solution as the sum of two terms: 1. **The homogeneous [solution]:** The solution of the associated homogeneous differential equation ($C\frac{dV}{dt} = -G \cdot V$), which is an exponential with the term in the exponent being the **minus** that makes me happy, then G/C. Because the standard, the label of that boring differential equation, requires that there is nothing here on the left [in front of dV/dt]. So you have to reduce it (mathematicians say it well) to the… formal case $\frac{df}{dx} = -\alpha \cdot f$. Okay, there was nothing here [in front of df/dx]. So this C, I must take it and divide both sides by C.

$$\frac{dV}{dt} = -\frac{G}{C}(V - E) + \frac{I_0}{C}$$

So here comes G/C. Which you know, because if it weren't G but R, it would be $1/R$. Dividing by C it would be the famous $1/RC$, the **time constant (**$\tau = C/G = RC$**)** of this equation. The homogeneous solution is: $V_h(t) = K \cdot e^{-t/\tau}$ 2. **The particular integral:** This piece here is due to the so-called particular integral. Which I solve… (as I've shown you, and if you want, watch those few videos on mathematical preliminaries)… I don't find it with the convolution integral, which would be the technique. I hope that you have already heard a little about these things; you should have taken math exams. But anyway, I don't do it with the convolution integral, but I do it with a heuristic method. I say: "But here, if this term is constant (the forcing term is $(G \cdot E + I_0)/C$), if this is a forcing term and it is constant, then the [particular] solution will also be constant." I can typically do this if the [forcing] solutions are a sinusoidal term, then the solution will also be sinusoidal. If the term has a ramp, then the output will also have a ramp. For a technical reason, mathematically (I don't know if you know, if they ever told you) it is said that a class of functions, the cisoidal functions (which are a combination of exponentials and sinusoids), are eigenfunctions of this class of differential equations. It means that if you put them in, they "spit" them out almost the same, as in the case of linear algebra, eigenvalues/eigenvectors. Okay, parenthesis closed. So if instead… so if you follow this path you could say: "Okay, V_p [particular] is equal to a constant." Which constant? I'll call it Q, or whatever you want. I substitute it, so by direct inspection, I put it in there and see what the value of Q must be. Since it is constant, the derivative of this Q is 0. So this term [dV/dt] vanishes. *[The professor returns to the equation not divided by C]…*

$$0 = -G(Q - E) + I_0$$

I have an algebraic equation where I have to write $Q =$ something.

$$Q = E + \frac{I_0}{G}$$

The C wasn't there… the C went away…

So this [total] solution is the two terms: $V(t) = V_h(t) + V_p(t) = K \cdot e^{-t/\tau} + (E + I_0/G)$.

Taking off the mathematician's hat: again, here is a term with minus and t. The exponential with the minus sign goes down, so at a certain point, it's gone. And what survives is E (which was the resting potential) plus (if I_0 is positive) some term that depends on the injected current and the conductance.

So I imagine that… if it has to arrive… so this is the [initial] value E (resting potential). I turn on the current here. I expect that I don't know what happens here, but at a certain point, after the transients have died out, I have this value here, which is $V_{ss} = E + I_0/G$. It looks like Ohm's law and it's not particularly complicated.

But, mathematically, rigorously, it can be done. As we did: from here (E) to here (V_{ss}), how will it ever do it? I certainly don't think it makes an exponential arc like this [concave], because it would have to be something that then has a discontinuity in the first derivative here, it would be strange. Presumably, it does something like this [convex], which is the famous **charging curve** of a capacitor. You don't know? Plot this equation here.

So, by identifying the value of the constant K with the initial condition ($V(0) = E \implies E = K + V_{ss} \implies K = E - V_{ss} = -I_0/G$), you formally have the expression for this exponential arc:

$$V(t) = \left(E + \frac{I_0}{G}\right) - \frac{I_0}{G} e^{-t/\tau}$$

But intuitively you would have arrived at more or less the same thing, you would have gotten there anyway.

Capacitor Discharging (End of Current) This solution holds, however, until you turn off the current, until you set it to zero (at $t = T_{pulse}$).

Phase 3: $I_{ext} = 0$ (for $t \geq T_{pulse}$) When you set it to zero, the equation transforms: this [I_{ext}] disappears.

$$C\frac{dV}{dt} = -G \cdot (V - E)$$

The only thing you inherit is the **initial condition** value for the new case, given by this solution [from Phase 2] when t is, [for example], 100 milliseconds. It will be what it will be, it will perhaps be $V(T_{pulse}) \approx E + I_0/G$.

What could this thing possibly do when I turn it off? Okay, it will go *to zero*… sorry, **it will go to** E [the resting potential].

And this type of… so the tangent to the exponential at these two points of connection and disconnection, intersects after one time constant ($\tau = C/G$) *the horizontal axes.*

So it's just to tell you: the **speed** at which this thing charges and discharges **is the same**. I'm telling you this because I will show you a system related to

synaptic transmission where this does not happen, where charging and discharging are not the same. But here, yes. Here the mathematical equation remains the same, the only thing that disappears is this term here [I_0], which at a certain point becomes non-zero (but still constant, if it's constant I know how to use the heuristic method and I can handle it), and then at a certain point it turns off and detaches.

So here it has detached. The value of *this*… I know it's 0… *[error,* $I_{ext} = 0$*]*. However, if I have to solve it, I solve it as I did before. Again, here too there is a forcing term which is the usual $G \cdot E$. Solution of the associated homogeneous equation ($K \cdot e^{-(t-T_{pulse})/\tau}$) plus the particular integral with the heuristic method (which is E). But for which I now know the initial condition, which is when $t = 100$ ms [T_{pulse}] it is this value here from before, which is probably $E + I_0/G$.

$$V(t) = E + (V(T_{pulse}) - E) \cdot e^{-(t-T_{pulse})/\tau} \quad \text{for } t \geq T_{pulse}$$

$E + I_0/G$ is reached mathematically only after an infinite time. So if this current step [T_{pulse}] is much smaller than this time C/G [τ], then it will not reach the *steady state*, the curve does not saturate.

Anyway, I suggest you do this once in your life.

Visualization with Python (Google Colab) And now I'll show you, before finishing (in 5 minutes), a way you have available to "plot" functions. In my day, there were graphing calculators; maybe you have never seen them. They were cool calculators that had a display where you could put the functions. Today you can even do it as a web page. I think in the math *refresh* module there is a site where you can put the expressions of mathematical functions and have them plotted.

What I'm showing you is a slightly more complicated approach, but it should be within your reach. We'll do this next time… to be able to get familiar, plot these functions, the results of this lesson.

The idea is to go to the course website, resources, *notebooks*, "To be or not to be…".

This, I hope you know, **Google Colab** is in the cloud. It's a kind of virtual machine that Google provides for free with limited computing resources, but all you need is a damn browser. You don't need to have, to install anything on your computer. And you can write code.

Here, for example, I'll show you what it's like. By writing **Python code**, I defined the numerical parameters necessary to plot the **Goldman equation** (that formula, I won't call it horrible, it's that slightly annoying formula that had exponentials, *capacitances*… that had the A's, the P's, etc., etc., etc.). I went to look up what the numerical parameters are, because I don't remember by heart what the Faraday constant is, I don't remember by heart what the charge of the electron is (1.6×10^{-19} C), etc., etc.

And I can compare… (which I don't see here)… I can compare… okay here, the expression. I can plot the expression of the current flux (J) as a function of the potential (V). And after a few moments, using a plotting library that you should know (or it would be beneficial for you to learn because it is the most widespread in *data science* and in any type of possible job that has to do with manipulation and… it represents one of the most important data manipulations). One is a mathematical library called **NumPy** and the other is called **Matplotlib**.

It's not connecting, obviously. But I'll trick it because I have already generated the graphs. And so you would have that for each individual ion species you can make a graph (this is because *flickery*), where I changed the potential with a `for` loop from -100 to 50-60 mV, and I plotted what the numerical value is that the Goldman equation or the ohmic approximation tell me.

Unsurprisingly, the **ohmic approximation is a straight line** (thank you) and [it is] tangent to the curve from the Goldman equation exactly at the point of… where the reversal point is… sorry, the Nernst potential, of equilibrium or reversal for that ion species. For potassium, it's -80 mV, for sodium, it's +50 mV, etc., etc.

You see that even for potassium and sodium, not moving particularly far from this gray range (which is this interval where the action potential occurs, so presumably what is worth taking into account), you see that the ohmic approximation is not all that great. It bothers me that the sodium current (which, however, I will show you… I showed it to you as ohmic) is at -50 or -70 millivolts (which is the [resting] potential), is already strongly different in the two approximations, Goldman or Ohm. For calcium, it's totally off, unless you are in a very small neighborhood.

Beyond Goldman, I am inviting you to use Python. Not to make database programs, simply to create a Python function that, given a variable (for example t), I put into Python `1 - exp(...)`, I put in the parameters, here I put t, and then I plot it and I have the graph.

So even if the knowledge or the requirement of analytical competence for the analytical solution bothers you in part, you have the possibility (and you must in this way get a minimum of practice with Python) to use Python to plot stuff. You have that example of the notebook that plots Goldman, but it's simply a mathematical function that is expressed as a Python function, and I plot it with a simple `plot` command.

I'll finish just by telling you: much of this code is my OCD, my compulsive syndrome, to make things look nice. So: "Absolutely, wait, let's put the gray," "change the axis limits so that it is zoomed appropriately," "make a part that is colored." But these are all things, these things here, that are practically irrelevant at the beginning. And today you can easily ask ChatGPT, or Claude, or Copilot, or Gemini, to help you write that piece that in the end is always the same, about which: "How do I change the line thickness?", "How do I change the color?".

I'll end here. Thank you, see you next week.

Neuronal Excitability: Ionic Bases

Alright, today I'd like to continue with the topic of neuronal excitability, and I'll probably open a parenthesis, a sort of preliminary on excitability, at the end or during the second hour. You can find both slide files separately; they are already on the site, in the repository. I'd like to start from what I mentioned last time would be intuitions worthy of a Nobel Prize. Indeed, even if I'm not certain this is what motivated or fueled the correct intuition of **Hodgkin** and **Huxley**—two Nobel laureate electrophysiologists in the '60s who, in the '50s, clarified the molecular basis, if you will, of the emergence of electrophysiological signals, particularly the action potential.

And I'd like to do this by considering an axis: this is the membrane potential axis, so I'm thinking about the electrostatic potential inside versus outside. Outside is conventionally zero for me, because you know that any other choice is possible; I choose to put the reference in the extracellular medium at zero. And I told you last time that, based on our considerations, there are specific consequences of the different ionic concentrations of the various ionic species inside and outside the membrane. For example, where is **sodium** most concentrated? Outside, thank you. **Potassium** is the opposite. **Chloride** is also highly concentrated outside, but remember that from the point of view of valence, having a negative sign, it counts as -1 in the context of the Nernst potential or reversal potential I'm thinking of.

Because, in a way, as I did at the end or middle of last week's lecture, I'm thinking that sodium, being concentrated… so again I'm thinking of the $\log(x)$ function, somewhere in Nernst, in the Nernst potential, there is the ratio of the concentration… sorry, the logarithm of the ratio of concentrations, and I always forget but I think it's *out* here and *in* here, because in fact, for sodium, I know it's positive. Do you roughly remember what value it had? It was positive because the logarithm is positive when the argument is greater than one, and the argument is greater than one when the concentration outside is greater than the concentration inside. And do you roughly remember the value? What were the units? Millivolts, so it's up here, it doesn't matter, the important thing is that it's quite high. Whereas for potassium, you'll remember, the situation is reversed, so it's definitely a negative value, and it was a value around -80 **mV**. You might also remember, for chloride, I showed in a forced, contrived way… frustration because it wasn't exactly the -70 **mV** I was looking for.

So, you have a reversal potential for potassium currents (E_K) that is very low, and a reversal potential for sodium currents (E_{Na}) that is very high, very depolarized and very hyperpolarized. And if you imagine, over time, taking an excitable cell and somehow, for example, you are the one injecting an ionic current inside the membrane, thus making the membrane more positive… because it's you, for example, with a glass pipette filled with a chloride solution and having that famous silver-chloride wire, which I remind you is what makes it possible, as a first approximation, to exchange between one world, that of metallic electrodes that have electrons as charge carriers (I'm also thinking of semiconductors, holes, but anyway, very different from the charge carriers in an electrolyte or an aqueous solution with charged ions). If you do this and give these *steps*, these current steps, you expect that unlike a passive cell, a cell that

isn't excitable, where the reaction is boring, it's the reaction of an **RC** circuit, a charge and discharge with exponential arcs. We did it together, and I hope some of you follow up on my requests when I say "try doing it as an exercise," in the end, it's Calculus 1 or at least elementary math of solving differential equations.

You know that the charging curve in this case doesn't look like that. This is a *sketch*, it's not exactly the shape of an action potential but it's very close, where okay, there's a charging curve but then there's a kind of phenomenon that isn't described by that physics, that biophysics of an RC circuit. And the thing one notices is that this behavior is more or less always contained between this maximum value around +50 **mV** (actually calcium, the Nernst reversal potential for calcium, since the concentration inside is practically zero and outside is a few millimolar, is even higher, anyway 100 mV or 50 mV here) and down here −80 **mV**.

So this *range* might suggest, and it did suggest to brilliant minds who figured it out when even from an experimental standpoint, the possibility of experimentally accessing a biological preparation like a neuron, a cortical cell from a rat, was surely very difficult if not impossible. They based their intuitions on these considerations, so on the biophysics of membranes and on the observation that at rest, unless the cells are dead, all cells are polarized, they are negatively polarized, and excitable ones can depolarize, meaning they lose their negative electrical polarization. It's all outdated jargon, if you will, because why not just say "the potential is negative, it becomes positive"? Oh well, okay, they say "the membrane is polarized," which is not trivial, it means that evidently, as we've tried to flesh out in recent weeks, there is some energy expenditure that changes the concentration, the distribution of ionic concentrations.

Excitable cells have the added fact that this electrostatic potential changes rapidly, it depolarizes, it becomes positive, it even reverses, not only is the polarization lost, on the contrary, it becomes positive, but it still doesn't exceed the values that are roughly those of the reversal potential due to sodium and calcium. And in the end, it repolarizes and even hyperpolarizes, because you see it goes lower, further down than the resting value, and even in this case, it doesn't go to an arbitrarily negative value, it touches that value.

So why the Nobel-winning intuitions? Because the endpoint of all the previous part that I titled "neuro-electronics" led us to formulate a relatively simple equation because it's formulated in the Ohmic case, although we know how to write it in the more general case where it's always an approximation (I'm thinking of the Goldman equation and the use made of that Goldman equation to write the ionic current terms when we don't want the simplification, the simplicity of the Ohmic approximation style). But when we settle for the Ohmic approximation, we can, in fact, write that the resting potential (now maybe I'll relax, I'll remove the word "resting," but anyway, under certain conditions, if there's a *steady state*, if it's at rest, not at equilibrium, because I wouldn't wish thermodynamic death on anyone) can be considered the weighted average of the Nernst resting or reversal potentials of the individual ionic species, so weighted by the membrane conductances for each species, divided by the total sum of the conductances.

$$V_{rest} = \frac{G_{Na} \cdot E_{Na} + G_K \cdot E_K + G_{Leak} \cdot E_{Leak}}{G_{Na} + G_K + G_{Leak}}$$

I urge you again to check the units, maybe I don't need to tell you, you are already very experienced. If I expect this to be millivolts, because here I measure millivolts, I have an oscilloscope, I have a multimeter that shows millivolts, the electrostatic potential… here I check that obviously $G \cdot E_{Na}$ must be… it's not millivolts, it's a current (millisiemens times millivolts) it must be divided by millisiemens, so it makes sense to me that there's an expression like this, because at least dimensionally it works, beyond the fact that we derived it together.

Staring intently at that expression and remembering the idea of a **weighted average**, perhaps you might have developed the intuition of the center of mass: if I somehow take most of my mass, I move it, the position of this center of mass moves. In this case, if I… it's the only thing I can do… given that I'm not changing the ionic concentrations (I'm certainly not changing them as rapidly as in a millisecond, I can't move the sodium that's outside, put it inside, and vice versa), so the thing that presumably changes, as intuited at the beginning of the last century, is that the **conductances** can vary, the ionic permeability of the membrane can vary.

So you might see that depending on the numerical value of these G_{Na}, G_K, G_{Leak}, the potential, for example, the resting potential, can lean more towards E_K and E_{Leak} (-80 or -65, whatever) rather than being $+50$. If calcium were also there, it would be even more evident. So, looking at this equation, you might intuit that if things start to move very, very quickly, with traces shooting up and down 100 mV in a fraction of a millisecond, clearly something is changing with these coefficients: G_{Na}, G_K, and G_{Leak}.

So what I'm doing is, again, invoking *steady state*, even though in general you'd tell me "but it's not *steady state* because the cell is firing, it's emitting action potentials." But if you'll allow me, this is still functionally useful for me to understand what can change.

So this was what we called the **Thevenin equivalent** or anyway the way in which, by hypothesizing that the membrane was passive, I could for simplicity write a single conductance term and a single resting potential term, by studying the limit, the equilibrium, the *steady state*. So assuming that, for example, as T tends to infinity, without having current terms (or by putting in current terms but stationary ones), at a certain point the potential tends to have a fixed value. And this fixed value depends on the combination of these Nernst potentials and these values, G_{Na}, G_K, and G_{Leak}.

If you love math, or if instead you love it more if you're visual, you could compare that case where what I did was set G_{Na} and G_K to zero. If I do that, it's as if… zero conductance means no more current passes, it means infinite resistance. And the circuit equivalent of a resistor with infinite resistance, or a resistor with zero conductance, is an **open circuit**, an open. A thing that has two terminals, one here and one there, and there's nothing there, it doesn't even have a capacitor, they have nothing, the current doesn't pass. An open circuit. But from this expression, mathematically it's easier, because G_{Leak}

and G_{Leak} cancel out, since in the denominator G_{Na} and G_K also went to zero. And so it's obvious that everything is shifted to E_{Leak}, which I remind you is simply an easy way to group all the ionic conductances that are not selective, not particularly selective, they are partly sodium, partly potassium, so with a mixed permeability. And you can also think of it as just the conductance due to chloride, it's not that crucial.

Suffice it to know that this quantity is on the order of -65 **mV**, -70 mV, it doesn't matter. Graphically, I've torn out those resistors, and if I look at this circuit, even if I understand nothing about electrical engineering, I can think that from here to here, I can theoretically disconnect the capacitor. Because in a regime like the one I'm studying, in the so-called **DC regime** (a regime where quantities no longer vary in time; DC traditionally stands for *Direct Current*, but it has come to mean a state where signals in an electrical circuit no longer change in time), as opposed to AC (AC regime, which again would mean *Alternating Current*, but alternating because it's necessarily *time-variant*), while DC means stationary, it means constant.

And this is a constant case. You know that the capacitor equation, the constitutive equation of the capacitor component, which ultimately survives here, $C\frac{dV}{dt}$... becomes $I = 0$ because when V is constant (this is why they say the capacitor "blocks" direct current, because when quantities become constant, the derivative is zero), $I = 0$ remains. And $I = 0$ is the constitutive equation of an open circuit, which means "no current flows."

So go ahead and use Kirchhoff's laws if you want, the current doesn't flow there anyway, it's a branch that doesn't exist. So that's why I've put this capacitor in gray or made it evanescent. What remains is that between here and here there is a potential drop that is the membrane potential under these conditions, when the sodium and potassium conductances are zero. So it's as if those (they are ion channels) are squeezed, are closed, they are closed doors. Sodium and potassium ions no longer pass, the only ones that pass, pass through that G_{Leak}. And it doesn't matter what G_{Leak}'s value is, if G_{Leak} predominates over the others, E_{Leak} wins and the potential is equal to E_{Leak}. Do you want to see it from a circuit point of view? Between here and here, the voltage drop is theoretically linked to the sum (Kirchhoff's loop law), it's linked to the sum of this ideal voltage generator and the drop across this resistor. But the current here is zero (you can apply Kirchhoff assuming you're not injecting current, this current is zero), so there is no voltage drop here. Therefore, between here and here is the same voltage drop as across that voltage generator. Two ways (if you prefer algebra, not even math, algebra, or if you are visual people for whom you've disconnected branches and this remains, this wins) you can probably understand where I'm heading.

So in this context, if I leave things as they are, the membrane potential across that capacitor stays around E_{Leak}. If you now suppose that I have G_{Leak} (so I leave this branch as it is, I don't touch it, I assume it's passive), while the branch (so this one) that represents the ionic permeability for channels selective to sodium ions, I now say is no longer zero. I'm thinking of opening these channels, I'm not telling you how much I'm opening them, it's not that important.

Again, mathematically, you see that here it's the weighted average between these two quantities, +50, +60, I don't remember what it was, +56 and something (it depends on the species, naturally), but anyway, it's on the order of 50 mV, and this value here, −70 mV. So it's clear that the potential is intermediate. And the more this weighted average is skewed towards this value E_{Na}, the more the membrane potential will tend to approach E_{Na}. In fact, what happens if I leave things like this and allow the circuit, or this equation, to reach (this equation here) the new *steady state*, with this amazing animation, it's as if I expect that with its dynamics (dynamics due to the time constants involved, whatever $\tau = RC$, time constant… obviously RC, okay, yes, the capacitance is fixed, the conductance here would become… the time constant would be C/G, G is obviously changing in time), so the way this system relaxed to E_{Leak} a moment ago might be different from the way, the temporal dynamics with which, the speed with which it now goes towards that other, this new resting potential. So it's clear it goes towards… it doesn't reach it because this G_{Leak} is not 0. If it were 0, then yes, this term would no longer be there, it wouldn't be in the denominator, G_{Na} would cancel out here. In reality, it's a bit below, it depends on how large G_{Na} is. Nevertheless, it goes up.

Now I'll show you what happens, the other case: if I turn off G_{Na} and turn on G_K. So I've ripped out this branch, set the resistance to infinity or the conductance to zero. The same thing happens here, now I have the weighted average between −80 and −70 mV, okay, it will go towards −80 mV. Again, it depends on the numerical values. Again, with this fantastic animation, the trace goes down, with its own dynamics. Again, dynamics due practically to an RC circuit. I repeat, the time constants obviously depend on the capacitance, but these conductances change in time, so it may be that the time constant is not strictly fixed. In other words, the speed with which I can go to some *steady state* (it's not guaranteed this *steady state* exists, but if it did exist under the conditions I told you, i.e., I rip out an element and then wait even an infinite time, I have patience) could be different, it could be much faster to go up or to go down, or vice versa.

For now, I'll do the following: I'll remove G_K again, put in G_{Leak}, and from this initial condition, the system evolves again. You see that simply with a stupid observation on the concept of a weighted average, imagining that these coefficients, these weights, have a biological meaning (they are biophysically the permeabilities, but they must have to do with some mechanism that allows ions to cross the membrane). Cross the membrane passively, in the sense that it's not against the electrochemical gradient. In other words, since there's a lot of sodium outside, if I open the door, some sodium will enter by pure diffusion. This is what I call displacement current, but it's passive, it's not against, it's not like if I have so many sodium ions outside that I have to spend energy to push more out against what would be the spontaneous gradient.

And in this way, somehow, I've intuited that if the shape of the action potential is that it starts from here, goes up, slows down, stops, goes down, but goes much further down than the value it was at before, and then stops, slows down, stops, and then comes back up, it means that somehow there must be some sequence of permeability changes. And this… this permeability change cannot happen at the same time. I can't have the sodium and potassium channels (the sodium

channels and potassium channels) opening instantaneously, synchronously, because otherwise, I wouldn't have this shape. The typical duration of an action potential is on the order of a millisecond, one or two milliseconds, it depends on the species. Cardiac action potentials, I think you'll see them if you haven't already, are much longer. Ditto for the action potentials of pancreatic beta cells, which you'll never see, but I'm telling you because I've studied them and they are wider, longer.

I'll tell you, I'll give you a couple of ideas why: they involve some sequence of permeability changes and obviously these permeability changes must be rapid. If the membrane potential at the beginning, in this phase that is often called the ***upstroke*** (the rising phase of the "stroke," it's ugly in [Italian], but in the literature, you read *upstroke*), if this happens in a fraction of a millisecond, it means these sodium channels must be able to open their gates very quickly.

Now, Hodgkin and Huxley, I told you, had no idea that these ionic conductances, these membrane permeability properties, were concentrated in space, were discrete, and were concentrated. I've already told you that they are proteins, I'll show you some animations, I discovered a guy on the internet who uses 3D modeling software typical of video games (I put it on Teams, for those who use Teams to read announcements, you will have seen the video). They are physically pores made of proteins that change their three-dimensional conformation, even very rapidly. It's not trivial that this change in conformation and consequent formation of a pore to let ions pass, as if it were the opening of a door to let ions pass… it's not trivial that this happens so quickly.

And you might think that some cells don't have this. Although they have potassium ions, they might not have these membrane permeability properties for potassium ions that are so rapid (a bit delayed, actually, delayed in the sense that it happens with a certain delay), it might simply not happen. The recovery from this point, which is called the ***overshoot*** (I don't know how to translate it, *overshoot* because it's like an extra elongation, a shot beyond the limit, *overshoot* because it not only reaches zero but exceeds zero. It's like in control theory, when there's an excess in the control of a variable, for example, of position: I'd like it to reach here, it exceeds, and then possibly comes back). In some cells, it might be that the return to the resting potential only involves these conductances that are always there, I never turned them off.

So the only thing that changes is that here the sodium, or the permeability that plays the role of sodium… you see I'm talking about sodium which is at +50 mV, but if you had, for example, calcium which is at +120 mV, from your point of view today it wouldn't change much, because you still don't know that calcium channels and sodium channels exist and that from the point of view of kinetics, some are faster than others. So, in effect, the cardiac potential of cardiomyocytes is wider because there are calcium channels, it's not sodium, calcium is the main actor, which open and close. Here it seems that, from how I'm telling it, which is the case for nerve cells, you have a system where not only do I have a mechanism to make the potential shoot up, but I have another mechanism to make it return very rapidly down. I could do without it, but then I'd be like the cells of the heart, where there's no consciousness, thought, emotions, etc., where this happens simply due to the passive RC properties, whereby from here, the potential slowly tends towards the resting potential.

Hodgkin and Huxley went further and tried to explain mechanistically how this kinetic of what they called ***gating***, from the English word "gate," could occur, whereby it's the opening and closing of a door. Although in '52 they somehow even provided experimental evidence that **multiple ionic species** were involved, multiple changes in permeability, it wasn't a generic change in permeability as proposed some 50 years earlier, or experimentally verified about 20 years earlier. They said, "no, no, it's different ions, and the membrane is specifically permeable in different ways, and from moment to moment, the permeability is not the same for all ions."

However, they couldn't measure the fact that these ion channels were doors, pores; they thought they were electrically charged structures (I'll tell you in a moment why they had this idea that they were charged particles), interspersed in the phospholipid bilayer and somehow more akin to a **transporter**. Something that grabs an ion from one side, obviously to pass it through the membrane it must be, as I told you, strongly hydrophobic and therefore in the lipophilic layer inside the membrane… to carry an ion from inside through this layer and out, energy must obviously be spent.

They thought it was a transporter and they thought, for a reason that will become clear, that this transporter had an electrostatic charge component, so much so that when the potential, for its own reasons, depolarizes or hyperpolarizes, these charged particles (since they are sitting in the middle of the membrane *sandwich* and the membrane potential is felt across the membrane) would feel an electric field and therefore a force pushing them to one side or the other of the membrane.

To make a long story short, they got very close, but they obviously didn't have the experimental tools to be able to say "no, look, it's not a diffuse property, there are no transporters, they are pores and they are discrete" and, as we'll see later, they are also so small that they are **stochastic**, they are noisy, they function randomly. It's not a door this big, it has nothing to do with quantum mechanics, but they are so small that they are affected by the conditions of a thermal bath at physiological temperature, 37 degrees, where there is thermal agitation.

What they did, in fact, was to propose an **equivalent circuit model**, and I repeat, this has always amazed me, they weren't engineers, they were physiologists, and not using digital computers, but using mechanical calculators, they were able to solve the (four) differential equations they proposed. You already know one of them, which is the charge balance equation. And they compared what was their experimental recording of an action potential which you see, yes, 0 is their reference, again it's just a reference, they translated the axes and set them to zero, but here it starts from -70 mV, goes up, depolarizes, and then hyperpolarizes and then takes a certain amount of time. This and this graph here are on two different time scales, you see one is between 0 and 2 milliseconds, whereas here it's *zoomed out*, it's between 0 and 10 milliseconds. So you see it goes up and down quickly, you understand why they called it a ***spike***, because it's very sharp.

And this is the mathematical model. And if I hadn't told you which was which, you might have said "boh, yeah, the one below, I mean, yeah, it does the same

thing." It's not perfectly identical, but it's pretty good for the 1950s… and this type of mathematical formalism and this type of mathematical description of permeability properties, we continue to use it to this day, 70 years later.

What I told you before corresponds to the phenomenological reality of how these sodium and potassium channels work. Today we know that sodium and potassium function in a **voltage-dependent** manner. The channels themselves have, within them, a portion of their three-dimensional conformation's domain that has a net electrical charge. Everything is electronic, I agree, but you know that the charge distribution in an amino acid sequence, in a protein, depends on the conformation, depends on whether or not it's neutralized by other molecules. These have a sensitivity to the transmembrane potential, to the electrostatic potential across the membrane.

And both, this shouldn't confuse you, **both open** when the membrane potential becomes more positive, when it depolarizes. Now I'll show you this sort of cartoon, this animation. I remind you that there's a lot of sodium outside and little inside, and there's a lot of potassium inside and little outside. So if I have these doors that are selective, for whatever reason… as soon as they open, the flow of transport current occurs according to the electrochemical gradient. So a lot of potassium exits and a lot of sodium enters for free. It's not because there's a difference in how these two activate or pass from a closed state to an open state. As soon as I switch to an open state, the permeability is non-zero, the conductance is non-zero, it's no longer zero, and so the ions that need to pass, pass. I believe the sodium ions are the green ones, they will pass from outside to inside, and the orange ones which are yellow, whatever, go from inside to outside.

Sodium is an exception, I ask you (I'll play it several times) to keep an eye on this sort of **additional gate**, it's like a door that has a double… and only the sodium channel has it, not the potassium channel. Keep an eye on this object too, while the action potential is somehow highlighted in real-time based on the redistribution of this charge across the membrane. I'm not saying the equilibria change, these always remain the same, but if I open it for a moment, a lot of sodium enters, but it's not necessarily that I've caused the balance of sodium inside and outside to be altered and become… it continues to be maintained. The quantity, let's say, the imbalance is such that if you open it for a while (I'll show you an exercise first), it's not that you dramatically mess up the ionic fluxes. So you eat bananas and chocolate to maintain those ionic concentration gradients which, if you left the door open for a long time, for several tens of minutes (you'll see), then the concentrations would start to change a little bit.

This is the animation. For some reason (which could be the experimenter injecting positive ions) the potential starts to become more positive, and the sodium channels have opened, and after a bit, the potassium ones have opened. I'll play it again because it's a bit fast.

So the potential, for other reasons, starts to depolarize. Sodium opens, then that hatch unfortunately closes, the potassium channels open. A third time: they open, they **inactivate**, and after a bit, because it's slower to react, the potassium opens. They follow that sequence I described as necessary to explain this rise, fall, and rise again.

This additional hatch wouldn't be necessary. This hatch, which is called… it's a *gate*, an **inactivation** gate, in fact, it activates with a certain delay after the channel is open. Even though (I'll play it again) the channel is open, this hatch closes at a certain point. So, like with potassium, it acts with a certain delay, not everything is instantaneous, rather it depends on the chemical kinetics. Now the channel continues to be open, but the hatch is closed. This causes the potential here at the apex, at the *overshoot*, to stop.

If this additional *gate* wasn't there, when the potassium channels open, they would find further conductance from sodium, because sodium, I told you, opens just like potassium. It starts to open when the potential is depolarized, is a little more positive than it was. So if I open and potassium opens, okay, let's see who's better, whereas I told you that what I need is *either* sodium *or* potassium. If they are both activated together, things don't work, in the sense that the potential would have some intermediate *steady state* value (assuming a *steady state* could be reached). Instead, I want it to go down, I really want to remove this depolarization. Ideally, I need this because I'd like to restore the system to a starting condition.

So evolution has not only created an object like the sodium channel, which is among the fastest biological objects in the world (it manages to activate in a fraction of a millisecond, tens of microseconds or fractions of a millisecond it activates), but it also has a mechanism to close it. While potassium, which is more delayed, is more *delayed*, because it inherently has a kinetic, it's clumsier, has a different mass, I don't care, I'll treat it phenomenologically, it has different kinetics.

This is, if it's of interest to you, a kind of small table showing the characteristic time of a lot of biological phenomena, expressed on a logarithmic scale, so they are like orders of magnitude, and you see that the activation of a sodium channel is among the fastest reactions there are, compared with the reaction of an enzyme, with the transit time of an ion passing through a channel. And it's what I often say here in this region: it makes sense, it's like the Ferrari (I'll say it a couple of times), like the Ferrari of ion channels, because not only is it an interesting channel, it's something that activates with the electrical potential.

Many, and it doesn't take a Nobel Prize, recognize that the fact that proteins exist that sense the electric field is the turning point of evolution for using an **electric code**, electromagnetism phenomena in information processing. This makes sense, I'm not sure if you can already grasp it, it's very complicated to study, but it's the key to electrophysiological phenomena. You have pores whose opening and closing depend on the electric field, but once they open and let ions pass, their opening or closing changes the electric field.

So the fact that nerve cells speak the language of electricity is because there are ion channels that open and close according to the value of the electricity. But this opening changes the electrical potential, and if the electrical potential changes, the permeability changes, but if the permeability changes, the electric field changes. So from an experimental point of view, it's rather complicated to be able to decouple the two things. I'd like to be able to understand when the door opens, because I see it opening and closing, as you saw in that cartoon of sodium conductance and potassium conductance. I see them opening and

closing, but who controls whom? It's not trivial to understand, especially how.

An Exercise: Metabolic Impact of a Single Action Potential Before the break, I wanted to return to that point where… so you can think of this as an exercise, but we can do it together… where: why do you eat chocolate and bananas? It's because you want to maintain the reversal potentials. You have ion pumps for that, obviously, but you might think that, okay, by opening the channels, opening the doors, I dramatically mess up the concentrations. So I have an extreme need for the ion pumps to always be working. The ion pumps are there, they work 24/7, and you need energy (ATP) to run them. But the single action potential isn't that dramatic in metabolic terms. Let me show you.

What I'm doing is, I'm imagining taking a small piece of a neuron's morphology. Here, for example, you experimentally see a **pyramidal neuron**. Pyramidal neurons are excitatory neurons, I've told you about them in the first lecture, in the cortex and also in the hippocampus. This cable you see is called a dendrite, dendritic tree, and here you can glimpse the axon. They have different colors because this, these cones you see, it's a microscope image (transmission microscope… actually it's a fluorescence image, so I'll tell you what it is in a moment). Inside these pipettes (here you see the horizontal section viewed from above in the microscope), I've already shown you a couple of images of a pipette exploring and poking or penetrating the soma of a neuron previously. So you have another one that, for those who are very skilled and gifted experimentally, manages to penetrate, to poke a portion of the cell membrane that is very small, a cable, which is empty inside (in the sense that there's a space so if I put an electrode I can measure inside versus outside), but it's very small and it's also complicated in the plane, in this case it's a brain slice, with a tip one micrometer in diameter to manage to hit it and not go above or below.

I don't know how many of you do needle and thread, or have ever tried in your life to put the thread through the eye of a needle: it's not trivial. In fact, few researchers manage to make these recordings, which are called *somato-dendritic* recordings, somatodendritic recordings. They have different colors because inside each pipette there was a different dye, and this is a fluorescent dye that when excited at a particular wavelength fluoresces blue and green.

So, I'm considering a piece of dendrite. I'm showing you that in the case of a whole neuron's morphology, rest assured that a single action potential doesn't dramatically change things. And so I'm thinking of a small cylinder with a **length of** $50\ \ \mathbf{m}$ and a **diameter of** $10\ \ \mathbf{m}$. By the way, it's quite chubby, quite large. It could be in the, let's say, proximal parts (because it's in proximity to the soma) of this apical dendrite. So a dendrite that extends at a distance from the soma, but judging by the value of 10 m, it's quite large.

So I have the volume, and you know that if I have the volume and I talk about concentrations, I instinctively want to multiply the damn concentration by the volume, because at least I'm thinking in absolute terms. It's up to you whether you prefer to count things in moles or count things in number of particles. The extra information here is that I know the membrane potential changes, there's a ΔV, and obviously ΔV makes me think of ΔQ, so a charge. Knowing what the elementary charge is, if you tell me what the ΔQ is… Since the membrane

is a capacitor, being a capacitor, if I know the ΔV I can perhaps infer the ΔQ. And if I know the elementary charge (1.6×10^{-19} Coulomb) I can work back to how many ions were exchanged in this process.

Anyway, things in order. This is the lateral surface area of this cylinder, $2\pi r \cdot L$. I've called $2r$ the diameter because I specified the diameter, and so it's this quantity here, $\pi \cdot 500 \times 10^{-8}$ cm^2. Why did I do it in square centimeters? Because the membrane capacitance that I told you, the specific membrane capacitance that I told you to memorize, is on the order of what? First of all, what are its units? A capacitance. Farads. And the only thing I ask you to remember is that… it's not one Farad because that would be a supercapacitor, it's one microfarad, but that's not all. Since it's a specific capacitance, it has this value when it's referred to the unit of surface area expressed in square centimeters. **One microfarad per square centimeter** (1 F/cm^2), you can remember that. That's why I converted micrometers to square centimeters. I know it can be boring, but once you understand or remember the trick from high school or maybe elementary school, you do the conversions, you're fine.

The capacitance (C) so I've expressed it in… so I've multiplied by the square centimeters and this is the total capacitance, in microfarads. $\Delta Q / \Delta V = C$, so I now know how to express ΔQ if the ΔV is 100 mV (from -70 to $+30$, about 100 mV, $+50$ mV whatever). And so I can calculate ΔQ and I get a quantity that is in Coulombs. I invite you to do it once, but the conceptual process is… okay, pardon, here it wasn't the volume, it's also the volume now, but the surface area is necessary because this is the capacitance referred to the unit of surface area. If it's a capacitance, it means it works, the law that holds for capacitors is: knowing what the change in potential is over time, I know what the change in charge is over time.

This is the change in charge, ΔQ. Is it a lot or a little? I don't know, I have to divide by 1.6×10^{-19} to get the number of ions exchanged, the number of particles. Or if you're a fan of moles, you just need to divide by the **Faraday constant** (F) which is the charge of one mole, simply a matter of units. If you divide by F you get it in moles, if you divide by E (by the elementary charge of the electron, 1.6×10^{-19}), you get it in particles.

So it would seem like 10 million, in reality, it's a negligible number, if you remember the example we did perhaps two lectures ago, where I showed you what typical millimolar concentrations corresponded to. So if you think about sodium, potassium… even sodium, of which there's a lot outside and less inside, is still… calcium would be a bit different, and in fact, there are reasons why even a single action potential matters. In this case, it's one nanomole, so okay, you can think that if now I can express not only the lateral surface area but the entire volume, and I think that inside there was 1 millimolar before… Here I've converted it for you into the number of ions. One millimolar, sorry, 1 millimolar means that in here there are on the order of 10^{17} ions, that is, it's 11 orders of magnitude more.

You don't deplete it, this tiny peripheral piece of a neuron's morphology, by firing just once. To empty that tiny little piece that has a 1 millimolar concentration of sodium, to empty it you would need 240 of these action potentials. That's a lot, typically, particularly cells in the cortex fire once or twice a second,

so you can think that this is on the order of minutes, and because the equivalent of ions is enormous. And there was another note I remembered, which is that if you consider a concentration of 50 millimolar, just to fix the idea, it means you have to fire for 6 minutes, generate action potentials at 30 **spikes per second**, which is something even I can't do. 30 spikes per second, and it is, despite not being a very high frequency, but in the central nervous system, it's very high and typically not sustained for 6 minutes continuously. Otherwise, you have an epileptic seizure, let's talk about that. And in fact, during an epileptic seizure, there is a change in ionic concentrations.

Digression: Kinetic Schemes and the Law of Mass Action

Let's restart with a digression. For the moment, I haven't actually told you in detail how these ion channels change, how they open and how they close. I've simply told you that phenomenologically they depend on the transmembrane potential, and I need to make a digression on what are called **kinetic schemes** or **first-order kinetics** or **Markovian schemes**. I don't know if you've ever heard these things, you've heard them in the context of chemical reactions, where you probably just swallowed them as a kind of formalism that, although it's not… doesn't contain the quantum mechanics that today we know is necessary to understand why chemical reactions occur. Simply, if I put some of those symbols that you know, with pluses and arrows, you know, "okay, this works for me because it predicts what I phenomenologically get, for example, in a beaker."

But it's not that the molecules add up $Na + Cl \rightleftharpoons NaCl$. This is a formalism, it's just that you've always accepted it as something qualitative. I'm making it quantitative, so it can be another potential nightmare. I hope not.

Everything that's at the basis of this description, which is phenomenological (I'm not telling you how the molecules combine, the various covalent bonds, the van der Waals interactions, no, I'm literally throwing all that out the window) and what I'm telling you is, at most, *how quickly* these reactions happen. $Na+Cl \rightleftharpoons NaCl$ simply means that for some reason, when I put these two compounds, two objects, these two states, these two conformations (obviously it's more correct to say objects), I put them together, there's a kind of transformation that I'm not describing, because it's not described here, the state changes and, at most, I tell you how quickly this happens. Often, perhaps some of you remember that coefficients, *rates*, kinetic constants were put in.

This formalism is due, it's the instance… the horrible instantiation… the consequence of a principle that I won't derive for you from first principles, it comes from equilibrium thermodynamics and is due to two Norwegian researchers from the… 1800s, and it's called the **Law of Mass Action**, which you've surely heard of. I'll repeat it for you in quantitative terms and in more abstract terms, where I'm not necessarily interested in chemistry, I'm interested in describing a phenomenon where I don't go into detail, but I want a way to describe how quickly this phenomenon happens.

And so, again, there are objects $A + B$. They can be chemical species, they

can be states. If it rains and I have the flu, I don't know, I become radioactive. There's no possibility of going back, and with a particular speed, a rate of change… and at the end of this, it's just a few slides, at the end of this, I'll tell you about it again in stochastic terms, in microscopic terms and not mesoscopic as we're talking at the moment.

This type of formalism literally means only that when there's an interaction between these two objects, the rate at which C appears is **proportional** to how much A there is and how much B there is. So if I write it like this ($A + B \rightarrow C$), I'm ignoring the mechanism, but I'm saying that C, the rate at which C is produced, appears, is converted (whatever, depending on the context), depends on the quantity of A and the quantity of B. It kind of makes sense because if I don't have A or I don't have B, C won't change, it won't be produced (or it won't disappear in the case of an arrow in the other direction).

And mind you, I'm not talking about absolute quantities, I'm talking about the **rate of change**. Okay, I made the unfortunate choice of calling it C, but it doesn't matter, A, B, C has nothing to do with membrane capacitance. The rate of change means I'm describing how quickly the quantities change over time. All of physics, biophysics is made of relationships… take Newton, take the charge balance equation… everything is easier when expressed in terms of physical laws that have to do with the change over time. It's not easy for me to establish a physical law to say "C equals…", "C as a function of time equals…". In this case, we do it, but the physical law tells me how the rate of production changes.

So written in other terms, the rate of production of C is linked to the concentration, the number, the moles, it doesn't matter. You know they are all equivalent concepts. Talking about concentration or density or talking about the total number of objects is the same thing, it means I'm as if I could multiply both sides by the volume. If I have reactions where these are concentrations, they are in millimoles, okay, I multiply all quantities by the volume of the beaker, I talk about the number of objects. And I'm saying again that the way in which, for example, $NaCl$ appears depends on the product of the concentrations. And the whole point is the rate of production, it's not "C, what is it now?". This is perhaps more intuitive than you think, but I wanted to stress it a lot.

$$\frac{d[C]}{dt} = k \cdot [A] \cdot [B]$$

So another consequence of this is that if this was $k \cdot [A] \cdot [B]$, it means that the rate at which C is produced is not dependent on the past history, it depends only on the current state, the state of A and B, how much concentration of A there is and how much concentration of B there is. Clearly, if you make it "reversible" it will also depend on C, but it doesn't depend on the past history. And I don't know how many of you know or are familiar with **Markovian models** in general. Maybe in *machine learning* you've seen something. In probability theory, there's a way to model phenomena that's called with a property that is Markovian, and it means "there is no dependence on the previous history."

Examples of Kinetic Schemes Here are some examples I'll give you. You'll see it's a little game where we write… you'll see I pull out some differential equations (I just pulled one out as a differential equation) and you'll see it's very easy. Here, for example, there's A which is converted into B, reversibly, k_1 and k_2 for the moment make sense, they indicate how quickly those reactions occur.

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$$

And to write this differential equation quantitatively, I'm in fact invoking the law of mass action. In fact, let's say, I look at each of these nodes (it's like a graph, it is a graph, in this case, there are only two nodes, there are transitions between nodes; Markovian models are exactly a graph with transitions). Here, for each of these nodes, I write a differential equation, which tells me how that species… ionic… [transcriber error, means "chemical species" or "state"] changes.

So here I have A, here I have B, I'll write two, $\frac{dA}{dt}$ and then another equation, $\frac{dB}{dt}$. When I set out to look at this A, I imagine it as if you had a kind of container of liquid, which has a kind of leak, a pipe, a drain, which is this little arrow going from A, away from A. It's a drain for me, this, of which k_1 is like the diameter. I'm reasoning in a light-hearted way, but not really. In the hydraulic case, there too the cross-section of passage (and in the end, we've partly discussed it) of the outflow flux would give a change in the speed of disappearance in this case. Creation or disappearance, production or disappearance or consumption, is simply a convention to say if the derivative is positive or negative, if it increases or decreases.

So I have drains and I have faucets. So when I think about A, I think exactly about the **balance**, the conservation of mass. A changes over time because there's this drain and because there's this faucet. This drain changes all the more rapidly the more A there is. This is the law of mass action. $\frac{dA}{dt}$ disappears as quickly as (you see, **minus**, because this is outgoing, here I put a minus) the coefficient I have and this $k_1 \cdot A$.

And it makes sense because if I don't have A, it can't disappear. In other words, if I had an equation where I write $\frac{dA}{dt} = -k_1$… here you don't make me too happy, let's say I'm not unhappy because… there are… there are no exponentials here. Do you know how to solve this differential equation? How do you solve the differential equation? You integrate… [confused text] So this thing here is a straight line, the solution is a straight line with a negative slope. If you don't know the math, review it, but you see that here it means that the speed at which A appears or disappears is constant. So it means that if I wait one minute, two minutes, it continues to decrease, it even becomes negative. In the case of chemical reactions or quantitative descriptions of objects that in the end are either in one state or another (but we're talking about the number of objects), it cannot become negative.

So the law of mass action solves this problem for you. It's natural that if I have, for example, a chemical reaction in which one species transforms into another but it's not reversible, at a certain point there's no more of it, it becomes zero,

it doesn't become negative. So that's why this law doesn't hold, it aligns with what is observed experimentally. So $-k_1 \cdot A$ (which was the level of the liquid in this possible container I'm imagining) and then there's the faucet k_2 which counts as a **plus**, because you see the arrow is incoming. Clearly, it must be proportional to B and the constant of proportionality is k_2. Here too it's as if I had two fluxes, each of which depends on how much stuff there is in this container, what the height is, how much mass there is in each of these containers.

$$\frac{dA}{dt} = -k_1 \cdot A + k_2 \cdot B$$

There's another differential equation to write ($\frac{dB}{dt}$) and it's easy to show that one is enough because these differential equations… you need $N-1$ of them where N is the number of states, because the Nth one is linearly dependent. In fact, I'll show you that, apart from the symmetry (you see that here $k_2 \cdot B$ appeared with a plus sign, here it appears with a minus sign; here $k_1 \cdot A$ with a plus, here with a minus).

$$\frac{dB}{dt} = +k_1 \cdot A - k_2 \cdot B$$

I'm tempted to add these two equations. Adding two equations means adding the terms on the left-hand side and the terms on the right-hand side. And if these two equations are true, their sum will also be true. If I do that, I discover that the sum of the derivatives, which I can, for example, rewrite as the derivative of the sum (thanks, invoking the linearity of the derivative operator), is equal to zero. Because this object here cancels with this one, and this one cancels with this one. I hope I don't have to do the little calculation.

$$\frac{dA}{dt} + \frac{dB}{dt} = \frac{d(A+B)}{dt} = (-k_1 \cdot A + k_2 \cdot B) + (k_1 \cdot A - k_2 \cdot B) = 0$$

And if the derivative of $A+B$ is zero over time, it means that $A+B$ **is constant**. And that's obvious. If I have A and B, in the end, nothing is created, nothing is destroyed. If I have sodium plus chlorine plus NaCl (this deserves a bit of attention and we'll look at it later), it's clear that at every instant there must be the same quantity of matter that was there before. If I put all this on a scale, the scale doesn't increase or decrease, it's simply a conversion. But the total is conserved.

So what we will do constantly, we will do frequently, is: since $A + B$ equals a constant, I can call this constant W. I can call it 100%, or if you want to split hairs you could say "I'll change the variables and each… I'll call for example $a_{small} = A_{large}/W$, $b_{small} = B_{large}/W$", so that $a + b$, with this change of variable, amounts to at most 1. So I can think in **fractions**: fractions of elements that are in a state A and in a state B. Maybe fractions of channels that are in a closed state, or in an inactivated state, or in a whatever state. Since they are discrete three-dimensional conformations (this is an assumption that we can discuss later if you like), and I'm interested in talking about a fraction of the total 100%: what is the fraction of open sodium channels at rest? Are

they all closed? Or is 25% open, 15% open? It can be convenient to think in terms of fractions.

If I do that I can… before doing that, to show you that the two equations… are… one is enough, they are linearly dependent, you can use $A + B = W$ (constant), and the constant is a given of the problem (it can be 100%, it can be 1, or it can be how many grams of sodium and chlorine you had at the beginning). And you see that I can express B as $W - A$. So this differential equation ($\frac{dA}{dt}$), I don't need to have another coupled differential equation, one is enough for me, because I get an equation in which only A appears.

$$\frac{dA}{dt} = -k_1 \cdot A + k_2 \cdot (W - A)$$

And I'll let you solve this because it's the usual, boring first-order differential equation, constant coefficients, and if you don't solve it at the exam, you'll tell me why, where I failed to awaken your interest in something basic.

Here the thing I can propose is… okay, let's use fractions. And to do that, if you don't like the change of variables, I can say: "okay, we have to do the change of variables anyway, but I can also tell you, let's divide both sides by W". So here, since the derivative of A… so $\frac{1}{W}$, which is a constant, I can bring it inside the derivative sign (since it's a multiplicative constant, it doesn't depend on t, I can arbitrarily bring it inside or outside). I want to bring it inside because I already have the change of variable ready. But I did $\frac{1}{W}$ here on the second term and also here on the left $\frac{1}{W}$. It becomes $-k_1 \cdot (A/W)$… and a_{small}. Here I have W/W, which leaves 1 (the 100%, thank God things remain consistent) and here again A/W which becomes a. So I can write it like this:

$$\frac{d(A/W)}{dt} = \frac{da}{dt} = -k_1 \cdot a + k_2 \cdot (1 - a)$$

And I can factor out a so that it becomes in the form I'm used to, $\frac{da}{dt} = ...$ (that constant that makes me nervous if the coefficient is positive or negative because there are exponentials that explode, or vice versa that dissipate, and all in all this is a dissipative system, there's no reason for it to explode) ⋯+a forcing term. And so I can write it by factoring as:

$$\frac{da}{dt} = -(k_1 + k_2) \cdot a + k_2$$

This is a number because k_1 and k_2 are given. I see with great joy that there's a minus sign in front so I relax, also because k_1 and k_2 I told you are the speeds, the velocities, the *rates*, the conversion rate. And the rate means "so many molecules per second," it's positive, it's not negative. So k_1 and k_2 are positive quantities and so here I have the guarantee… I know how to solve this differential equation which is in fact, apart from k_2 which is a constant term, it's an exponential… [correction] it's an exponential arc that has a transient part which then ends and then a constant term remains. How do I know there's a constant term? I assume there's a *steady state*. If there's a *steady state* I set the derivative to zero, and the *steady state* value (a_∞) is due to $k_2/(k_1 + k_2)$.

I don't know how many of you vaguely remember that this formalism here allowed (now clearly I'm wrong because I don't remember, I'd have to derive it in 30 seconds but I won't do it now) allowed from here, even if this was something almost discursive, almost phenomenological… it was phenomenological… to say "look, if I give you these *rates*, I'm telling you how at *steady state*… *Chlorine*… the ratio of concentrations ($[NaCl]/([Na]\cdot[Cl])$) …it could be the opposite, I don't remember now, we'll see later why… is equal to a function of k_1, k_2… something like that." So in chemistry, it made sense to use this formalism because you had very interesting conclusions about the *steady state*. In the end, chemical reactions happen at such a speed that you don't really care about deriving the transient. We do, we want to have the most general case possible (kinetic, Markovian schemes), we want to have both the *steady state* and the transient. Why am I interested in the *steady state*? Because in a moment I'll apply exactly the same language to describe ion channels and you'll see that I can describe and understand the action potential exactly based on those kinetic constants. So yes, it's the same differential equation, the usual one.

The $Na+Cl \rightleftharpoons NaCl$ Case and Mass Conservation Let's talk about this sodium $Na+Cl$. I think I put, okay, compared to what I wrote on the board, in that slide k_1 is inverted with k_2, but they are two parameters, they are two names, they don't have any meaning. So here I made a mistake precisely because here it was a ratio between k_1 and k_2. In any case, this is what perhaps some of you remember from chemistry, and I'm not sure if anyone ever showed you where this ratio comes from. And so allow me to write again for this kinetic scheme, again for each of these nodes (sodium, chloride, sodium and chlorine), I write a differential equation.

$$Na+Cl \underset{k_1}{\overset{k_2}{\rightleftharpoons}} NaCl$$

Here, to remind myself and to remind you that in this context they are concentrations, but I can easily think of them as absolute numbers, by multiplying both sides by the volume. So it's not that important. When I called them A, B before, here it could be $A \rightleftharpoons B+C$, it doesn't matter if you consider it as concentration, as a fraction, or as a total number, it doesn't matter. In the end, it's simply a change of variable. The concept is that you have a state: the state of the sodium-chloride molecule in a bound configuration, whereas in this case you have the individual particles, the individual unbound molecules. You can think in these states… I'm repeating this to you because if we know that ion channels have different conformations, different states (open, closed, inactivated… because there's that hatch inside which makes it explode, it's no longer two states "open or closed". It's open-inactive, open-active, closed-active, closed-inactive… it becomes four if I have that hatch, it's all the combinations of two elements, three elements, taken two at a time… that thing there, now I have to think about it because *combinatorics* was not a very interesting subject for me).

So reasoning in terms of either concentrations or molecules… [corrects himself] sorry, I was getting the order of the slides wrong… so for each of these, I write a differential equation. Here it is, okay, I consider it concentration, I write

$\frac{d}{dt}[NaCl]$. And again I have the drain and the faucet, so here there's a $-k_1 \cdot [NaCl]$, because "however much liquid there is in this container, it takes it away that quickly". At the very least, if you have a bathtub that is very full, a bathtub, a sink that is very full, the flow is much faster because the quantity of matter is much more… it presses on the drain much more than if you have a trickle. The reason why, in fact, the profiles with which the height of the liquid changes in a container, in a *reservoir* with leaks, are exponential arcs, it's not that it goes down suddenly, instantaneously, or linearly, they are exponentials. Where there are exponentials, it means that the state variable which is here also appears in the second term, remember that this is what makes me happy or unhappy, so it's the key, exponentials appear because they are the eigenfunctions of this class of differential equations.

So here it disappears with rate k_1, so **minus** (it disappears), as fast as there is $[NaCl]$. And it appears (or *apparates*? no, appears) as quickly as, proportionally to k_2, how much there is of both the concentration of sodium and chlorine, by the **product** as per the law of mass action. It makes sense that there's a product, the product is easy to nullify, it's enough for one of the two to be zero, the product is zero. I need both species to make this reaction happen, so the fact that there's an operation here that is the product fits with the intuition. There's a **plus** sign and so it expresses the fact that here I have the creation, the formation again, I have the passage into this bound state.

$$\frac{d[NaCl]}{dt} = -k_1 \cdot [NaCl] + k_2 \cdot [Na] \cdot [Cl]$$

If I take this equation only and consider the *steady state*, I say that here I'm not interested in solving it for the transient. Note, I don't know how to solve it for the transient yet unless I write the other equations, I have to write another differential equation for sodium and one for… [error, means chlorine], because here it's an equation where, okay, here this is the state variable, call it $f(t)$, $x(t)$, call it whatever you want, it's also here, but here there's another term that… they are other quantities, in theory it's a system of three coupled differential equations (not all three are dependent for the reasons stated). However, if I'm only interested in the equilibrium and I consider it by saying "a *steady state* exists". If it exists, it means the quantities don't change over time. There's a time derivative here, it means the derivative is zero. In this case, I can rearrange the terms, bring this to the first member, change the sign, and if I divide $[NaCl]$ by $[Na] \cdot [Cl]$ I get k_2/k_1 in this second member.

$$\text{If } \frac{d[NaCl]}{dt} = 0 \implies k_1 \cdot [NaCl] = k_2 \cdot [Na] \cdot [Cl] \implies \frac{[NaCl]}{[Na] \cdot [Cl]} = \frac{k_2}{k_1}$$

If you do this once in your life, you'll understand why chemists perhaps often proposed this to you as an extremely powerful method. In reality, the knowledge of this description with the language of differential equations is even more powerful because you can describe the transient, you can particularly, even in other contexts more related to chemistry, not be satisfied with what the final

result is, but you might need to understand how quickly the chemical species in a bioreactor, for example, are formed or not formed.

Was this thing about kinetic schemes known, or is it the first time you're hearing it? You already knew it? No, okay, okay. The idea is: am I boring you or not. This… if you can give me some feedback, if not.

This to conclude this part: the story of the conservation of mass. If you write it like this, it requires being a little careful, because okay, I have to write a differential equation for each species. And again it's a little game: I put myself here, I'm writing $\frac{d[Na]}{dt}$. You see it disappears (this is a drain), so there's a $-k_2$, proportionally to how much sodium there is… in fact, there is… sorry, how much sodium there is but also how much chlorine there is, otherwise the reaction doesn't happen, it doesn't disappear, I beg your pardon. So $[Na] \cdot [Cl]$, premultiplied by $-k_2$, which is this one here. And then sodium appears with a *rate* that is proportional to k_1 times how much $[NaCl]$ there is. So $k_1 \cdot [NaCl]$ minus… Chlorine is practically identical, you see it's practically identical, but here on the first member it's different, it's the differential equation that describes how chlorine changes.

$$\frac{d[Na]}{dt} = -k_2 \cdot [Na] \cdot [Cl] + k_1 \cdot [NaCl]$$
$$\frac{d[Cl]}{dt} = -k_2 \cdot [Na] \cdot [Cl] + k_1 \cdot [NaCl]$$

So, either you blast through a series of algebraic steps like "subtract the second and third equations", "add the first and second", so you algebraically manipulate what it becomes, imagining that there's a *steady state* (so setting all these terms to zero). You have, that is, a system of non-linear algebraic equations. And you know that a system of non-linear algebraic equations requires, eventually to make explicit "something equals something else", or to make explicit reactions, requires a bit of algebra, you have to sweat a bit. I've sweated for you, but just an epsilon, it's not a difficult thing.

Another way is: if you try to add all three of these equations, on the left you get the sum of the derivatives, which means the derivative of the sum. The derivative of the sum doesn't come out to zero if you add all three equations, because it's true that this term here $(-k_1 \cdot [NaCl])$ cancels with this one $(+k_1 \cdot [NaCl])$ and this term $(+k_1 \cdot [NaCl])$ cancels with this one $(-k_2 \cdot [Na] \cdot [Cl])$, but this term here still remains $(-k_2 \cdot [Na] \cdot [Cl])$ and also this term here $(+k_2 \cdot [Na] \cdot [Cl])$. I don't know if you see it, if you try to do it once you'll see it doesn't work out. "But how? That guy told us that if you add all the differential equations together you get the conservation of mass".

Yes, but in this case, to… to have the sum of the derivatives equal to zero, you have to add this equation **twice** to the others. That is, $2 \cdot [NaCl] + [Na] + [Cl]$ is constant. If you do it, you'll see that having a coefficient 2 here (and also a coefficient 2 here), here 1 cancels and here the other one cancels. This and this make 2, they are equal. And also this $-k_2 \cdot [Na] \cdot [Cl]$… they make 2 times. So if I want the first member to be zero (the sum of the derivatives or the derivative

of the sum to be zero), I have to put two times this quantity here… ah pardon, to the sodium chloride.

And this makes sense because if you imagine taking *snapshots*, instantaneous photographs in time, and you start, for example, in a condition where you have 10 (again I can do it as if they were molecules because I've multiplied by the volume, so yes this is a chemical reaction, in theory I should reason in concentrations, but you know that concentrations and absolute quantities are the same thing apart from a scale factor which is the volume). So I have 10 molecules of sodium, 5 of chlorine. I need one of sodium and one of chlorine to make one of sodium chloride. So in this case, after a certain time, the sodium has gone down to 5, because more than 5 don't have… more than 5 of chlorine. So each one of sodium reacts with this one of chlorine and… one of sodium and one of chlorine make… so they are two… one of sodium and one of chlorine make one of sodium chloride. So here this is gone, this has become 5 and this has gone back to 5 ($10 - 5$ makes 5). If I do it again, since the reaction is reversible, I can think that two of these have returned, they have two molecules, so it means that for each one you had 1 and 1. In fact, this from 5 became 6 and then 7. This from chlorine became first from 0, 1, 2. If you do the sum instant by instant ($10+5+0$, $5+0+5$), you always have the same number, except in this case that, okay… Except in this case, that is, pardon, you don't have the same number unless you multiply this quantity by 2 (2×3, 2×5). Another way to see it is that sodium plus chlorine is heavier, it weighs double, because it has two balls, one attached to the other. So this is another way to tell the story of the conservation of mass. When you have kinetic schemes (and we'll never see them) where you have $A + B \rightleftharpoons C$, it continues to hold with attention to the balancing discourse. There is anyway a balance because mass is conserved.

Exercises on Kinetic Schemes Now let me go back to this little scheme here, where I ask you to help me write the differential equation for each of these kinetic schemes. Here again, for each of these nodes, for each of these quantities, I have to write a differential equation, so I'll write $\frac{dA}{dt}$ and then afterward I'll write $\frac{dC}{dt}$.

Scheme 1: $A \xrightarrow{k} C$

Can you help me write $\frac{dA}{dt}$? I repeat, it's a little game. If you're not with me, it's either because you don't care, or because you're sleeping. So, $\frac{dA}{dt}$… You see there's only one drain? How do I write it?

(Voice from the audience: Minus k, A)

I can't hear you, sorry. Minus $k \cdot A$, perfect. And C, in the end, is the dual case, how do I write it?

$$\frac{dA}{dt} = -k \cdot A$$

(Voice from the audience: K times A)

$k \cdot A$. And indeed, I like it because if I add them, this and this cancel out and $A + C$ is constant (the derivative, the sum of the derivatives is zero, so the

derivative of the sum is zero).

$$\frac{dC}{dt} = +k \cdot A$$

Scheme 2: $A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C$

In this case, what do I need to change? Since it's no longer… not only is there the drain but there's also… there's the faucet from the other side. So there it was k_1, so I'll write k_1 here. Can you help me complete it?

$$\frac{dA}{dt} = -k_1 \cdot A \,...$$

(Voice from the audience: Plus k2 times C)

Okay. And here it will be the same but with a minus… [corrects himself]

$$\frac{dA}{dt} = -k_1 \cdot A + k_2 \cdot C$$
$$\frac{dC}{dt} = +k_1 \cdot A - k_2 \cdot C$$

…and in fact, if I add them, it works out.

Scheme 3: $A + B \overset{k}{\rightarrow} C$

In the end, this $A + B \rightarrow C$ (reversible/non-reversible), we've looked at it, so I won't stress you too much. In the end, it's almost sodium chloride. You also have the conservation of mass indicated, you see that here $A+B+2C$… [correction: the scheme $A+B \rightarrow C$ implies $dC/dt = k \cdot A \cdot B$, $dA/dt = -k \cdot A \cdot B$, $dB/dt = -k \cdot A \cdot B$. The conservation here is more complex, e.g., $A - B =$ constant]. And here too, again, it's not because it's reversible or non-reversible that the conservation of mass changes. I'm putting in some chemical species or states anyway.

If the channel is open… if I have a thousand channels, if 500 are open, the others will be closed. It's a kind of principle, not an exclusion, it's a conservation of states. If it's not open, it must be closed. Okay, if there's inactivation, there can be a third state, but there can't be channels that are in some "whatever" state that isn't… it's either open or it's closed, since there are only two states. Here it's either A or it's C.

Something that might be of interest to you, but I won't talk about it, is useful in **polymerization** reactions, where you don't have just one molecule of A. In fact, even in a context of electrophysiological signals, but we won't talk about it, in particular circumstances where multiple ions or multiple neurotransmitter molecules are needed to bind to a receptor. Actually, I'm thinking of calcium, but let's not talk about it.

Scheme 4: $nA + B \overset{k}{\to} C$

When you have a multiplicity, since this was a product $(A \cdot B)$, then in the second member of the differential equation, the law of mass action says that the rate of appearance, well… depends on the product $A \cdot B$. So you have N times A, so you have to do $A \cdot A \cdot A \cdots \cdot A \cdot B$. This is the reason why you have this exponentiation to the n. And it's typical of polymerization reactions.

$$\frac{dC}{dt} = k \cdot [A]^n \cdot [B]$$

In this case, watch out, beyond the fact of the… so you either get there with the intuitive heuristic that the quantity of matter must be equal, or rigorously, by working with algebra, manipulating these equations, you would realize that the conservation of matter, so the mass balance, takes a form that is no longer the previous one (it was 2 times C in the case of $A + B$…), so it's $n + 1$. Anyway, we won't see this in the course.

Stochastic vs. Mesoscopic Interpretation Before closing this premise, this *excursus* on these kinetic schemes, [which] were used by Hodgkin and Huxley to describe ion channels, as I've tried to insert several times in the last few minutes. I'll tell you, however, what another interpretation is, which is not a "population" interpretation. When I talk about a sodium concentration of 5 millimolar, I'm thinking of a system of 10^{18} ions, I'm thinking of a very numerous system, regardless of whether I express it as concentration or density, or multiply by volume somewhere. So I'm thinking of an enormous number of particles. I'm thinking of a system of many objects, **mesoscopic**. Likewise, when I told you a little while ago "if I have a thousand sodium channels…" and last week we discussed it, saying "these ion channels are interspersed in the membrane and from an electrical point of view I can… if it's possible, if they are all identical, they are independent (in the sense that they don't influence each other, for the moment I haven't talked about this, but one can imagine not), they all sit across the membrane and so they all experience the same potential". Kirchhoff, or the rules of electrical engineering for series resistors or resistors or parallel resistors, gives me those little formulas to group them. But I always have the idea that when I group them, I'm talking about a large population, so in that case, it's a mesoscopic description.

These differential equations written like this are **deterministic** equations. I solve them, I get the exponential arcs, and I go home, I have no stochasticity. In reality, the exact same formalism has a reinterpretation that is very important from the point of view of electrophysiological signals, because if you do an experiment with a biological neuron (I'll show you), it's not exactly a deterministic object. Today I'll show you an example of a computer simulation of the Hodgkin and Huxley model, and I'll show you the deterministic model and the non-deterministic version. If you plant an electrode somewhere in my brain, you don't see -70 mV, you see a fluctuation as if it were a stochastic process. And if you show me a photo of Swiss chocolate, Belgian chocolate, some parts of my temporal cortex recognize it and start firing, but they don't

fire like this [regularly], as instead I'll show you that the described neurons fire in a *pacemaker*-like way, like a metronome, regularly. They fire irregularly.

And so where does the stochasticity come from in this description? It's not there, they are ordinary equations (they could be partial derivatives, but they are deterministic equations). When it's written like this ($A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$) and you put the magnifying glass not on a population of channels, a population of particles, a population of ions, of molecules (sodium and chloride), but on a **single one**, it means that k_1 and k_2 are not the rates of appearance or disappearance, of formation or consumption. They are the **instantaneous probability** of being able to make a transition, of binding. So the single molecule can (in the case of an ion channel) has a certain probability from instant to instant of changing state, of jumping. And every time I talk about a phenomenon to which I attribute a probability, I'm implicitly saying "it's like flipping a coin". I have the coin, if I flip it… I'll avoid doing it, but who knows what came up… or a kind of free random number generator, because nature is a stochastic nature.

So in reality, it's the same notation but a different meaning, where A and B are no longer concentrations but are the state of occupation. In the case of an ion channel, I look at an ion channel and I see if it's open or closed. In the case of a sodium molecule, I can see if it's unbound or bound. And the correct way (but you'll see in a moment that my usual favorite boring differential equation, always first-order constant coefficients, always comes out), but this wording says that the probability of a transition between state A and state B in the unit of time, conditional on the fact that at state at time t (so at the current time) I am in state A (so the past doesn't count, only where one is at this moment counts). The transition $A \to B$ cannot happen if I am in B. If I am in B, the transition from B to A can happen. So the fact that I'm using these conditional probabilities… yes, I'm thinking of Bayes' theorem which you've perhaps heard of, but it's not crucial. I'm simply translating into a formalism where the transitions are two, either from left to right or from right to left.

And the important thing is that these probabilities are probabilities that are defined in a small interval. I don't know how many of you know or have a reasonably okay background in probability theory or statistics. I don't know how much you remember (and I'll probably revisit it, and if I don't revisit it, I'll revisit it in another course next year). There are two types of stochastic variables or really stochastic processes: discrete-time or continuous-time. The probability that this phone rings *right now* is zero. The probability that a drop of rain falls exactly on the center of your head is zero due to a mathematical fact of the null measure of the set over which you do a particular integration. Whereas the probability of getting heads or tails… [correction] not discrete-time, continuous-time… has discrete values and continuous values. A coin has only two discrete values, while the instant of a phone call is a random variable that I'll call T_0 and this T_0 is a number that belongs to the set of real numbers (positive reals, suppose), so it's continuous.

There's a fundamental difference, so that's why I'm saying here that I'm considering a Δt, otherwise it makes no sense to write "the probability of transition at the instant t". So in theory, I should write that the probability of transition

is given by these quantities, and these k_1 and k_2, if here they represented a speed, a rate (so dimensionally they are in units of second^{-1}), conversion per second… conversion can be dimensionless… "three times per second", "per second" is what interests me. So here k_1 and k_2 are not probabilities, they are instantaneous probabilities or **rates**.

So the correct description of this formalism is that the probability of transition is given by $k_1 \cdot \Delta t$. I won't draw it out, strictly speaking, this is actually the Taylor series expansion of an arbitrary function that I don't know but of which I'm only specifying the first order and I'm saying that the higher-order terms are negligible. But I won't tell you this, and I'll also tell you that the probability that in a time interval Δt there are two transitions is zero because I choose Δt sufficiently small. This is a thing about the Taylor series expansion, of a quantity that is in general an arbitrary function of time, that is, it's a function Δt. Because if I say "what's the probability the phone rings from now for the next two seconds?", it's clearly smaller than the probability "if the phone rings from now for the next five hours". You can intuitively understand this. The probability that that phone rings from now to 5 hours from now probably goes to 1. But if I take the Δt very small, the only thing I can do is say, "okay, if it's small, I think it's a function of the interval and I'll approximate it with Taylor". This k_1 wording is the first order of Taylor.

The Hodgkin-Huxley Model: Connecting Kinetics and Conductance

So, why is the story of Markovian kinetic schemes very interesting? Well, it's what Hodgkin and Huxley used. So, in fact, for them, the changes in conductance G_{Na}, G_K (and in their model, because they were focused on the permeability of a particular biological preparation, the squid giant axon) were everything. And they described this change in permeability in phenomenological terms. They thought there was some electrically charged mechanism, but in fact, they said, "for me, a channel is either **open** or **closed**." I don't know, the permeability is due to a number of microscopic objects that can be in two states.

(Pause, logistical comment about the power strip)

So, for the moment, for simplicity, we see the channels as open or closed, or let's see how they managed. The *link* between the electrical component and the component describing the states I'll give you now. And it's fundamentally that of considering the conductances that are called **active** (because they are not constant in time, they are not passive) as variables. They are variable, **voltage-dependent** conductances.

Now let's see where I put the voltage here, where I put the membrane potential. One possibility, thinking about that microscopic interpretation (and here too, it doesn't take a particular genius), is that if the channels are normally closed but start to open when the potential becomes depolarized, evidently it is, at least in this transition ($C \rightleftharpoons O$), from closed to open… here, what was a probability per unit of time (the probability of making that transition) or the *rate* in the mesoscopic case, in the deterministic case… it's here that the dependence on the potential will come out. And that's exactly what is done.

That is, the total conductance, for example, suppose you're talking about G_K, given that electrically we had grouped (again I mention Kirchhoff, I mention the little rules of electrical engineering for parallel resistors or parallel conductances, etc.), the total conductance is the sum of the individual conductances. So in a way, it's the number of these channels (N) times the conductance of the single channel (γ). Now, to be quicker, I'll say it's the number of **open** channels (N_{open}), because if they're closed, they don't contribute to the conductance. So the *link* is here. The conductance depends only on the occupation of one state; in the other state, there is no conductance. There could be five states with intermediate conductance values. In the simple case we're looking at now, in the description of these membrane ion channels, it's enough (it's sufficient, I'll show you in what terms it's sufficient) to assume that the channel is either open (fully open) or closed. It's not "slightly open". But intuitively, if they are changes in configuration, of three-dimensional structure, you might say "in one state there might be a lower conductance than in another". It seems that in nature, in most cases, only one state coincides with an electrically functional state.

So here it's the total number… sorry, the number of open channels (for example, of potassium channels) and this is the conductance of the single channel (γ), when it's open, obviously. 10 picosiemens, something like that, on the order of picosiemens. If I have 1000 channels and 500 are open, it will be 10 picosiemens$\times$ 500.

$$G_K = N_{open} \cdot \gamma_K$$

Maybe it would be more useful to think in terms of **fractions**. That is, to say: "I'd like to be able to know what the maximum conductance can be". So I'd like to write what the maximum conductance is… if you let me write it like this, $\bar{g}$. This here is $\gamma \cdot N_{total}$. So these are open channels, it means channels in this state. Here are all the channels, and at most, when they are all open, they will contribute to the conductance with $\gamma \cdot N$. Let's see how I write this for you. Having multiplied this quantity because I liked having this $\bar{g}$ which is therefore $\gamma \cdot N_{total}$. Now I have to divide because N_{open} is left here, but to make things work out (otherwise it's not an equivalence) I have to further divide by N_{total}, which I multiplied by here, this N_{total}, the total number of channels.

$$G_K = (N_{total} \cdot \gamma_K) \cdot \left(\frac{N_{open}}{N_{total}} \right)$$

This quantity (N_{open}/N_{total}) is exactly a fraction. So it's 500/1000 total channels, so that I can write 0.5. This is very convenient for me, also from the point of view of the experimental description, because it abstracts me from figuring out how many channels there are. So, the channels, I told you, are discrete entities, and they are quantities that are stochastic (and we'll see why later), but when I talk about them at a population level, and particularly in Hodgkin-Huxley's time when it wasn't understood if it was a distributed or concentrated property of the membrane, perhaps it's more congenial for me to talk about fractions. And so I define this quantity here, $N_{large,open}/N_{large,total}$, n_{small}. I'll

call n_{small} a quantity that is between 0 and 1. It's a fraction, it can't be negative because the channels… it's a number, it can't be a negative number of open channels. The open channels are from 0 to N_{total}. So at most, this ratio can be 100%, it can be 1.

So note, from here I've multiplied and divided by N_{total}. And so I like to write $N_{total} \cdot \gamma = \bar{g}$, g-bar, barred, times n.

$$G_K = \bar{g}_K \cdot n$$

And this thing here, in theory, I can do for all active conductances. Where am I heading? The story of fractions came up when, a little while ago, an hour ago, we were talking about kinetic schemes. Kinetic schemes like this one:

$$C \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} O$$

Allow me to call this α and this β. They are numbers, they are conversion rates, rates of opening or closing. If I want to write… if n is "number of channels in this state (O) divided by the total number"… can you tell me how I should write $\frac{dn}{dt}$? The usual little game, only that instead of k I'm now using α and β. Before it was A and B, now it's O and C. And now I'm thinking that here it's as if it were n. And there, obviously, you have to tell me. Remember that nothing is created, nothing is destroyed. How do I write $\frac{dn}{dt}$?

(Voice from the audience: Minus alpha n…)

I can't hear you?

(Voice from the audience: Minus alpha n plus beta…)

Perfect. Okay? So, okay, if you want, yes. We can do one more step.

$$\frac{dO}{dt} = -\alpha \cdot O + \beta \cdot C$$

And then… for C. Can I write it in a way… here I should also write an equation for C. Will you allow me to write that $C = W - O$ (a certain quantity minus the open ones)? So if it's like that, here instead of C, I'm using W because we used it before, $W - O$. How do I write… how do I get to n, where n_{small} is "number of channels that are open relative to the total number"? So if you like, sorry, but it's the same thing, it's O/W. How does this equation become? It becomes $\frac{dn}{dt}$. It remains practically the same, but I'm stimulating you to send oxygenated blood to your brain.

$$\frac{d(O/W)}{dt} = \frac{dn}{dt} = -\alpha \cdot \frac{O}{W} + \beta \cdot \frac{W - O}{W}$$

$$\frac{dn}{dt} = -\alpha \cdot n + \beta \cdot (1 - n)$$

(Voice from the audience: Minus alpha n…)

Minus $\alpha \cdot n$, okay? And then?

(Voice from the audience: Plus beta times one minus n)

Perfect. And if you want, I can factorize, it becomes $-(\alpha + \beta) \cdot n + \beta$. Okay?

$$\frac{dn}{dt} = -(\alpha + \beta) \cdot n + \beta$$

Okay, this is the "stuff" of the Nobel Prize, because α and β can be identified experimentally if you manage to see, for example, the *steady state* or the transient of this equation. And this equation, at least in theory, if α and β were constant, I'd know how to solve it. If α and β were functions that I don't know, that I can identify experimentally, if they were functions of the potential, okay, it would be a bit more complicated, in the sense that I can't do it with paper and pencil, I have to use a computer to do the simulation.

This thing here, I remind you that here it's the same thing as saying how the total conductance (G_K) changes over time, which is a maximum value ($\bar{g}_K$) times the fraction of channels (n). It's simply scaled. While n goes from 0 to 1, this quantity G_K goes from 0 to 200 mS/cm^2. Nothing changes.

Experimentally, Hodgkin and Huxley were able to verify (and now I'll tell you how), they were able to measure the conductances. In reality, they measured the currents. They measured the potassium current (I_K), for example, which is $G_K \cdot (V - E_K)$. I remind you, V is the membrane potential, and E_K is -80 mV. So I know E_K because maybe I was the one who put the concentrations inside and outside my bath and my squid giant axon. So V, maybe I know it because I control it. If I measure the current (I_K), I can get the conductance (G_K). But if the conductance is $\bar{g}_K \cdot n$ (apart from this quantity $\bar{g}_K$ which will be a number, it will be what it will be), I can deduce the stationary or transient value of n. And so if this is my model, I can determine α and β. And if I succeed, I can try to put it all back together in a mathematical model and see if what comes out is an action potential. And the answer is yes.

And this type of formalism is so successful that we still use it today for a potassium current with inactivation, or a current called muscarinic current, or (we'll see) it's used to describe the current that flows through an ionotropic glutamate receptor, which you should… I overheard one of you telling me that evidently Zoli is doing ion channels, so postsynaptic receptors, ligand-dependent (or ligando, I don't know how it's said). So not only voltage-dependent, but dependent on the concentration of a neurotransmitter at a certain point. They are still channels, they are still pores. I'll show you that exactly the same formalism works.

Let's go back here. Here it's a bit complicated. Potassium, if you remember in that cartoon, opens with a certain delay. You've never seen the equation written as $\tau \frac{dn}{dt} = -n + ...$ (something, for example, let me call it n_∞)? Have you never seen a differential equation written like this? It's simply the exact same equation in which I've put a quantity, a parameter that I'll call τ (tau). I put it here because if I divide both sides by τ, I get $1/\tau$ here. And this $1/\tau$ is exactly what goes into the exponential that makes me happy: $e^{-t/\tau}$ (thank God).

So τ tells me (you either put it here, or you put it here in the denominator) what the time scale is.

$$\frac{dn}{dt} = -\frac{1}{\tau_n} \cdot n + \frac{n_\infty}{\tau_n} = -\frac{n - n_\infty}{\tau_n}$$

This thing here $(\alpha + \beta)$, in the end, is the inverse of a τ, because you see it's in the numerator. And it makes sense to me, because α and β were rates, so "per unit of time", or "transitions per unit of time". So this is the inverse of a time. So $1/(\alpha + \beta)$ is a time. So this thing here $(\alpha + \beta)$ tells me how quickly… eventually the word *delayed* is no longer there… eventually if one channel or another has, simply due to these kinetic coefficients, a slower reaction capacity.

Again, the same stupid first-order differential equation with constant coefficients that you do in Calculus 1, anyway it's always that one, it's always… And from here I read something: that if a channel is later, is more delayed, slower to activate or inactivate, it must be because of these two numbers. Okay, they will be functions, but numerically those functions will be different, they will be (especially now that they are in the numerator) they will be small numbers. That is, the transition *rate*… 1 over a small quantity means it's a large quantity, so a large τ, so something slow, which has a slow time scale of a few milliseconds, instead of sodium (which I'll show you in a moment, for now we're not talking about it) which instead was very fast to activate, at least to activate.

So this is the type of formalism where n relates directly to the kinetic schemes.

Fundamental Assumptions of the Model Before sodium, however, I must tell you what the fundamental assumptions of this are. This doesn't always hold.

1. **Population Description (Mesoscopic)**: I'm thinking that I'm describing these channels as a **population**, so much so that I describe them as "fraction of open channels". I'm not describing *that* channel, there could be thousands or millions (we'll do that later), but for the moment we're describing a deterministic, mesoscopic system. A choir made of so many voices that even if one is out of tune, you can't hear it. The larger the choir (it's common experience), the less the individual voices count, because it's a summation, an average. The summation of sounds (because sound pressure waves reach my ear and add up) or the summation of currents or conductances (Kirchhoff) is the same thing. Saying "sum" or saying "arithmetic mean" is the same thing, the arithmetic mean is just further normalized but it too is a sum. I'll avoid citing some theorem from probability theory, but there too the irregular, stochastic behavior becomes more similar to the average behavior (in that case it's the ensemble average) when I have a large number of objects or attempts. What you may have heard described as the **Law of Large Numbers**: if I flip a coin a billion times, roughly the fraction will become $50-50$ if it's a fair coin.

2. **Statistical Independence**: We are anyway in a deterministic context and so collectively these channels, a population, undergo conformational changes and the only control variable is the electric field that enters here $(\alpha(V), \beta(V))$ because these channels are interspersed in the membrane. This is important because the moment we write it like this, we're saying they are **statistically independent**. They are identical and statistically independent, in the sense that if we are all channels, if I open, I don't directly influence you to open because my state has changed. At most, I can influence you because if it's a morphologically distributed structure, I open here, the potential changes locally, maybe this perturbation of the electric field is the only thing we have in common. But there's no phenomenon of **cooperativity**, i.e., the fact that if two or three or a hundred or whatever channels open, then the others open even more. It's a hypothesis that exists in many contexts of biology, it doesn't seem to be there in the case of channels, and it works because we can make this description which is the sum, the average. If they weren't statistically independent, there would be problems, we couldn't describe their fraction…
3. **Discrete States and Markovian Property**: Okay, there are these discrete states through which the population relocates, moves. These are the voltage-dependent coefficients I anticipated. And this, in the end, is the law of mass action: the reaction doesn't depend on the past, it depends only on the concentrations of the reactants, that is, it depends on the current state (if I'm in the open state or if I'm in the closed state). This is the **Markovian property**, it doesn't depend on the past. And it's exactly what happens with chemical reactions, where I'm not thinking that if two ions, sodium and chloride, bind, they influence (at least in those simple terms, if the solution is sufficiently dilute) how another two nearby ions combine or dissociate.

The Experimental Challenge: The *Voltage Clamp* The complication that required Hodgkin and Huxley not only to get their hands dirty from a mathematical point of view, but also electronic, paradoxically, is what I mentioned in the previous hour. That is, the fact that changes in conductance (G) cause (because they cause currents, I) changes in potential (V), because there's the charge balance equation that says $C\frac{dV}{dt}$ is equal to the summation of these currents.

$$C\frac{dV}{dt} = -\sum I_{ionici} = -\sum G_{ionici} \cdot (V - E_{ion})$$

…let me write it with I, okay, for how I've written it here, a minus is needed, for how I wrote $V - E$, otherwise they are… So if I change G, I change I. And by changing I, I change V. But by changing V, I change these α and β! And by changing α and β, n changes, and so G changes.

And obviously, like many phenomena in biology, it's exactly a **feedback** (positive or negative, it doesn't matter at this moment), but I'd like to decouple

them. If I have to measure quantities, it's very complicated to see that the current changes, so the conductance changes and the potential changes; changing the potential changes the conductance. I would need a way to isolate things.

And Hodgkin and Huxley invented a **feedback electronic amplifier**, capable (also helped by the particular geometric configuration, in truth, of the squid giant axon) of measuring the instantaneous potential, comparing it with a desired value, and, depending on whether… so creating an error signal. If the potential is −65 mV, but I have as my… I want to impose… now I want to study if the channels are closed or open at −70 mV, but the potential is changing to −65 mV, there's a kind of difference. And this error is injected inside in the form of current, a negative current. So if it was −65 mV, if I inject a negative current, the potential tends to decrease, maybe it becomes −72 mV. Now −70 mV (desired) −72 mV (actual) is a positive or negative quantity… whatever… positive. So now I inject… I change this feedback (which obviously must act on fast enough time scales, not too much otherwise it starts to have oscillations, but that's another story), I inject a positive current. Instead of −72 mV, it now becomes −70 mV.

So I'm making a **P** (proportional) control system. You've perhaps heard in engineering about the PID control system, for example. They are the simplest possible control systems: Proportional, Integrative, Derivative. Here it's simply based on an error, a feedback amplifier corrects and tends, as they say, to **clamp** (from the English *clamp*), it blocks the potential at a particular value.

I need to block the potential at a particular value because I have that damned $C\frac{dV}{dt}$. If V doesn't change in time (and it doesn't change in time because there's an electronic contraption attached to a biological preparation that acts electronically much faster than the biological preparation and keeps V constant), $\frac{dV}{dt}$ is 0. And what I'm left with is $\sum I = 0$.

So in theory, if I measure the quantity… so if by chance the membrane potential were to change (and it does for endogenous reasons, because these damned conductances woke up, activated, are closing, are opening), the potential changes and I have to inject a current artificially from the outside that *exactly* compensates for that current that caused the change in potential. So I manage to measure *exactly* the sum of the currents. And this is a first, very important point. So if I manage to nullify this, by looking at the feedback signal that I have to use to keep V constant, I can see what's happening at the level of currents.

The Use of Toxins to Isolate Currents So, this is a brilliant point from Hodgkin and Huxley. But it's not enough. They had to use, because otherwise, you would have had the sum of the currents… since both sodium and potassium each, suppose, change their conductance with similar equations (not identical, they aren't… you saw before, sodium activates first, then there's a kind of hatch that closes it from inside the cytoplasm, potassium activates with a delay, evidently this quantity α and β are smaller in absolute terms, blah blah blah blah). I'd like to break all the things apart, otherwise I see them superimposed and I haven't solved much.

The answer came from the availability of **toxins** that some venomous animals (including spiders, fish, and other species) produce precisely because they are neuroactive, precisely because they bind specifically to certain channels.

There's a toxin that is my favorite because when we buy it in the lab for experimental reasons because we need it (you'll understand why in a moment), it's called **tetrodotoxin**. It's a nice acronym: **TTX**. When you buy TTX, since it's something that is quite potent, you have to sign a declaration saying "I declare that I am not a terrorist, that I do not want to use tetrodotoxin for terrorist purposes." That toxin is a very powerful blocker, an antagonist, as they say, selective for sodium channels. It sets the total conductance to zero for me. It's a plug from the outside, it plugs the channels, the external mouth of the channels (maybe it's a bit more complicated than that), but it binds to a binding site in the extracellular part of the sodium channel subunits' domains and blocks ion conduction through that channel.

If that's the case, I might have solved it, and I could, with this amplifier called a *voltage clamp* (because it clamps, it holds the potential fixed), I could study the kinetics and I could identify the parameters of the **potassium current alone**. I repeat, I know that the current I measure (I_K) is $G_K \cdot (V - E_K)$, but this (E_K), I know it's -80, -90 mV because I measure it, because I know the concentrations I put in. V_m, I'm holding it fixed at a value I choose, thanks to the amplifier. This quantity $(V - E_K)$ is a constant value. $G_K = \bar{g}_K \cdot n$. In the end, if I'm only interested in the transient, I can take the current I measure, divide by this quantity that I know (because it's a quantity I can calculate), and I will have at most… I will have, in scaled terms, n. I'll see it as… up to a multiplicative factor. And I can do this because I no longer have interference from sodium.

There's another toxin, which at the moment I don't remember which beast it comes from. If I remember correctly, this one [TTX] comes from the *puffer fish*. The one that you can't eat in Japanese restaurants unless it's particularly… the chef… it's not sushi, but if he doesn't treat it well, because otherwise, it obviously blocks your sodium channels, so you stop breathing and so you die, there are no more action potentials.

There's another toxin called **TEA** (I don't remember what it stands for… tetraethylammonium, [correction: tetraethylammonium])… you can look it up on Wikipedia. This thing is specifically a toxin, a selective antagonist for **potassium currents** (for some potassium currents). And this is nature's gift, which has created extremely dangerous animals, but since they have to kill you in the most effective way possible, it blocks exactly that channel or this other one to create motor deficits and paralyze you in the end. So it blocks, by binding to the extracellular part of the voltage-gated potassium channel's mouth, and blocks conductivity.

So if I do it by putting in only TEA instead of TTX, I can study the **sodium currents**. And again, I do the same thing. I won't go into too much detail, this is just because the type of experimental work by Hodgkin and Huxley is not trivial, it's not that of "I just woke up and found the equations that work." They validated them and identified the parameters experimentally.

Parameterization: τ (Tau) and n_∞ (n-infinity) So for potassium, this is what we have, I've already told you. And if you allow me to divide both sides by $(\alpha + \beta)$, this is no longer here because I've moved it over here. But dividing and multiplying, I still have to remember that I have to divide it here. So that's why I have $1/(\alpha + \beta)$ here, here I have nothing left (but the minus sign reminds me that these things almost certainly don't explode, again it's a dissipative system) and then I have a quantity $\alpha/(\alpha + \beta)$.

$$\frac{1}{\alpha_n + \beta_n}\frac{dn}{dt} = -n + \frac{\alpha_n}{\alpha_n + \beta_n}$$

Does this fit with my intuition? Because $\frac{dn}{dt}$... n is a fraction so it's dimensionless, but $\frac{dn}{dt}$ has units of 1/time. 1/time multiplied by... [correction] τ (because these here are the inverse of a time) cancels with time [correction: τ has units of time, not 1/time]. So on the left of the equals sign, a dimensionless quantity remains. Here it's also a dimensionless quantity, and this too must become a dimensionless quantity. So since α and β have dimensions of inverse time, $\alpha/(\alpha + \beta)$ is again dimensionless because the numerator and denominator have the same dimension.

And I can think of this quantity as τ (tau), because it has the meaning of a time, of the time constant of that equation.

$$\tau_n(V) = \frac{1}{\alpha_n(V) + \beta_n(V)}$$

And this quantity here, I'll call it n_∞ (n-infinity), because it's what... as "infinity" for me, it's what, if a *steady state* exists (it might not exist, though), if it exists, that is, I think of letting $t \to +\infty$, of taking the limit for $t \to +\infty$, if a *steady state* exists, then n no longer changes in time. Then this quantity, the derivative, is 0. Here it's 0, on the left it's 0, and n, for it to be 0, must take exactly this value here. So I know that this here is the value n would take if the *steady state* existed. So I call it n_∞.

$$n_\infty(V) = \frac{\alpha_n(V)}{\alpha_n(V) + \beta_n(V)}$$

$$\tau_n\frac{dn}{dt} = -n + n_\infty$$

Why do this? Why do I do this? Because experimentally it might be easier to work with τ and n_∞ rather than with α and β. Both are functions of the potential, but it might be simpler to say "okay, you have this contraption, this electronic gadget that clamps your membrane potential. Well, fine... let's say, you set a profile for the membrane potential where you change it like a step." Okay, you'll have some... the current too, evidently the channels open, they close, they do something, but at a certain point, I go to a *steady state*. With those experiments, I can instantly understand what the dependence on the potential is, if I test multiple potential values, of this n_∞. If I have α and β, since α and

β are… it's a bit more complicated, a bit less easy. I can always go from the τs and from τ_n and n_∞, I can get to α_n, β_n. (I'm starting to say α_n and β_n because for sodium I'll have other quantities).

Analogy: The Low-Pass Filter An interlude that resonates with what one of you asked me during the break. Again, I'm not a mathematician, so I can loosen the grip of rigorous mathematical formalism, which, however, we have maintained so far. But every time I have an equation of this type ($\tau\frac{dx}{dt} = -x + x_\infty$), I don't know about you (you probably have healthier thoughts), but it makes me think of an **electronic filter**. It makes me think of a box, a black box where x is the output and x_∞ is the input. In particular, the way it's written, this thing here makes me think of an RC circuit, it makes me think of a **low-pass filter**. It makes me think of something that even when I throw something very fast at it, like an impulse, it attenuates it, it dampens it, it's a bit lazy, it has an inertia, it has these exponential arcs. But from the quantitative point of view of the hands-on electronics engineer, they say "okay, yes, you're smoothing out the corners for me." This is what a low-pass filter does: if you go too fast, I cut you off in frequency, and so only what is lower passes, the lower frequency components pass.

Beyond the fact that yes, mathematically you can write it with the convolution integral, in the general case where x_∞ is neither constant nor sinusoidal, it could be an arbitrary function. When it's like that… if you like working in the time domain, this mess of the convolution integral… well, it's a mess… it's obviously a mess in the case where x_∞ is generic. In the case where it's a known function, this convolution integral might be less… it might be less intimidating. If, on the other hand, you're more inclined to be in the frequency domain (Laplace or Fourier, whichever), you know that the convolution integral becomes a product in the frequency domain, because differential equations in the time domain have a much simpler algebraic form as their correspondence in the frequency domain, in the transformed domain. Here it's a differential equation, in the transformed domain it becomes an algebraic equation. This is why Fourier or Laplace is used in electronics or in systems theory. We won't be doing that.

It's simply to tell you that this filtering thing is universal. And the fact that this is low-pass filtering explains well why. If I, as an input to a system like the one we saw together, the passive behavior of a non-excitable membrane, am subject to an external input that changes rapidly like a step, it's as if I'm squinting, I'm narrowing my eyes… in reality, I don't see these transitions very well, I see things more blurred. In effect, the output is like the blurred input. This is to say that every time you see an equation like this (when it's like this, when it's written like this, first-order, it doesn't matter if it has constant coefficients or not… actually, constant coefficients, we know how to do analytically, if they're not constant, I'm stuck with this type of convolution), in fact, I can think of it as a way in which the output **tracks** the input, apart from a delay. Here, if you see this exponential arc that first rises and then falls, in effect, the output is mimicking the input, which was a DC input that rose and fell, apart from a bit of laziness, *sloppiness*, a bit of sluggishness. You see it doesn't rise straight up, it takes a while.

So, whatever the complex shape of this input, the output ultimately follows it with a delay (what I'm saying is wrong, obviously, but roughly speaking, not particularly). So when I have time-invariant inputs I can do it analytically, but when I don't, it's as if I can say (again) if the input varies very, very slowly, I can think of it as constant, so it's trivial to say "okay, x is instantaneously… it's always at *steady state*", because x_∞ changes very, very slowly. If x_∞ changes a little more rapidly, maybe x continues to track it, but it can't keep up, because x moves a bit more slowly because it's a low-pass filter. This is very useful information, and it will be useful for us to understand this thing about some channels being slow to activate. What does "slow" mean? It's slow in this case here.

The Activation and Inactivation Curves Now I'll show you, before the break, how Hodgkin and Huxley systematically analyzed these n_∞. Now here I'll give you both α and β, and n_∞ and τ. And I suggest you look at τ and n_∞ because τ tells you what the inertia is, how slow the system is to react. n_∞ tells you what the *steady state* is, what point this equation is heading towards, what it's chasing. Whereas α and β, yes, they have an interpretation ("it's easy for the channel to go from closed to open," and I told you "the channels open when the potential is depolarized"), but functionally I don't grasp it with the story of "I need sodium to open, because when it opens I have the first phase; if potassium also opened I wouldn't go up, I'd stay more or less flat, because potassium is strong, it wants to keep me at [negative values]". So first the sodium must open, then after a bit the potassium must open, so the potential has time to rise and then to fall. If I don't have this delay, things don't work. And the delay must come from here, sorry, from τ, not from n_∞. From τ, because it's τ that controls these dynamics for me.

Potassium Channel (Variable n) So, I only need to show you this because the others are sodium channels and I'll talk about them in a bit. You see that τ_n (so this graph was obtained by doing a particular experiment, systematically with this *voltage clamp* amplifier, fixed at -100 mV, then fixed at -98 mV, then fixed at -90 mV, then fixed at -80 mV… trying to cover this whole *range*). And you see that τ_n is a **function of the potential**, so forget about me being able to do things analytically from now on, because they are not constant coefficients. Yes, it's the usual linear first-order differential equation, but the coefficients are not constant. So I have to do it numerically, maybe I'll invoke Euler, or Runge-Kutta. I need a numerical method, which in the math *refresh* part (if any of you needed it) you found it. If not, I assume that roughly you all know how to implement a crappy numerical method like Euler's (but don't knock it, it's a useful thing).

So it changes in time, but roughly from the point of view of units it's on the order of **a few milliseconds**. It peaks at -70 mV at 5 milliseconds. So it means that when the channel is closed (if you'll allow me this *stretch*), the channel is closed, the membrane potential is at -70 mV, then the potential starts to depolarize… but so yes, n tends to follow n_∞.

n_∞ has this shape here, this **sigmoid**. The fact that it's a sigmoid is profound because it has inspired a large part of today's *machine learning*, where every-

thing… the units of an artificial neural network… has activation curves that are (nowadays only *threshold linear* is used), in other paradigms, in other contexts, perceptrons have a transfer function that is sigmoidal, that saturates. Here it must saturate because the fraction of open channels is either 0 or 1 or in between, it's not like it can become 1.5, it doesn't make sense, at most it's between 0 and 100%.

And you see that the thing I told you, i.e., "the [potassium] channels open when the potential is depolarized," is translated by this sigmoid because for potentials that are hyperpolarized (−100 mV, −70 mV), the fraction is practically zero (which is not exactly zero, you have to read this axis here). Suppose at −70 mV it will be 0.2, 0.1, 0.15… 15% of the channels are open at rest. But when I move the potential more and more (statically, obviously), I move it more towards depolarized potentials, you see that this grows and at a certain point, having reached… so the midpoint is around −50 mV. So if I exceed −50 mV, what happens after is further… it saturates, and from 0 mV onwards you can increase it even more, those are the channels, they are all open.

And the τ… so n_∞ is what n tracks. So G_K (the potassium conductance) tracks this curve here, depending on how the potential goes. Clearly, this tracking is… [limited] barring a separation of time scales, which is a simplifying hypothesis I made a little while ago for this thing that "x tracks x_∞". The output of a filter tracks the input of the filter, apart from a delay. How much delay? On the order of a few milliseconds. Yes, the milliseconds reduce, they become $1-2$ milliseconds, but it's still an object that is slow to react, slow as in $1-2$ milliseconds. That's why it's called ***delayed***, retarded. That's why it's called "the opening is delayed".

Sodium Channel (Variables m and h) In the case of sodium, of the sodium channels, which I'll finally tell you about before the interruption, you see that the activation curve m_∞ (I call it m instead of n, but the form of the equation is the same). You see that this too is practically almost indistinguishable, it's just a little more *steep*, a bit steeper, and maybe it's shifted a little more to the left, if I remember correctly. No, it's even shifted to the right, so paradoxically at slightly depolarized potentials, the potassium would open before the sodium, if it weren't for the fact that potassium is very slow.

Look at the τ_m, so the temporal dynamic of that equation for sodium (m), what values is it at? It's a **fraction of a millisecond**. I'm simply reading the axis. I understand that this too is not a constant but is a function that changes in time, in the end, it's a function that doesn't change too dramatically, it's a kind of Gaussian, a bell curve. Here it's on the order of a fraction of $0.1-0.2$ milliseconds. So that's why the sodium starts immediately and the potassium starts later, because the potassium has at least one or two orders of magnitude slower speed to activate.

But there are three of these graphs, because it turns out that because of that wretched (or not wretched, very useful) intracellular hatch that creates the fact that the channel can also be open, but it is **inactive**. And I told you this at the beginning of today's lecture, saying that "it's a useful thing to slow down and therefore make the duration of the action potential much *sharper*, much

shorter, more sculpted, more defined, shorter". To do that, I have to imagine that there's a temporal dynamic of that *gate*.

Currently, I'm thinking that a potassium channel, I imagine it like this, and here it has a kind of little gate. This little gate is a device that can jump between two states, open or closed. This is potassium, this would be selective to potassium, and since potassium (suppose here is inside and here is outside), the potassium ions go from inside to outside. For sodium, here too I imagine there's a little gate, but there's this thing that is also called the *ball and chain* model (chain and ball/sphere), or hatch or whatever you want, in which this little ball at a certain point blocks from the intracellular side.

You could also think that this object here is an additional little gate that, however, works with **inverse logic** (to use a term that might be familiar to someone in digital systems electronics). That is, instead of opening when the potential depolarizes (as the other one does), it works the opposite way, it closes. And in fact, if you look at this middle graph which has a state variable that isn't called n (for potassium), isn't called m (which is the activation variable for sodium), it's called h (for historical reasons Hodgkin and Huxley called it that), and it has to do with this inactivation *gate*. You see that this sigmoid works with inverse logic: that is, the more hyperpolarized you are, the more h is at 100%, that is, all these *gates*… are open. And it makes sense, because in that cartoon I showed you, when the potential was very polarized, very negative, the channel was closed (this is closed here, m is closed here), but the hatch underneath was open, it was wide open. Why? Because here h_∞ is at 100% or close to it. The more the potential tends to depolarize, the more (and quite rapidly) this tends to close.

With what speed does the sodium tend to track… well, the h variable tends to track its *steady state*? You see that τ_h, which is this dashed curve, is even **slower than potassium**. So roughly, let's say it's a factor of 2 slower than potassium, anyway it's on the same order of magnitude as potassium, relative to sodium. Sodium is a Ferrari, that is, at $0.1 - 0.2$ milliseconds it reacts quickly with that time scale, with that time constant. But the fact that then the hatch slowly closes and then the neighboring potassium channels also open has a latency, and this latency is given by this order of magnitude. Here on the order of a few milliseconds, here again from 5 to 1 ms, here from 8 to 1 ms.

So obviously the interesting things happen between -70, -80 mV and $+20$ mV, that's why this graph was made here. And with this description, I'll show you after the interval that we can make a detailed simulation of how it works, of how the action potential emerges. I'll stop here for ten minutes.

Assembling the Full Model

Okay, so here you see the story again of the hatch and the channel… the activation and inactivation part. So in analogy with what was said for the potassium channels, for the sodium channels (at least for their activation and inactivation), you have, in fact, a similar description, qualitatively but quantitatively, numerically different. Now these curves here, in a bit I'll show you, are ugly,

numerical functions, but it doesn't matter because anyway we're entering the condition where we don't do anything with paper and pencil anymore, we have to use a numerical method to simulate four differential equations (at this point, which I'll show you) and so we might as well throw in what's ugly because it was identified experimentally. There's a somewhat deeper component from the point of view of biophysical interpretation, but I won't talk about it, I'll just mention it.

Is There an Excitability Threshold? So a first interesting question you might ask yourselves is whether, having come this far, you could conclude if there is or isn't an **excitability threshold**, a discrete threshold that… that should motivate (if it's a motivated thing, and it might not be, it isn't) that describes a motto, a standard description in literature or textbooks, which says that neurons are **all-or-nothing** devices and that, once an excitability threshold is crossed, they emit (so if the input is large enough) an action potential.

Here it's not easy to pinpoint, there are no switches, there are no functions that depend on the membrane potential in a binary way. "Now the probability is zero, you stay in the closed state and shut up", or "now the probability is extremely high… the probability per unit of time is high to make the closed-open transition". There's a sigmoid that yes, is related to the step, is related to a *switch*, to an interruptor. In the end, the step corresponds, from the point of view of programming language, to that *statement* called `IF-THEN-ELSE`. `IF-THEN`: "if there's a condition then do something else". In biology, there are no Boolean laws or transistors, digital circuits, or at least they aren't like those made by man, so it's not so surprising that there aren't binary behaviors. And I'll point out that even in electronics, transistors don't have a binary behavior, they are used in a regime where the transitions are binary, but in some contexts of transfer functions, they are more similar to a sigmoid. This is a rather profound thing.

Is there a membrane potential above which all channels are open? No. Maybe around -50 mV, but there are considerations that I'll show you later. Yes, around -50 mV there's the midpoint of potassium channel activation at *steady state* (so imagine the transient), anyway it's not that the channels are… n is already directly equal to n_∞, it has to go there, it has to track it. And it's not just him in isolation, there's also sodium collaborating. But anyway, for sodium, you see that perhaps it's a little more… the midpoint is greater than this -50 mV. And for the h inactivation *gate*, it's even more hyperpolarized. So perhaps a value… from here, from this static analysis, of an excitability threshold (which you always hear about and which is even used experimentally, but in another context), there's no trace of it here. It's more of an analog system that has a propensity to make a transition. Here we're talking about a fraction of channels: "from this point on, 50% of potassium channels will be open", "here 10% of these inactivation hatches of the voltage-gated sodium channels will no longer be available, 80% or 90% are already closed", etc.

The Final Equations of the Model It's exactly the same thing repeated and written decently. I got carried away and wanted to immediately tell you this correspondence between time scales and *steady state* values that the state variable tracks. And here you not only find the n we discussed, but you have

by analogy the cases of which you experimentally have the graphs again, shown before, of the *m gate* and the *h gate*. I call them *gates* because, as I've indicated them here, beyond the fact that one works with a different logic, they equip the same sodium channel, they are not distinct channels.

How do I put these together? Because for potassium, we did it here, I know how to write the total potassium conductance, because I multiply it by… I have a total value… [text missing]… a maximum value $\bar{g}$ times the fraction of open channels, so times this n. So to the charge balance equation that was written here, $C\frac{dV}{dt} = \sum I$, I add three other differential equations, which are here. Ideally, one would want to add at least two more, one for sodium and one for potassium… one for the sodium channels and one for the potassium channels. For sodium, two are needed, because it has this strange characteristic of also having inactivation.

And we don't talk about it in this course (those who do the neuroengineering curriculum will meet me again next year), there's an enormous variety of voltage-gated channels, whose combination with similar equations (not exactly identical but similar, of the same form, with a τ, the variable $-$ variable$_\infty$) allows for describing the conductivity, the ionic conductance for many other channels. There are so many that some scholars call them… they define it as the **ion channel zoo**. So "if you are a pyramidal cell you have that set of 20 ion channels", "if you are a cortical inhibitory interneuron, which is called *fast spiking* because when you stimulate it, it fires very, very, very rapidly compared to a pyramidal neuron, it has another set of channels". As if nature had done what in video games in the old days you had the console and you put in a cartridge with a different game. Here, in the case of the squid giant axon, you only have sodium (voltage-gated) and potassium. So the correct name for these channels is: 1. **Sodium**: *Fast Inactivating*, voltage-gated (it inactivates rapidly). 2. **Potassium**: *Delayed Rectifier* (it's a delayed rectifier).

The "rectifier" part we don't care about, the "delayed" part I've told you now so many times that you're probably nauseated. There are many other channels, and the other channels can have the same type of complement, so every time you have a different current, you have one or two or three more differential equations. You understand why it's not trivial to possibly extract principles and, conversely, even to do accurate numerical simulations.

So, for potassium, we've seen it. Forget this exponent to the fourth power for a moment. For sodium, the way Hodgkin and Huxley experimentally found that things work (*fitted*), is by taking the two state variables that describe one *gate* (so a population of m *gates*) and a population of other inactivation *gates* (h), and **multiplying them together**.

$$G_K = \bar{g}_K \cdot n^4$$
$$G_{Na} = \bar{g}_{Na} \cdot m^3 \cdot h$$

Note that this product is not so trivial, not so unexpected, because when is the current and conductance active (non-zero)? When the channel is **both open AND not inactivated**. Since h works in inverse logic, when $h = 1$ it means the channel is *not* inactivated. The channel activates when $m \approx 1$. So 1×1

[correction: $m^3 \cdot h$]… will be different from 0. So the fact that the product is a mathematical operation that easily "kills" one of the two things when just one of them is small, is 0, accounts for a good agreement with the experimental data.

Here you see the individual currents and you see these exponents to the fourth (n^4) and to the third (m^3) only on this *gate*, on this state variable m and not on this one (h), because experimentally if you don't put them, you have a poorer agreement with the experiment. If you put 3.5 it's a bit better, but it doesn't go so well. If you put 4 here (n^4) and if you put 3 here (m^3) it works perfectly.

There is a comment, a deeper motivation for the fact that this is effectively… So, this is how Hodgkin and Huxley found it. In retrospect, with researchers in the '70s-'80s and subsequent years understanding the single, discrete nature of ion channels (before, it wasn't known they were ion channels), it was understood that both potassium and sodium channels are not the result of a single protein, but are the result of **four subunits**. As a first approximation, it's as if each of these subunits has a voltage-dependent domain and when you put them together… now I'll show you an image or maybe not, maybe next time… this is a kind of side section. I imagine it as a kind of object that has four of these "sausages" and when these four "sausages" get close, they combine (they are the subunits… surely Zoli is telling you this in a much, much more accurate way for synaptic receptors), but from a functional point of view, only when the "sausages" are close, then the pore forms in the middle of them. And their voltage-dependent properties are, as a first approximation, statistically independent and each of them satisfies a kinetic equation of this type ($dx/dt = ...$).

The fact that there is n^4 here and not $n_1 \cdot n_2 \cdot n_3 \cdot n_4$ is because these state variables are independent, since they all do the same thing, instead of writing $n \cdot n \cdot n \cdot n$ (there are 4), I write n^4. Here [G_{Na}] there's the same thing with the four subunits: one is responsible for inactivation (h) and the other three for activation (m^3).

There are obviously slightly deeper reasons related to another description that perhaps we'll see next week, we'll definitely see it next week, of a discrete, stochastic interpretation, which I anticipated is anyway allowed by the kinetic schemes. Whereby this n^4 or m^3, this product… the fact that there are products here, which is not a given (I've sold it to you now simply as a mathematical convenience from the fact that if you have four non-zero objects, you multiply them together, it's enough for one to turn off, you've turned off the product) is a very powerful thing from a computational point of view, whatever that means. From the point of view of probability theory, the product comes out because it's a matter of understanding that the channel is open when the joint event "all subunits are in an open state" (and there are four subunits, for example, of potassium). And the probability of a joint event where the individual events are statistically independent is the **product of the probabilities**. This has to do with set theory, I don't know if… you should have… those who have seen probability theory, you've seen that it was dished out to you starting again from set theory that you perhaps saw in some initial university math course. It has to do with the operation of union… and actually intersection. So the probability that "it rains today" AND "that I feel tired" AND "that the phone rings", since they are three independent events, is the product of the probabilities. And this

is why, for those who are interested, why there are these products.

The Complete System of Equations So: **four differential equations**.

1. **Charge Balance Equation (Potential V)**: The charge balance equation, to which we dedicated enough time to understand where it comes from, and to which today we can… we can complement with the fact that the conductances are anything but (in some cases, obviously for sodium and potassium; for this one [Leak] it's not voltage-dependent, so it has a passive, Ohmic, harmless form).

$$C\frac{dV}{dt} = -I_{ion} + I_{ext}$$

$$I_{ion} = I_{Na} + I_K + I_{Leak}$$

2. **State Variable Equations (m, h, n)**: But for the other two [Na, K], I need three other differential equations, because this is the Ohmic form where the state variable that says which channels are open and not inactivated for sodium and for potassium channels… [correction] …active… assumes… [pause, reorganizes] …so I lost my train of thought…

So the differential equations for each of these state variables:

$$I_{Na} = \bar{g}_{Na} \cdot m^3 \cdot h \cdot (V - E_{Na})$$

$$I_K = \bar{g}_K \cdot n^4 \cdot (V - E_K)$$

$$I_{Leak} = \bar{g}_L \cdot (V - E_L)$$

Whereas for the Leak, it's passive and doesn't cause problems. So to this differential equation (dV/dt), I substitute these currents which I can now finally write. And I add these other three differential equations which are of this type (dx/dt), which in the end, come from this little game of open-closed kinetic schemes, [including] with the difference that sodium has this exception.

$$\frac{dn}{dt} = \alpha_n(V) \cdot (1 - n) - \beta_n(V) \cdot n$$

$$\frac{dm}{dt} = \alpha_m(V) \cdot (1 - m) - \beta_m(V) \cdot m$$

$$\frac{dh}{dt} = \alpha_h(V) \cdot (1 - h) - \beta_h(V) \cdot h$$

τ has this form $1/(\alpha + \beta)$, x_∞ (where x can be h, m, or n) has this other form $(\alpha/(\alpha + \beta))$ that we've seen. It's simply a way of rewriting, but I was perverse because I had anticipated that it would be simpler to write τ_x and x_∞ instead of α and β. Oh well. This is for adherence to the Hodgkin and Huxley model.

Parameter Values (Squid Giant Axon) Here are the functions that are dependent on the potential, as found by Hodgkin and Huxley. Those of you with some biochemical or biophysical background might recognize something that formally has to do, in some cases, with a form of Gibbs free energy, where the electrostatic potential is the only field describing energy. But beyond this similarity that motivated Hodgkin and Huxley in their choice of the particular… You see an experiment, what functions do I put in? Nowadays, for example, one might put in a *DEEP* neural network, which is fundamentally a model to do a *fit* of a non-parametric function. It's like a Taylor series or sinusoid expansion… actually, it's a sigmoid series expansion in the case of a *deep machine learning* architecture. They had a biophysical background, so they said, "you know what, ion channels today, or as they thought of them, these molecules, these transporters, in the end, must obey the laws of biophysics and probably activate or deactivate based on Gibbs free energy." And that's why they wrote this type of formalism. But you can forget what I just said and just say "the *fit* of the experimental data was accurate when these were the waveforms".

Here are the values of the other parameters instead: * **Capacitance (C_m)**: 1 $\mathbf{F/cm^2}$ (let this be imprinted on your frontal cortex). * **Maximum Conductances**: * $\bar{g}_{Na} = 120$ mS/cm^2 * $\bar{g}_K = 36$ mS/cm^2 * $\bar{g}_L = 0.3$ mS/cm^2 * **Nernst Potentials**: * $E_{Na} = +50$ mV (or $+55$) * $E_K = -77$ mV * $E_L = -54.4$ mV (or -54)

You see they are all referred to the unit of surface area because we are describing a *patch* of membrane. If you tell me "no, it's a neuron that is spherical," okay, one puts in the values and you'll see that nothing changes, in the sense that in this equation you multiply both sides by the surface area (S). You would multiply here ($C \cdot S$), you would multiply here ($\bar{g} \cdot S$), here, and in fact, you would also multiply here ($I_{ext} \cdot S$). In the end, S is as if it cancels out, it doesn't change. The only thing that changes is if this I_{ext} is your pipette. Your pipette is no longer a distributed mechanism per unit of surface area, it's concentrated. At that point, you are injecting 100 pA of current or -100 pA of current. Anyway, this doesn't matter, it's just a consideration for the purposes of the simulations I'm showing you.

The Nernst potential is $+50$ for sodium, -77 for potassium, and -54 for the leak. These are the values one must use for the squid giant axon when wanting to capture both passive and active behaviors.

We'll see this next time, and I'll show you, also because you're tired, we're all tired, I'll show you something a little lighter, I hope.

Interactive Simulation (Google Colab)

On the site, if you go to the "Resources" section and go to the "Notebooks" part, you'll find at the bottom on GitHub (so here you find the notebook files, but I'm assuming you don't know… probably you know little about notebooks). Here you'll find, as I recommended you do in the first lecture, you'll even find buttons that, when pressed, open that notebook directly in **Google Colab**, which I remind you is a kind of cloud platform for, in effect, doing simulations

with Python, in effect, running Python scripts.

So the one I'm showing you now is called "Cell Excitability with Hodgkin and Huxley". And assuming it works, so I can use Google's computational resources for free, I'll first show you how I play with it and then I'll try to unpack the content, I'll show you the code, hoping to entice some of you to get your hands on it.

You are lucky today because you don't have to study a Python book, you can somehow use *Large Language Models*, in what is called, and is very fashionable, *Vibe Coding*. So even if you know zero about a particular programming language, but you roughly know the logic of it, you can get involved and you can write working code. The educational experience would obviously require your brain to stay attached. I don't care, and at the exam, it's not a programming course, but Bioengineering is a multidisciplinary field and the attempt is to tell you: either you're already equipped or I'm putting you in a position to do it. You should chew on math, biology, and *coding*, because the overlap of these, beyond the purely *data science* aspects, characterizes the bioengineer.

Since I'm a swot, in the first part of this Google Colab, I wrote, because I like writing equations with LaTeX (which is this formalism), it allows me to rewrite these equations. And they are written in a… they are rendered graphically in a nice way, let's say, with the exponent, as if you were using the Equation Editor in Word, but in a simpler way, especially one that doesn't crash like Word constantly does. I invite you to write your future master's thesis in Word and tell me whether or not you will suffer.

I'll run all the cells and the first thing I'll show you is this **all-or-nothing** behavior based on the Hodgkin and Huxley model, where I am the one changing the external current (I_{ext}) which is indicated by this current step you see here.

So note: to graph it on the same plot, I cheated, because the value of the current (positive or negative) has units of hundreds of picoamperes or actually in this case I think they are microamperes per square centimeter. I can read it here because in this slider I see -0.067. I believe, if I remember correctly, they are $\mu\text{A/cm}^2$, so that's 67 nA/cm^2. But to avoid having another graph next to it, I cheated and plotted on the same graph by multiplying or subtracting by a certain amount, so you have it exactly underneath. But it doesn't mean the current stimulus is -100 mV, it's simply… I'm the one who forcibly wrote that the plotted current was not the current I_{ext} (I used the same name as in the equations), but was $I_{ext} - 100$, so when I_{ext} is 0, you show it to me at -100 mV, and also multiply by 50 so you expand it a bit.

Let me hide the code right away, otherwise maybe… but I hope you don't get too scared. If you're brave you'll see it's not a particularly complicated thing. The other control parameter is not just the amplitude of the "flick" of the current step, but also its **duration**. It's indicated here I believe in milliseconds, so here it's about 1 ms (from 5… yes okay it could be, if these are milliseconds, 6 will be more or less here, so it could be). I can lengthen it, obviously, it doesn't work, but every time I touch this slider it should redo… okay, now it's redoing it. And I didn't want to show this, it's something else, so, okay.

What I'm doing here is giving a negative, **hyperpolarizing** stimulus, the am-

plitude of which I can change. And you see that the membrane potential which is… sorry, I have to go in the other direction… the membrane potential… (darn you action potential, emerging, now I'll tell you why it emerges obviously, afterwards, when I'm no longer stimulating).

When I set it to negative or even very negative, the membrane potential (which is this black trace) in fact does what a passive compartment, a passive RC, would do. It has a negative charging curve, if I make the duration longer it's an exponential arc exactly like the one we did with paper and pencil with the slides last time. I turn off the stimulus, the capacitor recharges.

The "Rebound Spike" Phenomenon So, I get an action potential here because by hyperpolarizing… In this graph here, you see that it's especially the fault of the **inactivation (h)**. When I'm around -70 mV (as I probably am here, I'm around -60, here suppose around -60, -70 mV), the inactivation (h_∞), so we're here, is not completely at 1. There's a little bit of inactivation. So all the other channels that are voltage-dependent adapt to a *steady state* where the sodium channels are [partially] inactivated.

If I abruptly bring the potential to about -90 mV and then let go (so I bring it almost here to -100 mV), I am forcibly removing all the inactivation from it. It's as if it were… I'm tempted to say, a withdrawal symptom, I don't know if it's a good comparison. I have constant inhibition (inhibition in the sense of constant inactivation) and temporally, for that input of mine which was meant to say "see, it's negative, I'm going in the direction of non-excitability," the neuron must not fire during my stimulus. Because during the stimulus, both sodium and potassium turn off even more, it's all *boring*, it's all dull.

Things are a bit more complicated because by removing… by hyperpolarizing the neuron, I **remove the residual inactivation** from it. And when I let go, I get a ***rebound***, an explosion. It's like when… again, it's similar to a withdrawal symptom where I always take some drug, something, when I stop taking it… [it's a] wrong comparison. It's more like a mechanism of constant inhibition and as soon as I remove… it's more similar to the brake. I'm driving a car with the handbrake slightly on, if by chance I release it, clearly the car can accelerate a little more. In this case, the car's acceleration leads me, after several milliseconds, to have an action potential.

This is an excitable behavior that is seen experimentally, and in the model, looking at the model you would have said "no, man, it's only when you put in a positive current that you at most get passive responses where, okay, here you're depolarizing a bit, but you don't get *spikes*." If you increase it a little, there's that sort of sigmoid, you're still not triggering this positive feedback between sodium, sodium inactivation, and potassium. At a certain point… not even now… at another point… nothing, the behavior is similar to the passive behavior.

And at a certain point, if the current is large enough, here a sort of… you see there's a discrepancy between the black trace and the violet trace (so you can think of the violet trace as the passive behavior, there's still something different but I'll introduce it later). Here there's clearly something **non-linear**, which isn't explainable by an RC circuit, so something has started to activate,

but it hasn't activated enough to recruit other sodium channels to trigger this explosion. Surely the potassium hasn't had a chance to intervene to bring the membrane potential back down. So this is the classic thing you would have expected at the limit. So okay, there's a charging curve, at a certain point, however, the potential is so depolarized that it's cascadingly recruiting the sodium channels which open, inactivate, and the potassium conductances bring the membrane potential back down.

One thing you could do would be to "tinker" (as it's technically called) with the code and instead of, at a certain point… this isn't where I wanted to do it… like this, okay here… instead of plotting the variable V… in the end, it's a system of four differential equations. Why V? Because V is what I measure. M, N, and H, I can measure them indirectly but I need an amplifier with this *voltage clamp* that Hodgkin and Huxley invented, which we have in the lab and use frequently for experiments of this type. But in a simulation, I potentially have the power to do anything, because I just need to, instead of saying "plot"… (I don't think I save it here), so if I say here… I'd have to change… instead of plotting the potential, I could plot m or n or h (there are three). And so simultaneously show you how, instead of just the potential, the variable h, the variable m, the variable n change in the $0-1$ *range*. Or I could combine the variable m and h in that $m^3 \cdot h$ to represent how the sodium conductance changes, if the sodium conductance opens and then closes, and the potassium conductance which opens and closes with a certain delay.

If you want, I invite you, and if you have problems I'll help you do it, it could be a stimulating thing. I'm convinced that you can do it by pure analogy just by looking at the code. And now I'll tell you about the code, I'm not giving you a computer science lecture but I'll tell you by pure analogy, even if you know nothing about Python and *coding*, how it can make a little bit of sense. The only thing that would be important on your part would be to review the Euler method, for the numerical resolution of equations. But for the moment, let me play with this system a bit more.

So, the story of the *rebound* potential wasn't there, it wasn't easily… it wasn't visible from the model, and it's, in a way, an additional prediction.

Frequency Coding and Periodic Activity One thing that the model (and a colleague of yours mentioned to me during a break) is: what if the stimulus were maintained? Now in this simulation, I'm only simulating 20 milliseconds, but in the next one, I do more. If I continue to maintain the stimulus, I don't give a *step*, a flick, but I continue to maintain the current sufficiently intense to be able to make the neuron fire… Because if the current is lower, okay, I can keep the current on all I want, but nothing happens. Yes, there's evidently a non-linear transient here due to the active conductances (sodium and potassium), but then it stabilizes at a new *steady state*.

If I change the current and the current isn't just a pulse, you see I get **periodic activity** of several action potentials. And by changing the current (you can't see it much here) the frequency changes. One thing you see here is that by changing the current, the amplitude of the action potentials starts to… suffer, it

starts to become much smaller. And this is what's seen experimentally because if I ask the neuron to keep firing, I'm engaging it in a dynamic regime where a part of that blessed sodium inactivation (h) is never removed. Even though potassium tries, the membrane potential never goes to particularly polarized potentials, and this wretched h, this h *gate*, continues to persist in this [low] region. Note: h_∞ is an instantaneous quantity. h evolves with this dynamic equation that has inertia, so it has this τ_h. So it's true that h tracks h_∞, but it does so with inertia, and this inertia also involves persisting and not allowing the potassium… [correction] …it continues to not be present… [correction] …it continues to not allow a reset of the previous conditions.

What I'm showing you here is the same thing, but I keep (and I probably change the scale of the currents) I keep the current on for an "infinite" time (here I stop the simulation after 300 milliseconds). When the current, the amplitude of the current is in this case 120 A… obviously it doesn't work anymore, damn it, damn it, the slider doesn't work anymore, there. If the current is hyperpolarizing, nothing interesting happens. This is a trivial thing, but it's only in one direction that you get excitation. So if you imagine the brain or a circuit of neurons as an interacting system, neurons talk and (you know from Zoli, and we'll see it too) exchange information only when they are excited. They are excited in one direction, that is, when they are depolarized. When they are not depolarized (with the notable exception of electrical synapses or *gap junctions*, which Zoli probably didn't tell you about) they don't communicate. It's a system, it's a network of units that are isolated. They only communicate if the neurons go "above threshold".

When the current is increased, has a sufficiently large value (so it's not enough for it to be positive, it must be sufficiently large)… for example, now it is… I don't know why it doesn't… okay… the behavior is not that of having sustained *spiking* activity, in this case, the current is too low, you get a single action potential and that's it. This business of the action potential is also due to the condition… the choice of initial conditions. It's a system of differential equations, it requires the choice of an initial condition for V, m, n, and h. This, obviously, can change things in the transient.

However, if the current continues to increase, you have an extremely simple example of **coding** a quantity (in this case, the amplitude of the current) into **frequency**. If I increase the frequency, beyond the fact that the amplitude of the *spikes* will decrease a little (I don't know if you can see it), the *spikes* become denser.

Type 1 vs. Type 2 Excitability I'll show you something remarkable, which is a, again, a prediction of the model that *fits* with reality. You see that if I (and I assure you this also happens when changing the current in arbitrarily small steps) pass from a condition where the output frequency is zero, it's practically zero… I define frequency, so a periodic activity of membrane potential oscillation which, for example, in a time window of 300 milliseconds, allows me to calculate the frequency as number of spikes/300 ms. (An alternative would be, since these *spikes* are regular, to take the interval between two successive *spikes* and take

the inverse, but let's just count them).

Here the frequency is still zero. If I try to increase it a little, you'll see that it doesn't go from zero to… [waits for simulation] …you see, it does little… that it passes from 0 to a frequency that, if I remember correctly, is about 30 spikes per second… let's see. Between 100 and 200 it's 1, 2, 3, 4, 5, 6, 7, 8. That's 8 spikes in 100 milliseconds, so that's 80 **Hz** (80 spikes per second).

If you can imagine it, a kind of graph where you have the current (the stimulus) on the X-axis and the frequency on the Y-axis (the famous Rosetta Stone I was hoping to have but don't, in the first lecture, the first or second week), I have a *mapping* between the stimulus and the frequency. And it's, for example, what some sensory fibers in my spinal cord are communicating to me. If you have an electrode and you put it in the dorsal part of my spinal cord, you would hear that the frequency codes for weight: I add weight, the frequency increases.

In particular, the excitability modeled this way for the squid giant axon seems to lack graduality. It passes from 0 to 80 Hz. Then if you change… (there were about 8 spikes in 100 milliseconds). So, if I count them here, you can see by eye that there are more, but it's 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15… 15. We've reached 150 spikes per second.

So there is a monotonically increasing relationship between stimulus intensity and frequency, but at the excitability threshold, at the so-called **rheobase** (which stands for… we'll see this term later: the minimum current value to get sustained oscillatory activity), there I have a first-order **discontinuity**, meaning it passes from nothing to 80 Hz. Not all neurons are like this; these are called **type 2 excitability**. And it's particular because it depends on the specific values, so on the position of these activation and inactivation curves.

I won't do it, but it would be enough for me to slightly change the position of this sigmoid, *shifting* it by about 10 millivolts, and you would have a **type 1** behavior, where the frequency-current curve would instead become capable of firing at arbitrarily low frequencies (3 spikes per second, 4 spikes per second, 10, 50, 80, 120). But it would no longer have a discontinuity.

Again, if you were brave, you could try to get your hands on it. And where would I think of putting my hands? It could be in the value… we said, of activation. In these curves, α and… unfortunately I gave them to you as α and β, you could rewrite them as m_∞, but you could simply play with these numbers here. +40 you could change to +50. Now I don't remember if… well… I have to shift to the left, so I have to… no, maybe I have to put +30 or +20. You would have to do it in both the numerator and the denominator and maybe also decrease this. You might discover that the behavior remains excitable but with different characteristics. These are all things at a more advanced level that should or could (the hope is that they do) stimulate your curiosity and make you try to get your hands dirty. I won't ask you this at the exam, so it's your idea whether you're preparing to get 30 on the exam or if you're preparing for a professional life. This is *up to you*.

The Refractory Period: Absolute and Relative Instead, I'll show you something more interesting, more important, that I will certainly ask you about at the exam, which is titled the concept of **refractoriness**. Again, this came up during the first or second break of today's hours, today's lecture, where a colleague of yours asked me: "yes, but doesn't a little time have to pass before the system is ready again to fire a new action potential?". In the end, here the excitable behavior… it's not like two *spikes* can be arbitrarily close. If I arbitrarily increase the current here, at a certain point the excitable behavior turns off. And it turns off because I'm no longer able to remove all the sodium inactivation, so the neuron remains stuck in a condition where part of the sodium remains inactivated, it never gets reset, potassium can't do anything about it.

I'll show you this in this other simulation, which has a more interesting paradigm to understand. Here I give **two stimuli**, very very brief, and they are separated by… in this case, it's about 30 milliseconds. It's these two flicks in red, and each one is controlled by a different slider. The value of these currents, as I've chosen them, is such that in both cases I give the flick, I momentarily manage to cross a kind of point of no return (which is around -60, -50 millivolts), so the action potential is generated because the sodium starts to open and a chain reaction is generated. And so I see that a *spike* is generated.

Personally, I could look at the profile of action potentials for hours because it's surprising that these impulses come out, which are more… engineering language. The concept of an impulse or coding with a binary component is more of a thing from electronics and digital systems, not from biology and billions of years of evolution, but this is a personal consideration.

Watch what happens if I narrow, if I decrease the interval between these two impulses. At a certain point… (nothing happens because Google Colab hates me)… at a certain point the second *spike*, this one, won't be there anymore because the system hasn't yet recovered from emitting the first *spike*.

So, we're still at 20 milliseconds. So the refractory period I'm looking for is now less than 18 milliseconds. It's less than… okay, at 16 **milliseconds**, you see that here this… and I haven't changed anything, it's exactly the same current amplitude. Is this **absolute** or **relative** refractoriness? Which means: if I increase the current of the second impulse here, can't I maybe manage to wake up the neuron? Yes, I wake it up. It's **relative**. It means that not all the sodium channels have de-inactivated (some still have inactivation), but if I push sufficiently, I can manage to make the neuron fire another time.

So, aware of this, I decrease it even more. Okay, I got to 16, suppose for time reasons I go to 13. At 13, I try to increase the current. Now, evidently, the current has to increase quite a lot. It might be that I can't do it anymore, that it's an **absolute** refractory period. Let's see if that's the case or not. There's something that seems to be generating, so I'm optimistic.

Here too, a consideration: here the shape of the *spike* is highly **stereotyped**. I've triggered the same cascade mechanism and the shape and duration are practically superimposable. I'm telling you this because in a moment, by decreasing the interval between the two a lot, you might think that when I increase the current a lot (at this point it was 3 $\mathrm{A/cm^2}$, now it's 13 $\mathrm{A/cm^2}$), you might

interpret this black *blip* as an action potential. In reality, it's not an action potential. The action potential, I showed you before, was similar to itself. So probably here I'm already in a state of absolute refractoriness. There's nothing to be done. Usually, Murphy's law dictates that now… nothing, okay.

Even at the maximum I can, for 10 **milliseconds** the neuron is refractory. If I do 1/10 milliseconds and multiply by 1000, I get a value that is similar to a frequency. In other words, if a neuron cannot emit (at least with these isolated flicks) two *spikes* closer than 10 milliseconds, it means that the **maximum frequency** is less than 100 spikes per second, 100 **Hz**. 100 Hz means the period is 1/100, which is 10 milliseconds.

Things may not be like this, but the frequency-current curve that I made you imagine in your head, might not only have this discontinuity (in the case of this type 2 excitability), but it might not be a curve that grows linearly, it might have a **saturation**. This saturation (we'll possibly talk about it in the next few times) is again at the basis of *machine learning*, of the analogy with units that function as *threshold linear*: they have a threshold before which the input doesn't make the unit fire, doesn't activate the unit, after which the behavior is not linear but is sigmoidal, it bends.

I'll finish here, next week we'll continue, suggesting you play with this. I don't think you've had many courses so far (or that you certainly will have at Modena) where you'll see the convergence of these things: the computational part, the data analysis part, the biological part, the electronics/amplification part (even if I'm not aware of how much… I don't remember what my colleagues G. Bertoni and G. Baldi… do with you… about amplifiers of objects), in a case that converges in the case of biological signals. I'll close here, thank you for your attention. All right.

Announcements and lesson plan Today, the idea is to conclude the part on **neuronal excitability**, continuing the introduction of some concepts related to higher-order phenomena with respect to the excitability described at its minimum, as I have introduced it to you so far, and also moving on to a non-deterministic, **stochastic** description. In the end, I emphasized to you that excitability is not due to continuous distributed properties of voltage-dependent ionic permeability of a membrane, but is linked to the characteristic of certain pores that are discrete and small. And when something is small, especially in a biological environment, at physiological temperature, it is associated with phenomena that are not deterministic, they are therefore… in particular, I am thinking only of thermal agitation.

And I believe in the second half of today's lesson, we will shift our focus to the description of **synaptic communication**. It is fundamental for the subsequent steps because the same formalism with which we described ionic excitability is related to the excitability of the membrane due to voltage-dependent ionic conductance, and it is related to what ionic conductance is linked to **ligand-dependent** permeability properties, not voltage-dependent.

Review: The first-order differential equation and filters This is again the model of four differential equations that **Hodgkin and Huxley** first de-

scribed in the 1950s in a series of articles. A book was published recently, a few years ago (I don't have the electronic version but the paper version, I can lend it to you if you want, I don't think the university library has it), in which the articles were collected and there is a *commentary*, there is a description, a comment on the various parts.

I wanted to revisit the heuristic concept I tried to convey to you last time. When in other terms you have a differential equation of the type dx/dt equals, *whatever*, $-x/\tau + f(t)$. Although rigorously the solution to this differential equation, where $f(t)$ is a known, time-varying forcing term, is rigorously the sum of two terms: the solution of the associated homogeneous equation (which means the equation obtained by removing this part) is a decreasing exponential, apart from the initial condition, apart from the constants to be identified; plus the particular integral which in the general case is not done in any other way except with the convolution integral.

Convolution between this quantity… involution or filtering, it's a filtering operation between the impulse response, which is a decreasing exponential (which is zero before and a decreasing exponential after), and the function itself.

But beyond this mathematical formalism, I have somewhat indicated to you that if τ is small, or conversely, if the speed with which f, the forcing term, changes over time is slow (it's a low speed), it's as if $x(t)$, roughly, is following f. This is a rough estimate, it's an approximate reasoning, but it's very useful.

Those of you who have an engineering background and have a minimum of sensitivity from the point of view of systems theory, recognize that this description is the same one that engineers love to death, where there are black boxes, where woe betide opening them, woe betide opening and writing the mechanism (albeit with phenomenological components), in which here I only have the impulse response, in time or as in some transformed frequency domain, in which the input $f(t)$ passes and is converted into the output. This is a filter and in the specific case, it's a **low-pass filter**.

Those of you who have some experience in electronics will recognize, beyond all the things we've discussed in recent weeks, this is the equation of an **RC** [circuit]. And you know the RC, in this configuration, when this input at this node is the current $F(t)$, it behaves like a low-pass filter, that is, the relationship between this current $F(t)$ and the potential across that capacitor is a relationship of the type that dampens fast changes.

The Quasi-Steady-State Approximation When obviously either τ is very large or conversely, when f instead varies very rapidly, it's not that there's much tracking by… the signal varies too fast, so this approximation of **quasi-stationarity**, in which I basically say it's as if the equation were always at *steady state* (that is, this term doesn't change), even if it's wrong because we are in a dynamic regime… but if this is zero, x is proportional to f.

This serves and is very useful for understanding what happens during an action potential, remembering that the individual gates of the sodium channels, you remember **M** and **H**, which are hanging there (why two? because there's the

hatch that closes from the intracellular compartment, inactivating the channel, while there are the gates, perhaps three gates, since it appeared as $m^3 \cdot h$, there are three gates that represent the activation of the channel), **N**, the four n gates of the potassium channels again, are all variables that satisfy a differential equation that ultimately is this one.

So n and n_∞, m and m_∞, h and h_∞, in the end, apart from different constants (perhaps written this way it would be dimensionally better), but anyway, apart from the different time scales on which the individual channel kinetics behave, roughly, sooner or later, the individual state variables would like to go to the value that m_∞, h_∞, n_∞ have respectively.

And these m_∞, h_∞, n_∞ I sold to you as identified experimentally, *fitted*, identified on what are experimental recordings made, okay, with a complicated **voltage clamp** setup using toxins to disambiguate, to decouple the currents. Okay, and they have a sigmoidal dependence on the potential.

The Activation Curves and Threshold Behavior You remember I tried to pique your curiosity, so m_∞, h_∞, and n_∞, roughly, except for H_∞ which does something the opposite (where this is **0** and this is **1**, the channels all closed or all open), M and N are roughly sigmoids, more or less of the same alignment, which have the intermediate midpoint at the same potential value which I remind you is roughly around **-50 millivolts**. So it may be that on the exam I will ask you to draw a graph like this and I will ask you "can you roughly put the order of magnitude?". And so here it will be more or less **-100**, **-90**, roughly, it's not **-2000 millivolts**, it's not **+500 volts** and here it will be roughly **20 millivolts**. I'm probably misremembering, but it's roughly just to make you understand that the *span* over which these proteins have evolved to become sensitive to the potential is exactly in the *span* in which the membrane potential varies, and conversely you could say "no, it's the membrane potential that varies in this *span*, in this interval".

So this is the dependence, okay, to finish the sentence… several sentences I left hanging a moment ago… I was trying to stimulate the fact that this is as if it were a kind of **threshold behavior**. If it were a rigid step, it would be "if you are below a certain value you are off, if you are above a certain value you are on". I tell you this because a large part of *machine learning* models based on artificial neural networks have that type of threshold behavior, they don't necessarily have a *smooth* behavior, smooth, "smoothed" (smoothed is ugly), sigmoidal. But nature makes sigmoids, it doesn't necessarily make switches/steps. Even transistors are not actually objects that are strictly switches, they are analog devices.

Graphical Analysis of State Variables (m, h, n) So, if I take, instead of just plotting V as a function of time, as you see here V_m (membrane potential, this is a graph taken from some book $V_m(t)$, we called it V, again this is the potential inside versus outside, but we set outside to zero because conventionally we can do that), I can not only plot V, I can plot $m_\infty(V)$. I know what the

instantaneous relationship is, it's an instantaneous non-linearity and so I can, if V changes over time, I can see how m_∞ changes over time, how h_∞ changes over time and so on.

And you have it here, the "infinities" (m_∞) are the dashed ones or *dashed* (I don't know how to say it), with the little bars, with the little lines. For example, you see that the m and the n which are the most interesting ones for me (one is for sodium, the activation of sodium, the other is the activation of potassium). It's true that M and N have a different time scale, so much so that that one is called *delayed rectifier*, voltage-dependent potassium channels, delayed. But when you represent m_∞, h_∞, and n_∞ there is no time, in the sense that there is no dynamic, there is no delay.

And it's a useful representation for intuition, for our intuition, because you see that if m_∞ has this behavior, it would have this behavior, because the action potential is changing from hyperpolarized values to depolarized values and then back to even more hyperpolarized values. So this yellowish curve (the one I always forget is that this light blueish one is called magenta and there's no way I can remember it), this yellowish one starts from **0.3**, **0.4** because evidently when the potential is at **-60**, **-65**, M_∞ is not **0** and starts to be at least around **0.4**, so the graph might not be accurate, my *sketch* on the blackboard.

You see that when the potential changes, M_∞ changes, it copies it. The same thing happens for… sorry, M_∞ was the magenta one, it's this one here. The graph is ok, in the sense that when the potential is more or less at the resting value, these sodium channels M_∞ are more or less closed. For potassium, the curve is evidently *shifted* more to the left, which is to say at the same potential value I have a greater activation for n_∞ compared to m_∞ and you see it starts from here, but more or less temporally it does the same thing because time isn't there, time is decided by the potential trace. Whereas H, you see the behavior is exactly the opposite, this violet one, it starts from values closer to unity, **0.6**, **0.7**, so even at rest there is a portion, a fraction of voltage-dependent sodium channels that are in an inactive state. At these depolarizations, it's already no longer **1**, it's a bit lower than **1** and it does the opposite thing, so when the potential increases it turns off, this H gate turns off and when the potential hyperpolarizes it returns to being not inactive, and then to later return to a more equilibrium value which is probably around **0.6**. During a firing, during a regime where there is a sustained succession of action potentials.

I'm showing you this because if you then look, if you compare M_∞ with M (so this magenta trace and this blue trace), you see that they roughly do the same thing. Why? Because the activation of sodium is practically instantaneous, it's very fast, I reasoned it out for you, it's one of the fastest biological objects that exist. In a fraction of a millisecond, this opens. It was due to these τ's, which yes, I agree, are voltage-dependent, but roughly it's a quantity, a small number, that τ_m.

Conversely, you see that if you compare the yellow to the red (so n_∞ to n), you see that n is lazy, it doesn't follow, I mean it follows, okay, it follows, so this reasoning is ok, but it follows with an even greater delay. And the same goes for h, h is anyway delayed, if it weren't, the action potential wouldn't exist. h you must compare the green to the violet. You see that roughly N (the red

compared to the yellow) and H (the green compared to the violet), seem to have a similar time dynamic.

This could have implications that we will not address in this course on **dimensionality reduction**. It's possible to write a system of differential equations that has two instead of four, in which in other terms the sodium m in the end is always at *steady state*, so I can throw the differential equation out the window, I can directly consider that substantially τ is so small that it's always at *steady state*, so dm/dt is gone. And the equation for n and for h in fact, you see, they even seem almost a mirror image of each other if there were an axis of symmetry around **0.4**.

So for those of you who will follow the curriculum on neuro, there will be a modeling effort of complexity reduction and from this graph here you further understand why the issue of time constants, particularly H and N, are fundamental to allow the action potential to go up and down.

The Biophysical Reality: Markov Kinetic SChemes In reality, in particular to revisit what I mentioned last time and a little while ago, the fact that from the point of view of an intuitive description, I imagine the sodium and potassium channels, since the currents… I'll take the example of the sodium current, which is a maximum conductance times… what? $m^3 \cdot h \cdot (V - V_{Na})$. I imagine it with these little gates, of which there are four, one of these is different, but… oh, I have the blue color.

It's not exactly like that, I sold it to you from a biochemical, molecular point of view, as the presence of different subunits, for example, seen from above, this object from a molecular point of view is the assemblage, the assembly of four objects that only when they are close create a pore and each of these has its own dependence, its own dependence on the transmembrane electric field, therefore on the membrane potential inside versus outside.

In reality, doing an experiment, the **kinetic scheme** that best represents the dynamics of potassium activation, the potassium conductance (so the study of the currents and their conductances), doesn't have a single *gate* and then the current has these exponents **3** and **4**, but in reality, there are many states: **1, 2, 3, 4**… there are **5** states of which only one state is conductive, associated with a conductance. And the same goes for sodium, you have **eight states** in which in fact it's as if structurally there were a symmetry and it depends, this branch above, this branch below, on the state… if you like, if I write them like this with states (state number one, state number two), there is no symmetry. If instead I put these M_0H_1, M_1H_1, in some way I'm thinking in these terms, but in space the structure is this, in which for each of these configurations H can be in two states (either active or inactive), and in each of the states for which each *gate* (M_0, M_1, M_2, M_3) are active, I can *flip* from one to the other.

I won't talk about it and I won't prove it to you, but this type of **Markovian** kinetic scheme with these specific coefficients can be shown to be equivalent to the product of the individual *gates*. So the model I told you about (open, closed, maybe repeated for each of these subunits, which are identical and statistically

independent) continues to work, but strictly speaking, the states through which the overall protein moves are multiple.

So in theory I should write a differential equation… I would probably write a system of **5** differential equations or **8** differential equations. Remember that in kinetic schemes they are all linearly dependent, so I can remove one because I can write a mass conservation relationship $n_0 + n_1 + n_2 ...$ equals **100%**, which means the protein is in one of those N states, it cannot be in another state. But strictly speaking, I could then put here only one number, a state variable which is the fraction of channels in that state n_4 or in the state $m_3 h_1$ assuming that is the conductive state.

I'm telling you this because in a moment we'll move on to the **stochastic description** and the stochastic description requires the most accurate, most extended description possible of the kinetic scheme of the channels we're talking about. So this is simply a slide to say: that type of kinetic scheme is reduced because for that choice of values that seem to be the ones that *fit* the most, it turns out that the four subunits are independent and so it's as if the open state were the simultaneous occupation of four subunits in the same open state. For sodium, there is one that, however, is an exception to the *rates* which are different.

Exploring the Model: Does a Threshold Exist? I encourage you (you have some figures, but you have the code, you have the Google Collab Notebook), even if you don't want to get your hands dirty writing numerical methods, changing the values… you have a damn *slider*, you can play with it and you can answer some questions that maybe I already stimulated last time.

Is there a **threshold**? Is there a value of external current, for example, and if one applies a value $-\epsilon$ (where ϵ is an infinitesimal quantity) the neuron doesn't fire, and if one applies that value $+\epsilon$ then the neuron fires?

The consideration is that there don't seem to be biological *if-then-else* statements, there isn't a kind of rigorous condition. Or conversely, the steepness of these sigmoids is not so extreme as to think that a threshold value exists for which, once exceeded, for example a threshold value in the potential (not in the current, but one influences the other), automatically, *boom*, maybe not suddenly but with a certain kinetic, all the channels switch from open to closed. No, it's a more *smooth* process.

And it's a *smooth* process probably because it's the collective effect, in this deterministic population description, of the effect of many, many units, which in themselves are gates that are either all open or all closed. If I have a door, right now it's either closed or open (even if it's ajar, it's open). Instead, a population of… you should think of windows in a large building with many, many windows. Probably when you have many windows the behavior, for example depending on the outside temperature (if it's very cold they'll all be closed, if it's hot all the people in that building, in those apartments open the windows), there will be some kind of gradation. But the question is: in the end, I see the electrical behavior of a neuron which is the result of this collective of voltage-dependent

channels. Is there a threshold? Try to play with it.

The thing you might encounter is that you would have to decide what the stimulus is. If the stimulus is a DC pulse, a constant current, which is constant (now here I've shown the transient but only to make you understand that I turned it on, from zero it became some value $I_{ext} = I_0$, something like that). This is one choice, but it's not the only choice.

In the simulations I prepared for you so that you could play with them or get your hands on them, modify them, break them, change the parameters, explore them (something you couldn't do with the same ease in a biological experiment), you also have other cases where the stimulus is a small pulse, a small rectangle of current, always constant, but for example, this is **10 milliseconds**.

So you would realize (I hope you will realize) that it depends on the stimulation protocol. Whether there is or isn't a threshold, what you would conclude "yes, a threshold exists", depends on the protocol. In a way, it also depends on the **integral underneath**. And the integral underneath shouldn't give you mathematical nightmares, but if you remember that in some way current means a charge per unit of time, when you take the integral of the current with respect to time, it's as if you were obtaining the transferred charge.

All these things are trivial for those who have an intuitive idea or a mathematically accurate one or field experience with a capacitor. When you inject current into a capacitor, it charges, and obviously, it charges all the more, the longer you are applying a current. Same thing.

Temporal Integration and Summation This concept of **temporal integration**, then, is partly linked to the capacitive properties of the membrane. Partly, it is obviously also linked to the active properties, to the fact that you have channels and conductances that are potential-dependent and so you could try to investigate what happens if you have a sequence of these little rectangles.

Now that I've told you the story of integration you would say: "ok, I get the game, here you're basically saying that if the integral, if the area under these little pulses, maybe they are very small pulses, but they are repeated in time, if they are repeated in time and if there's a sufficient number of them, then the neuron, hence the membrane potential, the capacitive properties of the membrane will sum up and the neuron will fire".

If you play with it (changing the stimulus in one of those simulations isn't complicated and you could easily resort to the various ChatGPT, Gemini, etc.), you might realize that it also depends on how much time passes between one stimulus and the next. In the end, it's a **leaky capacitor**. So if you do nothing, to my great joy, the potential decays exponentially because it's a dissipative system and it doesn't explode. So if you wait too long between one pulse and the next, you lose dynamic memory of what has been in the past.

So the story of temporal integration is seen not only from the point of view of charge transfer to make the neuron fire, but in general, it has a relationship with what is also called **summation of pulses**, summation of stimuli. In some

way, the membrane potential behaves like a variable that memorizes, keeps in mind, then forgets. And it's fundamental to forget things, even from a cognitive point of view at a very high level. If you were to remember in exact detail what you ate **12** years ago at **8:30** on **November 11th**, probably… at least this is what happens in an artificial system like a Hopfield network, you might have a catastrophe, that is, the inability to store any more stimuli. This thing about the learning catastrophe is a very interesting theme clarified by statistical mechanics, so by fields of research completely distant or distinct apparently from biology.

So even at a cellular, dynamic level, the fact that if I leave the room at a certain point (a time I hope is not so brief) you would forget me, has the effect of saying: "if the stimuli are frequent enough, I fire; if they are too sparse, if their frequency is too low, I don't hear them anymore, I forget them".

This you could play with on the generation of the action potential and the activation speed of the individual gates. In the end, you have the numerical values. You could simply multiply by **10%**, or divide by **10%**, the absolute values of those taus, τ_m, τ_h, and τ_n, and see if the thing continues to work. In some directions, you would find that the action potentials become very broadened, in others you would find that perhaps they initially become very, very narrow, but then it may be that when the time scales become comparable you don't see anything anymore, because for example, the sodium activates just as fast as the potassium and they cancel each other out as they go.

The Frequency-Current (F-I) Curve The interesting thing that I'll show you in a moment, and again you could easily do this, is to systematically change the value of your stimulus (assume for simplicity you do it when the stimulus is constant). And like last time I showed you I counted **1, 2, 3**, I think I did it here to pull out what the average firing frequency was and I told you that for the Hodgkin-Huxley model it's a **type 2** excitability model, it goes directly from **0 spikes/second** to a certain value, I think it was around **50, 60, 70, 80 spikes/second**.

What I'm suggesting to do, in other terms, is to systematically change the amplitude from zero. Each time you have to run a new simulation, just as experimentally each time we have to let the neuron rest, change the setting on the electronic amplifier or in the computer and say "ok, now I'll inject you with another current with an even greater amplitude, and even greater". So I'm thinking of a protocol that… increases, sorry, these should all be horizontal, so they are all constant values, for example, I can double them, each time I add **10 picoamperes** and each time if I do it over a sufficiently long time I can estimate how many spikes per second there are.

We'll see this together now because I'd like to give you the intuition for why when I increase the stimulus, I increase the firing rate. Maybe I said it here or I said it on Friday, I don't remember, maybe in both cases. There are some neurons in the dorsal part of the spinal cord that encode the weight of a proprioceptive stimulus that is, for example, in my hand right now. If you double the weight on me, right now I have a neuron in the dorsal part of the spinal cord that is firing at a certain frequency, if you increase the weight the frequency increases.

It's not necessarily linear (doubling the weight doubles the frequency), here too it's not necessarily that by increasing the current by a certain number of times, then the firing frequency increases proportionally, but it does increase, the behavior is monotonically increasing. I'd like to make you understand why, and it's relatively trivial, and it involves talking about the **first-passage time**.

Refractoriness and Ionic Concentration **Absolute and relative refractoriness**, we talked about it, I showed it to you (correct me if I'm wrong) in one of those stimulation exercises where with the *sliders* I demonstrated to you when a neuron became partially or totally insensitive to stimuli.

This we haven't done, I'll show it to you now. What happens if I change the **extracellular concentration of potassium**? Which you'll remember, potassium is highly concentrated inside, it's in low concentration outside. So if I put a lot of it outside, what happens to the **Nernst potential**? The reversal potential for potassium currents? In terms… in what terms? The number that comes out, how does it change? Normally it's **-80 mV** because if it lowers…

Perfect, perfect. It tends to become **0** because the logarithm of the ratio, if it were the same inside and out, would be logarithm of **1** which is **0**. So it's **-80** because there's this imbalance. If the concentrations become comparable because it increases outside, what do you think might happen if the reversal potential for potassium currents tends to increase, given that the role of potassium currents is to pull the action potential down? Any ideas?

I hope it doesn't happen to anyone, or that none of you suffer from this medical condition, but you would have an epileptic seizure, one of the many ways an equation [sic] can be triggered, because somehow the resting potential, even the resting potential itself, tends to become more depolarized and so sodium, which doesn't give a damn about potassium, starts to activate. Yes, it's true the potassium isn't sufficiently able to bring the potential way down, to hyperpolarize it, but I'll show you that it's sufficient, with no stimulus, to have a spontaneous, sustained, tonic activity, as in an epileptic crisis, in a *seizure*.

This we talked about last time and I'll show you that by setting a particular value to zero, I'll show you the change, what would happen if I had toxins that selectively block either the sodium channels or the potassium channels.

Optogenetics and Neuronal Control I mention it only because in the literature and also in our lab (but not for therapeutic purposes yet), people have developed, perhaps in the field you've heard mentioned, I think I told you about it at the beginning, it's called **optogenetics** (and they will surely win the Nobel Prize in the coming years, it's two researchers, one from MIT and one from Harvard). They put proteins, membrane channels or ion pumps that are found in other organisms, mainly in algae (or at least the main ones they started with were in algae), where the algae need to, for example, photosynthesize, they need to have some kind of information if there's light, a bit like some photoreceptors, some cells in our retina, which transduce light phenomena into electrical phenomena, into ionic phenomena.

And so in our lab, we have a way, with a viral vector, to convince real neurons to express ion channels that when we illuminate them with an orange, reddish light,

these channels open, they are channels that typically let… they are permeable to potassium [corrected: chloride, as mentioned later], and when these, actually chloride… and when they activate, when I turn on the light, the spiking activity of the neurons is completely abolished. So if one were to put them in the brain of an epileptic person, just before the onset of a convulsion, of a dramatic, synchronous activity, one could flash the light and it would reset all the neurons. And thus it would prevent this hyper-synchronization, hyper-activity.

From a control, engineering point of view, we've seen that when we hold the membrane potential because we have the light on, even for just a few tens of milliseconds, all the negative feedback mechanisms relax and we have a situation like this with that famous *rebound* I showed you earlier. Ok, we've turned off the activity, imagine this could be a little longer, and as soon as we let go, all the neurons are very happy to resume, in fact, they are even happier to resume their electrical activity.

And the key would be how do I, once I've turned on the light, turn it off in a gradual way or vice versa? How do I, like with the thermostat in this room, sorry it's too… How do I, like with the thermostat in this room, have a control law that prevents oscillations, prevents unstable behavior? Here, with your colleagues, we set it to **20 degrees**, I don't think the temperature has dropped dramatically. It's trying, the thermostat is trying in a very slow way, due to the dynamics of the system to be controlled, to activate the radiators, probably the hot air reaction [sic], to keep the temperature constant. We have to do the same thing, or one should, to think about neuroprosthetics based on optogenetics to treat diseases of excitability. Another example would be schizophrenia, but that's another topic, another story.

Simulated Pharmacology: Channel Blockers (TTX, TEA) In the last part, I'll simply tell you that it's possible to simulate **pharmacology**, this is a very simple thing, where in the code you see I have variables called $G_{\text{bar\_sodium}}$, $G_{\text{bar\_potassium}}$. I remember that one of these in the Hodgkin-Huxley model is **+120 millisiemens**, I don't remember what this one is, suppose it's **60 millisiemens**. I think there are more sodium channels, assuming the single-channel conductance is the same, there are more sodium channels than potassium channels, or the total conductance of sodium channels is much greater than that of potassium.

If I want to simulate the presence of that toxin **TTX** from the *pufferfish* (I don't know what it's called, the tropical fish that puffs up and that can be toxic because its toxin binds to the extracellular domain of sodium channels and blocks them), so not only do I no longer have an action potential, but I don't even have the ability to control my respiratory system and so I croak, I die, technically I die. It's incredible that nature has evolved molecules so selective to say "you are based on information processing, on sodium channels because you make spikes, I'll sabotage your sodium channels". It's what I was saying that to order it, one must declare they are not a terrorist. When you put it in an experiment where you have cells, as soon as you put it in, after a few seconds, when the substance diffuses and gets near the neurons, the impressive action

potentials disappear immediately. You practically go in time from one second where the neuron's response to a small pulse of current evokes a spike and evokes it, to a condition where there is no more excitability, and it's remarkable.

And it's a very useful experimental control to understand what depends on the electrical activity of the cells. If I remove, from a, if you like, chemical point of view, I turn off the sodium channels, if what I still see, if what I'm studying I still see, it means it doesn't depend on the electrical activity of the individual cells.

So here it's as easy as putting **0**, this variable equal to **0**, instead of where in the code it said this variable equals **120**, I set it to **0**. I can repeat the simulation. Ditto for… ok, I should have asked you which conductance you think I turned off, here I turned it off completely, I don't know why there are two graphs here, here I lowered this quantity, instead of **120**, I reduced the number of available sodium channels and I have a kind of aborted action potential.

In this case, instead, it's the dual case and it's interesting to see because I think it can answer the intuition. Here you have a toxin, this famous **TEA** [corrected: TITEA in text, but TEA is more common for Tetraethylammonium], which blocks potassium channels and so at a certain point you have the opening of sodium channels, their inactivation, but you no longer have anything to bring them back to **-70, -80 millivolts**, to hyperpolarize them, and so the trace of the membrane potential remains stuck in what is called **depolarization block**, so it's a block of depolarization, here the neuron does nothing more because it's never given the chance to return to an initial condition where it was ready to fire again. I think the difference between these cases is that here I put a little bit, so instead of setting this value to zero I reduced it, I put it at **50%** and so if you don't have enough sodium you don't pull the membrane potential down [corrected: up].

Again these are trivial things and in theory, it's very interesting to get your hands on them. Experimentally you have to buy expensive toxins and use them with extreme caution, in a computer simulation you don't have to wear gloves, you don't have to wear masks, you don't have to declare you're not a terrorist, you could even be a terrorist.

Beyond the Hodgkin-Huxley Model: The Diversity of Ion Channels
What I said before, and I won't expand on this topic in this course, is that in reality neurons don't only have voltage-dependent sodium and potassium conductances, so they don't have the famous *fast inactivating voltage-gated sodium channels* (so, rapid inactivation) and for potassium *delayed rectifier* (rectification and delay and delayed behavior).

There is a huge number of ion channels, right now I don't have the book or the figure with me, there are books just on voltage-gated ion channels, there are enormous families, they have a genetic counterpart, in different species there are variants and so not all channels have the same homolog in all other species. For the voltage-dependent sodium and potassium of the Hodgkin-Huxley type, yes, and that's why we're still talking about them.

Electrophysiological Phenotypes: Tonic, Phasic, and Bursting Firing It's possible that a neuron subjected to a current injection might either not fire (so, have no activity), or have **tonic** activity, or have **phasic** activity (as it's technically called). In particular, this has another name, it also has another name which is called ***bursting*** activity. *Burst*, I don't know how to translate it into Italian, it's a kind of shock, of period, of packets of action potentials that are fired and then there's an interval of several hundred milliseconds in this case without action potentials.

It's clear that no matter how hard you may try, when the current here is constant or it's a spontaneous activity, you will never have in the simulations I gave you or in the Hodgkin-Huxley model or imagining that there's only sodium and potassium of the type I told you about, you will never have a so-called **electrophysiological phenotype** like this. Why? Because it's the result of the presence of other types of channels and there are, as I said, different channels that depending on their… (I erased, here were the activation kinetics, sorry, the activation curves, the $m_\infty, h_\infty, n_\infty$), there are channels that have those curves *shifted* differently, or have different τ's.

Modulatory Currents and Spike-Frequency Adaptation And for example, in some cases, there are currents that are called, they are selective for potassium, which are called **muscarinic** (which always have the effect of opposing excitability). Any type of current you see that is selective for potassium, because of its reversal or Nernst, equilibrium potential (**-80**), if it activates it tends to discourage spikes, so it tends to slow down the frequency. Conversely, all the currents that are linked to sodium, to calcium, which have their reversal potential at depolarized values (**+20, +30, +100 mV**), tend to increase excitability. This is a very important concept that we'll see again in the description of synaptic receptors.

So again, depolarized or hyperpolarized, beyond one being negative and one positive, it's not that the algebraic sign matters, what matters is whether they are above or below the resting potential. Everything that is above tends to favor, to increase the frequency, to accelerate the frequency, what is below tends to brake it.

In this case, you see a phenomenon called **frequency-dependent adaptation**, in which despite the stimulus being constant, the neuron starts fast and then slows down, exactly like the ganglion cells and also the cells of the primary visual cortex that I showed you in the experiments of Hodgkin and Sido [?] tomorrow. The experiments of **Hubel & Wiesel** showed audibly, you could really hear a *trrr*, the *pitch* of the sound was representative of the frequency that was decreasing.

Here in this graph, I've simply put some names of conductances, for example of currents. There are some currents that tend to delay the onset of a spike, they are important because in some cases they are called **A-type conductances**, they tend to be potassium conductances but they have an inactivation. The famous hatch that seemed to be only a prerogative [?] of sodium, some potassium channels also have it, another family, another gene, another protein. And there are other currents, currents that are called *after-hyperpolarization*, which

come after the hyperpolarization due to potassium, which also contribute to modifying the shape of the action potential or the dynamics of the membrane potential after the action potential. For example, in this case, you see there's a progressive increase of the inter-spike interval, it starts fast and after a short while, it starts to relax. This is the possible mathematical modeling and subject of other considerations.

An Experimental Example: Human Neurons from Stem Cells *in vivo* To show you that this is something seen almost daily in the lab, I'll show you a trace recorded years ago by a researcher in my lab when we were in Belgium (he's now an associate professor at the Polytechnic University of Milan, a bioengineer). And the type of experiment was a very interesting experiment, but in this case, I'm simply selling it to you as if it were a neuron.

The experiment was interesting because it consisted of taking stem cells, embryonic or induced pluripotent, differentiated from biopsies. These cells were, in a Petri dish, differentiated into neurons, so we had human stem cells that were differentiated and transformed into, reprogrammed into neurons and were, as a conclusion to something already particularly complicated, were first transduced with a viral vector that made them express a fluorescent protein, the famous **GFP**, which perhaps you've all heard of (Nobel Prize many years ago to an MIT scientist, Tonegawa), Green Fluorescent Protein, so that Daniele Linaro could see them under the microscope when he tried to introduce that famous pipette I showed you and he saw the cells as if on the surface of the Moon with that microscopic technique that I told you is called **DIC**, *Differential Interference Contrast Microscopy*, differential contrast microscopy based on infrared. If he looked for the fluorescent cells, by changing the light source, he saw the green ones and the green ones were the human ones.

And he saw them in the cortex of a mouse where they had been implanted, in an attempt, in the hope of understanding what happens, in cell therapies where stem cells differentiated into… think of Parkinson's, where a portion of the nerve cells dies, and I put back identical ones from the patient, because I take them from the skin, I make them pluripotent, and then I reprogram them to be neurons.

The experiment was very complicated because implanting human cells into another type of organism is a mess, because there's a xenotransplant where the recipient's immune system doesn't recognize the cell as its own and so it attacks it, unless it's a transgenic mouse that is immunosuppressed, so it has almost no immune system. Except that these little immunosuppressed mice are extremely fragile, you look at them and they die, unfortunately, so keeping them for nine months, the time necessary for maturation, which recapitulated the human embryonic stage, nine months to reach electrical maturity in an animal that could die at any moment, was complicated.

Trace Analysis: Adaptation and Irregularity And when he injected a current for… I think this is about ten seconds, this is a calibration bar, as they

say, and it's **half a second**, so here it's one, two, it will be about fifteen seconds or ten seconds. If you squint, you'll see first of all that even though the current is constant, this isn't exactly a metronome. So the point I was making before, if you want to estimate the frequency here… you see, there are even some cases here, you can see by eye that there's a bit more space here, so the activity is regular, but like everything in biology, it's not exactly a metronome.

If you squint, you'll see that the very first action potentials here show exactly that **spike-frequency adaptation**, that adaptation of the spike frequency, which is also called **accommodation**, or *accomodamento* perhaps in Italian. And I may have mentioned it to you because it's a very typical thing for sensory receptors, i.e., the nerve cells that are linked to visual, auditory, proprioceptive, tactile, nociceptive (pain), etc., sensation. And I gave you the example that if a colleague of yours grabs you by the shoulders from behind, you probably have a reflex reaction called the *startle reflex* (I don't know how to say it in Italian), of jumping (Italian is not elegant for these things). But the clothes you've been wearing since this morning presumably don't make you jump, yet you have them on your shoulders, you don't feel them anymore.

This is because, evolutionarily, the world is not stationary, it's constantly time-varying, and perhaps I need to have neurons of all types, including sensory ones, that lose interest in what doesn't change over time. Here the current is constant. If, however, the famous tiger I've been invoking in class for years now were to enter (it hasn't entered yet), it would be a change, a notable non-stationarity, and so my neurons would have to sense the change and, for example, fire much more during the change, because the change contains the surprise, it contains information, and probably those of our ancestors who didn't have accommodation were eaten by the city [sic: tiger]… or by similar beasts.

Adaptation as a High-Pass Filter The interesting thing is that if you zoom in here, you can see this spike-frequency adaptation [corrected: frequency adaptation] better, and you can understand the story I was telling you about characterizing the frequency as the inverse of the interspike intervals. Paradoxically, here you could do it by plotting 1/(Inter-Spike Interval) as a function of time, and you would see that the concept of frequency adaptation is represented by a kind of dynamic of the frequency variable, of the observable frequency over time. It starts very high, almost **150 spikes/second** (you have one spike, two, they are the first two inter-spike intervals, they are these two points here), then things slow down a lot and within **two seconds** (which I'm not sure if it's this, no, it could be here). Within **two seconds**, so more or less here, you see that there's a kind of *steady state* of the frequency which is low, it will be on the order of **20 spikes/second**.

All neurons, particularly in the central nervous system, have a very low electrical activity because it would be metabolically extremely costly to fire at **100-150 spikes/second**. You see the little exercise from last time where I showed you how much a compartment of a neuron could eventually be messed up from the point of view of ion concentration. You keep eating bananas, potassium, and sugar, etc., for the ion pumps, etc., but… so metabolically it's expensive, and

this adaptation seems to make sense both from the point of view of metabolism and from the point of view of information.

In engineering terms of filters, of filtering, those things (band-pass, high-pass, low-pass), so those that favor a particular spectral content or that favor slow transitions, fast transitions, what is this, what filter is this? If this were the impulse response of the filter… Ok, no, it couldn't be because it would be… if it were… you have to think in terms of spike frequency. What filter could it be? It's one of the three I told you, so you have a **33%** chance of guessing it.

Given that the important thing is to detect surprise, change, and if instead it's something that's constant, I want to lose interest. It's either **band-pass**, or **high-pass**, or **low-pass**, one of the three. Try, I hope, to get an… Pass? No. Why did you say band-pass? No, it's low-pass. It depends on what you're considering. The low-pass… so you have to imagine that here the response, this is the response to a step, so the input is a step, which in theory contains all frequencies because it has an ideal, steep transition, and so in frequency, it's everything, let's say, in Fourier there's energy at all frequencies. And the output of a band-pass would mean that it only keeps a certain oscillation, a certain set of frequencies, in the output. Here a step has a response of going up and then coming back down. Is it a low-pass? Or a high-pass? Is it a **differentiator** or an **integrator**? Which are other ways to call, in the end, the usual differential equation that for heaven's sake I'll ask you on the exam.

If I have a step and the thing is, if you squint, the output follows the input, but more delayed, this is a low-pass. Beyond the fact that electrically it's a low-pass, there I have that the response is something like this. So, how did [?] you get close? It's a band-pass… I wanted to bust your chops because in effect, in the end, it's a **high-pass**, because for band-pass you would have had to tell me where it cut off, it was the fact of where it cut off. It's a high-pass, a differentiator, so the band-pass is a bit more complicated to see from the point of view of whether it's an integrator or a differentiator.

I call it a differentiator or integrator because if you mathematically take the integral, so I'm thinking of doing the integral from **0** or from $-\infty$ to t, where t is an independent variable and is one end of the integration. In fact, I'm trying to, for each t, to express, to calculate the area, so the area under here is **0, 0, 0, 0, 0, 0**. At a certain point here the area becomes… it jumps and becomes small, as time passes the area becomes larger and larger. So this curve here is the result of temporal integration. Whereas this graph here, ideally if this were an ideal step (so a function… I should call them distributions, I'll take a break now, so a Heaviside step), the derivative should be a **Dirac delta**. But if you think of this as perhaps a ramp, the derivative is non-zero only where the argument changes in time, so here it's zero and here it's zero, here it's zero, here it's zero, and only where… when it changes in time is it non-zero. So this mechanism of frequency adaptation does a high-pass, it does a derivative, and derivative means emphasizing temporal transitions.

I'll stop for ten minutes. Thanks.

Biophysical Mechanisms of Adaptation: Calcium-Dependent Potassium Currents … …

Good. So, the interesting thing about this phenomenon of adaptation or accommodation, which results from a functional point of view in a high-pass filtering, in a differentiator, in something that prefers changes (like in this case there was no current before and then there is current, here the response was "oh what a surprise" and then "how boring" and I go to *steady state*), depends on conductances, so on membrane channels, which are **potassium conductances**, and so far you probably could have gotten there, you could have anticipated it, because if at this particular current the frequency of the first spikes… I'm not getting the subjunctive wrong. So if at this current, the theoretical current without adaptation would be this, around **100-120 spikes/second** and then the frequency decreases, it means there's something that tends to pull the membrane potential down or anyway to oppose the current that Daniele Linaro was injecting, and it was a positive current. If he had decreased the amplitude of the current the frequency would have decreased, he didn't decrease it, the charge balance equation says that all these currents, including his, add up together, so there must be something that tends to have a negative sign, and the only thing I've told you about, besides chloride, that has a negative sign is the potassium currents.

And indeed this could have been due as a mechanism to a particular type of voltage-dependent potassium current, which is called the **muscarinic current**, it's also called I_M. Actually, in this case, we know that for this particular time dynamic, and perhaps if you take a look you might realize that here it's as if there were two time constants, two decreasing exponentials. There's a very rapid one that drops suddenly, in the order of **10 milliseconds, 20 milliseconds**, and then there's something that further brings it down over the order of seconds. So this is unlikely to be the I_M current and is instead a current, it's not voltage-dependent but it's dependent on the ion concentration, and it depends on the ion concentration of intracellular **calcium ions**.

So as I imagine it, it's a channel where it's selective only to [sic] channels, to potassium ions, that this is outside and this is inside, there's a lot of potassium inside, so if these open, and this gate opens to follow the electrochemical gradient, the potassium ions tend to exit. Am I wrong? Is there a lot of potassium inside? No, it should be right. And this gate doesn't open because this channel senses the membrane potential. It opens because there are sites here where calcium ions bind and the more intracellular calcium ions there are, the more this channel is active.

Now why on earth should calcium ions accumulate inside the membrane [sic] of a neuron? I told you that more or less the dynamics of calcium currents are similar to sodium currents, so from a voltage-dependent point of view. There are in particular two types of calcium currents, one is called *low-voltage activated* and another is *high-voltage activated*. Anyway, they have a… those activation kinetics are different. Anyway, it does more or less the same, it plays the same role that sodium plays. So every time there's a damn action potential, just as sodium ions enter the membrane, calcium ions also enter. You know that calcium inside

is practically zero, so as soon as some enters, it's a big phenomenon, so much so that calcium acts as a so-called **second messenger** in an immense quantity of biochemical reactions, of intracellular biochemical pathways.

And it's the key to linking the chemical world to the electrical world, because every time there's a spike there's an influx, a *puff* of calcium currents, to the chemical world. In this case, it's actually a kind of negative feedback, negative because these calcium-dependent channels are sensitive, selective to potassium and so the effect is to reduce excitability.

Detailed Spike Analysis: Changes in Shape Over Time One can notice… ok this would have been, but I should have removed it from the title, so I must remember that if I do this, then this can't appear here, so it's already a *spoiler*, either they are calcium-dependent potassium currents or sodium-dependent (the effect would be similar, there are both types, so calcium-dependent potassium or sodium-dependent potassium, it doesn't matter much), and so I would have asked you "what is it that acts as a brake on excitability?", and you would have answered, I'm sure, "potassium conductances". Chloride could also have been, but chloride is a bit particular because its reversal potential is roughly similar to the resting potential, and so even if it opens, the potential is there [sic], and so the famous *driving force* of the currents, $V - E_{\text{Nernst}}$, is practically zero, because V is more or less around its potential. It has another effect, but we'll talk about it later, we'll talk about it for the description of synaptic currents. In this case, therefore, it's the potassium conductances that have this blessed low reversal potential, at **-80, -90 millivolts**.

The interspike intervals increase [corrected from text: decrease] over time, because there's something that acts as a balancer, it subtracts from the current that Daniele Linaro injects. And another thing that happens, which is clearly visible by taking the first spike (this is a real spike, which looks a lot like a fake spike, and it's interesting as a thing, from a mechanistic point of view, that this is possible). If I compare the first spike to the last spike, you see there are several interesting things. The first is that the **duration** seems to broaden over time. I'm zooming in on this, I'm looking at the very first spike here, and the last spike, or one of the last. To the naked eye at this zoom, they all seem the same and in fact, they are more or less all very steep. But just like in the Hodgkin-Huxley model, if you simulate it, the shape of the spikes changes very slightly. Here too, it broadens. The *reset*, if you like the **hyperpolarization**, seems to be less marked, it doesn't reach down here. Here the Y-axis is always the same. And another thing you see is that the **slope**, the derivative of the membrane potential, dV/dt, seems to be steeper [corrected from text: less steep], so there's a residual depolarization. The *slope* also seems steeper at the beginning and bends later.

And I want to point out that when I talk about the *slope* of the membrane potential, in effect I'm saying of the action potential, I'm asking what is the derivative. I know that the derivative is a function of time and that there I should specify the slope of the tangent line at a particular… I don't care, I'm saying that I'm in fact considering the time derivative of the membrane potential

because I'm looking at it and because I'm not particularly surprised that it's more or less *steep*, so the angular coefficient of that line which, by Taylor, is the first derivative.

This thing here, for the charge balance equation, is exactly the sum of the currents. Now in the initial part, only sodium is open, so I can think that the *slope upstroke*, so the initial hit, the rising phase of the action potential for that time duration is only due to the sodium currents. So I, in effect, with the *slope*, with the derivative, am reading… this is a constant value that surely shouldn't change (it could change but on very long time scales if there were expression or synthesis, gene expression… genic, this expresses [sic], or so it either removes or inserts new copies of sodium channels, but this happens over the course of minutes, hours, days), what I see is the combination, this for me is a single quantity, so it tells me about this because $E - V$ is what it is but it's not so interesting, and this is the coefficient that changes particularly.

So looking at that I can imagine that after a while the **inactivation of sodium** has increased, so there are fewer available, non-inactivated channels. The famous H has become lower, it's no longer at **0.6** at the beginning of the spike, it starts to be a value like **0.5, 0.4, 0.3**. And so all these features that are seen in an experiment of progressive depolarization mean that the potassium currents, *delayed rectifier*, can't reset anymore (so they must also be inactivating). The sodium currents, because of the *slope*, are manifesting a residual inactivation that I can't get rid of, and in addition to this inactivation of the sodium channels, there's also this further calcium-dependent potassium current that generally lowers the frequency.

So this is a real neuron, and to dissect it without using toxins and identify the currents, there are also these methods. Another very interesting method is to try to take a more complicated mathematical model that includes other currents, other mechanisms, and try to *fit* it mathematically. Is there a set of parameters that gives me the model's response identical to the experiment? If yes, as a first approximation… there would be a very long discussion to have, but I won't give it to you in this course. Perhaps you are familiar with the *fit* of a mathematical model to data, you could have local minima, in the idea that you have for doing the *fit* you are minimizing a cost function. This is everyday in the context of *machine learning* where the cost function, or it's no longer called a cost function, it's called a loss function, is minimized for a particular set of parameters, but this set of parameters might not be the only one, it might not be unique and it might not be the global minimum. And so if you have a model that does the exact same thing, it might not be the explanation, you might then want to open the model and say "show me what the value of $G_{\text{Na\_bar}}$ was that the FIT procedure discovered", but it might not be indicative of what reality is. So what people do is with genetic optimization algorithms (which have nothing to do with genes, it's just a mathematical name for some styles of minimization, of optimization of functions, functionals), generate a family of solutions, which instead in *machine learning* is not done with gradient descent, but that's another story.

Ion Pumps: Active Transport The **ion pumps**, I mentioned them to you, are mechanisms of active transport, and we haven't talked about them, we don't talk about them because from an electrical point of view, they don't contribute dramatically. They might start to contribute dramatically in contexts of this type where I have the ion concentration of some species inside the membrane, inside the neuron, for which there are also ion pumps that extrude, that kick out calcium ions (and now I don't remember what they pull in to be balanced), and so in theory, one should put them in if one wanted to mathematically describe this relationship as well.

They are in general… they are modeled, they are considerable as fixed current generators. In particular, the so-called **electrogenic pump** (electrogenic means it creates a current), depends on ATP, it's called the sodium-potassium pump, it exchanges **three sodium ions** that are inside the cytoplasm, kicks them out, and pulls in **two charges, two potassium ions**, so it's not perfectly balanced, and obviously, it needs energy because there's a lot of sodium outside, so how do I take it and kick it out? I have to use ATP, and I think here there's a… I found this guy who uses a *game engine* for 3D games that you all like a lot (I like them less because I'm from the Pac-Man generation) and he uses them to do visualization, if you like, at a very high level, it's not a molecular simulation, so it's not exactly the truth, but it's a *cartoon* that I like a lot. I asked his permission… I think on Twitter, I asked his permission, he said yes, to be able to show it in class.

And I'll show you one now of the sodium-potassium ion pump, and then I have, I think, a couple of others of GABAergic synaptic receptors, where the quantity is chloride. I think his *commentary* voice is on, and if it's too loud, I'll lower it.

> *[Video Commentary] This is the sodium-potassium pump. It's a protein complex with a crucial job, to restore and maintain the neuron's resting potential. The pump has an alpha subunit, here shown in pink, and a beta subunit, here shown in yellow. There is also a small gamma subunit, but you can't see it from this angle. To start, three sodium ions from the cytoplasm of the neuron bind to the pump. Using energy from ATP, the pump undergoes a shape change and releases the yellow sodium ions to the extracellular space. This conformation allows it to bind two green potassium ions from outside the cell. The protein then gets dephosphorylated and the pump switches its conformation back to the original state. It then releases the potassium ions inside the neuron and the cycle begins again.*

The thing that particularly inspired me is to be able to give you a kind of *depiction*, a graphical, intuitive representation of what it is for a modification of a three-dimensional conformation, also due to biochemical reactions like phosphorylation and dephosphorylation (but I don't care), one could have, against a chemical gradient, by a kind of molecular motor, an ejection, an exchange of ions that actually pass through this structure that allows the passage of ions in a membrane structure that, as I told you the other time, the first few times, is strongly hydrophobic, energetically impossible, otherwise, for an ion to penetrate this layer of the membrane. So a lipophilic and hydrophobic layer, made of fats, of lipids, but where water molecules cannot go.

It inspires me, but it's clearly not a molecular dynamics simulation, which is a simulation technique where the atomic positions, at the atomic level, of all the molecules are used, and things move, however, for femtoseconds, for picoseconds. Here it would probably already be at several hundred milliseconds. And for example, there's no description of electrostatic interactions. In another of the videos, he says that if he had put in his green molecules, which are potassium here, they stand for potassium, if they had had to repel each other as they are positively charged, he wouldn't have been able to do the simulation. Because obviously, it's computationally extremely heavy. Anyway, if you like it, I think I put a link on Teams to one of these videos, and I hope this *cartoon* can give you a mechanistic idea of how these mechanisms, in this case, an active mechanism, allow this game, this electrical dance.

From Distributed Permeability to Discrete Channels Now before taking a break in half an hour… I'll take a look because the topic is a bit heavy… I wanted to go back to the microscopic aspect, a little less phenomenological, of membrane permeability. In the era of Hodgkin-Huxley, I told you, it was thought that there was some kind of transporter exactly like the sodium-potassium transport pump, the sodium-potassium electrogenic pump, something that had an electric charge, because somehow it seemed it was something that depended on the electric field, on the membrane potential, but it was thought to be a uniform, distributed property of the membrane.

We had to wait until the Eighties, when these two gentlemen, **Erwin Neher** and **Bert Sackman**, two German physiologists (they are still alive, they are now emeriti, they have been retired for several years, Sackman, but also Neher, are very high-level scientists, after the Nobel Prize for discovering the existence of ion channels as discrete entities, they continued to do research at the highest level, particularly Sackman, on cellular neuroscience).

Anyway, to cut a long story short, this is a very interesting thing, just like the excitement I showed you, my excitement I showed you for the story of digging up the *thickness*, the thickness of the membrane on a purely electrical basis (because I know the formula for the capacitance of a capacitor, so I don't have a powerful microscope to see how thick the membrane is, I make an electrical measurement and I see it), so too in this case, we didn't see the membrane channels. And reconstructed as I showed you just now with the ion pumps, we didn't see them the first time, the first time we got an echo, an electrical image of a consequence of them opening and closing, and it's all due to the invention of a technique called the **patch clamp** technique, where *patch* means patch and *clamp* means clamping, block.

The Patch Clamp Technique and the Giga-Seal Where instead of using a glass pipette, making it much, much more pointed in what is normally called *sharp electrodes* (they are glass capillaries, borosilicate, which are heated in the center and several cycles of heating and pulling, heating and pulling are done, because when it heats up, as you know from the glass masters of Venice,

glass, particularly borosilicate glass, melts… besides burning your fingers if you accidentally touch it), if you start to heat a lot and pull, you can have, as in some cases of artificial fertilization, *in vitro* oocytes that you often see or sometimes saw on television time ago (then for political reasons I think you don't see it anymore), instead of having a very, very pointed thing that penetrates the lipid bilayer as if it were a very fine needle into a wide-knit wool sweater (it doesn't break it, it goes right in), they had the idea of not making a *sharp* pipette, but of making it quite, not macroscopic, but quite wide.

Here the opening is on the order of one **micrometer**. We don't really talk about opening, but we talk about the electrical effect of resistance, when I take this pipette, I put it in a bath with electrolyte, I pass a current and I see what voltage I record. The wider the tip of the pipette, the lower the resistance.

It's called *patch clamp* because they had the intuition that if I… if one could manage to place it on the membrane **without penetrating** (so in all the previous lessons I told you poke, impale, penetrate, stab a neuron, thinking and hoping to give you the idea that the electrode is put inside the belly of the neuron). Here it's different, I leave it on the surface and this patch, this piece of membrane is blocked mechanically, it's a mechanical *clamp*, it has nothing to do with the *voltage clamp* technique of Hodgkin and Huxley. In that case *voltage clamp*, the *clamp* also had the meaning of blocking, but it was blocking an electrical signal, blocking the electrical properties, here *patch clamp* means mechanically.

And by doing so, there are different configurations they invented, but in the configuration that is represented here, they saw that simply by recording, as a first approximation, they saw that the current they could measure were very, very small currents compared to the currents one measures when penetrating the membrane, and one can do it because the signal-to-noise ratio changes and becomes very favorable, because here if I am a charge carrier, if I am an ion, I can't escape here in the gap in between. It is said that there is a **seal resistance**, that is, the mechanical contact between the pipette and the membrane is so tight that there is a resistance inside here to get back out (where I have the reference electrode) that is a resistance on the order of **gigaohms**, so *billions* of ohms, enormous, for all practical purposes infinite.

Single-Channel Currents: Stochasticity and Quantization If so, it means that if I have objects here that start to open spontaneously, since there's the electrochemical potential inside, sodium, outside, potassium etc., if it opens I see the electrical echo, I see a current that opens. And the surprising thing is that they saw two surprising things.

The first is that these currents occurred **randomly, stochastically, randomly**, it wasn't a deterministic thing, it wasn't a periodic thing, it was "here it opened, it was open for a bit, then here it starts from a zero *baseline* and the downward deflections you see…". It means it was an outward current in some way, but it doesn't matter, inward and outward doesn't matter. In some cases, the duration of the opening of this mechanism was a bit longer, then it was shorter, a little bit longer. Here it was almost a very brief opening that at the sampling time

of this amplifier, of this recording, it barely registered. Here it's much longer, a few tens of milliseconds, etc., etc. So stochasticity, which makes one think that they are single molecules, that it's not a population effect. They are sitting at body temperature, **37 degrees** or **35 degrees**, I think these experiments are anyway done at a temperature very far from room temperature.

And the second thing is that the value of… so when these signals occur, the amplitude values are **discrete**, it's either **0** or **7 picoamperes**, there's no **6.5, 3.1**, so contrary to what you would see… You would see if, using Hodgkin and Huxley's toxins for example, you measured the residual sodium current, then you would see that at a certain point this current, in the end, we plotted it, when I plotted M, H, N in fact it was that. There was something that varied continuously, here instead the experimental trace shows that there is no continuity, there are discrete [values]. In the end, it's like in quantum mechanics where energy is quantized, here the conductance is quantized, and it was such a revolution that these two guys won the Nobel Prize.

Because it was understood that these events, the fact that the amplitude distribution was strongly bimodal (meaning the histogram of the amplitudes is either **0** or **7 picoamperes**), meant that there were discrete states, it was either all closed or all open, and it was **one**, one channel they were seeing. Now, clearly, with hindsight, "yes, what else could it be?". But it took **30 years**, from the '50s to the '80s, for these guys, playing with pipettes over a Bunsen burner, pulling them, to get something that… for binding to the membrane, it's not a big problem. As anyone who has to wash dishes by hand knows, oil goes onto glass easily, you can't get it off. So when you put a piece of glass near a membrane, the membrane, for electrostatic reasons too, which are little understood, attaches easily. But the fact that there was such an accurate condition of a *giga-seal*, they say *seal*, *gigaohm*, for the gigaohms here, was revolutionary.

Patch Clamp Configurations There are actually different configurations of patch clamp that these gentlemen proposed. The one I showed you is called **On-Cell**, I lean on it, "eavesdropping" if you like, but "eavesdropping" there are holes, so something gets into my ear, it's not just vibrations.

But if, having arrived at this *on-cell* configuration, through the pipette connected to a small tube (in post-covid times we probably can't do it anymore, but normally the experimenter has their little tube attached to the pipette and sucks lightly) from a kind of mouthpiece, and this mouthpiece leads to the application of very intense negative pressure pulses, very intense because the cross-section of passage here is tiny, where I put my mouth is relatively large, it could be a couple of millimeters, then there's, say, a meter of silicone tubing and here it's one micrometer. So correctly I should say that the pressure is the same, but the force… pressure is a force per unit area, so if the area is very small, for the same pressure, it's an enormous force.

You actually manage to break the membrane, it's as if you were taking the cap off this patch, it simply dissolves and disperses into this pipette which I'd point out is enormous, it's like… it's the volume of a tennis court compared to a ball. The ball is the cell and the content of molecules, ions, etc., is that of a tennis

ball. The pipette contains liquid in a volume as enormous as that which could be represented by a tennis court. So ok, yes, there are pieces of membrane and junk from the cytoplasm that fly inside, but after a few milliseconds, they no longer matter.

And I see it electrically, I can realize the fact that I've arrived in this configuration, because while here the potential I was recording might have been the potential of the extracellular medium, as soon as I "uncork" this system, inside here it's **-70**, so on the oscilloscope, instead of seeing **0**, after I suck, I see that *boom!* the waveform of the potential of the pipette I'm recording goes down very rapidly and indicates to me that I've entered, that I'm in **Whole-Cell**. Typically an experimenter, especially at the beginning, gets very *pumped* when they manage to do this procedure here and the cell doesn't die, because the thing that sometimes happens is that the seal isn't perfect and when one applies a negative pressure, simply the membrane here breaks, but the cell isn't attached, so one continues to see **0 millivolts**, which is the potential of the extracellular medium.

There are other ways in which, without sucking, by simply retracting the pipette (the pipette is attached to three piezoelectric motors, which are along the XYZ axes, so it can be moved in space), one simply retracts it and a little piece of membrane remains stuck to the pipette. If one is lucky, one could take this pipette and put it in another container where the composition of the liquid present there has concentrations of ions or drugs controlled by my choice. And in this way, I can expose the intracellular part to the concentration I say, and so, for example, study this type of channel, potassium-calcium dependent, which sense the ions inside, but I inside a cell will never be able to change things, I instead want to change them at will. I can do it if I have this **Inside-Out** technique. The *inside* of the membrane is *out*. And there's another technique that's a bit more complicated that, in other terms, so after the *whole-cell* is established, one can have the patch of membrane that was removed in an inverted configuration where **Outside-Out**. The *outside* of the cell remains *outside* the pipette.

From Single Channel to Macroscopic (Population) Current This is simply nomenclature. The interesting thing is that these two researchers started to test not only ion channels, but they studied their voltage-dependence and they saw that what is normally a population characteristic, which you see below. Here, this is an example of a *voltage clamp* experiment that Hodgkin-Huxley might have done. You give a step, *voltage clamp* means you impose the potential and measure the current. You measure the current that the amplifier must constantly deliver so that the potential remains constant. If the potential remains constant, $C \cdot dV/dt$ is **0** and the current I have to inject is representative of the ionic current that the neuron is evoking at that moment.

And so you see here, this, even without knowing, this is… ok it says potassium, this is a response of the usual boring differential equation, etc., which is the dynamic equation of N, there's only N in the potassium channels, and I give it a step, these open, they have the usual charging curve with the delayed kinetics that characterizes potassium of a few tens of milliseconds or whatever it is. This

instead is sodium, so here I'm observing in effect $m^3 \cdot h$, I'm observing them together. In fact, I see an activation and a cumulative [sic] inactivation, sorry, an immediate rapid inactivation that follows. In fact, I see that when I give it this step, the sodium first activates and then inactivates. The potassium, instead, only activates. This is the trace of the potential step I'm changing, first I hold it at **-80 millivolts** simulating a resting condition and then I give it a "flick" [sic], let's say a step, and I hold it at **-30 millivolts**, for potassium I do it at **0 millivolts** and I verify that this is what Hodgkin-Huxley described.

If, on the other hand, I do it with these electrodes and these experiments by Neher and Sackman, where I can isolate, also due to the size of the *patch*, one channel or two channels, a very small number of channels, I can measure the so-called **single-channel currents**. And when I measure them, I see that the behavior is stochastic, and if I do it over and over again, I see that statistically this sodium channel, which is always the same (but you can think of it as representative, this is the **ergodicity hypothesis**, as representative of a population. If I take a single Italian, even if it's a flawed example, I can think that if I ask him a series of questions, the time average can be exchanged with the ensemble average over a set of Italian people I could interview by asking them only one question. This is called… this property that is hypothesized to exist, it's not easy to verify, is called the **ergodicity property**: a system is ergodic if you can exchange the time average with the ensemble average, just to be clear, the integral over time with the expected value for those of you who remember probability theory).

So here it's as if I were seeing a population of channels, because it's a single channel repeated over time, but it's as if they were N channels if they are independent. Here I get independence for free because now I do this trial, then I let some time pass, I do the trial again, there is no dependence, no memory in time, if I wait a sufficient time for the transients, if any, to be forgotten. And the same thing is therefore representative of statistical independence.

And you see that the opening of the sodium channel happens statistically only in proximity to this step, while that of the potassium happens repeatedly throughout the experiment in which I'm holding the cell depolarized or a bit more depolarized. It turns out that if I **algebraically sum** these curves, I get the ensemble average. So if I do the algebraic sum, which means the arithmetic mean, so I take the population average, I get the macroscopic behavior. And it makes sense, because I normally, from Hodgkin-Huxley onwards, I only measure the macroscopic effect of the population, I always see, *patching* a neuron, a choir of voices. But if I could isolate them one at a time, I would hear that, for example, this one is particularly out of tune, it's not that it has a characteristic that first it opens, then it inactivates with a kind of exponential time constant. This exponential time constant comes about because some of these occasionally open again later, but mainly they open here and then tend to stay closed. When I do the algebraic sum of many repetitions, I see that the sum of the events tends to become smaller and smaller in a gradual way.

Noise as a Fundamental Biological Feature If you remember, I mentioned to you that in the Markovian description of ion conductances, voltage-dependent or non-voltage-dependent, there are two interpretations. One is the deterministic interpretation, the law of mass action, the other… and differential equations come out that are always the same, but in the other interpretation, I have to think that those arrows between the states have the meaning of **transition probabilities**, not conversion rates. And I want to see if by chance I can learn something from this.

It's interesting to learn something from this because, for free, I see that I have a **source of noise**. This is a nice *paper* from several years ago, a *review*, a compilation of a lot of previous studies in which the authors summarized and listed all the cases where the functioning of the a system has, in favorable or unfavorable terms, a certain type of noise: the sensor noise in the case, for example, of phototransduction or mechanotransduction; a noise due to this opening and closing of ion channels, which are not deterministic devices. They *flicker*, like a screen *flickers*, like an untuned screen flickers, in an unpredictable way. Now I'll show you other examples.

Another example of noise is that of **vesicle release**. Professor Zoli probably told you the little story where when the presynaptic button is invaded by a depolarization, by an action potential, the synaptic vesicles (*kiss and run*, it's called) fuse from the intracellular side of the membrane, they release a diffusion of what was contained in the neurotransmitter molecules that were contained, etc., etc. This is on a microscopic, molecular scale, it's not that all the vesicles do the *kiss and run* (the *kiss and run* must also be the one at airports, where one *kisses* and then leaves). Here they *kiss* the membrane and then leave, it doesn't happen deterministically, sometimes things don't work, sometimes you have a *failure*, a failure of release, sometimes the neurotransmitter doesn't bind right away, it diffuses in the intersynaptic space and is dissipated.

Furthermore, there are a lot of conditions where, without doing anything, without a presynaptic potential, the synaptic button is as if it were incontinent and it leaks a little bit of neurotransmitter molecules, so the vesicles fuse on their own spontaneously because there are fluctuations in the concentration of potassium [sic], sorry, fluctuations in the concentration of **calcium**, of calcium ions in the synaptic button. The mechanism is so sensitive that it only takes a few calcium ions to enter to activate the mechanism, so it's a relatively delicate thing, it's not that you need very robust signals. So synaptic release is also subject to spontaneous activity, to noise.

And all this noise, even if it makes one think, particularly an engineer, that it's something unfavorable, it's probably a fundamental ingredient of the functioning itself. So the signals you go to record are not only noisy because the amplifier you are using is noisy, there is electrical noise (it's called, for example, **Johnson noise**, it's related to temperature, to resistance, there are noises related to the use of operational amplifiers), ok, but biology itself, one might say, "sucks", it's *sloppy*, it's imprecise. But this imprecision is probably a *feature*, not a *bug*, it's a characteristic, not a flaw.

Variability of the Neuronal Response: The Effect of the Stimulus And I'll show you an effect that was described several years ago and that is disarmingly simple. With a computer simulation, like the one I gave you of the Hodgkin-Huxley model, you don't see this.

Every time you take a neuron (in this case it's a pyramidal neuron from the somatosensory cortex of a rat, it's the same type as those we use in our lab for some experiments of another type), if you give a step of current (now you know it *ad nauseam*) the neuron starts to fire in a more or less regular way… maybe you can glimpse, maybe not, that there's frequency adaptation… here you see many jumbled traces, deliberately *ad hoc* to create an effect, because it's the effect of a **repetition**.

You do this stimulation which lasts about a second, about **700-800 milliseconds**, then you wait a few seconds so that the membrane potential has gone to rest, you do it again (you certainly don't wait an hour, you wait some fraction of a second) and you repeat it, for example, **25 times**. Each time you superimpose the traces and you realize that it's as if… you will never see this in a model because the model is deterministic, if you repeat the same simulation, the same thing comes out every time. In the case of a neuron, no.

I'm somehow trying to tickle you with the fact that beyond the fact that from one moment to the next you have other things to think about, you probably have stimuli that have consolidated in your memory, your foot started to hurt, your butt started to hurt because the seats are uncomfortable, so your state is not stationary. But if I were to repeat the same thing to you **25 times**, maybe with exactly the same words, the same intonation, etc., in your auditory cortex, I wouldn't see the same *spike train*, I'd see a slightly different spike train, as I see here.

The interesting thing is that the first two or three action potentials are relatively ok, reproducible. As you wait, there's a big… in the sense that the time at which, the instant at which they are fired (you can see it well here, in this, which is called a ***raster plot***. *Raster*, I don't know where it comes from, I think it comes from how old televisions worked, which had the electron beam that moved in lexicographical order from right to left). Here, each repetition, I put a little bar at the instant of the peak of the membrane potential, so where there's a spike, I put a bar. And you see that the time at which the first, the second, already the third, is fired, ok, seems to have a remarkable reproducibility compared to the last one. How many are here? One, two, three, four… I almost have trouble counting them. Suppose the tenth one is completely *scrambled*, out of phase, messed up (it comes to mind, so the word *scramble* is used because it has to do with gambling, like with a deck of cards that you shuffle to randomize it), almost on the same order of magnitude as the time between one spike and the next, of the same inter-spike interval.

So it's a remarkable thing and one says "but what the hell, but how is it possible? I have an absolutely advanced brain etc. and I have elements that are so *sloppy*, so irreproducible, and yet I seem to have a remarkable performance in the world".

If instead of giving a DC stimulus, so a constant stimulus, you give a stimulus that's always the same, but that varies over time (this is technically a, it's called,

realization of a stochastic process, or some way to generate random numbers, I fix the so-called seed of the random number generation and I generate the same thing). So I have something that fluctuates, as the reality of the tiger entering could be, of the fact that the world is non-stationary, so it's very different from the stimulus I've sold you so far as useful, because for this, for an RC circuit, I even have an analytical solution. When I do this, you see that it practically becomes an extremely accurate thing.

Noise and Creativity So it's a kind of noise generator, and the story of the noise generator, I like to think it could be linked (no one knows and it's very complicated to know) to **creativity**. How do ideas come to your mind? Beyond a deep cognitive aspect, partly linked to free will, partly linked to the unconscious, to awareness, whereby if someone tells you "think of a European city", something probably comes to your mind, can you introspectively describe the mechanism? No, it just came up. Probably there is no free will because you have sources of noise (for example, synaptic release, etc.) that provide an input similar to this and some random thought comes out, some… clearly on the basis, correlated with what the activity or the structure or the memory or the synapses are, etc., etc.

So this characteristic of surprising, of being able to give unpredictable answers, can be an *asset*, it can be a useful thing from the evolutionary point of view of survival. If I have something that always responds in the same way when the tiger enters or when I see a particular apple and I never eat it, ok, maybe I'll go extinct. The one of us, of the *ancestors*, of our ancestors, who by pure chance had a whim to take the apple and didn't die, started to propagate this characteristic of ion channel noise. In reality, I don't think there are ion channels without noise, I think it's the nervous system that has evolved to use, to make good use of the noisy characteristics.

Now let's take a 10-minute break, and in the last part of the lesson, I'll tell you how, by digging up that stochastic description, how to explain or how to describe this type of electrophysiological signal. Thanks.

Note on Transcripts and Feedback Thanks. …relatively easily the *transcript*, the *speech-to-text* transcription. When I do it, obviously it's something that isn't readable because I pause and it's not great, but I've seen that by processing them, feeding them to a *large language model*, threatening that if it summarizes (I've removed that from the *prompt* now but I would have been very angry), the result is that it even writes the equations for me with LaTeX, which is a formatting method, and it makes little paragraphs for me.

Take a look at it because at first glance, but I don't have time to review everything, it seems to be useful, it seems that the text is reworked, it's not bad. I don't think it can be considered a lecture note because it's at an informal level, there are no figures, etc. If any of you can take a look at it, you can tell me "look, after reading the first two or three pages it becomes complete hallucinations". I've glanced at it and it doesn't seem so, and I was particularly surprised by the

fact that when I speak and say… it writes the mathematical equation for me… it's surely not just a reorganization into paragraphs because I did *prompt engineering* telling it "you are a neuroscientist, you have bioengineering experience" so I gave it a lot of stuff. But if you can give me *feedback* on whether it works or not, because it's a potentially interesting thing. I've also read in the literature that the *transcript* is considered very useful by students, but that almost no one looks at it during the course. This is ok, but…

Cell-Type Specific Reproducibility: *Fast-Spiking* vs. Pyramidal Ok, the interesting thing is that not all cells do this trick. Here it would seem that on the left, for this stimulus, the noise generator is on, while here on the right the noise generator is off. There are other types of cells, which are cells of the **inhibitory interneurons** that are called ***fast-spiking*** because, for the same injected current, they have a spiking activity, an oscillation frequency much higher than a pyramidal neuron. So this one is excitatory and this one is inhibitory. For the same current, this one fires **4** spikes, this one here for the same current fires **3, 6, 9**, it fires about ten, and what is this? **25 milliseconds**… it must be **2.5 seconds** [sic] …so it fires probably **4** times as much, **3, 4** times the same frequency [sic]. So, by the way, their frequency-current curve is much steeper because then maybe it saturates too, but compared to pyramidal neurons, for the same current, at the same point on the x-axis, the number of spikes per second is much higher.

Here you see it's the same experiment, done among other things by a very, very smart Italian, Alberto Bacci, who is in Paris now though. So in this case, there are **15 repetitions**, unlike the pyramidal neuron, an excitatory glutamatergic cell, where by the second, third, fourth action potential the *jitter*, the flickering is dramatic. Here at the third, fourth, fifth, nth action potential the error starts to accumulate, as if there were an error accumulating, but it's much lower.

Why does this happen? It has something to do with the dynamics of the channels. Here, by comparison, the fourth spike, this is the *raster plot* of the fourth spike, practically all the times over **15** successive repetitions the neuron fires precisely. Here, no, here it's completely irregular, *scrambled*, altered, irreproducible, it doesn't reproduce itself.

Stochastic Models: The Role of the Number of Channels (N) So, the way to describe these things, to try to interpret them and eventually build theories or models that help us interpret electrophysiological signals like these, we remember that individual channels, like the ones I showed you, are discrete entities and when I consider them at the level of the sum of the membrane's effect, which you remember beyond the concept of Kirchhoff's laws, of the parallel [components], of the conductances, in the end, I'm summing the currents in this charge balance equation.

Now this graph here is slightly difficult to look at, at first glance you say "ok, here is one channel, here are 10, here are 100". Every time I make one of these graphs I'm rescaling the Y-axis, that is, here the individual channels have a small conductance, the current they pass when they open is a few **picoamperes**,

whereas when I have **10**, if all **10** open, probably here the majority are open, I should have **10** times that few picoamperes. Suppose **5 picoamperes**, here it would be **50**, so the distance between here and here is not the same distance as between here and here. I'm rescaling the axes to show you that, it's true, the quantities are noisy but very, very small, because if I have a good, very large population, the noise is negligible, the fluctuations are there but they are very negligible.

The important thing for me was to try to clarify that when the number starts to be… it doesn't need to be infinite, it's enough for it to be on the order of hundreds of channels, it's as if the fluctuations can be negligible. In black here I've put the case N tends to infinity, which I haven't drawn for N infinity, but for example, I invoked it using the equation I already know I can use, the deterministic equation. $N_\infty - N$. I used this one, which I know holds in the deterministic case, that is, in the case where there is an enormous number of channels, so much so that I even describe them with the law of mass action, just as I describe the reactions of sodium chloride binding, dissociating, etc., in a way, not *bulk*, it's wrong to say *bulk*, in a macroscopic way, with an enormous number of particles, of molecules.

***Channel Flickering* and Finite Number of Channels** And this **channel flickering** is worth trying to understand. This is not an experimental trace, it was me who used the Markovian kinetic scheme, and now I'll show you how, to describe it.

The idea is, if I can figure out, with paper and pen, more or less where this noise comes from, I could understand if by chance this noise, as it seemed to be here, maybe depends on the fact that here the trace of the membrane potential during this periodic activity doesn't get particularly hyperpolarized. Here, because of these fluctuations in the input, cases are frequent where there are these *swings*, these elongations, these stays of the membrane potential at more hyperpolarized potentials, where the membrane channels are predominantly closed. It's as if, I'm thinking, could it be that this noise is greater the more it depends on the state or depends on the membrane potential? Is it a noise generator that depends on the potential?

And if so, it could be interesting and intriguing to understand why *fast-spiking neurons* have a different behavior from pyramidal neurons. Ok, they have a different set of channels. Or they have a very large number of them, or maybe simply in the axon of these neurons, here there's a point, for example, in the **axon initial segment**, which is the initial segment of the axon, where the action potential is generated, where maybe here it's not that you have $N \to \infty$ channels. You have a number that's perhaps small, or you have some type of… in the axon or in the dendrites, you have a concentration of ion channels in a reduced number.

If they are reduced, they start to fluctuate, or rather, they always fluctuate, but if they are reduced, like a small choir, you clearly hear the out-of-tune voices. If it's an enormous choir, no, and it's always the same thing, in the choir too, the voices add up.

The Stochastic Markov Approach for Single Channels Summing algebraically, when I talk about an average, the average in my town is the summation from 1 to N (twenty?) of x_i and then I divide by N. Do you agree that even if I don't divide by N, roughly the mathematical operation is the same, I'll just have a scale factor. If the fluctuations of a choir, of a population of channels, somehow become a little less negligible when I take an average of an enormous number, it means that when I have the charge balance equation (which is gone), which was a summation of currents, there too I have a sum of effects. If the channels are few, it's as if I had an average but of few elements, so it becomes noisy. Now maybe it will make a little more sense.

There are two ways to approach this problem. The first is the **Markovian** one, the second I'll tell you later, maybe next time. And this is the simplest and it is: I already know that an ion channel is a system that goes through a series of morphological, functional configurations, for which a number of states exists with a number of transitions, and there is probably only one (in experimental reality, it's typically like this), there is one state in which the ion channel is open, it's conductive, and then the elements that are on these arrows of this Markovian kinetic scheme are either constants or they are voltage-dependent or they are dependent on ions, on ion concentrations, or they are dependent on the concentration of an extracellular neurotransmitter in proximity to the receptor. In the end, the receptor/ion channel are the same beast, more or less as a first approximation, not all synaptic receptors are ion channels, I'd be talking about the so-called **ionotropic** receptors, not metabotropic, but we'll see that next time.

So I already know this type of description, I've used it and I had told you "ok, here for each state write a differential equation etc. etc.", but wait, no. Here I think that now this is the description of **one channel**, not of a population of channels. In the chemical case, it's not a kinetic scheme that tells me how a solution in which there is sodium and chloride… no, I'm thinking of two molecules, one of sodium and one of chloride, they bind or dissociate. Even intuitively, you imagine it as something that *flickers*, that is subject to probabilistic, not deterministic, events.

So I can think of associating (I'll show you how it's done in the non-deterministic case, in the microscopic case), I associate with this channel… with this i-th state of the channel, I think of having N of these ion channels, suppose they are all identical, each of these travels between these states, jumps according to what the voltage-dependent properties are or whatever they are, and for each one, I have a binary variable that I call S, state (or I don't know why it's called S, I believe in accordance with a nomenclature and a notation from statistical mechanics, but it doesn't matter). This is a Boolean [sic] variable, it's **1** with probability… so it's a random variable, and it is… it takes the values **1** with the occupation probability of this state, otherwise (so with probability 1 - the previous probability) **0**.

And so far, one says "ok, in the end, I imagine I want to write the current through this channel and I want to put in it for a single channel, I don't want to put m^3h, I want to put this S, when S is **0** the current is **0**, when S is different from **0** the current is ionic, for that single channel, it's non-zero".

The Fraction of Open Channels as a Random Variable But I don't just have one of these channels, I have several. In this horrible graphical way, it's as if I'm imagining them as systems that are identical and work in parallel, they don't talk to each other, there's no so-called **cooperativity**, that is, the fact that ion channel number **22** out of a billion is in the open state doesn't change the occupation probability of another channel, even if nearby. They all read the same control variable, they are all intercalated in the membrane and they all "fire" [sic] at the same transmembrane potential, but there's no additional interaction. I think I'm telling you this for the second time in this course because the concept of cooperativity is generally very important in biology and it seems that at least ion conduction in vertebrates, in the central nervous system of vertebrates, rodents, primates, human and non-human, doesn't seem to have these characteristics of cooperativity.

So what I can think is that there isn't a single S, there are many, S_1, S_2, S_{1000}, S_N. Each one has a probability of being **1** or **0** according to the probability of that channel being open or closed, so they don't talk to each other.

So what I can do is that the famous **fraction of channels** in the open state, which I sold to you before in this way, a few lessons ago, now I write it according to the definition. How many channels did you say there are? There are N. Ok, so if I take these Boolean variables, these binary variables (sorry, not Boolean, binary), stochastic, random, and I sum them together, I have something that at most becomes N. But it's a random variable in turn, it's a sum of random variables, and $1/N$ normalizes the sum and makes it become a fraction. Ok, so in theory, if I had some contraption, some mathematical machinery that describes these random variables for me and makes them go from **0** to **1** according to the non-deterministic Markovian kinetics of this kinetic scheme, I put it inside, I replace where I had in Hodgkin-Huxley, I replace the N (N^4 if you like, but all of N), I put this in, and in theory, I should be able to simulate the excitatory activity when the channels are stochastic.

And in theory, it's the simplest thing in the world, you don't have differential equations, you just have to generate on the computer some kind of mechanism that becomes **1** with a certain probability and **0** otherwise, and it, let's say, *flickers*, it describes when this channel is here, if at the next step it stays here or it makes the transition, if it goes here, if it comes back… that is, you have to implement the Markovian kinetic scheme in a different way from the equation of mass action, from the law of mass action. For the rest, you put it inside the same currents.

Simulating Stochastic Transitions: Probabilities and Random Numbers I'll pick up this discussion on transition probabilities again. You see it's not particularly difficult. Here I told you one or two weeks ago (when you're having fun, time flies, I can't remember if it was last… whatever last week was), in which the arrows of that damn kinetic scheme, even the one from a moment

ago, are to be interpreted as **transition probabilities**.

Here you have the probability of making the transition from A to B in a time interval $[T, T + \Delta t]$, given that you were in state A at the previous time T. Conditional probability where we talk about an interval because continuous-valued random variables do not have non-zero probability over an integration interval of zero measure. If you ask me "what is the probability that the phone rings right now?", at this instant of zero measure, in this time interval of zero measure (because it's not an interval, it's a point), it's zero.

Here I say it's an interval, I call the interval… I take it in Δt, suppose one second, half a second, one hundred milliseconds, whatever it is, and I say that this k_1 and k_2 are the transition probabilities [per unit time], so that the probability of passage is given by $k_1 \cdot \Delta t$ + higher-order infinitesimals. This thing that seems a bit… that can be intimidating, simply means that I assume that this probability is a complex function as you please, but that it certainly cancels out when the interval… [it's] a function of Δt and when the interval is zero the function cancels out, because the probability for $\Delta t = 0$ is 0 (the phone didn't ring and even now it hasn't rung).

And this is its Taylor series expansion to the first order, and I say that what I put on the arcs of that kinetic scheme is the first term, the first order. The 0-th term is 0 because the probability for $\Delta t = 0$ is 0, so it's a function that, evaluated at the point where I'm doing the Taylor series expansion, is 0. And in a bit, I won't care about the higher-order infinitesimals, but an approximation… written like this, however, it's an exact expression.

This object here, I can simulate it on the computer in an ultra-simple way. You may or may not know that, for centuries now… no, for decades, computers can simulate… all the video games you play work with a **random number generator**. There is, obviously, an active academic research field, in particular also linked to cryptography, to the fact that you, in theory, must, to protect communications, you should try to be able to generate something truly random. And this is obviously very complicated because computers are deterministic, because von Neumann architecture, Turing machines, are objects described by laws, they are not *wetware*, they are *hardware*, it's not wet, it's a silicon thing that works, among other things in a bad regime where the transistors are… but it works and we even have *large language models* and *deep* networks that classify kittens or cute… dogs.

So random number generators are intrinsically deterministic objects that, however, have a behavior almost indistinguishable from the random case. One thing that enlightened me years ago was when a professor in class showed me this graph where he told me: "If by chance you have some way to generate a function $f(x)$, which is made like this, a periodic function (so these are lines), it's related to the remainder of the integer division… R… I don't know, some x-bar which is this interval". If you have something like this, it's a deterministic function, in which, that is, and it is a function because if I enter (so a function is a unique *mapping* where one element is associated with one and only one element, suppose it's here, one and only one element), so this is the output and this is the input. In this direction, it's deterministic, but if I tell you I got this value out, you can't go back to what the value was that generated it. So in

particular in the case of a sequence, a function similar to this or conceptually similar to this. I think these classes of random number generators are called congruential, something like that, there's this word *congruential*, and I don't remember anything else. It becomes, to all intents and purposes, unpredictable what the value of the previous sequence was. And typically these methods cause the output value to be recycled to the input and a sequence is generated. And this sequence is almost indistinguishable from a stochastic process.

Algorithmic Implementation: Generating Stochastic Events What you have in Python, Julia, Matlab, whatever it is, you surely have some function, either from a library or whatever, that generates a **pseudo-random** number for you. It's called pseudo-random because, unfortunately, complete randomness is achieved with hardware techniques that I won't tell you about. You have something that you invoke, and it generates a number between **0** and **1**.

When you generate it, you might ask yourself the following: suppose R is one of these random numbers and it's generated between **0** and **1** (suppose **[0, 1)**, it doesn't matter, they can be open as intervals). Could you answer the question, what is the probability that R is ≤ 1? Can you tell me what it is? **100%**, **1**, perfect. Ok. And can you tell me what is the probability that R is ≤ 0? It's between **0** and **1**, so **0**. Ok? Let's say this shouldn't be enough for your intuition to say, to say… "yes, it doesn't matter". No, it's open. You're right, but it's not essential. Here it is…

This ingredient should make you suspect, if I need to synthetically generate a probability that something occurs, so I can generate, pretend to simulate a coin, the toss of a coin whose probability is **0.5**. Can I simulate an event on the computer that happens **50%** of the time that I call that function? Here you have a clue that if the thing you put here, so if you use this, if you make a comparison $R \leq$ something, it would seem that what you put here is effectively the value of the probability of the pseudo-random event that you are synthetically generating. If I put **0**, it's **0**.

Now the next step would have been to ask you what is the probability that $R \leq 0.5$? I must add, I must emphasize one thing, that random variable is **uniform**, it means that the probability density function (here obviously you are more shy) is constant between **0** and **1**. It's not the probability distribution, it's the density function, its [sic] derivative, but anyway, it doesn't matter. It means that there's more or less the same probability that it will take any value between **0** and **1**.

And it's effectively true, if I run it, I take Python… no Python 3, it screws me now because I don't remember from memory… Obviously, it has to do the update now. Interesting. `python3`. Let's see what a fool I make of myself. And I do `import`, how do you say it, how do you do it… `from numpy`… no. `import`, how does it go, `numpy`, thanks. `numpy as`, how do you say it, `numpy` as, how do you do it, `np`, `as np`, thanks. Like this? Go, *sorry* thanks. And `np.` there's no completion… yes, there's `random`. Ha ha. `random.uniform`, damn you for changing the… I hate this, I hate Python, I hate Python. It's too annoying, that is `np.random.uniform` and probably I have to give it `0, 1`, *low, high*.

If I did it a large number of times, I accumulate these things in a vector (if I were cool I'd do it, but I won't because I'll definitely not make a fool of myself), and I made the histogram of all these terms, you would see that roughly all the values have the same probability of occurring. In fact, they are spanning between **0.1, 0.08** which was small, **0.9, 0.09, 0.2, 0.3**, etc., etc.

So this is the fundamental ingredient. And in this case, when I say "what is the probability that R is less than a certain quantity", in theory, graphically I should say, I'm asking what is the integral between **0** and **0.5**, so what is the area here? And the area here would be exactly **0.5**, so again it seems to work, whatever I put here is equal to the probability of the event I want to generate. So if I want to generate an event with probability $k_1 \cdot \Delta t$, I have R generated and then I ask, `if R <= K1 * delta_t`, then print "the event happened", otherwise "the event didn't happen". Remember that it's a probability of an event, I want, like a coin, I want for example to simulate if a coin tossed in the air results in heads or tails. Suppose I want heads, I must have some *if-then-else* that tells me, I compare R, the algorithm is simple, I take R, I generate it, I compare it with a quantity I call P_0. The times this logical condition is true, it happens with probability P_0, it happens P_0 times percent out of one hundred times. If you want, you can try it.

And it's linked to the property, so the probability density function is linked to the probability distribution function because it's its derivative, and so to go from one to the other there's an integration operation, and here it's assumed by convention that before **0** it's **0** because I don't have values of R less than **0**, and so it's this area. This area fills up proportionally, given that it's a rectangle of unit width… I forgot to say that this, being a probability density function, means that the total area under it must be **1**, because probability is one of Kolmogorov's axioms, it must be **1**, and so the height of this rectangle must be such that multiplied by the base, which is **1**, it must give **1** area, and so the amplitude is **1**. Knowing the amplitude, I can play this game of the integral and a fraction, and it happens with a certain fraction, so **0.8, 0.8** etc.

Simulating a Two-State Channel So this is the algorithm, and with a wonderful computer simulation from a few years ago, which I have to redo because it's pitiful, I can simulate… Simulate exactly the current, now I'll show you, but in the end it is: I generate the random number and depending on the state I'm in, if I'm in the open state I can only make a transition to the closed state, and if I'm otherwise… I'm not in the open state… I can only make a transition from closed to open. So α and β (you see here β appears and here α appears) are not all the possible cases.

And what I can generate in this very ugly, aesthetically very ugly, very ugly representation is the opening and closing of an ion channel, stochastic, which has two states, which has a single state, only one of the two that is open. And the thing I did between one and the other, maybe you can see it, maybe not, one of the two probability values per unit of time, so $\beta \cdot \Delta t$, in this case, is **0.01**, in this case, it's **0.1**, so I close much more easily, much more frequently, if I open, I close right away. In fact, you see that regardless of which one I defined

as open or closed, here the openings are sporadic, here instead, openings and closings are much more frequent.

Computational Complexity of Stochastic Models So in theory, I could put something like this, at this point I can only do it numerically with a computer and run a simulation, and simulate… here's the disadvantage, I have to simulate every single sodium channel, every single potassium channel. In each of the two cases, I'll have α and β that depend on the potential exactly with the Hodgkin and Huxley formulas, on those kinetics, α and β are complicated functions of the membrane potential, but I have to track them all, that is, in memory, I have to keep track of the state of all these damn… I can't use a single variable for all of them, I must have N variables, N_{sodium} for sodium, $N_{\text{potassium}}$ for potassium.

So computationally it's easier [sic, likely means 'conceptually simpler'], it allows modeling, describing, exploring the stochastic component, but it's extremely complex, extremely heavy from a computational point of view because if you want to explore a typical case you might have some tens, hundreds of thousands of sodium ion channels or potassium ion channels. And you have to do this algorithm hundreds of thousands of times per unit of time, because you have to do this at every time step. If the time step were **0.01 milliseconds** or even less, there you go, welcome to a world of considerable complexity.

Linking Stochastic and Deterministic Models I don't remember what I have to tell you… if any of you have had him, it means I'm old enough to have colleagues who were young, who are no longer young either.

I wanted to try to give you an insight, an intuition, and an explanation of why when you treat the deterministic case, exponentials come out, continuous things come out, but in particular, exponentials come out and time constants come out. When instead in the case… I have nothing that tells me there's a time, that there's a time dynamic with a time scale. I have N, **100,000, 10,000**, or even **10, 20**, entities, each of which *flickers* and fluctuates between, for example in this simple case, the open state and the closed state.

From Transition Probability to Occupation Probability (Master Equation) I want to do it in a simpler way, so that here, with paper and pen, we can manage to do something. So the kinetic scheme I have in mind now, I think I'll want to use A and B to be able to write dA and dB, to be able to say, pardon, not to write dA and dB, because I don't want to treat it deterministically, but I need to write the transition probabilities. But I want to do one more thing, I want to write the **occupation probability**, which is not the same thing as the transition probability. The transition is a conditional probability: I'm in a state, for example open, and I can only close, what's the probability? If the probability is **0.5**, it may be that **50%** of the time I don't close, I stay in the state where I am. It's different to talk about occupation probability, which means "but what is the probability of finding that channel in that state at time t?".

I'll show you where the exponentials come from. So here I've written it, this $P_A(t)$, I've written it as… I'm alluding to what is the probability of being in state A at a time t. And, furthermore, something that is very reminiscent of the conservation of mass, in the case of the deterministic, mesoscopic, non-stochastic interpretation, is that either the channel is in state A or it's in state B, so at a certain instant T, I either find it open or I find it closed. It's not that it's in some other third state, so the sum of the occupation probabilities is unitary, that is, if here it's **0.3**, here it must be **0.7**, if here it's **0.7**, the other probability $P_B(t)$ must be **0.3**.

And I do this bold calculation, I ask myself: suppose I know what the occupation probability of state A is now at time T, can I, as an exercise, calculate what the occupation probability is in the same state at time $T + \Delta t$? Apparently, it's complicated, but in reality, it's simple, because it's the logical disjunction [original text: conjunction] of two events, so it's either one or the other. From the set theory point of view, it's a disjunction [original text: conjunction], that's why you add. The probability that it rains *or* that the phone rings. This is the sum of the probabilities. [This is only true for mutually exclusive events; the speaker seems to be simplifying].

So the probability of being in A at time $t + \Delta t$ is the probability of having been in A at time $t + \Delta t$ AND of having also been in A at the previous time. *Or* the probability of being in A at this time AND of having been in B at the previous time. Obviously, this is a joint probability of two events, clearly, it can be written by the law, by Bayes' theorem, based on the transition probability. This one too can be written in terms of transition probability, because I didn't make a transition.

So the probability that I am in A now and I was also in A before has become the probability that now I am at $t + \Delta t$ *given* that the previous state was in A at the previous time. Multiplied by the probability of being in state A at the previous instant, this is Bayes. Plus the other case is the probability of being in state A at time $t + \Delta t$, *given* that I was in state B, times the probability of being in state B at the previous time.

This thing here is $1 -$ transition probability, because I didn't make a transition, while this one here is exactly the transition probability. I don't remember what it was, if it was $k_1 \cdot \Delta t$ or $k_2 \cdot \Delta t$. This quantity here is $P_A(t + \Delta t)$, this one here is $P_A(t)$, and this one here is $1 - P_A(t)$. Ok, here I've still written $P_B(t)$.

From the Probability Equation to the Differential Equation So you're starting to see that infinitesimals are popping up, and when infinitesimals pop up, if by chance there's hope for an incremental ratio, I take the limit, because maybe derivatives pop out and the exact same differential equation comes out, this time, however, it's not for the fraction of channels, it's for the **probability** of finding a channel in a certain state.

So what I do is write the occupation probability in state B as $1 - P_A$, since it's either one or the other [original text: or it's soup]… you're either in the closed state or you're in the open state, so something that is similar to the conservation

of mass but for probabilities, it's a probability sum to **1**, that the probability of the set of the total event, of the joint event is unitary, Kolmogorov's axiom. And what I'm left with is… and here I can factor out $P_A(t)$, because it appears here, it appears here, here there's $k_1\Delta t$, here there's $k_2\Delta t$. If I do it, it comes out, and I take the limit for $\Delta t \to 0$, I get exactly the same equation that I would write if I had done it with the kinetic scheme interpreted in a deterministic way. You can try, here it was $A \leftrightarrow B$ with k_1 and k_2, we had called them α and β, but in the end, it's always the same thing. Here it was $\alpha + \beta$ and here maybe it was β. And here, for example, I could redefine, write τ and P_∞ and I would be in exactly the same context.

The analytical solution of that thing there is exactly the same analytical equation as this. If k_1 and k_2 depend on the membrane potential, as k_1 and k_2 in this formalism did, I have exactly the same time evolution that I had for N, but I have it for the probability of finding that channel, a generic channel, in the open or closed state. So paradoxically, for the moment I haven't told you why I observe exponentials, but the exponential comes out here too, only we're in the world of probabilities, not in the world of determinism. This is the probability that something happens, it's time-variant. Now it's low, now it's starting to increase, now it's become certainty and so if I am a channel, maybe I make the transition, I know I'm in state A. For state B, it doesn't make sense to do it because it holds that P_B at every instant is $1 - P_A$ at that same instant.

This is the point where, but I think I'll do it next time. If you have any recollections of probability theory, you could try to calculate the mean value, the expected value of this stochastic variable. It's **1** when I'm in one state, in the open state, and **0** otherwise.

See you next week.

Markovian Stochastic Formulation of Excitability

Introduction to Biophysical Non-Determinism The idea I want to revisit is to conclude the part on **neuronal excitability**, pushing beyond the minimal and deterministic description introduced so far, to embrace a non-deterministic, or **stochastic**, description. I have emphasized multiple times that excitability is not a continuous, distributed property of voltage-dependent ionic permeability of a membrane, but is intrinsically linked to the discrete and microscopic nature of **ion channels**. When something is small, especially in a biological environment at physiological temperature, phenomena like **thermal agitation** dominate, and the behavior is no longer purely deterministic.

The Markovian Kinetic Scheme for Ion Channels Let's return to the fundamental concept that an **ion channel** is nothing more than a system that jumps between distinct states, for example, a **Closed** state and an **Open** state. These transitions are governed by probabilities, giving rise to a **Markovian kinetic scheme**.

Imagine a single channel, where the state variable s_i is binary, $s_i \in \{0, 1\}$, where 1 indicates the Open state (A) and 0 the Closed state (B).

- $s_i = 1$, if the state is A.

- $s_i = 0$, if the state is B.

The probability $P_A(t)$ of finding the channel in the open (conductive) state at time t is correlated with the transition dynamics.

The transition between these states is governed by the rates α (from closed to open) and β (from open to closed). In a small time interval Δt, the transition probability is:

$$Pr\{A \to B \text{ in } (t; t + \Delta t] / \text{state } A \text{ at } t\} = k_1 \Delta t + O(\Delta t)$$

$$Pr\{B \to A \text{ in } (t; t + \Delta t] / \text{state } B \text{ at } t\} = k_2 \Delta t + O(\Delta t)$$

where k_1 and k_2 are the transition rates, which in the voltage-dependent case are $\alpha(V)$ and $\beta(V)$.

Stochastic Simulation: The Random Number Method How can we simulate this microscopic dynamic? We need to generate a random event. We assume we can generate a **pseudo-random** number r, uniformly distributed in the interval $[0, 1]$.

To simulate the transition $A \to B$ in Δt, the condition is that the event occurs if the random number r is less than or equal to the transition probability in that time window:

- If the channel is **Open** ($s_i = 1$): the transition to Closed occurs if $r \leq \beta \cdot \Delta t$.
- If the channel is **Closed** ($s_i = 0$): the transition to Open occurs if $r \leq \alpha \cdot \Delta t$.

This mechanism produces the typical **"flickering"** signal of the single channel, which is the direct manifestation of stochasticity.

From Single-Channel Probability to Population Equation

The Markovian Derivation (Master Equation) Let's focus on the occupation probability $P_A(t)$ for a single channel. The event of being in A at time $t + \Delta t$ can only happen if: 1. One was in A at time t **AND** remained in A. 2. One was in B at time t **AND** transitioned to A.

Using the definition of conditional probability and considering that $P_B(t) = 1 - P_A(t)$:

$$P_A(t + \Delta t) = Pr\{A \text{ at } t + \Delta t / A \text{ at } t\} P_A(t) + Pr\{A \text{ at } t + \Delta t / B \text{ at } t\} P_B(t)$$

Substituting the transition probabilities:

$$P_A(t + \Delta t) = (1 - k_1 \Delta t) P_A(t) + k_2 \Delta t (1 - P_A(t))$$

Rearranging and dividing by Δt:

$$\frac{P_A(t + \Delta t) - P_A(t)}{\Delta t} = -k_1 P_A(t) + k_2 (1 - P_A(t))$$

And taking the limit as $\Delta t \to 0$, we obtain the differential equation that governs the temporal evolution of the occupation probability, known as the **Master**

Equation (or in electrophysiology, the form of the Hodgkin-Huxley transition rates):

$$\frac{dP_A(t)}{dt} = -(k_1 + k_2)P_A(t) + k_2$$

The Expected Value (Ensemble Average) This result is remarkable because it demonstrates that the equation describing the **probability** of finding a single channel in a given state is **identical** to the deterministic differential equation describing the **fraction** of open channels $n(t)$ in a large population!

Indeed, let's define the **expected value** $\overline{x}(t) = E\{x(t)\}$ for the binary variable x:

$$\overline{x}(t) = 1 \cdot P(x = 1) + 0 \cdot P(x = 0) = P_A(t)$$

Thus, the expected value of the microscopic state variable is exactly the occupation probability, and since the equation for $P_A(t)$ is the same as the deterministic equation for $n(t)$:

$$\frac{d\overline{x}}{dt} = -(k_1 + k_2)\overline{x} + k_2$$

The **Ensemble-average** is equal to the deterministic description.

Variance: The Essence of Channel Noise

Quantification of Fluctuations If the deterministic description captures the population average, it is **blind** to the fluctuations and **flickering**. The noise resides in the **variance** of the state variable.

The variance of a random variable x is defined as:

$$Var\{x(t)\} = E\{(x(t) - \overline{x}(t))^2\}$$

Since the variable x is binary ($x \in \{0, 1\}$), $x^2 = x$. The variance simplifies to:

$$Var\{x(t)\} = E\{x(t)^2\} - \overline{x}(t)^2 = E\{x(t)\} - \overline{x}(t)^2$$

Substituting $E\{x(t)\} = P_A(t) = \overline{x}(t)$, we obtain the formula for the variance of a Bernoulli variable:

$$Var\{x(t)\} = P_A(t) - P_A(t)^2 = P_A(t)(1 - P_A(t))$$

If we use the notation $\overline{x}$ for the open probability, the variance for the single channel is:

$$Var\{x(t)\} = \overline{x}(1 - \overline{x})$$

The Variance of the Open Channel Fraction (n) Recall that the macroscopic current I depends on the fraction of open channels $n(t)$:

$$I = \overline{g}n(t)(V - E)$$

where $n(t)$ is the average of the individual state variables, x_i:

$$n = \frac{1}{N_{\text{tot}}} \sum_{i=1}^{N_{\text{tot}}} x_i$$

Due to the property of statistical independence among channels, the variance of the average is the sum of the variances divided by N_{tot}^2. Thus, the variance of the fraction n is obtained by dividing the sum of the individual variances by the square of the total number of channels:

$$Var\{n\} = Var\left\{\frac{1}{N_{\text{tot}}}\sum x_i\right\} = \frac{1}{N_{\text{tot}}^2}\sum Var\{x_i\}$$

Since all channels are **identical** and **independent**, $Var\{x_i\}$ is the same for all, and the sum is $N_{\text{tot}} \cdot Var\{x\}$:

$$Var\{n\} = \frac{N_{\text{tot}}}{N_{\text{tot}}^2}Var\{x\} = \frac{\overline{x}(1-\overline{x})}{N_{\text{tot}}}$$

This is the crucial expression that tells us that **fluctuations are inversely dependent on the total number of channels, N_{tot}**.

Biophysical Consequences of Channel Variance

Inverse Dependence on Channel Number The expression $Var\{n\} = \frac{\overline{x}(1-\overline{x})}{N_{\text{tot}}}$ explains the intuition that "the larger the choir, the less you hear the off-key voices."

- **$N_{\text{tot}} \to \infty$:** If the number of channels is very large, $Var\{n\} \to 0$. The fraction of open channels n approaches its expected value $E\{n\} = \overline{x}$ (the average activation) with very high probability (this is the **Central Limit Theorem**). In this regime, the deterministic description is perfectly adequate.
- **N_{tot} small:** If the number is small (e.g., in dendritic **hotspots** or the **axon initial segment**), the variance is large and fluctuations are significant. These fluctuations can induce **spontaneous spiking** or cause **jitter** in *spike timing*, as observed by **Mainen and Sejnowski**.

Functional Dependence on Potential ($\overline{x}$) The variance also depends on the average fraction of open channels, $\overline{x} = P_A$. This function $\overline{x}(1-\overline{x})$ is a downward-concave parabola, which peaks at $\overline{x} = 0.5$ and is zero at $\overline{x} = 0$ and $\overline{x} = 1$.

Since $\overline{x}$ (the asymptotic value of n) depends on the membrane potential V_m via the activation curves (α and β are voltage-dependent), it follows that **the intrinsic channel noise depends on the membrane potential state**.

- **Extreme Hyperpolarization ($V_m \to -100$ mV):** All channels are closed, $\overline{x} \to 0$. The variance is near zero: $Var\{n\} \approx 0$. The trace is clean, not noisy.
- **Moderate Depolarization (near threshold):** Channels are in an intermediate state, $\overline{x} \approx 0.5$. The variance is maximal. Fluctuations are high, which can explain why **jitter** and **noise** increase when the neuron is depolarized under constant input.
- **Extreme Depolarization (spike peak):** All channels are open, $\overline{x} \to 1$. The variance is near zero: $Var\{n\} \approx 0$. This justifies why the peak of the action potential itself is not particularly noisy.

This dependence on the state V_m is the reason why creativity or the imprecision of *spike timing* is an active phenomenon and not just background noise.

The Langevin Method as a Mesoscopic Approximation

The Computational Alternative to the Markovian Model We have established that the single-channel **Markovian** model is biophysically accurate, but the simulation is **extremely slow** and **computationally expensive** because it requires tracking the state of hundreds of thousands of individual channels.

To overcome this hurdle, an approximation is sought that is **fast** while simultaneously retaining the **stochastic** component (the variance). This approach is known as the **Langevin Equation** (or effective mesoscopic formulation).

The Langevin method consists of taking the **deterministic** differential equation that describes the average fraction of open channels $n(t)$ and **adding** a stochastic noise term $\xi(t)$, whose amplitude and color (temporal correlation) are rigorously derived from the single-channel Markovian theory.

For the dynamics of the state variable n (which is $\overline{x}$):

$$\frac{dn}{dt} = -(k_1(V) + k_2(V))n + k_2(V) + \xi(t)$$

The noise term $\xi(t)$ is chosen so that its variance is consistent with the population variance derived Markovianly. In this way, the Langevin equation incorporates the variance:

$$Var\{\xi(t)\} \propto \frac{\overline{x}(1-\overline{x})}{N_{\text{tot}}} \text{ (dependent on } V \text{ and on } N_{\text{tot}})$$

Advantages of the Langevin Approach

1. **Computational Efficiency:** Only four differential equations (for V, m, h, n) need to be solved, just like in the deterministic **Hodgkin-Huxley** model, but now with a noise term that captures fluctuations. There is no need to track individual channels.
2. **Accuracy:** Despite being an approximation, the Langevin formulation is capable of faithfully replicating not only the average but also the **variance** and the **autocorrelation function** (the noise "color") of brute-force Markovian models.
3. **Predictive Power:** Being an analytically tractable model, it allows for the inference of biophysical properties (such as the number N_{tot} of channels) from the experimental measurement of membrane noise, as demonstrated by **Conti and Vanche**.

This dual perspective (microscopic/Markovian for the foundation, mesoscopic/Langevin for efficient simulation) provides us with the essential tools for analyzing the active and noisy behavior of a real neuron.

Stochastic Hodgkin-Huxley Models and Intrinsic Noise

I wanted to show you what happens when one takes the Hodgkin-Huxley model and transforms it, in the way I described to you, into a stochastic model. In this model, the two currents, the voltage-dependent sodium current—which is also called **fast inactivating** because it inactivates rapidly—and the **delayed rectifier** potassium current, are transformed and described stochastically.

The first thing I do is, as I did last time, apply a constant current stimulus. So, I turn it on at time zero and leave it on indefinitely. If the current, as in this case, is not particularly intense, it doesn't do much. The more astute among you might notice that there seems to be a little bit of fluctuation here. The trace does not seem to be exactly what would be expected; there would be some fluctuations. These fluctuations, if I take a current and make it even more negative (this is -0.5, which must be $\mathrm{nA/cm^2}$; the unit counts, but not the dimension because I am simulating per unit area, so I am not saying that the cell is of that size), might remind some of you of a phenomenon we discussed last time: the reproducibility of the firing **pattern**.

It was this figure here, and it was a seminal experiment by **Mainen and Sejnowski**, where, by stimulating a neuron with a constant current, a constant stimulus active for one second (on the order of 800 milliseconds or whatever), taking a pause and then restimulating it, then pausing and restimulating it, one could see that only the first **spike** of the series of 25 repetitions seemed to be reproducible in terms of **timing**. The others seemed as if there was a kind of noise source, of **sloppiness**, of imprecision that accumulated, such that perhaps the total number of **spikes** was maintained, but there was what is technically called **jitter** (I don't know the Italian word for it: a flickering, a flutter, I don't know if that's the right word). And you see that this flutter tends to become larger and larger the more time passes.

While, for the same neuron, a moment later, when the type of stimulus was not so boring, fixed, **DC**, constant, that is, but fluctuated (it was always the same current, always the same trajectory, it was generated like a set of random numbers but was always the same, somehow it was **rewound**, rewound, and the same form of stimulation was applied again), you see that it continued, instead, to maintain, except for very few cases out of 25 repetitions, a very rigorous reproducibility. It seems, that is, that there is a kind, as I said last time, of noise generator that, however, changes with the electrical activity of the neuron.

In particular, something I didn't emphasize last time, it would seem from here (I drew this red bar specifically): if the membrane potential remains rather depolarized, it seems that this noise generator is present, plays a large role; it's as if the variance of this noise generator, the intrinsic fluctuation of the membrane potential, were greater. If, on the other hand, the fluctuations bring the membrane potential, the input brings the membrane potential, periodically back to this slightly less depolarized bar, perhaps the noise generator tends to reset itself, tends to decrease.

I mentioned that this story was even more interesting than just the fact that perhaps creativity, perhaps the possibility for an animal to have responses that are not identical (we are not robots; if you do an experiment with a rodent or

a primate there is no deterministic consistency—besides the fact that even a robot would not have deterministic consistency because it would be a physical system, and in a physical system there are a lot of inaccuracies, non-idealities, you see this when driving a car, which make it impossible to execute exactly the same trajectory twice in a row). This is even more interesting because it seems to be state-dependent, activity-dependent.

And so the most acute, astute, no, acute among you, will see or would see that by lowering this current, the value of the current, the trace becomes cleaner. While, by depolarizing the neuron, the fluctuations tend to become consistent, even such as to emit spontaneous action potentials: creativity. It occurred to me… ah, and where was it? Meh. It's a fluctuation due to the intrinsic noise of the membrane channels that caused that neuron to fire, which, on its own, codes for the red color of a visual stimulus, but the red stimulus was not there in the visual stimulus, because I was looking at something else, yet the same neuron fired. And all of biology is like this, all of biology is noisy.

The interesting thing is that, having implicitly included channel noise, I did not write something **plus noise**. So, in the deterministic part, which I wrote here, where this current I_K was $G \cdot n(t)$, the fraction of channels in the open state, times the Nernst reversal potential minus the membrane potential, this is called the **driving force**. It's not that I took, as engineers do, who say: "You know what? I'll write this as $n(t)$ plus a white Gaussian noise, δ-correlated, etc., etc." No, I didn't do that: I went and took the biophysical meaning and, **for free**, I get a phenomenon that seems to fit, seems to be exactly what happens, and it is exactly what happens.

One thing that can be done is, here, for example, changing the number of channels. And if you remember the conclusion from a little while ago, it was this thing where the fluctuations, the variance, or the standard deviation (but it's the same thing anyway), are **inversely dependent on the number of channels**. If the number of channels becomes very high, the members of a choir become very numerous, there are no more fluctuations. So, if I greatly increase sodium and potassium, the number of channels, the fluctuations tend to decrease. In theory, if I were to put them… indeed, for example, the behavior of spontaneous **spiking**, of spontaneous action potential emission, is gone. If, instead, this number of channels becomes very small, the fluctuations tend to be very important, even resulting in many action potentials being fired.

Here you might object: "Wait, you are putting a small number of channels, so this term here is small, because this is the maximum conductance and the maximum conductance was the total number of channels times the conductance of the single channel." I am deceiving you because, to explain this concept to you, I am rescaling the quantities here; so, when I change the number of channels, I keep the maximum conductance value fixed. I do this because biologically, in the case of some conditions, for example morphological ones, like the **axon initial segment** of the neuron or a part of the proximal dendrites (so close to the soma), it could be that there is a very small number (now I write $n < 1$ but simply because for me it means a very small number, not that it's a fraction, but a low number: not a million channels, but $10, 15, 100, 500$). It could be that this **hotspot** with few channels can influence through electrical proximity.

You can imagine that the part… and we will look at this in depth… the dendrites to the **Soma** are as if they were connected by a resistor inside the cytoplasm. It behaves like a conductor, perhaps not ideal (previously, at the beginning of the history of excitability and equivalent circuit models, I sold it to you as an ideal conductor where there is no resistance, because I told you: "This resistance is negligible compared to the membrane resistance"). Spatially extended structures can start to matter, and this distal or at least proximal noise generator (but still not close to where action potentials are generated) can have this effect.

Response Variability and Stochastic Mechanisms

Another interesting thing is, for example, the same type of protocol where I keep the current constant, normally at zero. The deterministic case would be this: I keep the current at zero, there is an instant when I give a small current pulse, a small step, which here is given around 50 **milliseconds** and probably lasts 5 **milliseconds** or 10 **milliseconds**.

If I did the exact same experiment (here, anyway, this number of channels are such, there are many, but they are still not enough to eliminate all fluctuations, okay? Maybe at this level it is almost deterministic), the response to this step is more or less… in this case maybe it's not excitable. I increase the amplitude a little more. My apologies. The neuron is excitable, the response did not generate a **spike**, an action potential. I increase the current a little more, the amplitude of this step… nothing. I increase it a little more… **spike**. But, you see that (and this is what I wanted to show you) even with a very high number of channels, a protocol like this reveals… I am repeating it five times (as always, you have the code; you can take a look at the Python code and see how I did something like this internally).

The interesting thing is that by giving the same stimulus five times, **three out of five** I had a so-called **failure**, a failure of conduction, a failure of initiation, of the **triggering** of an action potential. Twice, however, out of five, I had a **spike**. And another thing you notice is that the instant at which the **spike** is generated fluctuates. So, again, this voltage-dependent characteristic of the membrane potential, because it depends on the number of open channels (I remind you, we are talking about these fluctuations of the variable n), and this variable n has a variance that depends on the number of open channels, on the fraction of open channels. Therefore, when the potential is very hyperpolarized, the channels are closed, this variance is low. When, instead, the number of channels is such that $x(1-x)$, which functionally is a parabola, is a parabola with concavity downwards (try drawing $y = x - x^2$; the minus… then there is no constant term, so it is centered, it is not centered, as you might say), it means there is a peak of the variance, a peak of the fluctuations. And it makes sense that, biophysically, when the potential is all hyperpolarized, no channels are open, there are very few, and that is why you saw, practically, when the current was negative, you saw the trace practically without noise.

Let's ignore **Kolmogorov**. Here, on these two **slides**, you have what I showed you live with that simulation. Well, this type of mathematical description, although very biophysically accurate, is, as said, quite complicated, it is quite

slow. You have to, for every single voltage-dependent ion channel (be it the type of sodium channel, or the potassium type; if there are other types of channels, you have to do it for every type of channel), keep a state variable in memory, okay, binary, **0, 1** (this perhaps costs nothing).

Yes, go ahead.

Relationship between Variance, Channel Number, and Membrane Potential

Yes. So, if I look at the expression for the variance, I realize that… therefore, if I try to **plot** that quantity there… so, that the variance gives me the magnitude… the variance, in other words, is the mean squared deviation, okay? And it should light up in your mind the fact that when you have a random variable that I call z, and this variable is Gaussian, the probability distribution density of this variable z, for example, is made… I mean, without example, it is made with a Gaussian, bell-shaped trend, it is centered at something you call the mean, μ, for example, and here (now it is not important exactly where it falls, but roughly) the magnitude, the width of this Gaussian is given by σ, the square root of the variance. The variance is often called σ^2, so σ is the standard deviation. I make this distinction because μ and σ must have the same unit of measure. The variance is squared, so if it is the average height, the variance will be meters^2. So that's why people also use the standard deviation.

So, mean and variance: you should visualize the fact that you have some **offset** and the more variance there is, the larger this magnitude is. In my mind, I imagine that this tells me that it is probable that when I extract or see people of a certain height, or see what is the instantaneous value of the current generated by a population of membrane channels, etc., they are around the mean but can, very rarely, be where the tails are, at values where the tails are, because the probability there is low, but where it is not low, it is easy for me to have events at these values. So, the larger the variance is, the larger the fluctuations are.

Another way in my head that I have to imagine these things is if you generate on the computer a set of Gaussian, pseudo-random numbers (not uniform, but Gaussian, so with this distribution), you **plot** them over time (so this is the beginning of my generation and this is the value of these numbers), they more or less do something like this. And if I squint, I see that this signal that goes up over time (I inverted the axes because I liked relating this bulge or the belly of the Gaussian to roughly the band in which it was contained, at least 68% I should say, one or at least two standard deviations, you tell me if it's 99% or three standard deviations is 99%), so for me it is how large the excursions of this stochastic process are.

There I have that the variance is dependent on the mean number of open channels, which is $\overline{x}(1-\overline{x})$. When $\overline{x}=0$ it is 0 and when $\overline{x}=1$ it is also 0. If you want we can do the function study, but the maximum is at 0.5 and it does something like this. The variance is not negative because it is a positive quantity and $\overline{x}$ only changes between 0 and 1. So this is the graph.

So, if the membrane potential, thus $\overline{x}$, changes by changing V_m (in addition to changing on its own in a transient), it changes due to the membrane potential

through those α and β, or k_1 and k_2 that we called them before, because these here are voltage-dependent. And they are voltage-dependent with that trend, actually it would be $\frac{\alpha}{\alpha+\beta}$, which would be n_∞, x_∞ here. Somehow I know that when the membrane potential tends to become more depolarized, both sodium and potassium channels begin to activate. So from here I am moving in this direction. Therefore the variance tends to increase, and I see it because when I tended to increase the current I pulled up the membrane potential. A lot of channels opened.

I believe you would not have flinched in the deterministic case: you would have said "I see that at a certain point the membrane potential deviates from the case of a simple passive **RC** because the sodium and potassium channels start to activate." Here it is the same thing, but also in the dimension of the fluctuations. True, the mean value would tend to integrate the current, would tend to change, the story of the concavity, I told you a colleague of yours from the second year last year was complaining to me, saying "Yes, but the concavity is different from what a passive **RC** is." Yes. Here, in addition to that, you also have the fluctuations, and these fluctuations begin to become larger and larger the more channels are in the open state.

The correct objection would be: "Okay, but after a while shouldn't it decrease?" Yes, indeed, during a **spike** (which is the only way the membrane potential is allowed to explore very depolarized points, unless you go in with a very notable current injection, you truly give a very strong current pulse and the membrane potential stays up there because you are injecting a lot of current)… I think technically the pipette, touching the membrane, would heat up a bit and you would lose the connection. But if that didn't happen, you would see that the noise would tend to decrease. And in fact, during **spikes**, during this behavior, the **spikes** are not particularly ugly, they are ugly and noisy here, but they are not here… in fact the membrane channel there is not particularly high.

What I can do on the fly… I'm really causing trouble… is to quickly change the simulation time. This is not enough. I would also like to change here, so not only the time but also the **range** of this figure. So I know how to use **Python** and **NumPy**, and so theoretically if I wanted to have a **zoomed spike**, it's not noisy here. Before, yes. So, when the membrane potential is depolarized, this is not particularly different from a potential generated by a deterministic **Hodgkin-Huxley** model. Can it be an answer? Did I answer? Well, what I'm thinking about is this.

State-Dependent Stochasticity and Experimental Implications

I am thinking, that is, I don't know if there was, maybe two times ago, I don't know if you told me you missed the lecture. Well, here I am thinking about these activation curves, and if you want, here I can reinterpret them (because the math is the same) as not the asymptotic value that the fraction variable would have, if there was time, but here it would be, in our context, the probability variable. But in the end the concept doesn't change: it is that in the case of sodium and also potassium, when I tend to depolarize, the number of channels in the open state tends to go from 0 to 1, to 100%. It is true, here I should actually multiply this m_∞ by h_∞ and it would have a kind of up-and-down

trend, but let's pretend there is no inactivation for the moment.

The more I am depolarized around $-70, -80, -100$, this is flat, it is zero. If it is flat, then the… then intuitively the channels are all in the closed state, and so good luck **flickering**. Yes, they can **flicker** but the transition probability is practically zero, so even spontaneously they do it once in a blue moon. When instead (because this curve here, or I can also interpret it as an occupation probability, but actually I prefer to think of transition probabilities), when the transition probability tends to increase (so α and β, which you also had and which are here)… now I should remember who is who, I think. Written like this, it means that α is the transition probability between closed and open. It is. Damn it, I don't remember things. So if it is open, closed and I say this is β, this is α, what do I write? $\frac{dO}{dt} = -\beta O + \alpha(1 - O)$. Yes, written like this, α is when it is closed, from closed to open. So, I start when I depolarize to increase this transition probability. So it means that if I have a sodium channel there in front of me (and potassium more or less does the same thing, in fact it's even a little more… no, the scale is not the same, it is slower, in fact it is delayed), I would see that at very hyperpolarized potentials it is practically always closed and rarely makes a transition, makes a fluctuation, **flickers**. If, instead, I start to increase V, this, which is the transition probability per unit time, I would see it much more often, okay, pushing the bottle means I am thinking that it opens and then closes. So I should reason about both α and β. And it is easy because you see that β practically, when the potential is very depolarized, practically becomes zero, that is, it is very easy for me to make the transition between closed and open and to remain open. The concepts conceptually hold even in the stochastic context.

Then, if and for those who like the quantitative and mathematical aspect, it can be useful not only as an exercise, not for intellectual exercise, but because (and this is what I am not telling you, I will only hint at it now) with the expression of the variance, I could in principle take a real neuron, stick a pipette into its stomach, and experimentally (and it is always like this) see that the potential is not fixed perfectly straight at -70, it would have a little bit of fluctuation. So, the experimentalists among you would say: "Yes, you said last time that the synapses (maybe it wasn't you, no, it was your colleagues on Friday), the synapses that we will talk about later, in the second half of this lecture probably, are incontinent, because they too are stochastic systems. Even if there is no action potential, in the end they are always ion channels, so they are also vesicles that fuse, so they are incontinent, they leak and every now and then they have spontaneous activations, what are called **miniature synaptic potentials**."

Experimentally I also see a fluctuation. If I block the synapses with a drug, so I make the receptors deaf, so I put something that blocks the synaptic receptors, the neuron no longer feels, no matter how much the other neurons release neurotransmitter. I have a selective antagonist that plugs the channels from the outside and so the channels no longer feel, no longer bind to the neurotransmitter. If I start to depolarize, I see more and more fluctuations, and if I have this formula, I can in one go measure the fluctuations and ask and answer the question: "But how many channels are there?"

There are 225. This was a revolution of sorts in the early eighties. There is a nice **review** article, by two Italians, **Franco Conti and Enzo Vanche**, both

now retired, who at the Institute of Biophysics and Cybernetics of the CNR in Genoa performed this experiment, saw the fluctuation, and had derived this type of description. It is exactly this, if you take their **paper** you see that it is this thing here that you study now, and from this expression they can infer what the total number of channels is. So how do I measure, if I don't see them, these ion channels? One way is with antibodies that bind, I see them fluoresce with some microscope, but if I don't have a microscope, with membrane noise.

This reminds me of a very brief anecdote, not from my childhood, but from when I was in Belgium. They had given me cells that were stem cells differentiated into neurons, and they told me: "Look, this cell here is a human induced pluripotent cell, I'm almost sure it was not an embryonic stem cell, so it was not taken from human embryos, it was instead a cell, for example from the skin, reprogrammed to become pluripotent and redifferentiated to be not a skin cell but a neuron cell." Molecular biologists normally say: "To be a neuron you must express these genes, these proteins. I have a specific stain for all these markers, for these proteins or genes or whatever, I tell you that this is a mature neuron." I am a pain in the neck and I say: "No, for me it is a mature neuron, it must fire **spikes**." And by putting the pipette inside the stomach of these neurons (so with the **patch clamp** technique, I mentioned it last time because **Neher and Sakmann** used it for this aspect), we saw as a function of time… so the experiment was practically the same thing we are doing together. A current **step** was given, a current step, and this current step… suppose this could be 500 **milliseconds** and the amplitude was increased. So a stimulation, 500 **milliseconds**, 500 **milliseconds** the neuron rested, and then I resumed with a greater amplitude. Suppose these **steps** could be 25 or 50 **picoamperes**, to give you some numbers, so that if I ask you during the exam: "Roughly, how many are they, pears, apples, are they megaamperes, what are they?" You can say that they are tens of picoamperes, hundreds of picoamperes. The membrane potential, which I draw here, suppose 70 **millivolts**. When the current was zero, it was a rather flat thing, there were no synapses, so all the fluctuations I saw were either membrane noise or amplifier noise. And since I paid $10,000$ **euros** for the amplifier, for it to be so bad as to make noise… I'm from Genoa, so I'm sensitive to these things. Then, by increasing the current, the fluctuations tended to increase more or less as I showed you and became high.

Now, these cells here never fired. So this made me think that I had proof that voltage-dependent channels existed without having done complicated experiments where I took the pipette, placed it on the membrane and then quickly retracted it so that a small piece of membrane remained. No, a silly thing, I put the electrode inside the neuron's stomach and looked at what it was doing and I saw that the fluctuations changed as the potential varied. So, from this formula, in theory, in fact, only because these fluctuations changed with depolarization, I could state: "Okay, yes, there are voltage-dependent channels, so it's not that these cells you give me are not excitable, but for me they are not neurons because I have to see **spikes**." Now, to be fair to that colleague, there were some ugly **spikes**, probably because there weren't enough sodium and potassium channels to create a real action potential. If you want and you have the **notebook** on **Google Colab**, theoretically you could see what happens if you take the **Hodgkin-Huxley** model and change the maximum conductance value for sodium and potassium, and so you are fundamentally playing (even

only in the deterministic case) with the number of sodium and potassium channels. You will see that if they are not numerous enough you don't have **spikes** and if they are too numerous it could also be that you can't have repetitive **firing** because the potential remains blocked under a stimulus. It stays blocked, if there are many sodium channels, sodium wins, the sodium currents win over potassium.

The Langevin Approximation: Computational Efficiency

Well, and yet, this Markovian, a complicated description, and I deceived you because the demonstration I showed you with **Google Colab** a moment ago has another approach. Another approach that we have shown to be very, very similar, is an approximation that has the name **Langevin**. Have you ever heard of this guy? It comes up in the so-called **Langevin equation** and we mentioned it when, in the case of the mobility of an ion, I told you that to do the derivation or to show you those stupid animations where I had balls moving, I had simulated the dynamics equations $\mathbf{F} = m \cdot \mathbf{a}$, okay? $\mathbf{F} = m \cdot \mathbf{a}$ in two dimensions, so the force had two components, okay? So the acceleration had two components, the velocity, which was the integral of the acceleration, the position, which was the integral of the velocity, etc., they had viscous friction, but they also had a stochastic term, because I wanted to show you the effect of diffusion.

The effect of microscopic diffusion is collisions, **Brownian motion**, the fact that water dipoles tend to agitate due to temperature and thus transfer kinetic energy to the particles, for example to the one whose mobility we wanted to calculate or infer. In that case, either you do the numerical simulation, as in this case the Markovian numerical simulation, or you try to write an equation where all the non-deterministic terms are enclosed in a single term which is noise. Exactly what engineers do, who say, in fact engineers do it for other reasons, they do it out of ignorance and they say: "On this communication channel I assume there is the signal I want to see plus a quantity that I don't know, which is noisy, which I hope has zero mean, I hope the variance is known and I see, I wonder if I can make some…"

Well, the same thing of writing a certain quantity plus noise is very easy, but what kind of noise do I put? I mean, I want to put a kind of stochastic process that fluctuates and it is an approximation because you tell me: "But no, you said just now, you have to follow every single ion channel to understand if it opens or closes," which is this novelty that you want to describe a kind of stochastic behavior of a population. It would be like saying: "In this room I have many gas molecules, but I give up because it is too complicated to track the kinetic energy of the single one (it would be exactly the same thing), and I want to have a formulation that, yes, respects thermodynamics, but also gives me…" (in the case of gas in a room, no, in the case of a flight doing turns tomorrow, so turbulence) "…I have the single particles, but the plane is not microscopic, it is quite large, so there are fluctuations, you feel the fluctuations when there are air pockets and turbulence. How the hell do I model them, how the hell can I capture them?"

The way to do it is to put… so for a number of years, people took the charge

balance equation and then said: "You know what? I'll put the noise here." And a priori I'll tell you, in fact I'll tell you in a wrong way, in an arbitrary way. A priori I tell you: "Look, here I put a noise whose variance changes with the potential." So, from a certain point of view, I can say: "Okay, yes, you're on the right track," but on the other hand, the person who proposed this could say: "But what's the alternative? Doing a simulation with Markovian models, first of all it is extremely **time consuming**, it is extremely long and computationally ineffective." Then, perhaps if you have, maybe you put noise here, some consideration, not for the **Hodgkin-Huxley** model, but for other descriptions of excitability, you could infer something without having to do a simulation, which is ultimately the goal of engineering, of physics. It's not that I have to build 25 bridges or 25 artificial valves to understand the one that works in the heart, I hope to be able to have formulas that tell me that they are **guidelines**, a guide to design. Here it would be the same thing, only that putting the noise here would mean that I am imagining that that term with n, which is non-deterministic, does not have, first of all it does not have the product between n and V, because this thing here, yes okay, changes with V, that also changes with V, because $n \cdot V$, so if V changes $n \cdot V$ also changes, but it is inside n that the variance changes, there it is an arbitrary dependence.

Another way is that in the deterministic equations you put noise here, and I'll make it brief because you can do this derivation rigorously using exactly the technique I showed you before. You have this n, now here I'm calling it x, you have this n, okay, I calculate mean, variance, actually here I have to calculate something else that perhaps you have never heard of, but in the end it is the color, which is called the **autocorrelation function**, but pretend you haven't. So it is possible to take a shortcut.

Comparison between Models and Noise Properties

So here, for the case of **Hodgkin and Huxley**, which is the one I simulated for you, I still have four differential equations, I don't have to track the single channels that are perhaps a hundred thousand, a few million, for which the single channels are then 0 or 1, no, no, it is exactly the deterministic model complemented by noise and here I put the variance formula that I derived, so that I derived analytically that I showed you before was $\overline{x}(1-\overline{x})$ divided by the total number of channels. So I continue to have a kind of knob that allows me to say if the number of channels is large, this term tends to be negligible, and since that variance was calculated with the formulas, with the dynamics of the single channels, this thing here somehow inherits the same characteristics. There are limits and I won't talk about them, it doesn't always work and perfectly, but I won't talk about them.

And the thing I'm showing you is the comparison between the **Markovian** case and the **Langevin** case, which is this effective, mesoscopic, still noisy, but approximated case. And in the end this is for 100 **milliseconds**. I am **plotting** the fraction of open potassium conductance channels and these of the sodium conductances for a certain potential value. I am keeping the membrane potential value fixed. And if I look at the black trace and the red trace, okay, I can say: "Okay, yes, it's a stochastic model, yes, it seems to fit both the mean and the magnitude of the fluctuations, both for potassium and for sodium."

And I do not only look with my eyes to see a temporal realization, but I make the histogram, that is, I flatten all these points onto the same axis and count how many times, how many points there are here, there are very few, here a little less, here inside there are very many. I make, that is, the histogram and the figure is **tilted** precisely to give the impression that if you half-close your eyes, if you squint, you see that here there is a band and this band is exactly the band in which this Gaussian… you say: "Where does this Gaussian come from?" The Gaussian comes **for free** because you have a lot of channels and you have heard of the **central limit theorem**. Have you ever heard of it? It is a very important theorem in probability theory, which says that when you have a lot of stochastic variables, random variables and you sum them together, the sum is distributed according to a Gaussian distribution. It is incredible. How the hell is it that if one has binomial distributions $0 - 1$ with a certain probability, you sum them together and it becomes a Gaussian? If you have a lot of magnitudes (I won't give the example of height again: if you take height, weight, sex, whatever), you sum them together into some other variable (here it makes sense biophysically, here it is a current because **Kirchhoff** currents sum up, in the example I gave no), however that sum of height etc. etc. is Gaussian, which is amazing, because the Gaussian should be a kind of primitive of the world of probabilities. And you see that it fits, that is, the **Langevin** approach, which is the red one, perfectly captures not only the mean but the variance, I couldn't say which is which. And it also captures this which is called the **autocorrelation function**, which I won't tell you exactly how it is calculated what it is, it has to do with how quickly these fluctuations change, how quickly, what their color is, the energy in the transformed frequency domain.

So the thing I can do is: if I can do the brute force simulations at least once with the single Markovian channels, with the single Markovian conductances. Give me a minute and I'll finish. I can do exactly the experiment as before and compare the times when there is, for example, a **failure** or there is an action potential, or vice versa compare what the **jitter** of the generated action potentials is. And I can see the model, so the neuron made with all the trimmings, with a hundred thousand channels, each described by a Markovian scheme, how it compares to a **Langevin** model which is very easy and very fast to simulate? Here I won't tell you which is which, if I didn't tell you that red and black are **Langevin** and **Markov** respectively, you would say more or less the same thing. The blue one is a guy who proposed another method and that in this article years ago we said: "No, the other method is really different, it's really poor. Why didn't this guy do the same simulation we did of the Markovian model? Did he want to make an approximation? And why doesn't he compare it with the real model?" So, simply, one can make quantitative indicators and transform that comparison, that comparison I hinted at by showing a current pulse, how many times it fires compared to the total. So this is the number of times it fires, between 0 and 1 is a probability compared to the stimulus amplitude, etc., etc., and things work reasonably well.

Synaptic Transmission: Electrical vs. Chemical

Okay, and the climax: if one does the experiment, if one reproduces that experiment from before, one sees that if the stimulus is constant you have this

jitter of action potentials. It is not made to be exactly identical to the one before. Here I am only showing the first four **spikes**. When, instead, the stimulus is with fluctuations, rapid excursions, here the **jitter** is very small. Paradoxically, the first one has more **jitter** than the second or the third. So it means that the **Langevin** description is not so bad. So many of the things that ultimately descend from this theory of these two guys, **Franco Conti and Enzo Vanche**, are statistical population considerations, so, pardon, linked to probability theory, they fit both experimentally and from the point of view of the equivalent description, when the simulation becomes too computationally demanding. This closes the part, I won't tell you about this, it closes the part on excitability. Let's take a ten-minute break and we'll resume on synapses, which you know a little about. Thank you. Okay, it starts, right? No, I'll continue until tonight. Okay. Yes, yes, okay, we'll finish at 6 p.m., or shortly before.

So, okay. So this part is relatively light. It starts with telling you, perhaps again, based on what you saw with Professor **Zoli**, but with the language of bioengineering and biophysics, synaptic transmission, because in the end synapses work, at least in the postsynaptic part, with exactly the same logic as voltage-gated channels. They are not voltage-gated, they are **ligand-gated**. You will see that exactly, I will torment you with open and closed, α and β will return in a moment.

So I will tell you that there are two types of synaptic transmission (perhaps **Zoli** told you about it, he told you about both). The first is called **electrical** synaptic transmission to distinguish it from **chemical** transmission. Not all neurons communicate or not necessarily communicate always and only with a release of neurotransmitter. Conventionally, "the brain is an electrical machine." In fact, this is also wrong: neurons convert, through synapses, electrical stimuli into chemical stimuli, then, **by the way**, in parentheses, they are further immediately converted into electrical stimuli.

There is an exception which is that of electrical synapses, and I will tell you in my way to try to give you, as usual, not a mnemonic nomenclature, but hoping to really be able to… to make you activate, to make some synapses appear, the nomenclature and the classes of these postsynaptic receptors in a way that I hope you won't forget, particularly also in the spirit of saying: "When is a synapse excitatory or inhibitory?" You would tell me: "Eh, it depends on the neurotransmitter." Maybe, yes, but it depends on the effect that neurotransmitter has when it binds to the postsynaptic receptor. Now it doesn't make much sense, it will in a moment.

The first type are also called **gap junctions**, where it means **gap junction**. **Gap** is a space, a small void, and they are synapses that you will understand in a moment why, or perhaps in a few minutes why, I define them as **bidirectional, slow, and signless** (where for me the sign, at this point, would mean that if a synapse is excitatory, somehow, with respect to the postsynaptic neuron it tends to depolarize it, it tends to facilitate the probability that that neuron can reach threshold and fire, while an inhibitory synapse tends instead to hyperpolarize, tends to discourage the… it is not universal but it is an excellent approximation… tends to discourage the postsynaptic neuron). So I get excited and by getting excited I release neurotransmitter which can have an excitatory or inhibitory effect depending on the identity of the neurotransmitter. In reality, the identity

and the type of functioning of the postsynaptic receptor are what matter most. But here nothing is released, but I wanted to tell you what the sign means to me.

In this case there are membrane channels, which I just forgot the name of, **connexins**, that when they juxtapose, and they must be in the presynaptic part, so in the membrane of neuron A and the membrane of neuron B, and the membrane of neuron A and neuron B must be close, they must almost touch, or at least be close enough to have a **gap**, a small space, and these **connexins** aligning due to binding reasons, I think electrostatic (I don't know this, I'm ignorant), form a pore that puts the cytoplasm of one cell in direct communication with the cytoplasm of another.

In the debate that I haven't told you about, perhaps I only mentioned it, between **Camillo Golgi and Ramón y Cajal**, it was interesting because in the end both were right. **Camillo Golgi** was convinced, even though he had invented the silver impregnation technique that allowed **Cajal** to not only make beautiful drawings but to infer the fact that neurons had a kind of polarization, in the sense that one part takes the input and another part, the axon, is the output. **Cajal** said that there was no continuity between the inside of one cell and the inside of another, but **Camillo Golgi** said: "No, it's like the heart, it's a **syncytium**, it's a continuum of excitable cells that yes are discrete, but they have communication between the cytoplasm of one cell in direct contact with the cytoplasm of the other." In reality I think **Golgi** thought there were no individual cells, I think, but that it was a kind of network, a kind of graph. Instead **Cajal** said: "No, a junction exists, I see it, there is clearly space between one neuron and the other."

Gap junctions in some way prove **Golgi** right, and they are, because, I repeat, they put the inside of the two cells in ionic communication. And the interesting thing is that it is a type of synaptic communication from a **phylogenetic** point of view (which means the evolution of different species), relatively ancient and very represented especially in invertebrates, especially in insects, which are invertebrates. And also **ontogenetically** (which means from the point of view of development, not philosophical, constitutive of being, but the development of the individual, from embryo to adult), **gap junctions** appear predominantly during the early stages of development. However, in recent years, they have been found in the adult cortex between different types of inhibitory neurons.

It seems that, since there is a diversity of cell types, "if you are an inhibitory neuron of type A, you touch and have **gap junctions** with other neurons of your same type, but not with those of type B." They are all inhibitory and in some way, there are reasons that maybe we will discuss together in the future, these **gap junctions** are specific. It is not difficult to imagine the reason for this specificity, it is in fact a consequence of gene expression. If I am led to establish, to express these **connexins** because I am inhibitory neuron of type A, it may be that there is an evolutionary reason why I am always led to express them and to establish an electrical synapse with another neuron.

Bidirectional because if I ask you: "Who is speaking, who is listening?", simply from this figure, from this **cartoon**, you tell me: "It's symmetrical," that is, an ion could pass. I haven't told you whether these **connexins** are selective or

not, let's assume they are not, they are not particularly selective to ionic types. Typically the calcium ion manages to pass from one cell to another, it could pass from here to here or from here to here, so it is bidirectional. There is no: "I speak, I spit out my neurotransmitter, then on the other side there is someone with a receptor, so with a hole that I spit into." Okay, the neurotransmitter binds and an electrical cascade continues. Not here, it is **slow** for a reason we will see (here there is no particular thing, there is only a hole, a pore), and from an electrical point of view, it is slow because this is analogous to two **RC** circuits, two **RC** circuits placed close together, they are two cells, they are two membranes. And it is **signless** for a reason we will see: it's not that if the one above is electrically active and starts firing action potentials, necessarily the neuron below tends to be either excited or inhibited. Maybe both, or, correctly, the answer would be: "It depends." Now I will tell you what it depends on.

Gap Junction Properties and Signal

This is an example of two… so they are not insect cells, it is a relatively recent article, perhaps, okay, maybe $10 - 15$ **years ago**, in which two types of **interneurons** of the rodent cortex of the **low threshold spiking** type are inhibitory neurons that have this connection, these electrical synapses. How did the investigators, the experimentalists, realize it? From the fact that by injecting, suppose, into the first neuron, into this one (you must have pipettes in both neurons, which is very complicated experimentally, because when you have the pipette in one neuron, you must first be lucky enough to hit the other neuron that is connected—it's not like you can see it and say: "Ah, these are connected"—and then you must ensure that when you establish the **patch** with the other neuron you don't lose the first one, because it only takes a little, only a breath). There are people who have abandoned research because finding connected neurons was something that required years, and after one year this guy had a number of… he had managed to lower the electrodes, to hit two connected cells only 10 times out of 12 months that he tried. Considerable frustration.

The interesting thing is that these experimentalists injected not only a depolarizing current, you see this constant step (here the calibration bar does not say how many **picoamperes** they injected). When the stimulus current is positive, it is depolarizing, this neuron does what we expect, I even see a beautiful frequency-dependent adaptation (you see that the **interspike intervals** of the first two or three are smaller than the others, then at a certain point it reaches the **steady state**). And here I see in the other neuron, by definition of synapse, I see some… not action potentials, they are called **synaptic potentials**. Why? Because they are the echo of these action potentials. When there is this action potential I see this hump, then I see another one, then I see another one, and so on. And these humps also have an amplitude that maybe depends on what it is, but above all I see that the time, the instant when they occur, exactly reflects the same **timing**, the same temporalization of the presynaptic **spikes**.

The strange thing is that when I also give a hyperpolarizing stimulus, where the neuron does not fire, this neuron here behaves like an **RC**, it becomes something like a sink full of a liquid with skin, it becomes something extremely boring. So

much so, even when the neuron is hyperpolarized, even if it does not emit **spikes**, you see an echo, an echo in the membrane potential of the other neuron. This membrane potential hyperpolarizes a little.

Here it is important to note the time scale. This calibration bar has a value of 30 **millivolts** for the upper trace, and indeed they are **spikes**. If you take this piece and put it here, you probably see that it fits $2-3$ times, so around 90 **millivolts**, 100 **millivolts** from the base to the top of the **spikes**, which is the **mantra** I tell you: 100 **millivolts**. So eat bananas, the voltage of a cell phone is not so different, $0-1$ volt, anyway. Instead the same length here (to avoid putting the two… from a graphic point of view it is a bit confusing, but it is a notable habit, a frequent habit), this calibration bar means that in the second graph it stands for 4 **millivolts**, so this thing here is 10 times smaller. And for the synaptic potentials you could tell me: "Well, okay, yes, I can imagine they are small, it's not that I fire and the postsynaptic neuron also fires." This does not happen or happens very rarely, particularly very rarely in vertebrates. But not only that, this hyperpolarization is also small, it is on the order of a few **millivolts**. The fact is that it is there, so it is as if there was a continuous coupling, not impulsive, that is, it's not only when I fire that there is communication. Whatever this membrane potential does, this other neuron follows.

Try to understand what the way to mathematically model or functionally, mechanically, mechanistically describe the way these two membrane potentials, these two cells, are coupled to each other could be. There is a wire in between, there is a resistor in between. So before I told you that **connexins** are pores, so it doesn't take a stroke of genius, and I believe it, I'll show you shortly.

The other way neurons talk to each other is a **chemical** connection, which you probably know well, and in this case it is **unidirectional**, because I am projecting to you, and this is my axon, and the axon, except in rare cases, is what spits out the neurotransmitter. And it typically spits it out not onto another axon (in that case it would be an axo-axonic synapse, which exists, but now I don't want to confuse you). I have a part of my morphology that is specialized to spit out the neurotransmitter and I can project to a neuron there, but that one in order to have the same impact on me must elongate its axon, maybe it doesn't elongate it, it elongates it elsewhere.

Chemical Synapse Mechanisms and Receptor Classification

So this structure, which is the termination, the synaptic terminal, the termination of an axon, which can also be, which forks, there are several **branchings** of ramifications, is called the **synaptic bouton** and it is, compared to **connexins**, a world of complexity completely, orders of magnitude higher. I think it is the most complex cellular organ, from the point of view of anatomy and cellular physiology, that exists in the human body and in the nervous system and in biology. It is extremely complex, there are a lot of players involved.

The final result is that when this terminal is invaded by a depolarization that travels in the axon, more or less always the same (for the moment I haven't told

you about these propagation phenomena, we will mention them, we will see them more from the point of view of dendrites, but that will be in the future), you can imagine that if the axon is excited and fires a **spike**, this **spike** can be like signals in a transoceanic cable, particularly digital signals in a transoceanic cable which are periodically, periodically in space, regenerated. This is why digital communications are used, because anciently **Lord Kelvin** in the nineteenth century had to, together with another guy, **Heaviside**, face and understand what the physics was of why if I had a cable of $10,000$ or $100,000$ kilometers (maybe $100,000$ is excessive), a few tens of thousands of kilometers, I spoke into the microphone on one side with an analog signal and on the other side the signal was extremely attenuated. You say: "Okay, thanks, it's like a very long wire of a resistor." Yes, but it also has capacitive and inductive properties and this leads to a deformation, what are called **transmission lines**. So at the receiver, even if they were only telegraph signals. If, instead, you imagine having to transmit only a logical level **0** or a logical level **1**, if at a certain point I put a station that instead of sensing 5 **volts**, **TTL** standard (which is something in electronics still very frequent), so instead of sensing 5 **volts**, it sees 4.5, it says: "Okay, this is very different from **0**. It meant 5," I regenerate it to 5 and this method of communicating digitally is extremely advantageous.

Here you can imagine that the propagation of an action potential along an axon, since the axon continues to have a lot of voltage-dependent channels, continues to self-regenerate. And ultimately it is the miracle of why I manage to move my toe from my motor cortex, despite it being one and a half meters, one meter eighty, whatever it is.

When the membrane is depolarized here, there are voltage-dependent channels particularly selective for calcium. Calcium enters because they are channels that open when the potential is depolarized and as I told you there is practically zero calcium inside, there is zero free calcium. Calcium actually exists in the endoplasmic reticulum, in a kind of compartment in a warehouse, but free to be able to trigger biochemical cascades, there is none, there is practically zero. So when it enters, what it can do it starts to do, and particularly what it does, this is the story, is that it makes vesicles fuse, vesicles which are pieces of lipid bilayer membrane that contain neurotransmitter molecules inside. An incredibly complex and fascinating mechanism of how a neuron can have, how biology and evolution can have compartmentalized a **quantum** of molecules. Something that resulted in a **Nobel** prize because this release of neurotransmitter is not continuous, it is not with arbitrary values, it is in **quanta**, it is in discrete units. Why must they be discrete units? Perhaps the operating principles of this thing here have to do with a digital encoding. Perhaps it is a coincidence. Try to make a system that makes analog spits, which can modulate the quantity of neurotransmitter. It might be more complicated, no one I think knows yet.

And these vesicles fuse and in what is also called here, or perhaps only here, **Kiss and Ride** (no **Kiss and Go**, **Kiss and Ride** at the airport), where in the intracellular part of the presynaptic bouton these vesicles are anchored and when there is free calcium, there are obviously proteins, **synapsin** in particular, that keep the vesicle anchored to the intracellular part of the synaptic bouton membrane, a pore is created and by **exocytosis** the content of this vesicle diffuses and at a certain point this one moment later seals itself again, having

become empty, and returns inside, no longer anchored, ready to be **replenished**, to be reloaded, resupplied again.

What happens in the postsynaptic bouton instead is relatively simpler. Note that this happens in a fraction of a millisecond.

Go ahead.

Calcium is outside, there is a lot of calcium outside and when channels that are voltage-dependent (so V here of this membrane must increase, must depolarize), when it increases there is an electrochemical gradient here, this enters and this signal is sufficient to do, to trigger a cascade. Things are a bit more complicated than this, the arrangement of these calcium channels is very close to this spatial arrangement, there are **microdomains** where they are. So it's as if… it's not simply "calcium enters and I release." It may be that there is a kind of modulation: "Now I release a lot, now I release little," so much so that people (we will see it later) have also described this behavior in stochastic terms, saying: "What is the release probability?" So the release probability is not all or nothing, it could be 0.8. Then there can also be a spontaneous release probability where there is no action potential but here fusion still occurs and this synapse releases neurotransmitter. So, for all intents and purposes, to the receiver it says: "Ah, but something happened." Maybe it released little. Okay? Did I answer?

From the postsynaptic point of view I say it is simple because it is fundamentally the same thing. I'll show you a couple of animations by that guy I saw on the internet who makes graphic animations. I repeat, they are not molecular dynamics simulations, they are just **cartoons**, but they are aesthetically pleasing to watch. If before we had in the postsynaptic neuron membrane… I repeat, even if it is funny, I have my axon elongation that spits out the neurotransmitter, the other neuron does not necessarily elongate its axon towards me, the other neuron has an ear, it has a kind of receptor. I call it an ear because, as I imagine it, I will show you, it is a membrane channel, so it is intercalated in the phospholipid bilayer and the only difference with the channels I told you about, which we have discussed so far, even from a stochastic point of view, is that it does not have the famous activated **gates**. So α and β are not functions of the membrane potential. They could be, but in general they are not. They are a function of the presence or absence, in some domain in the extracellular part, of the **binding**, of the chemical bond between a neurotransmitter molecule. Suppose this is **Glutamate**. When the **Glutamate** molecule binds to this part, to this mouth, to this site, the receptor opens (it is not always like this and we will see it), and when it opens, here simply due to an electrochemical gradient, this channel lets a current pass which is the one made up of ions for which it was selective. It is not necessarily said that **Glutamate** means that you are selective for sodium, potassium. It depends on the receptor and evolution obviously ensured that there was some alignment, but there are cases, especially during development, where a particular neurotransmitter, which is not **Glutamate** but **GABA** (I am thinking of **GABA**, gamma-aminobutyric acid), does not have the same effect as in adulthood. During the embryonic state, and in humans I don't remember when, in rats it is up to the first two weeks of life, after which the rat opens its eyes and things change, when **GABA** binds, the current here is not inhibitory, so it is not hyperpolarizing, but it is depolarizing. But how?

This is an inhibitory neurotransmitter. Yes, okay, but it depends on this one here. If this membrane channel acts on its own and has its idiosyncrasies, it is this that dictates whether you are excitatory or not.

So the postsynaptic part we have amply commented on, thinking of the voltage-dependent case. Here it is ligand-dependent. There will be in α and β, there will be some dependence on the number of these molecules around, if you want, or if you really want to know, **spoiler**, there will be a variable that is a function of time, the concentration of neurotransmitter in the so-called **cleft** (I don't know how to translate it into Italian, in the… I don't know… in the small space between one, in the interstice between, exactly, interstice between the synaptic bouton and the postsynaptic membrane). Here, suppose, for a few milliseconds there can also be a concentration of **Glutamate** or **GABA** as large as a **millimolar**, which is a lot. Why is it released here? The space, the volume is very small, so concentration or density are magnitudes that are the number of particles divided by the volume. If the volume is very small the concentration can be very large.

So this is **unidirectional, fast** (for the moment it makes no sense, I haven't explained why, fast or slow). In the end it depends on this. I told you that voltage-dependent sodium channels are among the fastest things, I compared them to **Ferraris**, taking the responsibility of saying something like that here, and they have the **sign**, in the sense that this neurotransmitter always creates more or less the same effect on postsynaptic neurons, unless there is some **shift-switch** during the embryonic phase.

This is an example of a heroic experiment performed with the **patch clamp** technique, but not with one pipette, with **four pipettes**. Keep in mind that for each of these pipettes there is about $15,000$ **euros** for the amplifier, another $30-40,000$ **euros** for the micromanipulator, and so if you have one pipette, or you have 4, or you have 3, etc., it means you have much more money for research. The world **record** was in a laboratory where I worked, they had 10, but I think it was paid for half, but this is a legend, I don't know if it's the truth or not.

This is an image in which four neurons, actually there are a few more, six, seven, eight pyramidal neurons, are visualized with a microscopic technique that I already told you about, the one that looks like seeing the craters of the Moon, which is called differential interference contrast microscopy, **DIC** (**Differential Interference Contrast microscopy**), with infrared. The infrared penetrates a little more into a tissue, this is a piece of rat somatosensory cortex tissue, 300 **micrometers**, and here I have four pipettes (now you don't see them here, there is one in neuron 1, one in neuron 4, one in 3 and one in 2). If I control by means of a current pulse, exactly like the simulation I showed you several times, I am the one injecting into neuron 1, I don't know if it is a presynaptic or postsynaptic neuron, because I told you I don't see them, that is, I don't know what the connectivity is between them. The axon of number 2 probably goes down and then perhaps comes back up and establishes a synapse with the dendrites of neuron 1. I don't know, I don't see it. So the only way is **blind**, so, okay, it's not completely **blind**, but I put these pipettes and start stimulating one neuron at a time and see if there is an echo, an echo of activation of these receptors which should show me that every time I make an action potential I

see an echo or not. Okay, here are the pipettes. Here you can glimpse with this fluorescent stain, you can glimpse the apical dendrites and also the basal dendrites, but the axon is honestly so thin that you can't follow it anyway. It could even be outside the plane of the slice, because it is cut. It's not that the plane of a section contains, it's not that the neurons are all, at least in the cortex, not all aligned in the same plane, so sometimes you might have two neurons that were connected *in vivo*, but when I slice the brain I cut the connection, which is what happens most often.

What I see is that if this is neuron 1, I see in one of the others, I don't remember which one it was, I see the echo, an excitatory postsynaptic potential. Note, here the calibration bar is 50 **millivolts** or 60 **millivolts**. 50 while here this small calibration bar is 0.2 **millivolts**, so the amplitude of these synaptic potentials is very small. Which should make you think: "It takes a lot of firing for a presynaptic neuron to eventually make a postsynaptic neuron fire." Perhaps a convergence of connections is necessary, where many neurons converge on one postsynaptic neuron. It is not one-to-one, it is not a chain: "I fire, then you fire, for you to fire," one **spike** is enough. No, this has a lot of very interesting considerations.

While the horizontal calibration bar is the same, and it is 20 **milliseconds**, which more or less fits because the **spike** is small, it's on the order of a millisecond, going down and up. For me it's only this that matters: the fact of this hump indicates that there are a certain type of channels, but it doesn't matter. When there is the **spike**, after some delay, perhaps due to the time it takes for the neurotransmitter to diffuse, about a millisecond, or at least the order of magnitude is that, it must diffuse in a very small space, which is on the order of a fraction of a **nanometer**, and it binds to this receptor. And this receptor will have its kinetics before opening. So I don't immediately and instantaneously see the echo of a channel opening.

Furthermore, I see that here, this is the resting potential of the postsynaptic neuron, I see that this deflection goes up, that is, it goes towards the excitability threshold, if you grant me that it exists. Regardless of whether it exists or not, the more I go up, depolarize, the more I increase the probability of firing the postsynaptic neuron. Now, this neuron here was sitting at -70 **millivolts**, so it is impossible for it to fire, but perhaps dynamically it could have been, due to other neurons that had excited it, it could have been a little more depolarized on its own, then this arrives and makes it fire. In this case, by injecting an action potential into the other neuron as well, into the postsynaptic neuron, it also had an, it elongated an axon and established a chemical synapse towards the starting neuron. So in this way one could say: "Look, even if with different amplitudes, these neurons, 1 and 3, were surprisingly bidirectionally connected," which is very unusual.

Now the thing here was interesting because the probability of simply finding a pair, I'm not saying one pair and the other one as well, simply two neurons A and B, A connected to B, in the cortex is a very low probability. That's why that colleague abandoned research, because it was on the order of 10%. It means that out of 100 times that you insert the electrodes, you spend several minutes, you probably spend $5-10$ **minutes** (unless you are very very **skilled**, very good), only 10 times you hit a connection. These guys have 4 electrodes, because

perhaps you can realize that if I am poor, I must have at least two electrodes, otherwise I don't study synaptic transmission. So at least two must be there. Every time I insert them and ideally manage to hit two cells, I hold them stably, I manage to do, I have that with $n = 2$ I have two possibilities. I could have A connected to B or B connected to A. I also have both, but conceptually there are two possibilities. Can you tell me what happens when $n = 3$? When I have 3 electrodes and therefore I can study 3 cells at a time, which can be connected for their own reasons, so what is the number of possibilities? The probability, I already told you, is very low. In the cortex the connection probability is on the order of 10%, 0.1, which means 10%. Probability is a number between 0 and 1 and so I like to express it as 0 and 1. If I have three, it means that every time I have to put an electrode on one cell, and I have two **targets**. Then I jump and I have two **targets** and I do it n times, I do it three times. You see how many possibilities there are? And for 4, for each one you put on and you have $n - 1$ possibilities because you are already… you could study **autapses** which are synapses that the neuron makes with itself, but they are not necessarily… they exist and are important even in the cortex, but they would not be seen with this type of electrophysiological technique. $n \cdot (n - 1)$. And if you see here 12 compared to 2 is an order of magnitude, it's a factor of 10 approximately. So if you have 4 electrodes don't leave science because more or less every time you insert the electrodes into 4 cells you find something, unless they are very, very distant because obviously this probability could depend on the distance. If you have two you need to sweat a lot. That's why this **dataset**, this article and then the data it generated was very important. And with 10 electrodes you can think that you have $10 \cdot 9$ (sorry, it's 12, the world **record** is 12, so it's $12 \cdot 11$ possibilities), so there it is practically certain that there are several connections. So in one go, 12, you manage to study a lot of synapses simultaneously.

So this is just to show you, beyond the aspect of so-called **connectomics**, a relatively recent, young field of research, of the last 20 **years**, in which people manage or would like to have the circuit diagram of the connections of a piece of brain. In this case the **connectome**, identified with this method, by brute force, was only between neuron 1 and neuron 3. Surprisingly it was a bidirectional connection. Could you tell me what the probability of this happening is, assuming there is no favoritism, it's wrong to say that, that there is no predominance of bidirectional synapses? The information you need is this. And it is the probability that there is one pair and that there is the other connection. Remember the story of the conjunction and disjunction of probabilities. Is it easy or difficult to find, if 10% is hitting one pair, to hit the reciprocal one? You could tell me that no, that's exactly the discovery, that there is an overexpression of bidirectional synapses, so that more or vice versa, an underexpression. Answer. But if you think it's simply that there is no dependence (I project towards you and you project towards whoever you want), you are projecting towards me, okay, there is no reason why if I establish a synapse with you because we are both pyramidal and I am spatially close, then you also contact me. If they were independent events, what would the probability be? Because it would be that you did $0.1 \cdot 0.1$, right? Yes. So it is a very small number and the whole story of this specific article is that it is more frequent than 1% to find bidirectional synapses in the cortex and they also have other properties.

Now, let me focus on chemical synaptic transmission and then briefly return to

electrical synaptic transmission. I have already introduced terminology, talking about **synaptic potential**, simply because it is not an action potential, there is nothing to understand. The **synaptic potential** is called the echo that is caused in the postsynaptic receiving neuron when there is a **spike** in the presynaptic neuron. And we talk about potential **P** or current **post-synaptic current, PSC** excitatory (so **Excitatory Post-Synaptic Current, EPSC** or **Excitatory Post-Synaptic Potential, EPSP**). It is obvious that I am considering and causing currents. The channels are in effect, through a change in conductance, in permeability, giving rise to an ionic current, so in effect they are currents. But if I have my electrode I measure the potential and it will be the cell with $C\frac{dV}{dt}$ that integrates that synaptic current. So I see a **blip**, I see a kind of change and I call that potential, but conceptually they are currents, indeed more correctly one should say a **change in conductance**. And this change in conductance can be excitatory-inhibitory.

The story of excitatory-inhibitory means only that these channels here have a **reversal potential** that is more positive or less positive compared to the resting potential. I remind you of the current formula, always in the ohmic world: $I = G_{\max} \cdot x \cdot (E - V)$. There is a maximum conductance, there is some fraction of channels, okay, these are ligand-gated channels, okay, always the usual thing, and then there is the **driving force**, $E - V$. This thing here depends on whether E is greater than -70 or less than -70. If it is 0 **millivolts** or 20 **millivolts**… When this thing here is something that changes from 0 and varies between 0 and 1, this activates, this over time probably does something like this, it activates and then deactivates. I interpret this rise and this fall. Then obviously the lion's share will be done by the conductance. If the conductance is very large it will have a very notable impact in terms of current, but the crucial term is this thing here. Where is V located? In fact where is V located at that moment? If V was at rest, so if this was -70 **millivolts**, if this reversal potential E is 0 **millivolts**, $0 - (-70)$ is a positive number, the current is positive, it is a depolarizing effect, an excitatory effect. If instead this quantity was not 0 but was -60 or -80, suppose -80, so less than -70 **millivolts**, now $(-80) - (-70)$, so -10. You just have to pay attention to the sign. So you have a current that is negative, a hyperpolarizing current, a current that discourages crossing a threshold and therefore the emission of an action potential. So it is an inhibitory synapse. It is the postsynaptic receptor that commands, that says what it is.

Then in fact there is a custom that in neurosciences is called a law, but we don't actually know if it is a law, indeed there are exceptions, which is called **Dale's law**, which says that if I take a neuron, the neuron always releases the same neurotransmitter. Particularly for vertebrates it is always true, almost always true. That is, if I take this neuron, it either releases **Glutamate** or it releases **GABA**, it does not release both. In insects and other animals it can be different. So it seems that… Whether you are excitatory or inhibitory, and it is still a matter of the reversal potential, so it is a matter of **Nernst**, of that particular ionic species for which that ligand-gated channel is permeable, but it depends on the identity. So if you release **Glutamate**, you release **Glutamate** for all your projections. Or if you release **GABA**, you release **GABA** to everyone. So if I have several, my axon projects, it branches out, they are all spitting out the same neurotransmitter. It's not that I excite one and inhibit another. At least

not in terms of neurotransmitter release.

Types of Synaptic Receptors and Equivalent Circuit Model

There is also an important distinction between receptors. I think you should have seen this thing here. The first thing was this, that it depends on the Nernst potential. Yes, it is true that **Glutamate** is associated predominantly or almost exclusively with excitatory synapses. **GABA** is an exception because sometimes during development it has an excitatory, depolarizing effect.

And the other thing that perhaps now, in light of all the… of how much I have bombarded you about ion channels, how they are modeled, how they are described, could be a piece of cake. And it is the following difference: there are **ionotropic** receptors which are pores. They are exactly the ones I told you about: the ligand attaches to a certain particular point in the extracellular part and the conformation changes and a pore is created and the ions that were there on their own, to which this channel is sensitive, is selective, by virtue of the electrochemical gradient that was there and characterizes that type of ions, flow or cross the channel either from outside to inside or from inside to outside.

Note, the story of the ligand attaching to a particular site is exploited for psychoactive drugs. When you, I hope not, take **benzodiazepines** or other neuroactive drugs that have a fast action, you are attacking, you are mimicking, not necessarily at the same binding site, the effect of a neurotransmitter that is not there. I take **benzodiazepines**, **Xanax** and the effect is what I would have if a particular neurotransmitter had bound, which would be the **GABA** neurotransmitter.

In the case of synaptic receptors, they are named after the chemical substance that was first seen to open these channels. For example, for **Glutamatergic** receptors there are two important examples. One is called the **AMPA** receptor (**AMPA** is an acronym, I don't remember what it means, it has a very long name). Another is called **NMDA**. You can find a bottle of **AMPA** on the market, not of **AMPA receptor**, but of **AMPA**, which if you take it and pour it onto **AMPA** receptors, these receptors open because these are **agonists**. **AMPA** is an agonist of this **AMPA** receptor and **Glutamate** is also an agonist. Agonist means that it tends to favor opening. Otherwise there are blockers which are called **antagonists**. For **GABA** instead they are called… these receptors are called **GABA A**, type A. So they have another type of problem… **benzodiazepines**, open **GABA** receptors.

There is another type of receptor, however, which is called, it is not **ionotropic**, but it is **metabotropic**. And **metabotropic** suggests cascades of metabolic reactions, where it does not necessarily have to do with energy consumption, but it has to do with a cascade of **steps**, at least that's how I… because there is potentially energy consumption but that is not necessarily it. Where, that is, the binding of the neurotransmitter molecule in a particular domain in the extracellular part of the channel does not directly turn on a pore, it does not cause a pore to form. Now I will show you an animation in which a change in the conformation of this, which is still a membrane protein (so it has a domain

part outside, in the middle and inside), moves and this movement activates a cascade of intracellular reactions, in particular it activates a protein called **G-protein** in the intracellular part. This **G-protein**, you will see it shortly, let's say it starts to interact with a potassium channel, which is there on its own, nearby, and activates it.

You can imagine that while an **ionotropic** channel immediately binds and the pore forms and the current exits, a **metabotropic** channel perhaps takes a few milliseconds, tens of milliseconds longer, because the conformation changes, this **G-protein** activates, moves, goes to bother the nearby potassium channel, binding from the intracellular side and the potassium channel opens. Now I have trivialized it and it seems fast. It can be particularly long.

This is the animation.

> **Video 1: GABA-A Receptor**
>
> In this video, we explored the **GABA-A receptor**. It's an important protein complex for receiving inhibitory signals from other neurons. This **receptor** can be found in **the membranes of the postsynaptic neuron**. **The inhibition works by changing the membrane potential**. **The membrane potential is given by the difference of the ion concentration between the extracellular space and the cytoplasmic side**. **You can see the ion concentrations here**. **The concentration of each ion varies depending on the location**. **The chloride ions are relevant for the GABA-A receptor**. **Their concentration is higher in the extracellular space**. **The number of green ions floating around here is about accurate**. **The cube marks an area of 10x10x10 nanometers**. **That volume of extracellular space would be expected to hold about 70 chloride ions**. **Let's focus back on the GABA-A receptor**. **This receptor consists of 5 subunits, 2 alpha, 2 beta, and 1 gamma subunit**. This is the most **common**, but other **subunit** configurations are available. When the **presynaptic neuron floods the synaptic cleft** with **GABA**, **then GABA can bind to the receptor**. **It changes conformation slightly, thereby allowing chloride ions to enter the cell**. **The opening of the channel is short-lived**. **The excess GABA is taken up by GABA transporters located on the surrounding cells within milliseconds**. **The GABA that is associated with the receptor can also leave the binding pocket again**. **Thus the channel closes, ready for the next signal**. Thank you.

It can be neuroactive, it could function or interact with the channels or with the nervous tissue in different ways, it could leave it open a little longer, etc., etc. One thing I didn't say is that normally the neurotransmitter, once he said it, once this interaction ends, which is still a binding, there is nothing magical, it's not that "now I'm bound, now I expel you," it either diffuses away into the extracellular space, or it is recaptured by the synaptic bouton, or above all it is recaptured by **glial cells**, which is another family of very important cells in the brain, in some species even more numerous than neurons, which we will not

talk about in this course.

Video 2: GABA-B Receptor

In last week's video we took a look at the GABA A receptor. This time we'll focus on the GABA B receptor. The name is similar, but as you can tell just by looking at the structure alone, the way they work is very different. GABA A is a ligand-gated ion channel. GABA B, on the other hand, is a G-protein coupled receptor. This animation focuses on the GABA-B receptor located in a synapse of a postsynaptic neuron. However, GABA-B receptors can also be found in other locations where they perform different functions. While GABA-A receptors use chloride ions to modulate the membrane potential, GABA-B has this effect by changing the potassium ion concentration. Potassium is positively charged and is more abundant in the cytoplasm compared to the extracellular space. Therefore, releasing potassium into the extracellular space makes the cytoplasm more negatively charged. Let's have a look at the structure of the GABA-B receptor. The receptor has two subunits, subunit 1 and subunit 2. These two subunits are held in close proximity by coil-coil domains in the cytoplasm. When the **neurotransmitter GABA floods the synaptic cleft, GABA can bind to the binding pocket of subunit 1. This changes the conformation of both subunit 1 and 2. A G-protein can bind to subunit 2. The G-protein consists of subunits G-alpha, G-beta, and G-gamma. Lipid modifications on the alpha and gamma subunits allow the proteins to be anchored to the cell membrane. When the G protein binds to the receptor, GDP and G alpha can be replaced with GTP. This activation causes the G protein to leave the receptor, and G alpha separates from G beta and G gamma. In this step, a signal amplification can take place as one receptor can activate multiple G proteins. G$\beta\gamma$ can then diffuse along the membrane and bind the potassium channel GIRK. GIRK is a tetramer and has four binding sites for G$\beta\gamma$. By binding, the G$\beta\gamma$ facilitates the opening of GIRK. Potassium ions can flow out of the cell. The channel is deactivated when Gα hydrolyzes its GTP and picks G$\beta\gamma$ back up. The final thing I want to touch on is GABA-B desensitization. KCTD12 can be bound to subunit 2 of the receptor.**

All these extra things are because this mechanism of a cascade of events naturally has (this is the phylogenetically most ancient way, in invertebrates many things work like this) automatically **for free** a kind of negative **feedback**. When many **G-proteins** are activated, then there are signals that tend to turn off or self-amplify. It is a very interesting thing that molecular biology has invented amplifiers, or thus an amplification with **positive or negative feedback**, which are used in electronics to perform computation. Here in particular, maybe there was only a little bit of **GABA** and I want to amplify it and I can amplify it

with this cascade of intracellular reactions, but at a certain point I want to stop, I want to inactivate because maybe I've been keeping the cell inhibited for too long, and again there are mechanisms that are **negative feedbacks**, they tend to block this **G-protein** activation.

Let's take a 10-minute break.

Well, the message that you must engrave in your mind is fundamentally this. For excitatory (and there will be another **slide**), inhibitory, the **Nernst reversal potential** of the ionic species to which those ionotropic or metabotropic channels, indirectly, are permeable, counts. If this is more depolarized compared to the resting potential, then it is a depolarizing excitatory effect, otherwise inhibitory.

And so this excitatory synaptic transmission can be **fast**, in the case of excitatory synapses, **fast** and these are **AMPA** receptors. Or it can be a little **slower**, but they are still **ionotropic** and they are… okay, no, here I should have said no, the fast ones are the **ionotropic** ones because I read the neurotransmitter, the pore opens and it's done. In the **metabotropic** case it is much **slower** because the receptors activate a protein, an intracellular cascade that activates an ion channel, etc., etc. So the difference is **fast**. Fast means that the activation is on the order of a fraction of a millisecond. So here there is the presynaptic **spike** and here there is the postsynaptic membrane potential, one can often write V_{post}, this would be V_{pre}. After a certain delay this goes up very quickly and the activation comes, the turn-off kinetics are fast. Here the time constant is on the order of $1-5$ milliseconds for **AMPA** receptors, they are **glutamatergic** receptors. While there are other receptors, still **ionotropic** though, that we will see because they are interesting, they are very important especially for memory, where this tail is a little **slower**, but slow on the order of $50-100$ **milliseconds**. These are called **NMDA**.

While there are much **slower metabotropic** receptors where this thing here becomes hundreds of milliseconds, that is, here the activation tends to stay up and go to zero very slowly. This is on the order of, suppose, 500 or 1000 **milliseconds**. These are called, for example in the case of **Glutamate**, with immense originality of names, **GLUMR**. **Metabotropic Glutamate Receptor**. It's easy to remember. Okay, another important receptor, particularly because it is also linked to the **neuromuscular junction** (this chemical release thing, makes muscles work, and is therefore something that I believe was so **successful** from an evolutionary point of view, from the point of view of movement, of electromechanical transduction, it has become ubiquitous from the electrical point of view of information signal transmission). So acetylcholine receptors are called **acetylcholine receptor**, with maximum originality. And all of these, even if they are… This means that it is activated by a substance called **AMPA** or also by **Glutamate**. This is a substance called **NMDA** or also by **Glutamate** and this is a substance called **acetylcholine** and it is **acetylcholine**. I don't remember the name of some selective agonists. Some are called competitive, competitive because they compete with the endogenous neurotransmitter right for the cholinergic receptors. I don't remember if there are substances, there certainly will be. And there are substances that block them, but I won't talk about them.

While the **metabotropic** ones you have, sorry the M goes first, it's **mGLUR**, not **GLUMR** but **mGLUR** (**Metabotropic Glutamate Receptor**), sorry. For **GABAergic** inhibitory synapses, it's simpler. There is **GABA A** and **GABA B**. **GABA B** is **metabotropic**. More or less the kinetic constants are similar. The **metabotropic** one is on the order of tens, hundreds of milliseconds. **GABA-A** is on the order of a few milliseconds, a little slower than **AMPA**.

In this case, all these receptors (this **glycine** receptor is representative of the central nervous system in the spinal cord and is activated by a neurotransmitter called **glycine**, because in the peripheral nervous system there is no **Glutamate**, **GABA** or at least there are other neurotransmitters that are important). I'm having doubts about whether the **spinal cord** should be called the **central nervous system** and not the **peripheral nervous system**. I believe it should be called the **central nervous system** and the **peripheral nervous system** is the periphery, not the spinal cord. However, this **glycine** receptor is typical of the spinal cord.

In all cases, these receptors or the channel that is then activated by the conformational change of the **metabotropic** receptor, are such that they are sensitive, selective to an ionic species whose Nernst reversal potential is lower than the resting potential and that is why they are hyperpolarizing, **alias** inhibitory. It's not because this molecule, **GABA**, the **GABA** neurotransmitter is particular, sorry, the neurotransmitter is particular, it is the receptor that says it all.

How do you combine the two worlds, the electrical world of the charge balance equation with the story of synaptic transmission? Nothing could be simpler: it is as if you had as many branches as there are ionic species or ion channels selective to different ionic species, ion channels of the same type. Here are the **fast inactivating** ion channels selective to sodium, here are the **delayed rectifiers** selective to potassium, here are the chloride channels which are not voltage-dependent. Another branch, and this other branch does not necessarily have this arrow that graphically means: "Look, the value of this resistance or conductance, which is the same thing, changes over time depending on the potential." Here it changes over time depending on whether or not there is a neurotransmitter in the **cleft**, in the intersynaptic space. If there is, then these receptors begin to open, if there isn't, they stay closed. So here this conductance and ultimately this current, this magnitude here, will have a kinetic scheme, open, closed, which we will see now.

So nothing could be simpler, paradoxically in the context of what I have obsessed you with so far, the story in particular with the ohmic description, which is more than adequate for this type of current, in the case of chemical synaptic transmission.

For the electrical one?

For the electrical one it's always the same thing, but in the sense that there too I change, I add another branch, but here you did not flinch from the fact that there is also a battery, an ideal voltage generator, which is the Nernst potential of the ionic species for which that particular ligand-gated channel is selective, to which it is selective. In the case of electrical synapses it is a bit different because here these **connexins** could be selective to different ionic species, maybe to multiple

ions they could have less pronounced selectivity and simply be permeable to different ionic species. Do you have any intuition? It is literally a piece of wire.

Do you have any intuition about what the expression to describe the current due to a **gap junction** could be? First of all, as said, it is something that concerns both the (I call it) presynaptic neuron and the postsynaptic one. In reality there is no big distinction, in the sense that they are both instantaneously **pre** and **post**. So somehow I have a charge balance equation for neuron 1 (this would be the current due to the **gap junction**), and then I have another equation (I am neglecting the various terms for sodium, potassium, chloride, **leak**, blah blah). Here you should tell me what the heck I put. I repeat, here you have a membrane, you have a resistor and you have another membrane. I'll make it even more explicit. Here the potential across this membrane is V_1 and here it is V_2. What is the expression of this current? What should I put here in the other one? Great, perfect. Exactly, exactly.

And here fundamentally this potential difference, literally here it would be from here to here, yes it's true, I should apply **Kirchhoff's** loop rule, but I see with my eyes that here it is $V_1 - V_2$. This difference across this resistor is $V_1 - V_2$. Your colleague simply invoked **Ohm's law** and said that yes, the current inside a resistor is this difference divided by R. He wasn't necessarily thinking about the direction of the current, but if I do things with all the trimmings, imagining, remembering the consumer convention, for which if I take this arrow, this positive potential in this direction, then **Ohm's law**, $V = R \cdot I$, or $I = \frac{V}{R}$, is positive in this other direction. So, here, since the current is entering, I actually write this thing here:

$$I_{\text{gap}} = \frac{V_1 - V_2}{R_{\text{gap}}}$$

where we call R R_{gap}. I think R_{gap} will appear now. And up here, however, you see, here it enters neuron 2, here it is leaving neuron 1, so here I must put the same current with a minus sign. I put the same current apart from the minus sign.

Then I get carried away by my usual paranoia and I say: "Wait, I am… it's true that here I have a differential equation where the state variable appears here with the sign okay and here it's true that okay because there is the minus sign, so it doesn't make me explode, it doesn't make positive exponentials that explode come out." And from here I see two things: the first is that there is this symmetry, in the end it's the same term apart from the minus sign, but the fact that the synapse is excitatory or inhibitory (because this term here and or this term here, clearly cannot be simultaneously, this one is positive or negative) depends on what those two neurons were doing, V_1, because $V_1 - V_2$. If the two neurons are, for example, synchronized in the emission of their action potentials, if they were exactly two exactly identical cells and exactly synchronized, this term would no longer be there, because it depends on the difference. So if two neurons are exactly synchronized, roughly speaking (because obviously they will be different, they will necessarily have different capacitances, they will have slightly different surfaces, they will have sodium, potassium, etc. conductances, they are not exactly the same thing). but if they were, two exactly synchronized neurons doing the same thing on their own, they no longer influence each other through a **gap junction**.

Conversely, and this is the thing that is easy to understand once you remember **Ohm's law**, which is therefore useful also in biophysics and biology, if $V_1 - V_2$ is positive, then the synapse for this neuron 2 is excitatory, because the current tends to be positive, it tends to be depolarizing, while in the other neuron it is the opposite. But as long as V_1 is instead less than V_2, things change. So here effectively, I am connecting them, let's say, with the equivalent circuit, it is perhaps better seen, but in the end even with the two **blobs** I hope you can swallow it without too many objections. And here I don't have a **Nernst** potential because in the end it behaves more like a **continuum** between the cytoplasm of one neuron and the cytoplasm of the other neuron.

So I understand the **signless** part now because it depends on the sign, it can be positive or negative, depending on the activity at that moment. The fact that it is **bidirectional** I see because I cannot escape if you are making a **gap junction** to me and I am making one to you and we have the **connexins** aligned. And the fact that it is **slow**, this is not immediately easily seen, unless you are electrotechnical experts for whom you could say: "Stop, here you have the capacitance and here you have the resistance, somewhere you have RC." So this thing here if it is on the order of tens of milliseconds it is slower than the **AMPA** receptor that I erased just now which went up quickly, it went up in a fraction of a millisecond. Here what does a filtering, what conditions the coupling of signals are the **RC** properties of this contraption. This contraption here is an **RC**. That is, if I imagine having some interesting signal here, yes, I see it at a certain point here, but I have a resistance here and a capacitor here. So if you consider one of the two neurons as a signal generator, you discover that the membrane potential in the other neuron is in the **low-pass**, filtered, **low-pass** version, simply due to the capacitive and resistive properties of this contraption. So here I can be as fast as I want, but this is slow to integrate signals, particularly because this **gap** resistance is very large, in the sense that **connexins** are not excellent pores.

And in fact we saw here that the echo was quite small, this was something like perhaps 2 **millivolts**, so yes it works, but it's not that this kinetics is particular, it reflects what the kinetics of the first neuron is in the case of **spikes**, that when the second neuron were to have, in this case I am the one injecting a negative current and it goes down. My goodness, it goes down slowly and this guy follows, follows very reduced with a factor, called the **coupling factor**, very low, it is not one-to-one, these **connexins**, this resistance is not zero, and it is slow as much as the membrane is slow to integrate signals.

So probably evolution favored the development or constitution, particularly perhaps for the **neuromuscular junction**, which works (the **neuromuscular junction** requires that there is not necessarily, yes but yes, a geometric proximity between the synaptic bouton and the muscle), but it is not said that the muscle must express the same **connexins**. I am the one spitting **acetylcholine** at you and you contract because you have cholinergic receptors. And it is faster. Perhaps chemical synaptic transmission was adopted precisely because it is much faster than this one and so many of us are perhaps better than insects.

This is exactly the same formula and you notice that I am… here I have simply written the **Thevenin** equivalent circuit without worrying about whether it

is sodium channels, potassium, etc. The thing that reassures me is that this state variable, as mentioned, appears here also with the minus sign and this also appears here and it is another minus sign. This is a way, if you cannot remember this electrotechnical aspect, you can remember that I, on the other hand, get agitated if you make exponentials that explode, because nothing must explode here, it is a dissipative system, there is nothing that self-feeds that can amplify here amplification is not there. So the synaptic current term in this case again also becomes an arc… sorry a branch in this circuit. It does not have the **Nernst** potential but it has the potential of both neurons, so it depends on **pre** and **post**.

Before telling you this story, I wanted to insist and say: if you are brave you could try to implement not one of the **Hodgkin-Huxley** models, but two. Simply copy, paste and change the name of the variables. Here I couldn't call it V and V, otherwise you would have said: "But it's the same V." So you could try to take two independent **Hodgkin-Huxley** neurons and couple them with an electrical synapse. You might discover that if you inject the same current into both these neurons, for example, but use different initial conditions, if the **gap** conductance were zero (so the two neurons were not talking to each other), you would see the two **spike trains** out of phase. As soon as you connect this, so when you redo the simulation with this, you would see that progressively over the course of a few **spikes**, perhaps one or two, the neurons synchronize. The reason why in invertebrates, in particular (I told one of your colleagues, I forgot to say it), the heart cardiomyocytes work like this with **gap junctions** and what is the reason? They must be ultra synchronized to contract at the same time. Yes, it's true, there are spatially distributed contractions, exploited for example by **pacemakers** that make one part contract and then the other, otherwise the heart would not have the effect of a pump, if everything was synchronous. But beyond this aspect of propagation and which is also linked to the activation of the nodes, so of the nervous system of the heart, the tissue must react as a **whole**, even though it is made up of distinct cells. This is why these **gap junctions** are there.

And in the experimental case that I told you about at the beginning, where but perhaps only invertebrates, but okay, perhaps also vertebrates, but only in the initial phases of development. No, even in the adult there are in particular subpopulations of identified neurons, in particular they are families of **interneurons**, so **GABAergic** neurons that spit out **GABA**, and therefore presumably have an effect, when they are excited, they inhibit the others, they are connected by **gap junctions** probably because inhibition, in addition to having a role in information processing, is very subtle which I am not going to tell you about now, but it's not simply that excitation does everything and inhibition only serves to prevent epileptic seizures. But from that interpretation of containing excitation in the end inhibition is a brake. It might be useful to have that even if spatially distributed, in my cortex they are several millimeters in all directions, it's not that all inhibitory neurons are there at that point, they are distributed, it might make sense that they must behave globally in the same way, that is, they must be synchronized.

A term used by some researchers is that inhibition can function like a **blanket**, a duvet, a blanket, a blanket that therefore has an instantaneous effect on an

entire network. Imagine that different points of your visual cortex represent, by activating, different points of the world. Now, apart from seeing your faces illuminated, I see them distinctly. Now I'll say something silly that might not be so stupid, if in particular, even without staring at one of you, if one of you particularly, I don't know, I say: "Damn, I must have run into this person at the supermarket the other day," it may be that due to this effect of increasing contrast, which means, in my cortex, since you are in different points on the retina, even in my visual cortex you are, due to **retinotopy** you are distributed, you are represented in different points, your faces. It may be that if I have an overall, global inhibition, not local, but global, I can make, let's say, stand out, for example, the one of you who has the whitest, palest face (because now I'm being silly), because maybe it reflects better, I don't know, because you got less sun, I don't know, because I don't know. In this case the effect of inhibition makes me turn off all the competitors, all these **foci** of excitation and perhaps makes the most important one win, what from a computational point of view is imagined (it's not certain, let's say it doesn't always exist, we don't know if this mechanism exists exactly), is called **winner takes all**, which yes is an **ABBA** song, not an equation, I wish it were an **ABBA** equation, but it means that it is a method to increase contrast and only the strongest one wins because the others are suppressed. To do this I need inhibition that is common and global, even if I have one inhibitory neuron and I have it here. No, I want all inhibitory neurons to be synchronized. I'm not saying that **gap junctions** have this as their only role, people don't know. In particular that type of inhibitory **interneuron** population has both **gap junctions** and chemical synapses between them and so it is a big mess and so **bottom line** we don't know.

One interesting thing, so to try to link it to something engineering-related which, in addition to the clearly modeling, mathematical, descriptive, mechanistic aspect, I would like to quickly present a concept that goes in the direction of this **connectomics**. As for chemical synapses, it is hugely important to be able to understand what the connection diagram is, and this is done in a brute-force, very difficult way, by putting in a lot of pipettes, making one neuron fire, seeing the echo of the others and so on. When there is a connection, I know there is an anatomical connection, when I see the echo, when I see for example an excitatory postsynaptic potential or an inhibitory postsynaptic potential.

In the case of **gap junctions** I can imagine that, considering the **steady state**. And if I inject a current here, if I have a pipette here, if in the middle there is only one jump, so there is no chain of cells connected by a **gap junction**, I might be connected to this cell, but in between… what if there are two others. The only thing I do is I put an electrode in one, an electrode in another, I depolarize, I hyperpolarize and I see that in both cases I have coupling, but I don't know if it is a very, very weak coupling or a coupling… so it is very weak because the **connexins** are few, or it is very weak because in between, and I don't see them anatomically, there are two other cells that are intermediate.

And intuitively, this is something I understand here. Here I only have this conductance of this neuron, then I have this other resistance, in the end it is in series, they are all in series, this is in series with this, which is in series with this. So if by chance there was another **gap junction** and another cell and another **gap junction**, it would be a series, a chain of resistors. So from the point of

view of the amplitude in this **DC** regime, where capacitors disappear because transients disappear, if I see a very, very weak coupling, so I hyperpolarize or depolarize and I see a very, very small echo in the other neuron, I could say: "Ah, damn, so from a topological point of view there are several neurons in between," or, and I couldn't discriminate this, "there is a single **gap junction** but with this resistance value which is very high, so it is poor coupling." This is what I have in mind.

I put in pipettes randomly, I don't know exactly if this, assuming this is a chain, is a simple case of a one-dimensional network, the dimension doesn't change much. I don't know that 2 and K have proximity, so they are connected but not directly, they are indirectly. So it's as if I could, I would like to infer a distance between the connections, because if I have the distance I could perhaps reconstruct the connectivity, I could reconstruct the topology. It could be interesting. I could see as if I opened a cell phone, saw the electrical diagram, I could infer, in the end it might be almost impossible. If I open an **iPhone** I can't get an idea, maybe roughly, of where the battery is and where the **CPU** is, but theoretically I could infer function from structure.

And for what I told you, in the **DC** case it is quite complicated. The difficulty is this ambiguity. I could have, in this simple case, a cell in between that attenuates it a lot. Suppose I inject into 1 and record in 3. I could do the opposite, but I also have 2 in between. Or I have 4 and 5 or 6, whatever it is, they are with a single **step**. If you want I could define a kind of distance. Here the distance between 1 and 3 is 2, because there is an intermediate jump. Here the distance is unitary, there is immediate proximity, but you see that here it is done on purpose, here the resistance is very small, while here it is a bit larger. So I might, when interpreting the two situations, not be able to distinguish the case of a network like this, of a topology like this where there is an intermediate neuron, if I only look at the difference between V_1 and V_3, I say: "Okay V_3, you are attenuated, but you are attenuated because 2 was in between." Suppose I don't see 2, I see V_1 and V_3. Here I see V_4 and V_6 and here I see practically the same thing and so I could conclude and say: "There is a cell in between," but no, they are directly connected.

Interestingly, perhaps it is not particularly interesting for you, if I look instead at the **transient** and not just the **steady state**. The **steady state** I cannot distinguish, this is an example that I constructed specifically for this ambiguity. If I look at the **steady state** I realize that the curves, the time constants, the exponentials have slightly different kinetics, because here I have a capacitor here, I have a capacitor here, I have a capacitor there are only two capacitors, not three. So if I could, I did it, analytically write the differential equations that go from one to the other, I would see that I have more terms, more capacitors, a first-order equation is not enough for me, I have to write a second-order equation. I don't like working in time, so I don't like seeing these **transients** like this, because they are very difficult. Experimentally it is impossible to see something like this, because of that noise, the amplifier noise but also the channel noise. Yes, I see that there is a minimal difference, now I don't know which is which anymore, the dashed part and the continuous part, but the curves are not superimposable, so much so that I see that the dashed ones are slightly different from the continuous ones.

What I can do is, since engineers do that for a living, I inject **sinusoids**, so I move to the **Fourier** domain, I study a **permanent sinusoidal regime** because I know, or hope, in particular I don't inject one frequency at a time, I inject a **chirp**. It is called a **chirp** because if you listened to it on a speaker it would have a whistle that increases in frequency like a bird's chirp. I do this here, you see that it becomes more and more, the oscillations are visible, they are of the same amplitude and they are visible at the beginning because they are slow, then it accelerates, accelerates, accelerates. It accelerates up to, I think, 50 **cycles per second**, so it doesn't go to kilohertz frequencies, but it is enough for me to induce in a linear, **RC** system, which some of you will know can be written, so the differential equation can be written as an algebraic equation in the frequency domain, as a different phase relation. If I look at the two situations, I compare V_1 and V_3 or V_4 and V_6, I don't have the same, so if I have a cell in between I have an extra **phase shift**. Technically this is true at infinite frequency, in reality it is also seen at relatively high frequency.

And what we did experimentally is, since these inhibitory **interneurons** are connected to each other with **gap junctions**, if I **patch** two, can I by chance know or have an experimental method to say: "Are they directly connected or is there an intermediary?" Something even more interesting, but I won't talk about it, is that neurons are not balls, unfortunately they are not these circuits, that is, they are not structures that are single compartment, with **lumped parameters**, they are distributed objects. What happens if I have my axon or my dendrite and they are two dendrites that touch distally, distal from where I have the pipettes, it's as if I had a cable, an electrical cable, an ohmic connection there and I realize it from here. Anyway, to make a long story short, this is a method literally taken from electrical engineering and describes a technique to infer the **electrical connectome** of a network of neurons connected by electrical synapses.

Receptor Kinetics and Synaptic Transmission

With today's lecture, I would like to finish the part on synaptic transmission. And so, the topics I would like to treat are, once again, **kinetic schemes**, this time applied to the quantitative description of synaptic receptors, post-synaptic receptors, which are therefore operated, **gated** by ligands, not necessarily by potential. I would like to show you how a simplification of the model allows one to acquire an intuition regarding the physiology of synaptic signals and tell you, at a somewhat quantitative level, about both so-called **short-term synaptic plasticity** and **long-term plasticity**, and we will see the two differences.

So, in the conclusion of the last lecture, the element, therefore the **take-home message**, the element to take home, was that the same formalism, the same conceptual scheme applies also when describing how two neurons are connected together and one adds in parallel, due to the conservation of charge, of the balance… Therefore the conservation of charge, of mass, the charge balance equation: another branch where basically this type of branch represents currents that are mediated by receptors, which are activated by the presence of neurotransmitter in the **cleft**.

Here, with this formalism, I am actually reasoning by having a neuron as a

point-like element, and this is not the case. I believe that by the end of the day we will have moved on to another description where spatial extent, the morphology of the neuron, counts, but for the moment everything is in a point. And so, if in this point there are also receptors that are turned on when there are neurotransmitter molecules, well in this case this branch would have a change in the **conductance** of that path, of that **branch**, of that component.

And as I insisted quite a bit last time, the type of value of the Nernstian potential or equilibrium potential is what depends, clearly, on the ionic species to which that type of receptor or channel (so remember, **ionotropic** or **metabotropic**) is selective, and it is what decides if the synaptic coupling is excitatory or inhibitory. And we saw that in the case of an electrical synapse, which we will no longer consider, things were slightly different, in the sense that it was not a branch only in the receiving post-synaptic neuron, but there was also a resistor joining the two independent circuits which were two distinct cells.

Description of Synaptic Current

So, concentrating only on the description of chemical synaptic transmission, I would like to solve, with the same formalism, the problem of how I describe the current. I describe the current as the change over time of a conductance, for example for simplicity dictated by a difference between two conformational states of a protein, of this membrane receptor, which for simplicity can be either **Open** or **Closed**.

And by doing so, in fact, I am making explicit what the value of the conductance should be; I call it **synaptic conductance**, which is what changes over time. And it changes over time when, if and if, another presynaptic neuron decides to "spit out" (*sputacchiare*) neurotransmitter molecules, because in the synaptic bouton, which is juxtaposed to the membrane of the post-synaptic neuron, vesicles fuse and the neurotransmitter molecules contained within these vesicles diffuse. But all this mechanism does not interest me for the moment; we will examine it slightly later when we talk about so-called **homosynaptic** plasticity.

Here I just want to understand what the dynamics of these receptors is, and therefore this conductance will be again linked, proportional, to the number of post-synaptic receptors in an open state. So it is always the same little game: there will be a maximum value $\bar{G}$ and a state variable representing a fraction. I have resumed describing things here in a deterministic way, in a mesoscopic way, as a population; here there are very many post-synaptic receptors and this is a description without noise if you wish, only in the deterministic case (you know how to eventually translate it into the stochastic case).

> *Student intervention:* "Could you put the code back for a second? Which code? Yes, naturally. Oh, I apologize. The… I believe they are… The first one might be an O and the second a zero. O, zero. What is the probability that out of 25 letters and 10 digits the first two were exactly those? You can… It is the same probability as any other combination of digits, but obviously… Thank you."

Transition Rates and Dependence on Neurotransmitter (T)

So, in the simplest case, channels can be **Closed** or **Open**. I am taking the values of the transition **rates** in this case already with the idea that I know how they must describe things. In particular, they will not be voltage-dependent; they could be, some are, in some cases there is a dependence on potential, but it is made explicit in a different way and I will tell you about it (it is the reason why in this period of the year, with your study fatigue, they foist things rich in magnesium, magnesium ions, on you, and we will comment on why briefly), but for me the transition **rates** are effectively constant, unless, in the case of the transition from closed to open, there is in the **synaptic cleft**, in the inter-synaptic space, there are neurotransmitter molecules.

So β is a constant, it is a number, it is the inverse of a time, in fact it is a **rate**, it is a speed, so many transitions per second. And $\alpha \cdot T$ is a product. While before, in the description for example of voltage-dependent conductances, we wrote (then I will pull up the screen, it doesn't matter, I'll write it here), we wrote $\alpha(V)$, so we said it is some very unsightly function in the case of the Hodgkin-Huxley model of the potential, here I make the dependence explicit. I say that it is a linear dependence, proportional; so it is a product between a number that dimensionally is not only the inverse of a time, but is also the inverse of a time times a concentration (because multiplied by the concentration the unit of measurement terms, concentration-concentration, cancel out and it returns to being a **rate**, a probability of transition per unit of time, a transition speed) and with this symbol T, with these brackets, alluding to the extracellular concentration of neurotransmitter molecules (T-transmitter).

So it is slightly different from what we saw before, but here too α and β, in this case only (sorry, only this transition probability which is α times something), this thing here depends on time, because the concentration of the neurotransmitter in the **cleft** depends on time. In most cases, if the presynaptic neuron does not speak, does not emit action potentials, these will not give, will not **trigger**, will not initiate any synaptic release and therefore the concentration of neurotransmitter in the inter-synaptic space will be zero.

When instead an action potential arrives and the vesicles fuse and the diffusion of neurotransmitter molecules begins, then there will be a change over time which, however, is not necessarily… it's not that it then remains constant, because the synaptic space is small, it is on the order of a fraction of a nanometer (or pardon, tens of nanometers), but it is open. Furthermore, therefore, the diffusion of neurotransmitter molecules continues and the neurotransmitter diffuses away from the receptors. And furthermore, there are glial cells that are there ready to recycle, suck up, manage the recycling of neurotransmitter molecules.

Neurotransmitter Dynamics and Flow Modeling

And now we will see one of the ways to describe it, because otherwise this neurotransmitter profile in the inter-synaptic space might have a… yes, let's imagine it can rise rapidly and then decrease. So the increase is probably due to the diffusion kinetics of the vesicles and the diffusion coefficient of these neurotransmitter molecules in the medium, the extracellular *medium*, but at a certain point it turns off: the concentration returns to zero with some dynamic

that I cannot indicate a priori. It depends, as said, on free diffusion and *re-uptake* by, for example, glial cells.

But the strategy is this: I say that the conductance is active only in the **Open** state, by convention (by my convention), and again with the same little game I would write that $\frac{dO}{dt}$ (you see, there is a small arrow exiting this state O) exits with a speed β. And this flux depends on how much O there is, so it is minus because it exits: $-\beta \cdot O$. It is a proportionality, I remind you, by the law of mass action, but it is reasonable because if I don't have O (if here I don't have a term that is proportional to O) it wouldn't make sense for it to be a negative term, because integrating each member I would have a quantity O that over time tends to decrease, even going negative. While this way I have the famous exponential arcs: you see the minus sign, I am calm, I have no problems and therefore there is a saturation effect, of shutting down to zero that maintains physical consistency.

Now, physical consistency, in the course of probably this hour and the next, at a certain point I start to loosen it because I need to be able to simplify this description to extract something that I don't see instantaneously from here, I don't see immediately.

The other term, you see, in this container (again I imagine them as if they were containers, tanks of liquid) there is this tap, this flow, which is all the more intense the more C there is, the more channels/receptors are in the **Closed** state, proportionally to $\alpha \cdot T$. You see that T acts as a kind of tap: when T is 0 the tap is closed, here this arc from C to O is no longer there; it is there only when T is non-zero. And when T becomes/changes (it can have some gradation, suppose from 0, 0.5 millimolar, 1 millimolar, which is the peak neurotransmitter concentration in the inter-synaptic space), then this transition speed, of increase of O, obviously tends to increase. I call it speed of increase because here I am describing the time derivative, which is a speed of appearance, of apparition.

Rewriting the Equation: Canonical Form and Time Constants Actually I can write it for C as well, but these two equations are linearly dependent, so in the end it suffices for me to remember the conservation of mass ($C+O=1$), so I write C as $1-O$. Same thing we did in the generic case of voltage-dependent conductances and we can arrive at this form:

$$\frac{dO}{dt} = \alpha T(t)(1-O) - \beta O$$

which is related exactly to the same rewritten form where I had a state variable quantity, "infinite" version (as was $m_\infty, n_\infty, h_\infty$) and I also have the τ. In this case I called it O_∞ and τ_O.

$$\frac{dO}{dt} = \frac{O_\infty - O}{\tau_O}$$

This is the time constant with which this equation changes and O_∞ is the steady-state value. I told you as a practitioner, as an engineer: I can consider this system a box where the output is the state variable, the input is this O_∞ and in the end O follows O_∞. How does it follow it? With this kinetics, with a little bit of delay.

The interesting thing is that this time constant, moreover as it was also in the case of voltage-dependent *gating*, is concentration-dependent. When there is no neurotransmitter concentration, the denominator is smaller… No, I'm wrong. So, pardon. If T is 0, this time constant is $1/\beta$. When T is non-zero (yes, that's right), when T is non-zero, the denominator tends to increase ($\tau_O = \frac{1}{\alpha T+\beta}$). So 1 divided by something that is larger tends to be smaller.

It means that in the rising phase, in the so-called **onset** phase, of opening, the currents (which are ultimately proportional to O, the synaptic currents) go up much more rapidly. It is always an exponential arc, I am calm, you see here there is the minus sign, but this τ is much smaller when T is non-zero. T, note, can only be positive, so when T is non-zero, here the denominator becomes larger, so this τ_O becomes small, so it grows very rapidly. And then when it decreases (suppose it decreases because T turned off, because T is zero), then in fact only β remains and it is a quantity, seeing as there is no longer this additive term in the denominator, it is a slower quantity: it goes up rapidly and goes down more slowly.

Which, if I recall correctly, is similar to the type of **synaptic potential** (here it is the potential we are recording and actually it is not the current, but in the end synaptic potential and synaptic current are the same thing except for an integration, integration by the charge balance equation, the work of the equation satisfied by the membrane potential). You see that here the rise is much steeper, much faster than the descent; the descent, or also called turn-off kinetics, is much slower. Here with these considerations we have this consequence effectively **for free**. And again it is implicit in having made one of the two kinetics, the opening kinetics, concentration-dependent.

"Square Wave" Approximation of Neurotransmitter

You could have said: "Okay thanks, it's intuitive. In the following I have a bit of a problem solving a differential equation of this type". Which yes is indeed true and is first order, but it is not with constant coefficients, because by definition τ_O, which is a coefficient, and O_∞, which is the known term, the forcing term (from which the particular integral actually descends), are functions of time. And as said I don't know what the profile of neurotransmitter in the inter-synaptic space actually is.

I can measure it; in some cases there are synapses that are so massive that somehow I can measure the neurotransmitter concentration as it varies over time. I could even use the receptors themselves as **reporters**, as antennas: since they open and close depending on whether there is neurotransmitter or not, I could see from them what the profile and duration is, doing a sort of deconvolution operation, of **reverse engineering**.

However, for simplicity, to fix things, I say that this T, function of time, is a particular function of time, simple for me: and it is a **piecewise constant** function. Piecewise constant means that suppose the activation of the presynaptic neuron (so the moment the presynaptic bouton "spits out" the neurotransmitter) starts at one millisecond, goes up very rapidly in zero time, reaches a maximum value, T_{max}, which can be one millimolar, and then goes back down after a millisecond. So one millimolar for one millisecond. This is the way I have to, again, try to

understand something and extract intuitions, simplifying my life.

In fact, as said, this would be something that changes gently, it goes up maybe rapidly (but certainly not in zero time) and goes down in a slightly slower time, probably because it is linked to lateral diffusion and to a *re-uptake*, a recovery with endocytosis by the neuron itself and by other cells like glial cells.

If I do this, however, this differential equation also becomes one with piecewise constant parameters. So I know I can solve it from here to here, where in fact τ_O is only $1/\beta$ and O_∞ is 0 (you see, at the numerator: if T is 0, O_∞ is 0), and if T is a constant value T_{max}, again, this is a number and this other is a number. So I can write the solution as two distinct terms and in the end I always find the same exponentials, it is not a big surprise.

Comparison with AMPA Receptor If I compare, therefore, the solution of this equation with piecewise constant parameters (and this black trace you see) is compared in blue with the current, obviously normalized, which is in fact a real **AMPA receptor**. A receptor that, as I told you last time, is a glutamatergic receptor: it binds to glutamate, obviously it also binds to the substance called AMPA with which it was selected. Glutamate binds to many receptors: it binds to AMPA receptors, NMDA receptors, it also binds to metabotropic glutamate receptors.

For AMPA (and I recognize it from the fact that the turn-off kinetics is fast, it is on the order of 1-2 milliseconds, perhaps I called it the **Ferrari** of synaptic receptors), it is an ionotropic synaptic receptor: rise and fall are immediately consequences of an ionic flow through the pore that is created in that receptor. And you see, the agreement is not perfect: in the rising part yes, in the falling part no. But it might not be so crucial to capture exactly the waveform of this potential, of this temporal change of synaptic conductance.

And anyway, to make it short, do you want something more accurate? Now eventually I will provide it to you, but the "open and closed" type of description is probably not the best description. It may be that, as also in the case of voltage-dependent channels, there is something more (remember it was $n^3 \cdot h$, it was n^4, assuming therefore accounting for the existence of different subunits; now in that case the subunits were independent). In short, it can be arbitrarily complicated if one has the mission to understand and replicate the experimental behavior exactly.

Advanced Models: AMPA Receptor Desensitization

If one has to do something like this, one might prefer a much more accurate description. This is a description of the **AMPA** receptor which, as you can see, is not "closed-open": there are three states indicated by the letter C and they are **Closed**, in the sense that the channel is not permeable to ions. By the way, the AMPA channel is permeable to a mix of sodium and potassium ions and the reversal potential is 0 mV, I believe I said that last time. In states C_0, C_1, and C_2 the channel is not open, the conductance is 0. The only state where the conductance is non-zero is this state O.

And there is an additional state called D, where again the conductance is zero,

and it has this letter, this label D, because experimentally it was understood that there is a need for all these transitions. Some of which are dependent on the presence of neurotransmitter in the inter-synaptic space, but not all; for example, these where from the Closed 2 state one escapes somehow into this sort of trap, are not dependent on the neurotransmitter concentration, they are fixed. And this state is called the **desensitized** state.

Indeed, when you take this kinetic scheme and activate it (so simulating, imagining that there is a train of these little pulses, of these little rectangles of neurotransmitter concentration in the inter-synaptic space, which are regular: here I believe they are one every 10 milliseconds, so 100 times per second, 100 activations, 100 **Hz**), it means the presynaptic neuron is firing at 100 Hz, which is a lot, it is a very high activity frequency. Perhaps a cortical neuron could fire at 100 Hz for a very short time, do a **burst**, a packet of spikes. Forget about seeing 100 Hz for a neuron, for example a pyramidal one, holding it for a few seconds. It doesn't happen: the neuron gets tired, it turns off, there are frequency-dependent adaptation phenomena, as I mentioned to you, etc.

Comparison Between Simple Model and Model with Desensitization
If you use this [simple] scheme, the amplitude of these maximum peaks of the synaptic conductance remains practically the same. Again, the important thing is that you don't go and activate this faster receptor with a frequency on the order of or faster than β. β is a frequency (a rate), $1/\beta$ is the kinetics, it is the turn-off time constant. So what I am saying off the cuff, by eye, is that this β somehow tells me what the time scale is in which the tail is exhausted. Today we will talk about tails (I don't know if I talked to you about my bank account at the university that I pay myself every month… Yes, we will resume it. Exactly, it was about adaptation, **spike frequency adaptation**. Here I am basically thinking of the same thing). So the tail is finished here and if there is a further pulse of neurotransmitter in the *cleft*, so another spike, ok, the system is ready again.

In this case instead [with state D], if you (and it is not particularly complex, the only difference is that here it is a single differential equation, here it is 1, 2, 3, 4, 5 states; they are $5-1$, they are 4 simultaneous differential equations that you have to solve numerically. You can potentially write it, you know that it is possible to describe a system of more than one differential equation as a single differential equation not of order 1 but of order N, in this case it would be of order 4, but it doesn't make much sense), in the end you would want to have a value to put inside here and to simulate this synaptic current. In this simulation V_m remained fixed because here the membrane potential is not changing, it is as if it were a **voltage clamp** experiment.

And here you see that, since I am going so fast (so I repeat, here it is on the order of a pulse every 10 milliseconds; I notice it because here out of 20 milliseconds there are two, so roughly it must be that), then the inactivation makes itself felt and therefore the amplitude of these conductance peaks (this is the post-synaptic conductance, I emphasize it because we will see something similar in another context) shows a kind of "tiredness".

The interesting thing is that it is possible pharmacologically, for the specific

case of the AMPA receptor: there is a substance called **cyclothiazide** which, if you apply it, you remove the occupation probability of AMPA receptors of the desensitized state. Effectively the AMPA receptor, even at 100 Hz, continues to function without getting tired.

Physiological Meaning: Runaway Excitation

Probably nature thought (beyond cyclothiazide, which I believe is an artificial compound, I don't know if it exists in nature, or perhaps it is an agonist in this case, instead of an antagonist, somehow it tends to potentiate the effect of these currents), but in general the fact that glutamatergic synaptic receptors, therefore excitatory, have perhaps evolved in some cases a kind of fatigue, could be as a possible interpretation (this interpretation might be worthless) be due to the fact that if I am a glutamatergic synapse it is a big responsibility. Because if I activate myself and propagate the excitation signal, it may be that this excitation signal can become pathological, can be explosive, can be what is called in jargon **runaway excitation**, excitation that "runs away" uncontrolled, or a **seizure**, an epileptic crisis: a global synchronization of all the cortical tissue.

Because the cortical tissue and many other areas of our brain and our nervous system have connections that are recurrent. I'll give the stupid example I often give, since you are particularly close and maybe you are a bit edgy today (I don't know why, again because of the fire alarm): if I were to, a stupid example, were to slap one of you and a **burst** of excitation… you turn to your colleague next to you and you start beating each other up particularly. If you were much further away there wouldn't be a brawl. It's a stupid example to say that if by chance there was a kind of muscular fatigue in your arms, in your synapses (actually it should be in this case in the receptors, so it would be the receiving part), but if there was a kind of fatigue in this sense, it could be that the collective effect of this reverberation, of this recurrence of excitation, could be sedated. And this seems that it could be one of the reasons for these mechanisms of **spike frequency adaptation** and desensitization.

The thing is different on inhibitory synapses and we will talk about it, because it is very interesting, because it is the exactly dual effect. If I am inhibitory, maybe there is a sense why I am receiving a lot of spikes, because the spikes evidently were generated by excitation. And this excitation perhaps puts me in the condition of having to inhibit the others and maybe my inhibition must not lower the voice, but raise it: my inhibitory effect must become more and more prominent if there is someone who keeps saying "fire, fire, fire". But it will be clear later.

Intuitive Interpretation of the Kinetic Scheme Here it is simple to understand because, again, I imagine it as if there were here a kind of plumbing between four tanks of liquid and here in fact there is a kind of leak. Okay, actually the leak… the arrow can also go up, but it depends on the values of this R_D, of this rate that returns up from the desensitized state. Because only when I am in C_2 can I open, otherwise no. So if I have something that makes me fall… I imagine it again with a lot of channels, of receptors that all work in parallel and many are at a certain point moving from left to right because T has become

non-zero. So these arrows are possible, but here one falls into the desensitized state, subtracting oneself from those that can instead reach the open state.

You have to imagine it like someone flipping a coin. Ok, the probability there is 0.5, but it's not that heads comes up every time. So suppose the probability is 0.1. One out of 10… what does it mean? Time? Ok, they are probabilities per unit of time, so it makes no sense to say 0.1; I should say 0.1 per millisecond. Every millisecond there is a probability of only 10% of making the transition and so maybe the majority of these receptors (which I imagine all in parallel, independent, etc.) don't see the desensitized state, but someone does. So collectively the fraction of channels that are available to go into the open state reduces. It is even simpler than the case where the kinetic rates are controlled by an external variable, like the potential or like the concentration of a ligand. Simply the transition happens.

How does it happen spontaneously? The fact that here it closes spontaneously if there is no more… Actually it closes even if there is neurotransmitter, but in fact one sees perfectly: when there is no more neurotransmitter there is this **recovery**, this kinetic closing of the channel.

The Role of Magnesium and NMDA Receptors

Why do you take magnesium? Or why is magnesium given to hyperactive children? The time I said: "Is it possible that it's like this?", that is, psychiatry in general (so I take responsibility for this), psychiatry gives molecules randomly like this, saying: "Yes, but because I know… I made up the little story (now I'll tell you another one), the little story of AMPA receptors, and so if I put a little… so I lower the glutamatergic tone and people are less stressed." In the end, for the moment it is like this: besides obviously medicine and psychiatry having a huge base of observations, trial and error (not even the "trial and testing" of Galilean memory). I have a thousand patients in my office, I saw that those to whom I gave benzodiazepines of that type or the antidepressant, ok, are a bit better, and so I say: "Ok, you seem similar to me". But there is no knowledge as an engineer would want it, to say: "That parameter should have been 20%, now it is 26%, I have to reduce it". There is not this in biology yet.

With bioengineering, particularly with **neurotechnologies**, this is getting closer. One says: "Ok, I don't know what the right temperature is, but maybe I put some neuroprosthesis that with a closed-loop control dynamically adapts an activity, brings me back, removes the Parkinsonian tremor because it tends to decrease the affiliation of a particular population". I don't know which one would be physiological, but I can measure the tremor; the more tremor there is… so this is a direction more based on principles than based on experience.

So, does anyone know why you take magnesium if you are hyperactive or to calm down? You might think, and you wouldn't be far from the truth, that it is because there is something to do with the **glutamatergic system**. Ok, but damn, it is only one way, one mode of communication between cells (the most important mode of the nervous system perhaps, agreed), but it's not that my whole nervous system, if you lower $\bar{G}$ a little bit, then I become a different person. It's a mess, we don't know.

The Voltage-Dependent Magnesium Block Anyway, the particular fact of magnesium, do you know it? It is due to the fact that there are glutamatergic receptors, still **ionotropic**, called **NMDA**. They are called NMDA receptors because there is a substance called NMDA (of which, as for AMPA, I don't remember what the acronym stands for) which acts as an agonist. They are glutamatergic receptors though, so when there is glutamate in the inter-synaptic space, these open. But they have, in the part… in their domains (it would be wrong to say in the intracellular and extracellular part because it is everywhere), they have a **sensor of the membrane potential**.

So I repeat: they are receptors that are placed in the post-synaptic neuron, they are intercalated and therefore on their own (like the membrane channels sodium, potassium, calcium, etc., are voltage-dependent) this one too somehow has a dependence on the potential. The potential is of the post-synaptic neuron in which they are placed, but they open when there is glutamate.

An easy way to describe them, without complicated kinetic schemes, is to think that there is certainly a state variable between 0 and 1 (the fraction of open channels) and this for example evolves with a simple two-state open and closed kinetic scheme. But the maximum value ($\bar{G}$) is not a number: it is a number times a quantity, and this quantity is an instantaneous function of the membrane potential and the presence of **extracellular magnesium**.

That is to say, if you have 0 magnesium (these are the small circles in the graph) or 0.01 millimolar of extracellular magnesium, you have that this $\bar{G}$ is not constant, as you would expect (given by the conductance of the single channel times the total number of channels). Well, in this case, in a phenomenological way, in an effective way, you see that it depends (I am looking only at this curve here for the moment) it also depends on the **post-synaptic transmembrane potential**.

If this is very depolarized, then yes, basically it is constant. However, as it starts to be hyperpolarized, this value of the maximum conductance of post-synaptic NMDA receptors tends to decrease. And it decreases badly, it decreases down to 25% or to zero. So it means that if there is no magnesium there is a very notable dependence on the membrane potential… Ok, no, said like this it is wrong. The receptor also depends on the membrane potential and the more magnesium there is (passing from circles to diamonds, to squares, to triangles, so the extracellular concentration is passing from $0, 0.1, 1, 10$ millimolar), this dependence tends to be there anyway and tends to shift towards more depolarized values.

And the **range** of this sensitivity is exactly in the range where synaptic integration and the action potential occur. It's not that it has a range for which physiologically it doesn't happen; it is right there. So evolution made it there.

Coincidence Detection I'll say it again in another way, so maybe you can anticipate: but what the heck is this for? The conductance… suppose there is a necessary physiological quantity of magnesium. So if you take magnesium orally, you are moving on these curves, so you are at resting potentials for example, you are **turning off** glutamatergic synaptic transmission, regardless of whether there is neurotransmitter in the inter-synaptic space or not. The interesting

thing is that this changes: so this curve is no longer 0, it becomes 0.75, 1, etc., up to a maximum, when the potential is particularly depolarized.

So, for this receptor to conduct a current: 1. There must be neurotransmitter molecules in the inter-synaptic space, so that the "closed-open" game flips and that variable R (or O) becomes non-zero, because otherwise this current is null. 2. And then here there is a product, so logically it is a kind of **conjunction** (AND): two things must hold for one to see an NMDA current. There must be the neurotransmitter (so the channel flips from closed to open) **AND** the post-synaptic membrane potential must be depolarized (to remove the magnesium block).

So I am the presynaptic neuron and I "spit", I speak, I am excited and I speak, and speaking I spit glutamate. And the post-synaptic neuron hears it, it binds to the receptor, but on its own, to have this current, it must be depolarized, it must fire (or almost). It occurs to me: what is this thing here? It is an operation, it is a remarkable computation. It is a **temporal coincidence detection** of two events. The presynaptic neuron is firing **AND** the post-synaptic neuron, on its own business, is depolarized.

So this correlation is probably not a coincidence, because the NMDA receptor is extremely permeable to **calcium** ions, it also has a reversal potential around 0 mV (so anyway it is certainly excitatory because it is greater than the resting potential, -60), and it is involved in a whole series of phenomena linked to **synaptic plasticity**. This detects coincidences between pre and post activity.

And obviously, if by chance every time I, the post-synaptic neuron, am active when the presynaptic neuron wants to talk to me, perhaps there is some reason why the two of us become connected, why we move from a **correlation** event to a **causation** event. Maybe it is necessary, as for two concepts in psychology that are similar and co-activate, it is necessary that maybe if they always co-activate, that a kind of reinforcement exists between the two and that it becomes that when the presynaptic neuron fires, then the post-synaptic neuron also fires.

But this thing here in theory precedes, because again the membrane potential can or must be depolarized and could be depolarized for other reasons, for the activation of other synapses. It is not necessarily that I speak to you and every time I must excite you; you can be excited on your own and if temporally there are these co-activations, that is this synchrony, perhaps it is good that our synapse too, our communication channel, becomes privileged.

However, it has always made a big impression on me that one gives magnesium to a hyperactive child because this way this sigmoid **drifts** (shifts) and therefore tends to be even less excitable. If you don't give magnesium, so if there is little magnesium, either this curve is practically horizontal or it is anyway very shifted to the left and therefore even at resting potentials, if there is a little bit of glutamate, this has a depolarizing effect on the post-synaptic neuron. Does it make sense? This starts to require a bit of attention because, again here I am talking about the presynaptic neuron exciting the post, and the post on its own business being depolarized. I know from experience that people get a bit confused: "But how? Glutamate gives a depolarizing effect…". NMDA, given this reversal potential, is a depolarizing current. Yes, this is an extra thing, it is an extra coincidence detector.

Metabotropic Receptors and Complex Kinetic Schemes

Ok, now in the case of **metabotropic receptors**, the discourse, even if we won't deepen it that much (you can try to write the equation), nothing is… It allows, with the same language (it is very powerful, because it is phenomenological: in the end I don't have to specify that this is the G-protein that got activated, it is simply G_0). A quantity that describes a density, a concentration of objects in a particular state that can be free, or here it could be bound to the receptor.

And I can translate into a kinetic scheme what is for example the metabotropic behavior where the neurotransmitter, when it binds with the receptor in a *naive* state that is not bound, not only gives (so it doesn't open a channel, the conductance here… the O pops up here, so yes, in the current expression I will put the fraction of channel in that state)… before getting there I have to struggle a bit.

[Image of G-protein coupled receptor signaling cascade]

Here R represents the state of the receptor (for example I am thinking of the **GABA-B** receptor or the metabotropic glutamate receptor, **mGluR**) when it is bound, in a state that is bound to the neurotransmitter molecule. And on its own business, you see here, it can even go into a desensitized state and flip and can also go back, can free itself (since there are no… since all these transition probabilities are constant, so they are not further dependent on voltage). And I need receptors in this state **AND** there must be intracellular **G-proteins** in the free state to be able to go into a conformational state of "receptor and activated G-protein" combination, which after a while detaches.

And this G-protein in an activated state decays on its own business and returns to a ready state, but binds to a channel that was (a channel for example potassium) that was in a closed state. Maybe N G-proteins are needed (see that three-dimensional animation I showed you last time by this guy on X or Facebook or Twitter or whatever): only in this case does one pass to an open permeability of a potassium channel.

And so all this mess translated into a system of differential equations represents for me and allows me to describe and understand metabotropic activation. The only thing that happens is, imagining that each of these steps has one or more differential equations, you can imagine that every time there is a differential equation you have some kind of time constant, of temporal kinetics, therefore a kind of **delay**. Which rightfully justifies the fact that ionotropic receptors are Ferraris (the neurotransmitter binds, the pore opens and ions pass immediately). Metabotropic ones are the most ancient from an evolutionary, phylogenetic point of view (and they are probably… phylogenetic means on species, on different species of mammals, insects, invertebrates, vertebrates, whatever you want, they are the oldest) and they are **slow**.

So if the tiger enters, perhaps it is convenient for me to evolve synapses in my visual system that can pass information quickly, rather than stuff that here maybe activates after 50 milliseconds. The tiger might get the better of me.

The interesting thing here is that they could, for these schemes (which you could also read as biochemical kinetic schemes that some of you might have

done), it lends itself, and in biological reality it is so, to a whole series of **positive feedbacks**, amplifications. So I have maybe only one molecule of neurotransmitter. This is particularly interesting for the olfactory bulb, for the olfactory system where maybe even just one molecule of jasmine odorant (of the flower, oh well, of the rose) I take only one and I have to amplify it. And instead of doing an amplification job that exists in the central nervous system at the level of spikes, at the level of electrical activity, I can do it at the level of biochemical reactions. Just as I can make a kind of *safety* mechanism, of safety, of a brake where if I have too much activation I can block it. And so this with AMPA… the AMPA receptor doesn't have it (ok, it has this desensitized state, but it is simple from the point of view… it doesn't allow… it allows only maybe to disfavor, not to amplify weak signals).

After the 10-minute break I will show you how, to try to simplify things, what advantages there are if, instead of thinking of a train of these little pulses, of these little rectangles (each placed at a time when there is a presynaptic action potential), I swap them, I replace them with **Dirac deltas**. And you will tell me: "But this doesn't exist in nature, concentration doesn't reach an infinite value in zero time". Yes, but mathematically the effect is similar, it is very similar. And we will see it later.

I'll stop for 10 minutes, you know where to find me if you have questions.

Approximation with Dirac Deltas and Impulse Response

Good. So, here I have resumed this kinetic scheme of the post-synaptic receptors of chemical transmission where there are only two states, **Closed** and **Open**. Again, this is the equation I write when I describe the change over time of the fraction of channel in the open state. And the thing I can observe is that if I, instead of putting T, an arbitrary function (which somehow accounts for this graphical summation, graphical superposition of functions that are practically zero everywhere, except where the little rectangle is), I can write it instead as a sum of individual functions, where I have to change the argument anyway depending on the index of the summed element. That is, I can imagine that that train of pulses I showed you before, of rectangles, is describable as a summation and then here there is a translation, a *shift*.

Those of you who had the courage to watch those few introductory mathematics videos will have seen that in a very intuitive way I show you (perhaps it was even with some Jupyter Notebook or Google Colab or whatever it was), I show you that it was possible to understand that if you put, change the argument of the function, subtracting or adding, the function moves rigidly on the X axis; if instead you put plus or minus something here, the function translates from top to bottom, so towards or along the direction of the Y axis.

Here however I change, I am no longer interested in using the little rectangles, I want the **Dirac deltas**, because I like them. Or rather because I know how to behave when they are put on the right hand side of a differential equation. I know this because engineers, when they calculate the impulse response, are in fact having an impulse on the right part of a differential equation of a system of this type, and they have one. Ok, here instead of being one they are an arbitrary number, they are N, and they are each placed at a different instant

t_k $(t_1, t_2, t_3 \ldots)$.

So the situation is this, in which I have different instants. Not necessarily for the moment… not necessarily these times representing the activation times of the presynaptic neuron, not necessarily must they be uniform, that is integer multiples of some quantity; they can also be random. As long as I call them t_1, t_2, t_3, have written somewhere where they are, I can write concisely. So writing sum of these terms, $\delta(t - t_k)$, in fact I am expressing in one go the graph of this function that has 4 (in this case there are 4), 4 Dirac deltas. This is a very convenient thing and it holds because where the Dirac delta is not present (like where the step was not present) the function is zero, so I can literally sum it. And doing the summation, unless I am… so I have to *shift* things, but if not… here it is a bit more difficult, but if I am careful to put them at instants that are more distant than the interval of that millisecond of this duration of the neurotransmitter, of the neurotransmitter profile in the inter-synaptic space, the amplitudes do not overlap for me. The sum always happens between a quantity that is 0 everywhere and a quantity that is 1 (or T_{max}, where was it? T_{max}) only in one point. I'm making it very long for those of you who perhaps haven't seen Dirac deltas, but this is very convenient for many reasons.

Multiplicative Terms and Receptor Saturation What I heard written here and C I wrote as $1 - O$, which by definition (so in this case the equation is still exact) and T_{max}, which was the amplitude of these Dirac deltas, but since the amplitude was the same for all, I brought it outside the summation.

Looking at this equation I don't know how to solve it because here it is not a system… beyond the fact linear or non-linear (in this case it is a linear system, it has constant coefficients), but here the input is a **multiplicative** input, one says, because the state variable O, here multiplies the input. While I am used, in systems theory, to having a term that exhausts (which appear as they wish) all the terms that are the state variable, plus something, and this something does not contain the state variable. This is typically the simple version, of which I know how to manipulate, I know how to write the impulse response, I know how to solve analytically with the convolution integral or with various heuristic techniques. When I have something like this I don't know how to do it because, or rather I could do it but it is more complicated, because it is a multiplicative input term.

I make you reflect that this term $(1 - O)$ has the function of, for free (it emerges from this kinetic scheme), taking into account what is the **saturation of post-synaptic receptors**. As I told you, the presynaptic bouton "spits out" the neurotransmitter molecules and these bind to a number that is however limited and finite of post-synaptic receptors. When a neurotransmitter molecule approaches a post-synaptic receptor and finds it occupied because there is already another molecule that is bound (so if they are almost all open, open in the sense that they are bound to a neurotransmitter molecule), $1 - O$ is very small. And so this term here, whether there is this input or not, whether there is or not, is not taken into consideration, there is no space, there are no other *slots*, since the majority of channels is in a state (of post-synaptic receptors) is in a state bound to neurotransmitter molecules.

However, a situation like this happens in two circumstances. The first is that there are so many vesicles in the presynaptic bouton that in an instant I flood you and saturate all the receptors (as if they were seats, perhaps this is a more fitting example, as if they were seats in a classroom: a huge quantity of students enters, the seats are those and in one go they sit down). Another possibility (this is usually not the case, also because metabolically it is complicated to regenerate in the presynaptic bouton a notable quantity of neurotransmitter, so most of the time the quantity is limited, it is small of neurotransmitter; on the other hand it is an electrochemical conversion and so it could be, and it is, metabolically expensive to synthesize the neurotransmitter, package it in vesicles, it can be tiring).

The second scenario, which is a bit more realistic, is that the doors open repeatedly with a certain frequency and every time new students enter. So if the *firing* activity of the presynaptic neuron is at a certain frequency, this frequency again has to do with β, with β is a kind of term of comparison. Then obviously I say it because β is the *rate* with which the neurotransmitter unbinds, so with which you perhaps have finished sitting and leave. So if I start to have an activation that is on the order of β, then again I can have that I saturate, there are no more free seats. So this $1-O$ is absolutely convenient for me. But this happens again not so frequently, because again it means that the presynaptic neuron is firing at frequencies comparable to β, which putting the appropriate… putting the appropriate numerical values means on the order of a few hundred Hertz, a few hundred spikes per second, which is practically very very rare. Yes, it could be for very short… for packets, for *bursts*.

But from here on I am making assumptions, approximations. So I am aware of the fact that not only was this a simplified view, but here I am saying: "You know what? I'm just throwing this away". I say there is only 1. So it's as if I said: the majority of receptors are almost all free, so they are almost all closed, always. And you could tell me: "But no, they are not". Yes, because anyway the fraction of receptors in the open state, when it increases, increases and becomes at a peak value for a short time. So if the presynaptic activity is not very frequent, the neurotransmitter always finds free receptors.

So I am writing that I approximate and I am putting here a term that finally is quite simple to handle. Have you ever seen a differential equation of this type where the forcing term is a Dirac delta? Electronic engineers, those who have the idea of the impulse response should give a positive answer. Maybe you have never seen it with a train of pulses, so with an adder of many shifted pulses, what is called a pulse train.

I will show you shortly, comparing it and doing paper and pen (or marker and blackboard, if the marker writes), I show you that if you allow me this simplification I can extract some information that is basically here. That is, I can have on one hand an expression (but this is not particularly interesting: instead of solving this differential equation, I can have an algebraic, iterative equation, which tells me what the amplitude is at the instant of the $k+1$-th spike, knowing what the amplitude was at instant k and knowing how much time has passed). But this could have a value of the type: if you want to run simulations, you want to have a method to accelerate times. Because if a pyramidal neuron receives something like 10,000-100,000 synaptic inputs from

other neurons, it might be hot to have 100,000 (even maybe there will be 50,000) differential equations of this type to solve numerically instant by instant.

Dynamic Steady State and Frequency-Current Conversion

But the most interesting thing, of which I just want to show you how one arrives at it, is this expression here which says that more or less at **steady state**, at the regime state… And you can tell me: "But what regime? I have presynaptic spikes and from the post-synaptic point of view the potentials (so the currents or the post-synaptic potential, the change in conductance) is something that goes up and then goes down, goes up and goes down. Where is the regime?". There is no fixed regime, it is similar to what is called permanent sinusoidal regime, in which there too there is no fixed regime, there is no constant regime, but it is a kind of dynamic regime that I show you.

And from this (now I tell you what this *steady state* is) I obtain this information which is very important: apart from α, T_{max} and β^{-1} which are numbers, here it tells me that at *steady state* a synapse, a post-synaptic receptor, a family of post-synaptic receptors, are producing a current for me (ok, this must be multiplied by $\bar{G}$ and multiplied by the *driving force*), but anyway on its own it is a current **proportional to the presynaptic frequency**.

Which is a very interesting, very powerful thing. Somehow, despite being a strong approximation, it tells me that **a synapse is an object that converts frequencies into currents**. Which is what it does. And this conversion is linear, it is proportional. And a light bulb might light up in your brain and say: "Ok, if I have to develop some intuition on what a synapse does in a large network of neurons, maybe I could say that its value is testifying to me what is a value… so it testifies to me with a continuous analog value over time, of what is the frequency of a digital event and extracts the frequency for me".

So this is what I am saying and we haven't talked about it, we will talk about it next year, combined with what neurons do. In the end we saw it only from the numerical point of view, we saw this when I showed you that for the Hodgkin-Huxley model, for example increasing the current (my injected current, but it could be the synaptic current injected by another neuron), when this current is increased the firing frequency increases. It doesn't matter that it increases linearly.

So in a certain world (for which this slide makes half sense now and the other half makes no sense because we haven't seen it in this approximated way), the so-called frequency-current curve, which perhaps I asked you to try to play with, perhaps I asked your predecessors of the second year, converts a current into a frequency. So it converts an analog level into a pulse train. While the synapse does the opposite effect: it converts a pulse train into an analog value. And so maybe at a certain point one might (but the temptation doesn't come now, it will come in a year) to say: "But ok, what if by chance I connect this arrow to this…". Because in the end synapses are objects that have an effect on other neurons and let's say the frequency activity of this arrow actually I could attach it here, because in the end it is neurons that produce these action potential trains, so it's not that synapses invent these action potential trains for themselves. So I could see and study (and it is done in a self-consistent

way) a kind of combination between a synapse and a neuron which could be representative of many neurons.

The Bank Account Analogy So, what I would like to do is, before moving on to synaptic plasticity, I would like to put a damn black slide because I don't want to turn off the projector which I don't know how to do, so give me a second. This is not very elegant… ok… ok. It's brief, it's a little game, but once in a lifetime the story of my bank account must be clarified mathematically.

So you have to allow me to make an approximation, a simplification. I am thinking that the presynaptic spike train over time is at **uniform frequency**, that is, this time yes, the Dirac deltas must be equispaced. So if I were "cool" I should write that t_k is proportional to some ΔT, for example. It is an integer multiple of some period. And this period, ΔT, could be described as $1/f$, since frequency and period are one the inverse of the other, in a context where there is a uniform frequency, one says, because it doesn't change over time.

So the equation was something like this (it doesn't matter about α, it doesn't matter about T_{max} and β^{-1}, it doesn't matter), it was something where there was, as a function of time, a differential equation describing dx/dt, there was a $-\beta x$ plus, let me call it, A, then there was the sum over k of these $\delta(t - t_k)$. It doesn't matter much, it is much of a number, now you will see because it is not particularly important.

So the first thing I can say is that when I am not close in proximity to a Dirac delta, this quantity disappears, because it is zero everywhere, practically it is zero always, almost always, except when there are exactly those Dirac deltas. So in that case, when t is not… is different from t_k (k from 1 to whatever is n), the equation becomes very simple, and it is the usual, very boring equation:

$$\frac{dx}{dt} = -\beta x$$

And I know how to solve this because x (apart from the initial condition, which here I leave indicated the constant to be identified) is $e^{-\beta t}$. I am happy because there is a minus sign and so there is the minus sign in the exponential, so it means it is a decreasing exponential.

So somewhere here (so this would be the graph, so this is somehow the presynaptic input appearing in that equation, presynaptic input, and this is for example the value of $O(t)$, or of $x(t)$, whatever it is; in the end x and O are the same thing except for a scale factor which I don't have now, it is not important to recover in its precise value). So at a certain point every time there is a space between two, so the interval where there is no Dirac delta, starting where I start (whatever the initial condition will be, I don't know, fixed at whatever it is), I know how to write the evolution over time because it is an arc of exponential that goes down.

Here perhaps you see the little game I want to play. It's as if it were… so that is the university gives me (because there is a plus) a quantity of money at the end of every month. But fortunately I don't behave like this: I don't spend proportionally to how much I have in the bank account. I have fixed expenses, sometimes I exaggerate with Amazon, but surely it's not that I do it

proportionally: "Now I have [a lot], so I double". No. Here instead by the law of mass action yes: I am… the higher the value of x, the more rapidly it must go down, in fact I have an exponential.

In the case of my bank account, here it is as if I no longer had the dependence on the value of the bank statement, on the total value of money in my current account, and so the solution would not be this, it would be a straight line, it would be something of the type constant $(t_0 - t)$, something like that. So it is something that instead of going and then slowly saturating, would go linearly, so much so that I can go not only… I go into the red, I can go down arbitrarily, my debt can increase arbitrarily, it is proportional to how much I spend. So it's not this, but the story of the bank account perhaps remains in your mind.

Note: I made this same analogy for the accumulation of calcium in the cytoplasm of a neuron when talking about frequency-dependent adaptation mechanisms. Here I am talking instead about the fraction of post-synaptic channels in an open state. The math is the same, but they are two different concepts. In a first case it was a concentration of calcium accumulating (this could be a kind of influx of calcium, calcium channels opened, there was a *puff* of calcium). Here it is another thing: that is an opening, a quantity of neurotransmitter that arrives all at once in a moment of time and between one moment and another this is the kinetics in question because it is a synaptic receptor that from open becomes closed.

Integration of the Dirac Delta The second step is to say: but when I am around t_k, how should I behave? So here I can apply the integral on both sides and I apply it across a Dirac delta. I apply it between… suppose this is t_k, the k-th instant. Using this nomenclature, this formalism of mathematicians, I write (removing this), I write that I do the integration between t_k^- and t_k^+. What I should say more correctly is that I am imagining doing this integral, t_k, between two points, so in an interval, between two points that are across a Dirac delta, any one (then you see that the thing always repeats). After that I should do a limit procedure, saying that this on the left, this left extreme goes to t_k from the left and this on the right decreases going to t_k from the right. But it doesn't matter that much, because the only thing I have to reason about is that here I have the integral of a derivative. Integral and derivative cancel out because one is the inverse operation of the other.

So let me cancel this… so here by the fundamental theorem of calculus, I know how to write this because I know what the primitive is. The primitive x I calculate at one extreme minus calculate it at the other extreme: so $x(t_k^+) - x(t_k^-)$. I like this because in the end I know what the value of this x is immediately before and I imagine that immediately after there is some kind of jump. In the end the university pays me a salary, here I certainly go up, by I don't know how much, but I go up (unfortunately I always have little, but I go up), that is, after minus before will be some quantity.

This piece remains [the term $-\beta x$]. This is the most annoying piece, because I have to invoke a thing that annoyed me as a student, which is a fairly heuristic thing, that is, I invoke the **continuity** of this function x. You tell me: "But you haven't solved this equation, how do you know that x is continuous?". I assume

that x is continuous and here basically I am saying that I am doing the integral, that is, I am calculating the underlying area of a function that is ok, that is not pathological. This [the delta] is a pathological function, but fortunately it cancels out with the integral, we will see it in a moment. If it is a normal function, when I do the underlying area it is easy, and it is easy to understand that when I restrict the integration extremes a lot (mathematicians say "when I do the integration on a domain of measure zero", the same thing), the area becomes zero. Because there are no particular discontinuities; at most there could be a discontinuity of the first kind, like this one, but for mathematics this is not particularly dramatic, there are no Dirac deltas.

So this quantity here I know is zero. But there is obviously a heuristic that can disturb you because again I don't know it in advance, so I have to assume it and then I have to verify that it is so. Another way to see it is that this class of differential equations ($dx/dt = -$blah blah + something) have the output that is said to be always more continuous than the input, because in between there is an integration operation. The integral, unlike the derivative (which highlights transitions, highlights discontinuities, highlights changes), the integral softens everything, let's say it does *smoothing*, it "smooths", as they say, slows everything down. So that's why I assume that if the Dirac deltas which are ugly beasts (they are strongly discontinuous, go to infinity in zero time; worse than this there could be multiple derivatives of this Dirac delta, they are even worse, but they are not here), if it is a Dirac delta, the x, which is in fact a kind of integrated version, at most will be a step. That is, I am imagining in another context, hoping that someone can resonate, that I should talk about distributions, which are these special functions which are the Dirac delta, the step (often called, indicated with θ, the so-called Heaviside function), they are all functions that are… they are not special functions, and I know that in fact the integral of a Dirac delta becomes a step, and the step is a function that has only a discontinuity of the first kind.

Anyway, to make it short, in that case I removed that term which was quiet, quiet, so here I literally write 0. Plus this: it is simple because the Dirac delta is by its definition a function (there are various ways to define it, even one way is using the inverse Fourier transform, or the inverse Laplace transform, or taking a step whose amplitude is inversely proportional to the duration, so if you do the limit, when you shrink the duration, the amplitude goes to infinity), but anyway, there is another definition that I like more because it is axiomatic, where there is not much to understand, which says: the Dirac delta is that function which, if you put it under the integral sign, and the integration extremes comprise it (comprise where the term t_k is, where the Dirac delta is centered), extract the function that premultiplies this Dirac delta. One. Another way to say it is that the integral of that Dirac delta (sorry, here I remove the summation because I am considering only one Dirac delta at a time), another way is that the Dirac delta is that function whose area is always unitary, that is the integral across where the Dirac delta is defined is one. However you want to put it, here you write A.

And this is a dramatically easier thing to do than solving, even if it is linear, piecewise constant, the equation satisfied by O for when the pulse, when the concentration of a neurotransmitter is not described by a series of Dirac pulses,

but by those little rectangles. So here I gained that I have a simple rule, because this means that **after** (so bringing this part to the right), **after = before + a factor that is always constant A**.

So the university pays me the salary, or vice versa, they are the vesicles full of neurotransmitters that have flooded the inter-synaptic space and have bound to these receptors of which I am describing the fraction. After which, however, here between one and the next it continues to decrease, it continues to decrease exponentially. Here I continue to have the *income* from the university and here I decrease.

Dynamic Steady State At a certain point (and this obviously holds strictly only if the Dirac deltas are equispaced, now here graphically I made it return because I am lazy, I made it return after two or three… after two or three Dirac deltas), at a certain point you see that the next Dirac delta happens, arrives more or less always at the same moment. So it is as if there is a repetition, so it is as if this goes down and then comes back up. In this time, which I know to be $1/f$, this value here I know to be the value that was… the same value that was here. Suppose I call this… what do I call it? I call it $\bar{x}$ (x-bar). I call this $\bar{x}$ because, again, it is a kind of dynamic equilibrium. It continues to repeat in the same way at a certain point. It does so because it continues to insist on the residual tail, but at the same moment. So this point here I call $\bar{x} + A$. This decays to $\bar{x}$ and this point here will be again $\bar{x}$ plus… point… plus A.

If you want this is a hypothesis: I am hypothesizing that there exists a regime where this thing goes up and down and at a certain point it always does the same thing. That is, dynamically it is as if these peak amplitudes (or you could see the amplitudes instead of… the minimums, or you could see the intermediate amplitude), more or less are the same thing. And the only question is: but what is $\bar{x}$? $\bar{x}$, I know how to write it, because it is $(\bar{x}+A)$ (which is the initial point… I am thinking of this… this k is the value that the function assumes when t is 0; t in this case is the interval, it is as if it were a… I had taken this variable t, now this is 0 and this is $1/f$). I know that $1/f$ has passed, and $1/f$ milliseconds before this value was $\bar{x} + A$. And so here I write: times $e^{-\beta \cdot (\text{how much time has passed})}$, $1/f$.

From here it suffices for me to make $\bar{x}$ explicit, because for example I want to write what the expression of this bottom value is, $\bar{x}$. But I could have written it differently, I could do x again, the peak or the intermediate value. So what I do is write:

$$\bar{x}(1 - e^{-\beta/f}) = A \cdot e^{-\beta/f}$$

I brought this $\bar{x}$ to the left side, factoring it out. This multiplied this exponential, so I brought it to the left and e appears here in this parenthesis. And on the right I write, remains A times $e^{-\beta/f}$. So $\bar{x}$ becomes a kind of function of f:

$$\bar{x} = \frac{A \cdot e^{-\beta/f}}{1 - e^{-\beta/f}}$$

Now I won't stress you further, even if, believe me, if you try to do it yourselves, to reason… Theoretically you could also try to write some code to realize the fact that with this rule (so 1 plus 2), which incidentally is similar to what you

would write for intracellular calcium in frequency-dependent adaptation, but it is a different thing… here we are talking about synapses and we are not talking about my bank account because I don't spend as much as, proportionally to how much I have. With these two rules you could realize that when there is a uniform regime, a uniform frequency of presynaptic activation, at a certain point this transient, if you squint, you see that there is a kind of band. And it is shown that this band is on the order of $1/\beta$, but it doesn't matter. At a certain point you have a regime and you could describe this regime as the mean value, the peak value, whatever it is, is a function of f.

I won't do this, but if f is particularly large, if the frequency is high (which in your mind might say: this phenomenon occurs rapidly, because high means high compared to β, that is it means that the new Dirac delta arrives before the tail has exhausted itself completely)… Fortunately I am thrifty, so the next salary doesn't arrive finding me exactly at… it finds me that… so that's why I manage to save, I hope these stupid things can be useful. So here in fact it is telling me that I am saving at a certain point, but not that much… it's not that my bank account increases… Ok, my bank account could increase if the income was higher than the expenses. But here I have that the loss is proportional to how many receptors are in that state, by definition of mass action. That is why there is a steady state.

And so when f is sufficiently large compared to β (you see here that you have β divided by f), you can approximate the exponential with the first Taylor term. I won't do it, you can try and you see that you get an expression where $\bar{x}$ is equal, is proportional to f. And this is a thing, I repeat, interesting because it tells me that this band, roughly, goes up or down depending on how much f is.

Obviously in the real case, it is not true that synapses are always driven, are always activated by a train of action potentials at uniform frequency. Indeed they never are. However something similar in a regime that is not deterministic but is stochastic, so where moments are unpredictable but happen with particular probability distribution conditions, the same type of reasoning can account for the fact that the synapse somehow responds proportionally to what the frequency is. This is a more advanced topic so it makes no sense to stress you now. Is the green light still on? Yes? Thank you.

Synaptic Plasticity and Learning

Now, it seems (by now we have known this since the 70s, so it's been more than 50 years, from the 70s and also from relatively recent discoveries) that synaptic transmission is, compared to all the biophysical and biological things I have described to you up to this moment, one of the sites where experience or electrical activity in general changes the structure. So I am talking about **plasticity**, I am in fact talking about… I am evoking the theme of **learning**, which is not necessarily equivalent to synaptic plasticity.

This is a very interesting, very profound thing, because learning memories, concepts, and being able to recall them, does not arise immediately from the consequences of saying: "Look, T_{max} could change over time, or $\bar{G}$ (which was the maximum conductance or the maximum number or the maximum conductance of post-synaptic AMPA receptors) might not be fixed, it might change

over time." Maybe it changes very slowly, it doesn't change on a time scale of milliseconds, it changes on a time scale of minutes, of hours.

You see me, you learn (I hope) and something remains in your mind (if nothing else the story of the bank account, which I hope, together with the story of licking your sweat, is by now indelible). That certainly did not happen instantaneously. It may be, however, that now you have dedicated a gene expression to me in which new proteins, new synaptic channels, new synaptic receptors have been inserted into the post-synaptic membranes, because that concept had to be strengthened.

At the basis of this (which I repeat is not so obvious based on these considerations) there are two phenomena called synaptic plasticity (so it is not necessarily equivalent to learning, but it is its probable cellular substrate). And they are of two types; the ones we examine today are of two types: it is called **homosynaptic** and **heterosynaptic** plasticity. It has nothing to do with sexual preferences, but as in that case, here it has to do with something that does not include the presynaptic neuron… sorry, does not include the pre- and post-synaptic pair, while heterosynaptic means that it includes the activity between presynaptic and post-synaptic neuron. It will become clear in a moment.

1. **Homosynaptic plasticity** is also defined as **short-term plasticity** (STP). And I will show you and we will comment on and study together what short-term depression and short-term facilitation are.
2. While for **heterosynaptic plasticity**, it is also called and attributed to characteristics of *long term plasticity*, **long-term plasticity**, and it has to do with a (or it is necessary, a necessary condition for its expression) a co-activation or a relationship, a correlation between presynaptic and post-synaptic activity. This is not present in homosynaptic plasticity, which is more solitary, while heterosynaptic means that there is the one who speaks and the one who receives must do something (like the story of the NMDA, of the NMDA receptor).

And so we will talk about long-term synaptic plasticity dependent on *timing* (depending on the timing of spikes) and what is called redistribution of synaptic efficacy.

Short-Term Plasticity: Inertia and Limited Resources

One important thing to realize (probably an engineer doesn't realize it because for him synaptic transmission is a gain, it is a value similar to an amplification factor, or to the value of a resistor on a wire, the resistance of a wire which is that, and ok, I put a signal, the signal at the destination becomes amplified or attenuated, but it doesn't change over time, what changes maybe is the signal). Instead synapses, which are objects, as said, are probably the most complex organelles that exist in biology, out of all living beings, have a certain dynamics, latency, inertia. It takes some time for neurotransmitter to be expressed, synthesized, so these vesicles are packed, are then anchored in the presynaptic membrane.

A disgusting and stupid analogy again is that of the **llama**, the animal llama which I believe (some do it, I haven't seen it done) spits. The synapse spits. If

you ask the llama to spit with a certain frequency, and the frequency is very high, the llama might have a dry mouth and simply have no more saliva to spit (a disgusting thing, with respect for llama animals), to make you participate in the fact that this is not a system… it is not a wire, it is a dynamic system.

Then just as I could be with my voice (I have a bit of a cold but I think I'm managing), where now I am ok, but at the third and fourth hour my voice with activity will be much lower. This regardless of whether you speak, are attentive or not. It is only my problem, a problem linked to my ability to *replenish*, to restore my resources in generic terms (I won't say what they are), the resources for my neurotransmission.

Synaptic Depression (Short-Term Depression) And so these synapses have fatigue, inertia or **depression**, therefore depression of responses during repeated activation. So if the presynaptic neuron, on its own, has an activity, a frequency of, I don't know, 50 spikes per second, it may be that they are too many for the synapses. They may be too many for the synaptic vesicles even before the receptors. The receptors maybe desensitize on their own, but I might not have any neurotransmitter left to release because I really [lost] my voice.

Synaptic Facilitation (Short-Term Facilitation) However, as sometimes happens to me (surely it happens to you too), the same dynamic system can instead show a dual behavior of not a depression of responses, but a **facilitation** at times. And I imagine that the hope is that you can do it also during the colloquium course in bioengineering, where I ask students to make a presentation at the exam, to organize themselves in groups during when speakers come and therefore to start putting yourselves out there, to give presentations. It is useful, job interviews and other things, I don't have to tell you. It might have already happened to you for example in your bachelor thesis defense that at the beginning you were quite nervous and then you warmed up.

So this context of warming up or depression has only… it is on the short term, because if you leave me a little bit of time without speaking, my voice returns. Vice versa, if you are nice and pumped up because you are in the middle of your presentation and I put you to sleep, you return to a certain baseline value of anxiety versus confidence. So it is independent of what the *audience* does, it is independent of what the post-synaptic neuron does. If it were dependent it would be a process of heterosynaptic plasticity, because it would involve both the speaker and the listener. Here it is only the speaker: if the speaker speaks too much, speaks too frequently and gets depression or vice versa gets facilitation.

Experimental Evidence: Pyramidal and Interneuron Connections

This thing of short-term depression is actually a relatively recent observation from the years 90-2000, but it is not a surprise because people who studied the neuromuscular junction knew it all along, since the 70s, that when the release of acetylcholine produced a muscle contraction, at a certain point the nerve fibers of the neuromuscular junction could simply run out of acetylcholine. You can try, even if from the muscular point of view you also have the onset of lactic acid, and therefore you also have other mechanisms, even other desensitization mechanisms of nerve fibers (so somehow also of axons), but somehow if you try

to perform a muscle action "give and give", at a certain point you can't do it anymore. I repeat, in that case it is more due to lactic acid in the muscle, but the concept might be familiar to you.

So this is an example, not in the neuromuscular junction, but between glutamatergic synapses between two pyramidal neurons of the rat somatosensory cortex. Here you see two cells, one is green (they are three-dimensional reconstructions), one is green and one is black, they are very very close, their somas are very close. The experimenter had two pipettes, one in the soma, in the belly of the black neuron and the other in the belly of the green neuron and in one of these (as in the experiment I showed you last time), instead of just giving a "flick" (*schicchera*) to produce a presynaptic spike, the experimenter gave a train. I believe they are ten, three, whatever it is, plus he let elapse perhaps 800 milliseconds or thereabouts, and then he gave a further flick to emit a further action potential. And he measured the post-synaptic membrane potential.

The thing is more interesting compared to last time. Last time one could only see that, basically by making a spike fire, you saw if the post-synaptic neuron was connected or not (which had notable importance, it allowed in part to infer the connectome at the cellular level, I told you). Here, surprisingly, people (I don't know why it took until '97, the late 90s, why people didn't just give a pulse… people maybe gave two, but give a train and maybe change the frequency too, so maybe you can study the frequency-dependent behavior of the synapse).

To make a long story short, what is seen in the post-synaptic neuron is a progressive decrease. These are excitatory post-synaptic potentials, here the cell is simply recorded in so-called *current clamp*, at -70 mV, at -60, whatever it is, and again here the amplitude of these events is tiny tiny, they are synaptic events, they are very very small. It gets tired. But if you wait a bit, so there is a pause period, you see that the amplitude of the response to the nth spike, the subsequent one, is much greater than the amplitude of the last one of the train, not yet completely restored, not completely recovered compared to the amplitude of the first one. The amplitude of the first one is higher because the experimenter had waited a few minutes before resuming stimulation. So in fact this trace I am showing you is the average of several repetitions and between one repetition and another the experimenter waited maybe not a few minutes but certainly a few tens of seconds.

Target Specificity: Depression vs Facilitation The interesting thing is that if this same neuron (suppose the black neuron, which is this one here that I made fire, the one that is presynaptic) by chance projects (here I don't think it is here, maybe it will appear on the right, but anyway), if it projects not only to another pyramidal neuron, but projects to an **interneuron**, to another neuron in particular they are called **basket cell** (they are basket cells, like a basket, I don't know), in their vicinity, they are typically inhibitory neurons. I am a pyramidal, I am excitatory, I project (I repeat this is **Dale's law**: if I am excitatory, glutamatergic, all my synaptic boutons are glutamatergic), so I release, I spit to that other neuron with a synapse to that other pyramidal neuron and the synapse however depresses.

And it has another post-synaptic target (it's still me, so I still release gluta-

mate), but the synapse towards that other neuron, look what it does? In fact it **facilitates**. It is not so much different in amplitude with respect to the same order of magnitude of a fraction of a millivolt, but it is a completely different behavior: it tends to be, to facilitate, as… so not to turn off progressively due to fatigue, but to "get pumped up". And if one waits a certain interval of time, practically the amplitude of the response (so the response to the nth spike) becomes comparable or roughly comparable to the first one.

- **Depression (Pyramidal-Pyramidal):** Here I have dry mouth and I have no more saliva, I have no more resources, so the neurotransmitter vesicles have probably run out, they are empty, they haven't had time to restore themselves yet. Yes, the mechanism is very fast, but it might not be so fast at this frequency (which I believe is… Well, there will be 4 or 5 spikes in 200 milliseconds, so it's 25 Hz, it could be. I am thinking if this was instead 1000 milliseconds it would be easy… so it could be, so from 200 to get to 1000 I have to multiply by 5, so they would be yes, around 20-25 Hz). So we are not talking about hundreds of Hz as in the previous case where AMPA desensitized. Here already at physiological frequencies, 25 Hz is ok, actually even 20 Hz is ok, synapses have a strongly time-variant behavior.
- **Facilitation (Pyramidal-Interneuron):** And in this case ditto, here it is not dry mouth, here it is probably (this I am giving you two explanations) here it is probably the **accumulation of intracellular calcium**. You know that the release of these vesicles depends on the influx of calcium, it may be that there is residual calcium in the synaptic bouton, so it persists and if it persists it might make release easier when there is a further influx of calcium.

The real question is: but why do two synaptic boutons coming from the same axon, of the same presynaptic neuron, in one case do depression, in one case do facilitation? No one knows. There seems to be a specificity of the type of synapse based on the identity of the target, of the interlocutor.

Functional Interpretation of STP Specificity

Again, with an interpretation partly probably superficial: if I am excitatory and I project to another excitatory neuron, perhaps it is better that my communication channel, since it is glutamatergic, loses strength. Because in the case of recurrent connections it could easily explode; this could be a **positive feedback** and could explode. So if I have a mechanism whereby I get "dry mouth", my voice goes away immediately, it may be that I manage to interrupt eventually a **seizure**, an epileptic crisis.

Perhaps this is exactly for the same reason [that the opposite happens with the interneuron]: I, glutamatergic, excite you who are inhibitory, who are GABAergic, and your role is probably to act as a peacemaker, to inhibit everyone. Because maybe I might have gone crazy. Also again, if these frequencies are not extremely high, it might make sense that I continue to facilitate response after response. Because while here I discourage excitation (the emission of a spike by the post-synaptic neuron), here honestly a 2-3 mV EPSP does not make a neuron fire; at most if I am at -60 mV it makes it arrive at -65 mV. Ok, if there

were more neurons simultaneously maybe I would be close to threshold and could fire, but it may be that if this first EPSP made me arrive at the threshold, the last one certainly won't [in the case of depression], so I can interrupt like this, even without going completely voiceless.

Here [in the case of facilitation], if the EPSPs are, again, are excitatory post-synaptic potentials (they depolarize the neuron), but the post-synaptic neuron is inhibitory, at a certain point (and this is seen experimentally), if I continue, at a certain point a synapse… I manage to make the inhibitory neuron fire. So I have to fire many times (and I am not talking here about temporal summation, but I am simply talking about this facilitation). It may be that therefore it is evolutionarily profitable that, when I am perhaps gone crazy, the synapse with an inhibitory interneuron is particularly activated, so the GABAergic inhibitory neuron activates, fires and since he by trade releases GABA, he silences the whole network.

Again, the interesting thing is that this is calcium-dependent and seems to be again… here is the graph where the pyramidal neuron with respect to the interneuron has even potentiation and then even a spike; in the case of pyramidal-pyramidal a depression.

The Experimental Challenge: Connectivity and Multi-Patch

This was quite an important discovery. I repeat, it is trivial in the case of the neuromuscular junction, but no one for some reason had observed it between neurons of the central nervous system. One of the reasons is that, as I told you last time, since these pyramidal neurons (or the pyramidal neuron and this cell which is probably not a *basket cell*, but is a **Martinotti** cell, it is called, it is a GABAergic interneuron that stays near layer 5; this is layer 5 where the somas of these cells are), the **connection probability** is very, very low.

So people at least must have two pipettes, two electrodes, two amplifiers, two micromanipulators. And I told you last time that if you have only two micromanipulators, two pipettes, two amplifiers, you are destined to find few connected pairs. If instead you have 4 or 6 or 8 or 10 or 12… if you have 2, 3, 4, 5, you have a number that changes quadratically [exponentially in speech, but implies combinatorial] of possible pairs: $n \cdot (n-1)$. If with 2 you have only 2 possibilities, with 3 you already have 6, with 4 they become 12: you have already increased by an order of magnitude.

This guy, **Henry Markram**, who was my advisor at my second postdoc at EPFL in Lausanne, had a setup… he is an artist, he has the hands of a violinist. Because to have this ability to (despite them being controls, so you perhaps would be more impressed by a *gamer* who plays rapidly), the same class, he managed to put the pipettes very rapidly into the soma of these two neurons without losing the other *patch*, without introducing vibrations. And he had four, and so he had more than the others and managed to find easily and describe this phenomenon that maybe the others saw once every two or three months.

Dendro-Dendritic Synapses in the Olfactory Bulb The interesting thing is (maybe I have to take a break, so I simply tell you that) also in the **olfactory bulb**, in cell types called **mitral cells**, one has the same thing. A mitral cell

has a target, has a behavior (which is poorly seen here because it is crappy) depressive, and in others it has a facilitative behavior. The interesting thing about mitral cells (maybe I was talking about it with one of your colleagues last time) is that here the synapses are **dendro-dendritic**: so they are not the neurotransmitter vesicles at the end of the axon, but they are in the dendrite. Simply another example.

I'll stop for ten minutes, sorry if I have enthusiasm for synapses. Thank you.

Kinetic Modeling of Synaptic Depression

So it is worth it, why not, with the usual kinetic schemes (this time applied in another context) to understand something about it. Because again, if we manage to put in a slightly more formal way the phenomenon I described to you… Up to now I simply told you what people saw: the fact that it is known that it is partly linked to the depletion of vesicle resources (that there are no more vesicles because they are in limited number) or there is an accumulation of free calcium in the presynaptic bouton that changes the offset, makes release much easier; these are things that came later. And the advantage of being able, for example with kinetic schemes, to give a quantitative representation, because then I can open it, I can do everything, I can partly simplify it and extract again some intuitions, like the one that a synapse converts a frequency into an analog level proportional to it.

Here I am thinking, so I describe only **short-term synaptic depression**. And in fact, even if it is wrong (because we don't know if the phenomenon I sold you up to now as short-term depression is totally a presynaptic fact… intuitively I told you that vesicles are limited, so I am stressing the fact that it is only a fact linked to the presynaptic bouton; currently we don't know if there is also a dependence on the post-synaptic neuron, in particular on the population of post-synaptic receptors; probably they are two combined things).

The Three-State Model (R, E, I) So much so that in the literature people said: "You know what? I generically describe what are the **resources** for neurotransmission". You will tell me: neurotransmitters. I call them resources, so the *reviewers* of an article cannot attack me, saying: "I didn't say it is a presynaptic phenomenon". For simplicity we can imagine that we are talking about synaptic, presynaptic vesicles.

And so these presynaptic vesicles, or rather the neurotransmitter contained in them, can be found in three states (again here you see the phenomenological approach, I am not describing in a biophysical way). I am simply saying: look that they can be in a state: 1. **Recovered (R):** Also called *ready*, *releasable*, ready to be released, restored. 2. **Effective (E):** In an effective state, where they have been released and are available to bind to post-synaptic receptors. 3. **Inactive (I):** And then they can be in an inactive state, whereby, for example, they are diffusing laterally in the synaptic *cleft*, in the inter-synaptic space or are in the *re-uptake* phase, of fishing back, to be recycled and put back inside, re-packaged in vesicles.

There are therefore three states and three transitions, they are not reversible. So if you are ready to go, when there is… this is an activation probability, the release

probability, or a release *rate*. Historically it was called U as **Use**, in a barbaric way, but it was so. So when a vesicle is ready for use, if with a probability of use (clearly this probability is normally zero and becomes non-zero when there is a spike)… In fact this thing I am telling you is the story of the neurotransmitter rectangle, but with all the trimmings, much more sophisticated: there it was a rectangle that goes up, reaches one millimolar, T_{max} and goes down. Here I am saying: with this thing here, I make that T_{max} dependent on previous activity, on previous history.

So here when it is used, when there is a spike, it passes from "ready" to "effective": the neurotransmitter is released in a very very fast way. And then in an equally fast but slightly slower way (with a kinetics that here I am writing as one divided by a time constant to be able to skip a step and in fact introduce because it is convenient for me intuitively to speak of time constants, of time scales), with which an **inactivation** phenomenon occurs ($1/\tau_{inact}$). Or even with which a phenomenon occurs whereby vesicles that are in a state no longer functional, no longer useful to bind to post-synaptic receptors, have a period, a **recovery** time scale, *recovery* ($1/\tau_{rec}$), which again accounts for this transition with a *rate*, which is a rate, so it is not a time, it is the inverse of time, it is a speed.

The thing I notice is that obviously (otherwise we wouldn't have seen it if the llama or if Michele Giugliano didn't need a certain long time to restore the neurotransmission vesicles, saliva or voice, we wouldn't have seen, we wouldn't have appreciated short-term depression). So it is reasonable to assume, because it is a consequence, that this arrow here, this transition is much slower. So the value here of this speed is small, that is the time constant is large: the time scale is long, 500 milliseconds, against this one, the inactivation time scale, which maybe is 1 millisecond, 5 milliseconds. The neurotransmitter disappears immediately, but then it takes a lot of time, 100 times more slowly to be recovered.

Differential Equations of the Model

So the first thing I do, now I write a differential equation for each state. But you will see that it starts to get a bit hot because they are three differential equations (actually they are two because one is linearly dependent on the others), but they are ugly. I would like to have some expression that tells me: "Here, this is the amplitude of a neurotransmitter pulse based on past history". But let's start.

The first is, again, they are as if they were water tanks.

$$\frac{dR}{dt} = \frac{I}{\tau_{rec}} - U \cdot R \cdot \delta(t - t_{spike})$$

So does R decrease or increase? It decreases with this arrow, proportionally to how much liquid there is, and increases, for this other incoming arrow, proportionally dependent on how much I there is. So it is minus U times R plus I divided by τ_{rec}. So I wrote this as "1 over" because now it is easy to make approximations.

The second equation is for E. E appears because this arrow enters and disappears because this arrow exits. The exiting arrow is easy: minus E times

$1/\tau_{inact}$. And I plus is U times R. Again you start to see that obviously there is some symmetry. What exits here enters here.

$$\frac{dE}{dt} = -\frac{E}{\tau_{inact}} + U \cdot R \cdot \delta(t - t_{spike})$$

So I increases again because E enters proportionally to $1/\tau_{inact}$ and disappears proportionally to how much I there is with this time scale. So dI/dt plus $1/\tau_{inact}$ and minus $1/\tau_{rec}I$. But for the moment I leave them like this.

Simulation and Separation of Time Scales And I show you what happens if I take those three equations and simulate them. Obviously the thing I have to do is take (otherwise the system goes into some *steady state* and does nothing interesting), I take U and instantaneously make it become a certain value and then bring it back to zero. This simulates the arrival of an action potential for me and simulates the fact that the release probability, synaptic release which in the end is how much is described by this U, is very rapid. The release probability changes, normally it is zero (normally in reality it is not really really zero because there is a basal level… synapses are, I told your colleagues, they are a bit incontinent, they leak, so they release vesicles even when there is no presynaptic action potential). But at this level and in this deterministic context the lion's share is done by the so-called synaptic release evoked by presynaptic activity.

So what I did is set U to some function that is 0 always, then at a certain point becomes a certain value, the numerical value doesn't matter, and then goes back down. And what I saw is that for free this system of equations brings me reason for one thing that is very simple: that is there is a **very rapid emptying of** R, of the R compartment, and then there is a **slow recovery**. And I imagine it: the synapse has these vesicles, I let a few out, it is obvious that those remaining [are] decreased, they are no longer 100%, they are decreased. And due to this cycle, which I have for free with the kinetic equations equivalent to this kinetic scheme, it starts slowly, with the same time scale of τ_{rec}, starts to return towards 100%. I expect it to reach 100%, because I would like, I expect that there are no other branches here. When U is 0, ok, if I wait a very long time, 100% of the (so the occupation probability becomes unitary for R or in other words, all vesicles, so there are many, independent, identical) return ready to act.

The thing I see is for example that the effective ones (E) are the echo of this decrease of R, because E and R have similarities. You see however that it is a little slower compared to U. U is an impulsive signal, because I made it and I wanted to make it impulsive because I wanted to understand what would happen if I said "now release the vesicles and then I step aside". What you see is that the rising phase of E is steep and then the falling phase is not instantaneous. It is not instantaneous because this time constant $1/\tau_{inact}$ is always the usual little game, the same differential equation is always that, they are decreasing exponentials. Ok, it is quite faster than this time constant, than this exponential that takes a long time to return to 100%, but surely it has a finite time, it is not the Dirac delta which is instantaneous.

So again, the thing that interests me is only… there are two things that interest me for my subsequent approximation: the inactivation, the inactivation process

are very fast and the recovery, the filling of neurotransmitter vesicles is very very slow. Translated into mathematical terms, this τ has a numerical value much larger than τ_{inact}. And the other element is that the activation of vesicles, this one here, is very rapid.

Since I am in a mood devoted to Dirac (who by the way was a very curious guy, very interesting, there are books on Dirac's life, he was a bit peculiar), I say that it is a **Dirac delta** centered, so shifted, with the minus (this means that I centered it at the generic instant of a presynaptic action potential). And then I specify the area of this Dirac delta: I say it is big U, so usage factor, but big U, because it is a constant, while $u(t)$ is a function of time, big U is a number. I have to put it because otherwise I would remain with the area of this Dirac delta being 1, because it is exactly 1 and not 1.2 or 0.65 or 45. So this way I fix the idea and that is the area of the Dirac delta.

Reduction of the Model: Separation of Time Scales

I need these two pieces of information and I manage to reduce three differential equations into a single differential equation, which I manage to unpack. Ok, to finish, this is I. I is quite boring, it seems somehow to follow the same dynamics as R. What I am inactive, I remain inactive for a long time, just as much time as I take to recover. So it is as if there were only two mechanisms here: one, that of black and green which are very fast, and another time scale which is that of blue (ok, of red, but blue and red are roughly similar). This is a technique that is often used in various fields of physics and biophysics called **separation of time scales**. Here I am realizing that there are two phenomena that are on different time scales, so maybe I can consider one immediately at *steady state* and the other instead that still has to start doing the dynamics. Now I show you in what terms.

So I resume the equations and start considering this differential equation here, that of E. Here I simply transcribed it and one thing I do simply for convenience, I multiplied now both members by τ_{inact}, so that here I get that type of form $\tau \cdot df/dt$, which can be typical, because I like seeing that here there is a time in the numerator and there is a time in the denominator. In reality, what I am saying is that if τ_{inact}, compared to all other time constants in question, in particular τ_{rec}, which is the most important one, if it is very very small, it means that this equation here (remember the engineer "squints", says this is a *black box* whose output tracks the input and tracks the input with this time constant). If this time constant is very very small, output and input are practically the same thing. In other words, this equation is already always at *steady state*, that is it doesn't change anymore, there is no more dynamics, I can cancel this derivative and I can therefore write that E, so this second member, is equal to zero, that is $E = U \cdot R \cdot \tau_{inact}$.

And I got rid of this differential equation. I repeat, it is an approximation because E was not instantaneously a copy of U, it had a certain little tail, but here I, with this reasoning, say that it is sufficiently analogous to represent a transition.

If I want to put, I want to link the two things, the kinetic scheme, open/closed post-synaptic receptor, T_{max} to this world, I must probably say: wait wait,

before I do other things, T_{max}… and I must probably put it in analogy or link it to the peak of this little neurotransmitter pulse, because this has the meaning of neurotransmitter in an effective state, active, ready to bind to post-synaptic receptors. It is exactly T, but while there I had the little rectangle, here I have something that is a transient with an exponential that you saw numerically. So what I could do is I could say: I take the peak. So if you have a function that mathematically is written like this, then I see here now that the peak is the factor that premultiplies this mess here. But if I didn't have it, I could say, to put T_{max} here, I could say that I take $E(t)$, which might not have that form after my approximation, I integrate it and then divide by τ_{inact}.

If you take this expression here, do the integral from minus infinity to plus infinity, do the underlying area, you know how to do the exponential because the integral of the exponential is still an exponential, apart from a factor that cancels what one would get by differentiating the primitive function, and this is the term I put to compensate for the term you get by doing the derivative. You discover that by doing the integral and dividing by τ_{inact} you find exactly the peak, because in other words if you take just an exponential like this divided by τ_{inact} it has an area, it is a function that has a unit area.

In other words, I want to put this peak here, and here I am thinking: this looks a lot like a single exponential, but in general now here I made a mess, I no longer have an exponential, I have U, which is a Dirac delta, or R, which is a function of time, ok, this is a quantity. So what do I have to put here at T_{max}? With this trick I can say: do the integral of E. Here the integral is only… so it is only… ok, it is not very trivial, it is not very trivial. I say that, since here there is a Dirac delta, the integral pulls out the value of the function R, which is under the integral together with the Dirac delta, and gives me the value of the function R at that point. This is again… is used is a heuristic made on the basis of the definition of Dirac delta and relies on a graphical interpretation. If I had written directly suddenly: "put here instead of T_{max} put big U times R at the moment of the spike", you would have said: "Ok, where does it come from?". It comes from here. If you do this, despite this approximation, it continues to have a link with what is the peak of the neurotransmitter described instead by this three-state kinetic scheme.

The Resource Equation (R) This removed from the way (so again here I have the way to link the two worlds), I return because I was left with two differential equations, I would like to have one. So here is what remains, obviously it is an approximation, remains from the differential equation that was dR/dt. Now I use the famous, usual expression of conservation of mass. In all kinetic schemes, one differential equation is a linear combination of the others, because in the end either you are in R, or you are in E, or you are in I: they must sum to 100%. Sorry if not I don't see…

So I write 1 as… One of those quantities I write as 1 minus the rest. So I I write as $1 - R - E$. And I know how to write it, R I leave indicated, simply becomes $1 - R - U \cdot \delta$ ... because E is approximated. Obviously this continues to be an approximation because this is an approximation, but actually this is an exact expression, I equals 1 minus the complement. So, in the last equation that remained, I had R, which decreased proportionally with U (U times R,

here now I wrote big U times Dirac delta R), and I had an incoming term that was due to I. But now I I write as $1 - R$ (neglecting E because it is very small in time), and so I manage to factorize and write a single differential equation where on the right hand side I have only R.

So I have no other unknowns, R is enough for me, which is the quantity of neurotransmitter in the recovered state, which has a certain slow dynamics and which behaves… if you imagine that there are no spikes, this stuff here you remove it, I am distant from where there is a spike, so that term is null. So if this null term multiplies by 0 also this R… it is slightly more annoying than the bank account equation etc. that I showed you before, because here the input is multiplicative and here I have no escape, the input I have to keep multiplicative. What remains is:

$$\frac{dR}{dt} = \frac{1 - R}{\tau_{rec}} - U \cdot R \cdot \delta(t - t_{spike})$$

Looking at it I can say: "But yes, sure", apart from the minus which makes me happy because in the end nothing explodes, it is a differential equation that normally has arcs of exponential (I don't know if they go down or go up, but they are always arcs that then saturate), and at *steady state*, if there are no inputs, this goes to 1. How do I know? If there is the *steady state*, R is constant, so the derivative is 0, and this term here vanishes when the numerator vanishes, that is when $R = 1$. In other words, this piece here accounts for the fact that now R starts where it must start (R always between 0 and 1 because it is a fraction, and this is a constant that holds when I work with kinetic schemes). It starts from where it starts and then slowly, with the time constant τ_{rec}, returns to 100%, which is what I saw in the trace (I don't remember if it was red or not, it was blue before). R, even if before it was the complete model, without having any approximation, that's what it did. It started, it was immediately, dramatically decreased due to use and then tended to recover.

Now, this term here, beyond the fact that you could say: "Ok, I turned off my brain as you told me to do, this thing came out so I believe it is equivalent", but intuitively this nevertheless makes sense. I cannot trivially do, to realize it, do the integral of both members. Here I would know how to do it (fundamental theorem of calculus, the primitive, ok); here I would do exactly the same thing (continuity, the integral on a set of measure zero and therefore it is zero). Here I am screwed, because here is the integral but there is R and it is not done like this. It is done by separating variables, but we won't do it.

What you see is that here is a quantity all positive. The Dirac delta is stuff that goes to infinity. U is a release probability, presumably it is a positive quantity. R is a quantity between 0 and 1. Here there is a minus: when there is the Dirac delta, here there is a very strong subtraction of quantity and therefore R, which before was traveling at 100% because it was the *steady state*, starts instantaneously to lower. But there is R, that is, it does it proportionally with how much R there is. That is if you imagine that there is not only one spike, but many presynaptic spikes, it is not that here you remove, remove, remove like Amazon expenses from my bank account. Here the expense depends on how much money I have: if this R which is between 0 and 1 tends to become small small small, the quantity I subtract becomes small small. In the end if I have few neurotransmission resources in my synaptic bouton, here he says:

"Look, you have to give…". Another way to read probability: the probability of 0.5 means 50% you have to give. No, it is 50% of those remaining, because it is multiplied by R. And it makes sense. If I don't have any, I give at most a fraction. It's not that I give an absolute number. The fact that there is "times R" means that I am giving a fraction of it.

Dynamic Steady State and Frequency Dependence

Ok, this was the intermediate step that should have appeared earlier. Having done this reduction, a single differential equation (I won't bore you with doing pen and paper to see what it's like), I can take it and in an analogous way, similar to that of the bank account with this tail, I can that is say: if the presynaptic activation is a train of pulses at constant frequency, of which the period is $1/f$ (the frequency f), I can calculate what is at the **dynamic steady state**.

Before I had called it O_{ss} (*steady state*) and it was the fraction of post-synaptic receptors. Here, somehow, I am thinking about what is the amplitude of these little pulses of neurotransmitter due to this mechanism of synaptic depression. And what I see comes out (I won't do it for you), an expression comes out that is not proportional to the frequency, although I can eventually do the Taylor series expansion here; it is a bit more complicated than before and accounts for the fact that if the frequency… (so first of all somehow, however it depends on the period; now sorry here I indicated the period T and it is disturbing, I have to change this slide and put $1/f$ because otherwise you think T is the neurotransmitter concentration).

This R_∞ is the regime value, and it is the one, note, that I was putting here. So I put $U \cdot R$ here at T_{max}, so R counts. And if you do exactly the same transition story (it is slightly more complicated due to that fact of this multiplicative term, where the Dirac delta cannot be done trivially with that integral), you find that however it is a dependence on frequency. So, and this is not surprising, the amount of neurotransmitter itself depends on the activation frequency: it is not a surprise, the slower you go, the more this amount of neurotransmitter will be ready, will be at 100%; the higher the frequency, there will be less and less neurotransmitter released into the synaptic space.

But if you look at the specific dependence on frequency, and you actually make this graph where on the x-axis you have the frequency (that is 1 divided by the period) and on the y-axis you have this quantity, you see that there is a region for low frequency where there is a dependence of the amount of neurotransmitter on the frequency. So it is a synapse that tends to become, to grow with frequency. However, there is a certain frequency in which a certain regime (here they call it **supralinear**, **linear**, and **sublinear**), in which the slope of this curve tends to decrease and at a certain point saturates. There is a frequency, for example, at which the synapse becomes insensitive to frequency, at least from the point of view of the amount of neurotransmitter.

The Synapse as a High-Pass Filter (Differentiator) This for example is seen here: this is an experiment in which the frequency of the presynaptic neuron was changed from 0 to 15 spikes per second, 30 spikes per second, 80

spikes per second. And you see, besides the behavior indicated here (and this accounts only for the *steady state*, for the fact that here it seems a behavior that has an integrative transient, here it seems to be a behavior, ok, proportional to the frequency, fine, there is as much more neurotransmitter the higher the frequency is, and then this neurotransmitter will further activate the synaptic receptors which also have a dependence on frequency).

But see what very interesting thing happens at a frequency, at a frequency jump, from a frequency of 30 spikes per second to 80 spikes per second. You have a… so practically the amplitude changes, but it changed little. But the transient signaled the variation in the neurotransmitter concentration. What I was obsessing you with last time saying: look that frequency-dependent adaptation is a filtering and it is a **high-pass** filtering, because nature is, I was saying, total, always continuous change, so the nervous system has accustomed itself, has evolved to discard things that do not change over time.

Here you have in fact the same system at the level of the single synapse, which is a crazy thing. Because not only is it crazy in itself (because at the level of the single synapse), but because every single synapse will potentially have a different history, a history dictated by the presynaptic neuron that controls it. And anyway this *high pass* behavior, of high-pass, of differentiator, changes as the frequency varies. If the frequency is low, here it is more similar to a slow integration.

So to make a long story short: synapses (whatever the *Large Language Models* and *Deep Learning* architectures say), in vivo, biologically, are not weights W that are constant over time. They themselves have filtering properties. For those of you who are particularly interested (I am not sure if in the various *machine learning* courses you are told this), try looking for **KANs** (**Kolmogorov-Arnold Networks**). It is a different architecture proposed by people at MIT very recently, a few years ago, two or three years ago. And somehow the same concept of having filters and of… So it is not synapses as weights that change gains, but they are temporal computations that underlie the possibility of learning, the possibility of approximating functions. I am not saying that Kolmogorov-Arnold Networks implement exactly synaptic depression and facilitation, but they are similar.

Ok, here simplifying things it becomes a further law: for very high frequency (but I was interested at all frequencies) it becomes $1/f$, which is this $1/f$ trend, power trend, but which I won't talk to you about.

Network Phenomena: Up-States and Down-States

Before taking the break I wanted to tell you briefly that this frequency dependence of the amount of neurotransmitter (which we have now seen thanks to this reduction of the model in a very in-depth way): practically we have extracted what is potentially a **computational primitive**.

If you imagine it simply as something that when there is a lot of activity turns off the voice (and therefore the synapse no longer transmits), you can immediately imagine or understand why if I plant (or rather if a PhD student who is now a researcher at the University of California in San Diego, Joao Couto), if I

put **silicon probes**, electrodes, in the medial prefrontal cortex part of an anesthetized rat (and since each has more than one recording site), what I record is an alternation between epochs where there is activity (**Up state**) and epochs where activity disappears (**Down state**). Up, down, up, down.

And the same thing if I take a network of neurons *in vitro*, so not an *in vivo*, complex system, but I take dissociated neurons, the famous cell cultures, I put them in a Petri dish (maybe a Petri dish plus… maybe I already told you, with electrodes like a "bed of nails", where many electrodes detect the activity of many units). I can somehow intuitively appreciate why the activity consists of epochs in which all neurons fire, then the synapses get tired and these neurons detach. When the synapses start to *recover*, the neurons can connect again and the activity is no longer disordered but is synchronous (everywhere that kind of fireworks represents the top view of these electrodes). You see it well now that a so-called **super burst** arrives.

This is a network phenomenon, it is not the individual neurons that have on their own the property of being *bursting cells*, *intrinsic bursting*. It is at the network level. This thing of the violence of the slaps, of the smacks that I would start, here they start it by themselves: at a certain point here there is a lot of activity, but the neurons detach and are silent for a while, because the synapses got tired, they ran out. And you see spontaneous activity, you see that there are these waves of synchronized activity, which arrives spontaneously because: 1. The system is at body temperature, 37 degrees, so there is channel noise. 2. Synapses are incontinent, so there is synaptic release anyway and so post-synaptic neurons are a bit activated, a bit stimulated, even if there is no presynaptic activity. 3. And last thing, connectivity (in the cortex I already told you about it, it is around 10%, etc.): in these cultures the probability of having two random neurons connected here is higher, and it is 30%, and it is a recurrent connectivity. I connect you, you connect… but at a certain point somehow it returns. It is a **positive feedback**.

This positive feedback thing, with simply the element of which we have potentially also examined the dynamic component (low pass, high pass filtering, which I put there for you, I don't expect you to digest it so easily), but simply the story that I get tired and I have no more neurotransmitter in the synaptic bouton to spit out, explains to me for free, instantaneously these phenomena that are very important. Because be it a network of neurons *in vitro*, but be it more importantly the cortex just now *in vivo*, it is a mode of operation of all parts of the nervous system during development and every night when you sleep (not when you dream, but when you do long-wave sleep, **slow wave sleep**). Your cortex synchronizes, synapses get tired and it turns off, it resynchronizes, etc. etc., and you sleep.

Let's take a 10-minute break. Thank you.

Mechanisms of Long-Term Plasticity (LTP)

(Interval / Answer to questions)

I believe generally no, ok. Ok, so, in today's lecture and the last one, we basically brought up, even from a quantitative point of view, several concepts. The last

of these was the **release probability**, which is the most ethereal one. What does release probability mean? Yes, ok, I can define it mathematically, but to what do I attribute it? This is more complicated.

Surely I talked about vesicles, I talked about the number of, or anyway I talked about the quantity of neurotransmitter that is released (so somehow how many molecules of neurotransmitter are inside each vesicle) and I talked about post-synaptic receptors.

In the case of **long-term plasticity**, it is conceivable that they can change in a persistent way (not dynamic, not activity-dependent like the story of short-term, homosynaptic plasticity, where somehow I told you that there is some kind of relationship between the frequency, the demand for use, and the quantity of neurotransmitter that is ready to be released). There can be instead a persistent, dramatic, structural change, which could have to do with:

1. **Number of Receptors (N_{post}):** For example, going from 1 to 2, the number of post-synaptic receptors. This would lead to a change in synaptic efficacy, a change in the maximum synaptic current, because $\bar{G}$ (G-bar) would change, the total number of post-synaptic receptors which is encapsulated inside that $\bar{G}$ would change.
2. **Number of Vesicles (N_{pre}):** Another thing that could change is, passing for example from here to here, is the number of vesicles containing neurotransmitter. So it could be that there is a structural change where in fact T_{max} will change. True, T_{max} is a dynamic quantity that depends (in terms of short-term plasticity) on the past history, but ok, you would have more. The quantity would change, a scale factor. Therefore the quantity would change or, if you want, we could say the release probability could change; you have more vesicles so it could change.
3. **Single Channel Conductance (γ):** Another thing that could change (which here I don't remember anymore in this review… I believe it is, I don't remember where I took this graph from, I don't remember who is who, what the passage was, it was very elegant), you can also have a change not of the number of receptors, not of the number of vesicles, but of the single channel conductance, due to what is for example called **phosphorylation**. So some persistent structural change of the receptors that causes the pore to be somehow wider.

In all these cases this change has a counterpart of a persistent change that can somehow be considered a **substrate of memory**, a substrate of something that accounts for the fact that an animal (us) can memorize things and keep them for even 80 years. 90 years, less, depending on when one kicks the bucket.

Hebb's Law: Causality and Correlation

A very important thing in the context, let's say, and in the end if you want a kind of guiding principle that came out, is that of the proposal, of the theory of **Donald Hebb**, at the end of the 40s (or 50s). He was a psychologist, but he had a clear understanding of the physiology of the nervous system, which back then at the time was not complete (it is not even complete today, but it was not as complete as now).

And Donald Hebb is remembered for a principle that in the end is distilled in this way (partly horrible, because it does not account for what could have been both his intuition and what was then the instance of things). He was dealing exactly with, he had in mind speaking of concepts, of memory, of cognition; he had an idea that there must be a neuronal correlate, and somehow he concluded: **"Cells that fire together, wire together"**. This doesn't work in Italian, that is "se sparano assieme, si… spagan… spaghettano", it doesn't come out in Italian. That is: **if you fire together, you connect together**.

And that speech I made before: it could be that there are mechanisms whereby if two neurons (which on their own are not even connected, or are connected but not necessarily the synaptic efficacy of one neuron is such as to recruit or influence, exciting and inhibiting it, the other neuron)… If by chance there is some temporal dynamic where there is a correlation, a co-activation (maybe in a certain temporal relationship, because if I activate now and another activates half an hour later, perhaps the causal link in our universe might not be useful to remember), and this could be a way to create memories by noting co-activations, correlations that could become, as said, **causation**.

He did not necessarily speak of concepts, despite being a cognitive psychologist, but he was literally linked [to biology]: he spoke of the axon of cell A. When this axon is close enough to the threshold to excite neuron B and repeatedly, persistently is involved in the process that leads cell B to fire, some growth process must happen. What I showed you before: for example an insertion of new post-synaptic receptors (in the end they are proteins, they are linked to expression: genes are expressed, proteins are trafficked, *trafficked*, they are brought to the right place). Or it has something to do with a persistent change, another type of mechanism of growth process of the number of vesicles. Or even a metabolic effect, a change, such as can be the phosphorylation of a receptor, of a membrane channel that changes its conductance, increases it, decreases it.

Discrete States and AI Analogy The interesting thing is: but if it changes with this story of phosphorylation? Does it change gradually? I am thinking again of **machine learning** where you have synaptic weights that in theory (maybe those of you who are more competent will know that it is not so), in theory are real numbers, they can change continuously. Now I potentiate you a little bit, I potentiate you even more. The story of phosphorylation could be something binary: either the synapse is not potentiated or it is potentiated. Or regarding post-synaptic receptors, in the end I can integrate an integer number of them, so from the point of view of efficacy a continuity might not hold.

Perhaps you know that with **Large Language Models** there is a memory problem. If you tried to play by downloading some weights, you download hundreds of gigabytes onto your computer and the fact of changing their numerical resolution is being explored. They are numbers normally represented with floating point at 64 bits (or 32). What happens if instead of having that type of numerical resolution that allows me to represent the value 51.68462… ok, integers? 51 or 50, you don't have the intermediate value, deal with it. It seems that it is possible. And it could be exactly what happens in the nervous system, where synapses in these persistent changes it's not that "I potentiate you synapse, I potentiate you in an analog way"; it may be that there are **discrete** states.

Memory and Erasure (Kung Fu Upload?)

Anyway, whatever it is, this guy got very close. To this day we don't know exactly… we don't know for example how to alter memories. Some time ago (and I believe I have to give a talk for the general public in a few… maybe next week) I was asked: "Could we then learn new concepts like **Matrix** when they do the Kung Fu upload?". It is a fundamental problem that the representation of memories or representation of information is distributed: I don't have *here* the day of my graduation, *here* the marriage and *here* I don't have it. It is all distributed. I said this because we don't know how to create new synthetic memories or erase other memories.

Having said that there are (and if you want I can give you references) several scientific articles where, in the particular case of the limbic system (hippocampus and particularly amygdala), a rat, a mouse that has been exposed to what is called **fear conditioning** (Pavlovian type conditioning of fear)… obviously this has a very notable impact for PTSD syndrome, post traumatic stress, *whatever*, so anxiety disorders, panic and traumas. The possibility of erasing, of mitigating the activation, the co-activation of a particular environment (I get panic in a particular situation, I am in that situation and I suppress the amygdala) and in that way I tend to disfavor, or I tend to do *reversing* of what was instead a consolidation of a plasticity. We are very far from doing the Kung Fu upload, perhaps less so from curing anxiety disorders in that way.

Lømo's Experiment (1973): Tetanus in the Hippocampus

In the 70s there was a key experiment (I don't know exactly if it was by chance or not). Norwegian researchers (**Bliss and Lømo**) took slices of cortex… if I remember correctly, they did it also on the intact animal, a rabbit, but typically they took a slice of **hippocampus**. And in a particular combination of the hippocampus you have basically a circuit that is **feed-forward**: you have fibers projecting to a population of post-synaptic neurons and there is no particular recurrence, reverberation, etc.

So if you place an ugly electrode (not a pipette: two twisted wires, two microscopic twisted wires, but they are still two wires) and you apply (here there is a kind of symbol of a coil, because in fact it is what is called inductive coupling, it is a galvanic stimulator where there is no continuous connection, because it would bring in noise; there is no continuous connection between the signal source and the electrode, there is a transformer in between, this decouples the DC for those who know what I'm talking about) and you give, you start giving a stimulus. In fact you are activating, and these guys did it at high frequency.

Normally if you activate it only once (again this is not a pipette in the stomach with those very fine experiments of pairs, of *pairs*, of couples that I showed you; here it is: I place an electrode that records from a **population** of neurons; today we do it in the belly of the neuron, it is also done outside, it would be like a microphone placed here and if you activate yourselves the microphone hears the integrated, summed version of all your voices). So if I with this "flick", a small electroshock, start doing it once a second, I measure in your population reaction the effect of this electrical stimulus that generates activation of synapses and this activation of synapses in some cases also generates spikes, but predominantly I

manage to read from the extracellular point of view the so-called **Local Field Potentials**, which are an integrated synaptic activity.

And so I see that for 15 minutes, every second, every two seconds, I give a flick and I see an amplitude. Here it is normalized, it is millivolts or whatever it is. I normalize, I see that it is always the same. Then at a certain point, at time zero, I start giving a stimulus always exactly with the same channel. This for me was a bit complicated immediately to realize, but what does it mean? So it is something homosynaptic, because if before I activated the synapses with the same channel, now I start activating at high frequency: I am discharging maybe a facilitation or a depression, but it is certainly short-term stuff. Due to the particular anatomy of the hippocampus however, by stimulating these fibers, one activates both some neurons and other post-synaptic neurons and creates temporal correlations.

What is seen is that instantaneously after giving this **High Frequency Stimulation** (this high frequency stimulus, is also called **tetanus**; I believe it is called tetanus because tetanus has to do with the tetanus disease… now I don't remember the nervous disorder anymore, maybe you can help me, it should be some tonic activation. I don't remember where tetanus comes from, look it up on Wikipedia so you can know). So after that I resume giving a stimulus to read the system (here in the end it is always: I write and I read, so stimulus and I see, stimulus and I see). But the tetanus here was a high frequency blow and then afterwards I resume at one every second, one every two seconds, every ten seconds.

Post-Tetanic Potentiation (PTP) vs Long Term Potentiation (LTP)
What I see is that instantaneously there is a kind of… what you would call facilitation. It says: "Ok thanks, here the synapses have facilitated and you with this reading pulse are continuing to see the effect of this accumulated calcium". And you wouldn't be too far off, it is called in jargon **post-tetanic potentiation** (*Post-Tetanic Potentiation*). So this disappears very quickly, over the course of minutes or tens or hundreds of seconds.

The interesting thing is that this potentiation, this facilitation, **persists**. And it persists for hours. Now here it is up to 60 minutes, so about an hour later (in fact you could object: "I want to see that after three hours it is still here"). And significantly, the amplitude of the synaptic responses is significantly greater than the *baseline*, than what it was before starting. So in a causative way I wrote into a piece of brain and this information persists.

This thing here was also done in vivo and it was the first, in the 70s, the first demonstration that there is an activity-dependent plasticity (because here I am the one who induced activity) and a mnemonic change is maintained… probably not structured… pardon, not mnemonic, a persistent physiological change of a physiological observable is maintained, which could be correlated to a type of memory.

If I tell you a phone number, you are not reverberating it in your head, but simply after a while you know it: probably an **LTP** (*Long Term Potentiation*) has been created in your hippocampus. There are mechanisms, now we know, of consolidation that also involve the cortex (and parenthetically *slow wave*

sleep, sleep, and the activity of information exchange between hippocampus and cortex during sleep phases, during some sleep episodes, is precisely to consolidate and transfer to even longer-term memory the episodic memory of which the hippocampus seems to be the seat).

Spike-Timing Dependent Plasticity (STDP)

So this plasticity was found everywhere. And in more recent years (and again it involved this researcher **Henry Markram** and also a Chinese researcher who had been in the United States for years at Berkeley, **Mu-ming Poo**), they are more or less at the same time, they published articles, one in *Science* and one in *Nature*, again resuming the story of synaptic plasticity, but in an extremely finer way. They used pairs of electrodes. So in the best way, so obviously only few musicians, experts, talented people also from the motor point of view, also of patience, of aptitude for experiments (which is not trivial, you can compare it with the aptitude for cooking: maybe some of you will have the recipe, but maybe they are poor, or vice versa they excel, they are intuitive, maybe they add salt… and these maybe placed the pipettes in a way: "In my opinion I won't take this cell, I'll take the one next to it because it seems more vital". How to know? And ok, they are Henry Markram).

And they found that this writing characteristic depends on **temporal information**, temporal information on a time window of milliseconds (now I will argue better and tell you what it is). And it had been partly anticipated by a theorist, by a physicist, who said: "In my opinion synaptic plasticity, also revealed in this hippocampal LTP with tetanus, must have some kind of time-based correlate, because otherwise I don't explain why I can learn motor sequences, sensory sequences, why I manage to learn a language, why I manage to learn to listen to music and interpret it, when the information is temporal".

And this is called **STDP**, **Spike-Timing Dependent Plasticity**, and it is both a potentiation and a depression, and now I will show you why. It has been found in many, so in the neocortex, in the hippocampus, in the olfactory bulb and in other parts, and surprisingly it depends on the **causal relationship** between presynaptic and postsynaptic activity.

Again, I am a pyramidal neuron in layer 5, I am excitatory, I release glutamate, I have my synapse projected there (it is a heterosynaptic plasticity, so what the receiver is doing also counts, regardless of whether he has his own axon that maybe returns to me, it doesn't matter, it is only the synapse, my synaptic bouton is here) and it depends, in a way that I hint at the possible biophysical correlates, it depends on the precise timing of when my spike was emitted (note: the spike was emitted but it takes some fraction of a millisecond to propagate to the synaptic bouton, but close parenthesis). The exact *timing* of my spike and the exact *timing* of the other spike, which again can be caused by me (because I have a nice effective synapse that is every time I fire he also fires) or he fires on his own.

Causality (Pre-before-Post) and Anti-Causality (Post-before-Pre)

1. **Potentiation (LTP):** If these two spikes are close, and they are close not… ok, I said it before, it's like NMDA receptors are coincidences, no,

they must be close in a particular temporal causality relationship. **Pre first and then post**: then the synapse potentiates. I don't know if it potentiates on the presynaptic or postsynaptic side, but it potentiates.

2. **Depression (LTD):** If instead the **post-synaptic spike arrives first and then the presynaptic one arrives**, there is a kind of anti-causality relationship. Because anatomically if one looked at the circuit, if Ramón y Cajal saw the circuit, he would say: "Information travels in one direction, so when the presynaptic activates, maybe the postsynaptic activates later". If instead it activates before, the synapse depresses.

As if there were a recipe to say: causality (causality which means cause-effect, the cause must precede the effect) is intrinsically hinged in biology. Which is cool.

And this is a graph taken from Mu-ming Poo's article in '98 (I met him, I was about to go do a post-doc with him at Berkeley, but… life's mistakes, several life's mistakes). What they did is: they had two pipettes, one in the soma of the presynaptic neuron and one in the soma of the postsynaptic neuron, and they gave flicks to make the neuron fire. So in the end they had two ways of using the system. Giving a flick in the presynaptic neuron, they could see what the echo was in the postsynaptic neuron, they measured the EPSP (excitatory post-synaptic potential). But they also had the electrode there, therefore they used it to read. So in a plasticity induction phase, it is said, they could inject a very intense current in both the pre and the post and they could do it at will. They could go "tac-tac" or vice versa go "tac-tac" with a temporal resolution of several tens or hundreds of milliseconds.

That is, they really buckled down on many experiments (not just one experiment: each dot, if I remember correctly, is a pair, is a separate experiment) and for that synapse, between two connected neurons, so quite complicated, they did that same particular temporal relationship. Pre before post, which is here, or post before pre (you see that post precedes pre) and they did it with "wait, do 200 milliseconds before, do 150 milliseconds before".

The cool thing is that you have an inversion around zero with a… let's say, these curves you see, these solid lines are in fact an exponential fit (it doesn't matter that it is exponential because in this case here it is not a time variable, it is an interval variable). If the interval is somehow negative, you have an **LTD**, a depression. Here is the change that was expressed after having done the induction many times pre-post, pre-post or post-pre, post-pre, it determined in normalized terms a subsequent expression of the EPSP that was smaller, at 80%, 70%, even at 40%. While simply changing the sign of this interval (so instead of post-pre, pre-post), it was instead a potentiation. Again, the interesting thing is that across zero, within very few milliseconds, you have a huge LTP or a huge LTD.

Safety Mechanism (LTD > LTP) The thing you might notice is that there seems to be more LTD than LTP. Again this is a thing perhaps linked to evolution, it is a kind of safety mechanism. I don't want that for some reason neurons fire trains of spikes pre-post and that it prevails in a completely random way. If you imagine making neurons fire in a completely random way, you can imagine

that it is… it is equiprobable that it is pre-post or post-pre. So in that case there it could be: "Look, but if it goes wrong for you, potentiation wins; and if there is more potentiation there are probably more spikes, and if there are more spikes there is more potentiation and it ends up in a seizure".

So the fact that here the area subtended by these points in the depression side curve is wider is perhaps a safety mechanism to say: "Fire even randomly; I tell you that on average I make you turn off synapses a little bit, I don't make you potentiate them at will, so that there is a kind of perhaps homeostatic control that is rather safe".

STDP Variability: Neuromodulation and Development

So the surprise is that there is a causal relationship and that it is timing, and that LTP and LTD are two sides of the same coin: so very little changes in the induction of plasticity.

There are over the years (this is a *review* article from a few years ago where it is called "canonical"), in other parts of the nervous system it is found that these curves, this dependence on timing (again this horizontal axis is always the pre-post interval: so if pre-post is positive it means that the pre… sorry, if it is post-pre, because post-pre if the time of the post is greater than the time of the pre, so it is subsequent, then I have potentiation; if instead it is negative I have depression)… However, if studies used **neuromodulation**, they simply poured dopamine or dopamine receptor antagonists, the type of profile completely fell apart: it became wider and it doesn't seem to have the LTD component anymore. Now I don't have the slide, but I don't remember if it is in the cerebellum, there is an STDP in reverse: that is, in this part where LTP should be there is LTD and in the left part there is LTP. So there is a zoo here too.

And an interesting thing is that during the developmental phases, it is not that this curve appears when the first neurons form during embryonic development. At the beginning (also these P13, P21 stands for days after birth, *post-natal days*; we are talking about a rat)… typically rats open their eyes and thus reach maturity of the visual system around P14, two weeks after birth. P13 and P21 are what are called very young rats, 21-30 are *juvenile*, 35-42 are also to do with the onset of puberty and therefore the hormonal effect. Again neurotransmitters, neuromodulation: the profile changes from being predominantly LTD to becoming LTP, even in a way independent of timing. So it seems that there is something, there is something very interesting.

The Arrow of Time and the Eligibility Trace And so synaptic plasticity is not simply the consequence of a co-activation. It depends how, it depends on the direction of causality. Years ago I read a book titled *"The Arrow of Time"*, written by physicists; it had to do with the irreversibility of the laws of physics. I'm not saying there is food for thought, but the fact that there is a direction of time here would seem to be captured by a biological mechanism.

So how does it work? It works that if you have two neurons (this is presynaptic and this is post-synaptic), if there is a *firing* activity of this type, somehow the synapse between the two must be able to read. It reads some information

perhaps linked to residual calcium, perhaps linked in part to the coincidence between action potentials (which, now in this case there is no morphological structure, but from the Soma action potentials *back-propagate*, retro-propagate into the dendrites where the synapses are). But, however it does it, the synapse somehow reads that "pre before post" must be a potentiation.

So it is as if every presynaptic spike had a kind of temporal trace which is called **eligibility trace** (*traccia di eleggibilità*) which at a certain point loses memory. This is truly a hypothesis: it is a decreasing exponential, whose time constant here is the same time constant of this curve (of this curve here). So this arc of exponential changes because it is a dependence on the interval. Same thing if I take it and assume that there must be some biophysical mechanism: it is as if the synapse, once the post-synaptic spike arrived (so this neuron here), the synapse manages to read and says: "Ok, but was it still in time for this eligibility window?". If yes, then potentiate yourself (in fact the arrow should become chubby).

If instead the causality relationship is inverted, the eligibility trace must perhaps be associated instead with the post-synaptic neuron. And when the pre-synaptic spike arrives at the synapse, it looks at the eligibility trace that the post-synaptic neuron has and sees that yes, they are still legible, but it is another dynamic, another technique. So in this case depression occurs and the arrow becomes thinner, more slender, because the efficacy of the synapse (for example $\bar{G}$, or the release probability, or the phosphorylation of receptors, I don't care, one of those phenomena) is activated or depressed or potentiated.

Beyond Pairs: The Triplet Rule

So the interactions are for pairs of spikes. If I ask you when (and this is the most appropriate situation) when neurons have a *spike train*, a train of spikes of this type, I just by eye cannot understand who is pre and who happens first. Well, I know who is presynaptic and who is post-synaptic, this yes. The blue one is always presynaptic (anatomically it has a synaptic bouton projected and touches the post-synaptic neuron, so there is no escaping this). What I don't know is if, for example, this spike here, the third one, happens before or after… do I have to consider it as having happened before or after these spikes of the presynaptic neuron.

So, in this case, people doing experiments (doing the experiment) saw that, for example, in this circumstance, the synapse potentiates. And obviously the question is: how come? Before it seemed to be an interaction of pairs of action potentials and now one of the proposals (I simply tell it to you like this, briefly, without going into detail) hypothesized that actually the synapse is not based only on two spikes and on two eligibility traces, but counts **triplets**.

So if you think that every post-synaptic spike somehow interacts with a pair of presynaptic spikes (and you represent this sentence mathematically and put it inside a simulation), you would manage to express, to explain the experiment.

Dependence on Firing Rate The snag is actually resolved because things do not stand so simply on an aspect of only temporal causality. There is also an influence of the **firing rate**. Now I would like to get there. So, when you

are in this type of regime, instead of in a type of regime where the frequency is much lower… and in a regime where the frequency is much lower, probably the temporal information makes sense, because there is sufficient silence to say: "This arrived before and this arrived after, and this is in particular referred to this, because it is too distant from this other spike". If instead the frequency is higher, it is not.

This mechanism was (so either timing or *firing rate*) was again expressed not in words, but in a mathematical description by two researchers, **Pfister and Gerstner**, years ago. And they showed (this is a graph that I plotted some time ago) that the time dependence… the two curves are obtained by keeping the pre-post interval fixed (I keep this always fixed, suppose a millisecond, or post precedes pre). So here there is temporal information. And at low frequency (at low frequency means that I do this pre-post event, I do it rarely)… but I can do it also at higher speed, maintaining the same phase between the two stimuli.

If I do it and go slow, around a few pairs of spikes per second, the black and the dashed remain different. One is potentiation and the other is depression. The interesting thing is that at a certain point, at frequencies not particularly so high (around 30-40 spikes per second), what was supposed to be an LTP starts to experience a dependence on frequency; so at the *pairing* frequency, LTP also starts to experience a dependence on the *pairing* frequency. But in the end, exceeded a certain critical frequency, **everything makes LTP**.

So, in some cases, if synapses are of this type, when neurons fire (for example in the visual cortex), if the firing activity is very high, you have that both from the presynaptic point of view and from the post-synaptic point of view, I always have a potentiation. So if by chance anatomically there are both synapses in both directions, both potentiate and you have a bidirectional *motif*. Vice versa, in other areas of the cortex where the code is said to be sparser (in other words neurons fire at much lower frequencies), you continue to work in a regime where timing counts.

This thing here, which is called **triplet rule**, has partly reconciled (and there is still a piece I won't tell you about, which further reconciles with a lot of experiments) where people started to say: "But wait, timing is not enough for me, it must be frequency too… but how?". Simply considering triplets captures both timing and frequency in a way that *fits* the experiments. So it is a very powerful description. Again, here is a case (of which however we do not go into mathematical detail) where having systematized, formalized, modeled the phenomenon, allowed to say: "Look, but it doesn't work… however if I add this it starts to work and I see and explain the experiments". Then with the same model I can say: "Wait, I have never tried it with that other stimulation protocol, for example Norwegian, with high frequency tetanus… let me try". It turned out that it *fits* and works even in a context that was never the one on which the parameters of this model were identified.

Interaction between LTP and Short-Term Plasticity (STD)

So, now, the interactions between long-term plasticity and short-term plasticity are particularly interesting because so far, let's say in the last hour, focusing on long-term plasticity, basically I told you: "Dunno, this changed, change this… $\bar{G}$

(G-bar) changed". So, whatever the train of presynaptic action potentials and the temporal dynamics, anyway changing this (halving it, doubling it), I change the amplitude of all the peaks of a spike train, of a PSP train. I don't change the temporal structure, I don't change how if it gets tired, if it facilitates: I am scaling. This is literally a scale factor.

So if this tells me: "Ok look you inserted another 100 post-synaptic receptors in that synapse that had to be potentiated", so I changed, or vice versa the conductance of the single receptor increased because there was phosphorylation, I change here and I change a scale factor.

It is different if I change the maximum concentration of neurotransmitters, actually if I change U (the release probability). Because you remember that U determined how much I subtracted during a spike from this container R of vesicles ready to be released, of neurotransmitter in a kind of state ready for action. And if U changes for me, and I have a train of 10 spikes… while before it was small (yes, between the first… depending on the frequency obviously, the depression would have been a little bit gentler, almost interceptible), if instead U becomes ten times as much for me, at the first use I almost have no more neurotransmitter available. So it changes the temporal profile completely for me, altering what is, again, in *machine learning*, not a change of scale, it is a change even of *processing* over time.

Dean Buonomano's Experiments: Hippocampus vs Neocortex These are experiments done by an American researcher, who is a great one, he also wrote a very interesting popular science book on the representation of time in the brain (I don't remember what it's called), and he is not Italian, he is of Portuguese origins: **Dean Buonomano**. I met him several times and a couple of times I said: "But you have…". No, he is not Italian.

He did that type of *paired recordings* experiments, and he did it both on hippocampus slices, and on cortex slices (so in the end it is always on rat, but on different parts of the cortex), and he applied a particular protocol, the same plasticity induction protocol. And what he saw is that, comparing what was obtained before induction and after induction (before is this dashed trace and after the trace [solid]), it differed notably if it was in the hippocampus or if it was in the neocortex.

1. **In the Hippocampus:** If you manage to see (unfortunately it should have been in color), you see that basically from the dashed part to the darker part there is a scale factor. I doubled everything. So the amplitude of this event here, which was smaller than the second, anyway is doubled; and the amplitude of the second, which was a bit more [large], is doubled. So in the end all these IPSPs (which are a consequence of the response of a train of 10 potentials… 3, 6, 9, whatever they are) changed uniformly over time. For example, if I were able to potentiate sufficiently, enough to, at a certain point, make the neuron fire, I would have that before this train, this echo of IPSP is not able to make the neuron fire (you see it doesn't go above threshold). Afterward however it may be that it goes above threshold. So here there is a small **temporal summation**: to make a long story short I increase, induce potentiation, not only does the

synapse potentiate, it potentiates in a temporally indiscriminate way, but it makes me fire in correspondence with the presynaptic spikes towards the end of the spike train.

2. **In the Cortex:** If I do the same thing in cortex, where there is this much more pronounced short-term depression, apparently I don't change $\bar{G}$, in cortex I change U. And changing U you see that instead of scaling everyone in the same way, I scaled the first one: the first one became a nice quantity, but the second in proportion (given that there were no more resources or there were much fewer available) did not increase by the same quantity, in fact it is indistinguishable from what it was before, and so on. So what before was a train where there were no depression effects, now there are: given that the first one was a nice blow, a notable subtraction of quantity of neurotransmitter, the amplitude of the subsequent IPSCs is actually smaller. And if by chance I were in a regime such that these amplitudes were sufficient to make the post-synaptic neuron fire, I would see that the first, maybe the second presynaptic spike, would give rise to an excitation to make the post-synaptic neuron fire.

This and this are very different. In terms of signal coding, of some information coded in time, here perhaps I am saying: "I want to increase the gain of all temporal information". Here I mean, perhaps: "I want to turn the system into a system that signals only the change, only in the transition". The transition is when I turn on and then I want the system to forget, to get tired, to empty itself.

Redistribution of Synaptic Efficacy

These are other examples (pardon, the one before was a cartoon), these are examples where this (and we are finished, and I leave you) where in the hippocampus there is this scaling that is revealed before and after normalizing. So what you see here, *scaled* and *subtracted*, are versions where I basically demonstrate that if I multiply or divide by a certain quantity two trains, the two traces become equal. This doesn't happen, doesn't work when I do the same scaling, the same normalization [in the cortex], why? Because the synaptic efficacy of the single ones changed.

This phenomenon here was (and I say goodbye) was for the first time described by **Markram**, who did not call it synaptic potentiation due to the interaction with depression: he called it **redistribution of efficacy**. Because in his mind he saw that before the efficacy was on the first one and now, changing, it is as if this efficacy distributed over this train of events redistributed itself, shifted. He had imagined a kind of conservation of amplitudes and simply a redistribution: now you don't have it here anymore but you have it in the middle (or at the beginning). And it has a lot of notable implications.

See you next week! Thank you.

Introduction

Before we begin, I'll make a very brief mention of a notebook that I made available to you regarding the previous lecture's section. Anyway, today we approach the problem of the generation and characterization of **extracellular signals**.

For the moment I showed you, and we discussed extensively, the biophysical bases of the generation of intracellular signals, or those recorded from the intracellular point of view. Today, effectively, we start to see what is needed to understand extracellular signals.

Notebook Analysis: *Short Term Synaptic Depression* Before doing this, I'll go to the usual GitHub and there is a notebook called **Short Term Synaptic Depression**. In fact, it traces and resumes the description of the kinetic Markov model of short-term depression, therefore of the temporal dynamics of the vesicles, of the resources for neurotransmission that we saw last time. Besides having the text and the little formulas, you theoretically have the Python code to generate some figures that I showed you in class. Some are like this, they are not interactive. If you are curious, you might want to look at how it is made, where the equations are.

This thing, which shouldn't particularly disturb you, is that for each of those states, for each of those variables that describe the fraction of vesicles in a state R (*recovered* and effective) or I (*inactive*), **Euler** was used, the numerical method of Euler, for which the time derivative is approximated with the difference quotient. It is not particularly complicated. The potentially interesting part was to show you from an interactive point of view, particularly for the combination of short-term and long-term plasticity.

What I do here is, again, simulate exactly the same thing, however, I can change the frequency. I can change the arrival frequency of this train of presynaptic action potentials with uniform frequency, so they are intervals between one event and another that are identical (again the university that pays me the salary every month). I can change the frequency and I can change two other parameters. You see that if I slow down, if I increase the distance between one spike and another, the so-called presynaptic **inter-spike interval**, the dynamics of the amplitude of the post-synaptic excitatory currents change a little bit.

But here I have two other parameters. The first is called τ_{d2}; I don't remember why I called it τ_{d2}, or rather I remember it and it is another context, but it is the time, the time constant of recovery from depression. It is the time constant that returns from state I to state R and now is 290 milliseconds. If I make it very, very rapid — think of the llama that spits, "sputters", and now has a capability to restore the vesicles containing the neurotransmitter very rapidly — you see that although I am asking the synapse to release, release, release at a frequency of 47 spikes per second, the amplitude is not so dramatically different from the first compared to the responses to the other events.

And if I make it even shorter, even more rapid, in fact, it is a synapse… here maybe it gets pissed off, it gives me an error because at a certain point there might be somewhere a division by zero, somewhere there is an $e^{-\ldots t/\tau_{rec}}$. So in a numerical simulation if I put τ_{rec} to zero someone has to get angry, has to get upset. Anyway, when there is no phenomenon, when the *recovery* is instantaneous, you see that the amplitude of subsequent EPSCs practically does not change. Instead, when there is a certain inertia, a certain dynamic, for example 190 milliseconds ($100 - 150$ are typical biological values, at least in cortical synapses), there is this attenuation of responses.

The third parameter you can change is U, which was the **release probability**, or the quantity of vesicles that are released at every instant, at every event. And if I lower it dramatically, you obviously see that the amplitude decreases: I am releasing much less neurotransmitter, true. What happens is that, however, since I released less, little, I depleted the reserves little. In fact, you see that here the amplitudes are not so different. To see an effect with this value of U I have to go precisely to a very, very rapid frequency and perhaps it is almost not seen, it starts to be seen here. If you see here I see it from the tail, that here the first and second are all interrupted, but it seems a little higher and then tends to decrease.

This can perhaps develop your intuition on the interaction between parameters, so what is the presynaptic frequency, the demand for use, the recovery time constant, the time scale on which recovery occurs, and the release probability. What for example is seen is that a particular interaction between long-term and short-term plasticity could change precisely U, it could potentiate precisely the parameter U. This would be the behavior before a long-term potentiation and, if I change U, I have again a kind… what is called a **redistribution of synaptic efficacy**. It is true that some events are potentiated, but others are much more depressed. To recover exactly the traces that I showed you in the presentation last time, in the slides last time, I would have to put inside also synaptic facilitation and all synapses probably show both depression and facilitation at the same moment, however, they have different parameters. Anyway, I hope someone can find this notebook interesting and useful.

Extracellular Signals: Overview and Techniques

So, for this part of extracellular signals, the reference text is again this one here, *"Electric Brain Signals"*. I hope there are other texts, as I believe I said during the weekend or whenever it was in recent days in response to a colleague of yours: you can find part of the information (which anyway is in a very, very reduced manner compared to this book) also in this chapter on other texts.

I start by telling you that, to motivate the story of extracellular signals, not everything is intracellular, in the sense that not all current technologies allow measuring the electrical activity of one neuron at a time and on time scales that are those of milliseconds, so they would allow us to see single action potentials. Although this is a goal, the Holy Grail of the research field, for which I would like to be able to listen to the voices of the single units that make up a network and listen to the voices with good temporal resolution, so have a very low temporal and spatial resolution. So in this graph, which is a description of the resolution of the spatial scale with which different techniques are resolute, the desire would be to be here.

Limits of Intracellular Recordings In fact the *intracellular recording*, intracellular recordings with an electrode that is called *sharp* (pointed) or *patch* electrode, which does not necessarily go inside the belly like a knife but stops at the membrane (you remember, then a negative pressure is applied, etc., etc.), are in this part of this graph. In fact, they allow measuring events that are very rapid, of the order of a fraction of a millisecond.

I will ask you at the exam, roughly, how much is the duration of an action potential? What is the duration of an action potential, roughly? How much is the width? The amplitude you know more or less all. From the resting potential to the peak how much is there? How much? 100… 100, excuse me? 100 volts? 1000 volts, ok. And more or less how long does it last? Seconds, days, months, microseconds, milliseconds, nanoseconds? Milliseconds. And roughly? Four, one… about one. It depends on the cell, but typically it is a millisecond.

So ideally I would like to be here and I would like to be here also to see one. How big roughly is the belly of a neuron, the soma, the cell body of a neuron? They are micrometers, but more or less how much can it be? The order of magnitude? Tens of micrometers, yes. Different cells can be different and certainly, and today we will start to look at it, the dendritic tree can change things dramatically. In humans, a pyramidal cell of layer 5 of the cortex has an apical dendrite almost a millimeter long. In a research project that ended a few years ago and in which I collaborated, in which I was involved, it saw at least in rodents cells that projected from the thalamus to the cortex for several millimeters, because they had an axon that was very long, however the soma roughly is the one indicated.

So I would like to be able to play the keyboard or hear the single notes and the moment in which they are articulated, so I would like to stay here, both in space and in time, however, it might not be possible. An intracellular or patch electrode in the soma of a neuron, aside from being a thing that requires a research laboratory, an anti-vibration table, a Faraday cage, a piezoelectric micromanipulator, ok, it is unlikely that this is ever extrapolatable to a clinical context. But above all, once a neuron has a pipette inside the belly, there occurs — I have said it other times — a dilution of the cytoplasm, a dialysis of the cytoplasm, because the pipette is an enormous volume: you have to imagine it like the volume of a football stadium in comparison to a ball, for example. The ball could be the intracellular volume, the football stadium is the entire volume [of the pipette]. So there is certainly a diffusion and a *washout*, a dramatic change of the cytoplasm and therefore after half an hour, an hour the cell is dead. Certainly, even if I retract the pipette the cell is not that it is particularly well, in fact, it dies, after a while it dies.

So it is necessary to move also because if you have to have some diagnostic question you cannot think of having a neurosurgery operation every time, of exposing a part of your body; it is not feasible. So typically unfortunately one stays in this part: I am thinking of EEG, MRI, they are time [spatial] scales of centimeters, if not tens of centimeters and therefore it is extremely macroscopic stuff.

Macroscopic Techniques and Temporal/Spatial Resolution From the point of view of temporal resolution, I could have a macroscopic resolution like someone could be usually outside this room, could hear us talking, could… now you are silent, if you made noise a generic clamor would be heard and one could, ok, not distinguish single voices, but could trace it in time with great accuracy. And this is true for EEG: EEG is poor in space but good in time. Then interpreting EEG signals — we start in some way to play with it — is another pair of sleeves [another kettle of fish], it is complicated to interpret, to make sense of that, of the voices, of the confused clamor of a large group of

people, of neurons. It could be confused, but I can hear it.

There are other techniques like functional magnetic resonance imaging (fMRI) that have a very poor temporal resolution, of tens of seconds, so forget that there is any correlate with the emission of action potentials; it is more something, as in the case of fMRI, metabolic, particularly due to oxygen.

There are other techniques here indicated as **intrinsic optical imaging**, which means that without doing anything, due to properties of oxygenated or non-oxygenated tissue, if you illuminate with a light source a part of the cortical tissue, you would see that it has a different optical reflection characteristic. They were pioneering studies several years ago.

In other cases, there are organic substances that change their conformation and start to become fluorescent when the membrane potential in which they are intercalated changes. They are called **voltage sensitive dyes** and inside a plasma membrane they have a lipophilic domain, so it loves to stay among lipids, so it intercalates in the membrane and feels the transmembrane potential and changes its own conformation depending on the electric potential. So in theory you could, doing with an imaging technique, see as an optical correlate little lights that turn on correlated with the membrane potential.

Another thing that happens, I don't know if it's here, yes, there is **calcium-based imaging**. Again, substances that can be either put from the outside or genetically engineered, so that it is the cell that expresses them directly as if they were its own membrane proteins, which are not sensitive to voltage but sensitive to calcium. When there is calcium they bind, change and fluoresce. But it is difficult that from the clinical point of view people inject these dye molecules *calcium imaging*, *calcium dye*, *calcium sensitive dye* or *voltage sensitive dye*. What is much more common is the part of electrophysiology, even invasive, but without the use of these compounds.

In this case, you would have with *calcium imaging* or *intrinsic optical imaging* you would have a very low spatial resolution, in the sense good, of the micrometer (you could look even at a single compartment of a very complicated neuron), and a temporal resolution however of some order of magnitude slower compared to electrophysiology, because these objects here are slow to react, or because intracellular calcium — now we know it because in particular for the story of frequency-dependent adaptation, the bank account, etc., I made that example also in that case — takes time for a molecule of free intracellular calcium to go away, to be extruded or internalized in the endoplasmic reticulum. So all this leads to having a slow temporal scale.

Local Field Potential (LFP) In the middle, there are the so-called **Local Field Potentials**, which we will deal with, which are a technique, an invasive measure and it is in some way electrophysiological, with an electrode, and depending on the type of positioning, also technology, shape, they have a temporal resolution that varies from a few milliseconds up to a few tens of milliseconds, or pardon, tens of seconds or minutes. They are relatives of the EEG and we will understand what they are. Just to make it brief, not all recording methods are the same. There are some methods that are for example based on magnetic fields or on positron emission, positron emission topography (PET), and they

have advantages and disadvantages, but above all, unfortunately, they are located in a part of this graph that might be unfavorable to the characterization in detail of the electrical phenomena of the nervous system. Moreover, these temporal scales are multiple, they are heterogeneous and they are multiple.

Anyway, I wish to dwell on this part here, which are the Local Field Potentials, also called *low frequency part*, even if it is wrong, in the literature you don't find it yet. It means that compared to spikes — they are indicated here as *sodium spikes*, they are the ones we know, these are extracellular spikes, they are also called **multi-unit activity** — they are capable of… so Spikes and Local Field Potential are the type of signal, they are two faces of the same signal that you can measure if you insert an electrode in depth, or on the surface like the electrocorticogram, or on the scalp like the EEG. Anyway, what it is requires a description at different spatial scales and obviously the question remains on what it means that spikes have a frequency characteristic of the order of hundreds or thousands of cycles per second while Local Field Potentials have something slower. Beyond the definition that I say, that the Local Field Potential is the low-frequency part, what do they tell me about the biological phenomenon? Maybe if these are slow they are talking to me about synaptic activity, while these that are fast are talking to me about action potentials? All very beautiful but it is necessary to be able to understand it somehow, to be able to verify if this is the case.

Relationship between Intracellular and Extracellular Signal

This is an example of what is, in an extremely intuitive way, the situation of the techniques we have seen so far. Normally we can, with intracellular access, access everything that can… well actually it is not even everything, anyway [we access] with maximum temporal resolution recording the so-called **sub-threshold** and **supra-threshold** activity. It means the synaptic activity, which I see here sub-threshold because the neuron is integrating it, and I also see the so-called supra-threshold activity because the neuron fires in some conditions and I see these signals that are much larger in terms of signal-to-noise ratio.

What I said anyway — this intracellular electrode is not the end of the story in theory, going in the direction of maximum accuracy — is true because the neuron is not a small ball, it is not a sphere. It has an incredible, in some cases incredible, dendritic tree and axonal tree; what is generally called **neurites** (means dendrites and/or axons, it is a generic term). These arborizations have — and we will see it today — they are not **isopotential**, they are not at the same electric potential. If they were, and the soma was too, it might make sense that I record in the soma: "Ok, what the synapse is doing over there, the compartment of the dendrite where the synapse is insisting, is the same thing". The potential instead no [is not the same].

As you can imagine, also for example the axon is a structure morphologically, electrically distributed — it is said **electrotonically distributed** — and would require having a recording mode with many simultaneous intracellular pipettes, or with many of these voltage-dependent dyes that are distributed over the entire membrane of the neuron. So in theory I could see it like a Christmas tree,

I could see it in different points, I could see the activation, the wave of what is the electrical activity propagating in the same cell.

The problem is that those objects there could be toxic. You could do an experiment that lasts a few minutes and then there is a toxicity phenomenon that kills the cells; there could be light-induced toxicity (you have to have a strong light source and a progressive inactivation of these objects occurs which is called *bleaching*, like bleach, whitening, it is called **photo-bleaching**). So what might seem without problems, in reality anyway is an experimental technique that maybe lets you see, gives you the possibility to see a few seconds and then you see nothing more.

Extracellular Recordings: Multi-Unit vs Single-Unit The thing that evolved first, actually simpler even than intracellular recordings, is the technique of **extracellular recordings**, in which [one talks about] measurement of **multi-unit** activity. Which units? Neuronal units. "Multi" because evidently, like this microphone — you are not talking, but if you were talking, this microphone would hear multiple voices simultaneously — because in the extracellular space, to hear only the voice of one person, I should put it in your throat, and then there you would hear only my voice, the voice of a single unit (**single unit**). Which could be possible, certainly it is with an intracellular electrode; with an extracellular electrode it is more complicated.

Associated with this extracellular technique there are other terms — again I repeat it — these **Local Field Potential**, corticograms, electroencephalogram. And in the end you might think that from the extracellular point of view, in a very banal way, you hear only the echo from the outside and of the strongest signals. A very simple thing you could think of is that when a neuron, inside compared to outside, emits an action potential, it means that the inside becomes more positive than the outside. So it is as if positive charges here, which are for example sodium ions, entered abruptly because sodium channels open and therefore left a void of positive ions outside.

So outside you would see that the potential locally could dramatically change: it would change to negative. So the dual, the opposite compared to the intracellular action potential which instead is a positive deflection. But to make it brief, you would see only dramatic events. Because yes, it is true, synapses when they activate the neurotransmitter binds to the post-synaptic receptor, there is a charge flow (in the case of AMPA/NMDA it is a mix between sodium and calcium; it could be instead for GABA-A chlorine, GABA-B potassium; for metabotropic receptors I believe it is again sodium and calcium etc.), however these currents could be very small and so I, from the extracellular point of view, could simply not be able to measure them, or rather I measure them but they are covered by noise.

Signal Filtering: Spike vs LFP It turns out that an extracellular electrode yes "kind of works", but it makes you see only spikes; what you lose is the whole world of integration of sub-threshold synaptic signals. And so the question is: is it so? If a neuron never fires could I see nothing? In reality, things are even more complicated.

In the sense that if you take an extracellular electrode and put it in distinct points of the morphology of a neuron — now morphology is important, and it is important because it is something that is extended for tens of micrometers, up to hundreds of micrometers in the case of larger cells and larger mammalian cortices — you would have in a case like this (which is a simulation, and we get to see how this was done, of the extracellular electrostatic potential… I continue to call it electrostatic because we are not in a regime… quantities change in time, but we are not in a radiofrequency regime, we are not in a regime where Maxwell's equations would predict the generation of a magnetic field concatenated to the electric field; for this reason it continues to be called electrostatic case. It's not that static means necessarily *steady state*, it means at low frequencies and we will see it: the fastest things that are are action potentials, they have a Fourier content of frequencies up to $1000 - 2000$ cycles per second and although you can have radiofrequency emission at 1000 Hz, one kilohertz, hardly this is electromagnetic activity, a true electromagnetic propagation).

To make it brief, in this simulation one wants to represent what is an **ECoG** electrode, so of electrocorticogram (so that is placed on the surface, so on the pia, a membrane that isolates under the bones the brain tissue from the rest), or above the scalp, an **EEG** electrode. What would it see when a neuron — and in this case I am not indicating what this neuron is doing from the point of view of synaptic inputs; I only know that intracellularly this neuron seems to receive synaptic inputs, integrates and makes a spike.

If I put an electrode close, extracellular, close to the soma, I see what I told you: I see a strong negative deflection. But careful with strong or weak. Here, as your colleague said, there is a bang of 100 millivolts, which is a remarkable thing. Outside, despite during these huge bangs, I first of all see only… it almost seems that I don't see the subthreshold behavior, as I anticipated before. So it is as if there were a kind of **high-pass filtering**, like a kind of **derivative**. That is, it is as if I, if I take the derivative here, maybe I change the sign… clearly the derivative of something that is very very steep is large, a large velocity. You know that if I take the derivative of a certain quantity the absolute value has to do with the change in the unit of time, so the more something is rapid, the more ample it is in the world of the derivative.

In fact here these transitions, this dynamics [subthreshold] is rather slow, it is some ten, hundreds of milliseconds; here [in the spike] it is a fraction of milliseconds. That's why roughly I say: you must know that roughly a spike exhausts itself in the span of a millisecond, so if I ask you "but what is the *upstroke phase*?", you could say: "Ok, half a millisecond, 0.25 milliseconds, a fraction of milliseconds". So outside I see a signal that is negative and fundamentally I see nothing of the sub-threshold activity.

If however I move the same electrode to different points, for example here, where presumably synaptic inputs were arriving… pardon there is one thing I wanted to say, careful speaking of strong or not strong. Here is millivolts; here it is true they are 100, but they are **microvolts** (μV), so 10^{-6}, so a thousand times smaller for a neuron. So tens or hundreds of microvolts, which is the current situation that for example we see in the laboratory when we use extracellular electrodes on top of which we grow neurons: at most we see a few hundred microvolts these signals.

If you move the electrode elsewhere (neglect the magnetic aspect, this is just to say that in theory with first principles I could also derive and understand, from the point of view not only electric, what happens), moving here, anyway the scale hasn't particularly changed. Actually this has become a fraction of microvolts, stuff that is a hundred or a thousand times smaller than what was the amplitude of the extracellular spike I saw here. Here however I see something that seems to be a kind of strange signal, which becomes first negative and then positive. Perhaps this thing here has to do, although it is extremely small, practically almost impossible to record… perhaps it is the extracellular echo of the opening of an AMPA channel, an AMPA receptor. It lets positively charged ions enter, so it leaves a negativity from the outside. I probably see this negativity. Here in the dendritic tree, at the top of the dendritic tree, I see a similar signal.

Local Field Potential and EEG Note: in EEG I can do the same thing imagining that, from the electrostatic point of view, to model the scalp, model not only the distance… here in the end we are in the world of electrostatics where it matters if I move away from the source: signals change as $1/R$, $1/R^2$, distances matter. If I have an electrode in the belly of the neuron no, because I record what the whole soma from the electrical point of view is doing. Here it matters. And if you see here we are at a fraction of **nanovolts**. And it is… I wouldn't be able to do as I would have liked during the first lecture (for a long time I tried with electrodes, the same electrodes, to put them on my forehead), simply it is impossible: there is too much electromagnetic noise. I told you about the famous 50 Hz and other intrinsic noises of the amplifier, so signals that are nanovolts… it is true, I don't have a single neuron, I have a few billion, nonetheless it doesn't matter, signals are obscured by noise. This would be the correlate of a single neuron.

It would seem interesting then that the spatial extension must be taken into account and something of the type: now that I know the excitable electrical behavior of the single neuron, perhaps I can combine it with what is a more classical electrostatic treatment. If I have a distribution of charges in space, then I can calculate what is the potential at this point, for example seeing all distances. Do you remember the electric potential? It was the weighted sum, weighted by distance, of single charges, it went as $1/R$, point charges. Here they are not exactly point charges because I am imagining precisely like here there are holes, pores, ion channels that activate sucking… they are like wells into which if I have a charge it disappears in there, or they are sources, they spit, they have a breath of ions, of charged particles, that is they are currents. So perhaps the treatment must be slightly more complicated, more refined, but it makes me think that if I know everything about this neuron, then I can predict what is from the extracellular point of view in different points the electric potential outside.

And this can be crucial to say: look that if you from the point of view on the EEG, on the scalp, are recording signals like this, look that it doesn't mean that neurons activated at this moment. Here one even sees the echo of the spike, but again it is a fraction of nanovolts and if you also have a billion of these cells, a few million maybe underneath (also because the human cortex is convoluted, so

it is difficult that there is a flat part… this is to scale more or less, so this neuron could be accompanied by a few million other neurons nearby), it is unlikely that they fire at the same identical microsecond. In that case yes, you would have a summation of these little peaks and you would see them on the EEG. And, *by the way*, it is seen during pathological states of epilepsy, where spikes are seen at times… they are called spikes, not necessarily are they [intracellular] spikes. Conventionally this is called action potential and from the extracellular point of view it is called spike. Anyway spikes are seen because there is pathological synchronization.

Interpretation of Raw Signal: High-Pass and Low-Pass Filters So how do we extract sense from this extracellular thing? I show you before starting two characteristics. We concentrate on this part here, so the **raw signal** that you would see from a metal electrode of a fraction of a micrometer, so there is a metal part of a few square micrometers of surface exposed to the extracellular environment placed close to some neurons. This is the raw trace. Note here there is no unit of measurement but they are microvolts and you see that there are slow deflections and then every now and then there are little peaks. Leave aside now that these little peaks go up instead of going down as I showed you, it is not so crucial, here it is the technique, simply the recording technique that is different.

Here is just to say that if you take this trace and do a **high-pass filtering**, that is, in fact you take a frequency component (probably something that you are… tell me if it is something that you are not seeing with Gibertoni and Gibaldi… this could be crucial), you should do something like filtering. Here I don't want to throw away noise, I don't want to do a low-pass or high-pass filtering to throw away noise; I want to isolate different characteristics.

If in fact I throw away the slow components for free I have that this *baseline* that oscillates I don't have it anymore. In fact the filtered trace is not exactly the same (this anyway is a trace recorded in our laboratory): it is flat by definition because I removed the DC, I removed the Fourier components at very low frequency. In fact the trace doesn't… what remains are these very very rapid depressions, they are negative, they are spikes. They are phenomena that if I zoomed (and I will do it in the continuation) have a duration that is very similar, a little smaller than what is the duration of an intracellular spike. Well, thanks, they are the extracellular correlate, now I know it. But if I look, if I… so this is the theory, if I record a signal and I see it like this, I have to play it to be able to convincingly say: "No no, these are spikes of a neuron".

Is it only one neuron? Because I see here that there are events that are equally big and then there are others that are a little smaller. Maybe the electrode is here and I have a closer neuron and a farther neuron. The farther one makes sense that it might be attenuated in its effect it has on the electrode. While, so these high frequencies means between 100 and 100 Hz or 100 cycles per second and 5000 cycles per second, so between 100 Hz and 5 kHz.

And instead if I take with a **low-pass filtering** only the slow component I don't have the little peaks anymore, they are too fast, I removed them and a low-pass filtering only the slow signals pass. And you see that I have a signal

that by definition, having filtered between 0 and 100 Hz, 100 cycles per second (I say cycles per second because Hertz at times can make you think of spikes per second and here is another world, here is the world of analog signals in which I am talking in effect of a frequency component in the sense of the frequency domain, in the sense of Fourier), and if I do it here by definition I have signals that have frequencies that are lower than my *cut off*, than my cutoff frequency which is typically around 100 Hz.

In parenthesis if you go maybe to an epsilon to look at the introductory part of this course I told you something on EEG and I told you if I remember correctly that in EEG in a phenomenological way, in a totally descriptive way slower oscillations are described, oscillations a little faster, conventional names are given: gamma frequencies, alpha frequencies, beta frequencies. Do you have… do you remember something? For example **gamma** was between 50 and 70 and 100 hertz, **alfa** and **beta** are associated with some sleep states, **delta** and further are slow, are between 0 and 100 hertz, if nothing else because they come out of such a filtering and so thanks, the signal cannot anyway be, since I threw away the higher frequency part it doesn't contain other oscillations.

This is what is called the **Local Field Potential** and these fluctuations, these frequencies, these speak of anyway neural activity, only that I don't know… while spikes are simple to understand, ok, there must be the soma somewhere and I am observing the result of synaptic integration, the emission of a spike. Here I have the impression that it must be something in a much coarser way, probably at the population level, not single unit, of maybe synaptic activity, however I have to try to understand it.

And *that's it*, here the part on which I wanted to insist is that LFP often, now one is starting to see, particularly from the didactic point of view, by definition one speaks of low frequency component, because Local Field Potential has implicitly the concept that it is an electric field, a potential of an electric field that I am measuring and that is local. Local where? Local in the sense of some ten, hundreds of micrometers around the electrode. Yes, but it depends on the electrode and perhaps, again, it is more correct to speak of the action that one had on the signal, rather than already jumping on what eventually might not be it, the spatial scale.

Here for the **multi-unit** or **single unit** activity… I repeat, I believe this is multi-unit because here units are more than one, these and these have distinct amplitudes. It is true that, you saw it also with the Hodgkin and Huxley model simulation, the same neuron could fire action potentials that were the first of the train, they could be very steep, the others could be a little degenerated. This *slope* tended to bend when I asked the neuron to fire in a very rapid way. And if in between V intracellular and V extracellular there is some derivative with respect to time (I have to show you because this is not…), if this is a derivative it could also be that the same neuron has steeper spikes, others less steep and I see it with lower or higher amplitudes depending on steepness. So there is intrinsically an ambiguity: is it the same unit that maybe fired twice in a row so the second spike is slightly less *steep*, less steep, more *sloppy*, more scarce? There it's not that I notice, amplitude could be equal but the rise is a little lower because voltage-dependent sodium currents are not so intense, because voltage-dependent channels, voltage-dependent sodium are not 100% ready and

available, some are inactive and if in between there is a derivative, yes amplitude does [count] for me, but *slope* also counts for me, the inclination. So everything is: can I extract some sense on what I see extracellularly? Because like this it is a big mess, it becomes heuristic: "these signals are fast, so they are action potentials", but how many are they? Ok, it becomes a little complicated.

Joao Couto's Experiment: Simultaneous Patch-Clamp and Extracellular This is a heroic example, and I keep it because I am fond of it, of a PhD student who is now a researcher at the University of California in San Diego I think, or San Francisco, I don't remember San Francisco, **Joao Couto**. When we were in Belgium, heroically he put in the prefrontal cortex of an anesthetized rat not only a series of extracellular electrodes, but he even shoved a pipette, roughly — he didn't hit exactly the same, but roughly with an angle for which the tip of the glass pipette, a *patch* electrode in vivo, so further heroic — was roughly in the same region where the extracellular electrodes were.

And what you see is that here are multiple traces and they have artifacts because I don't have the right trace, so this is an image and I wanted to stretch it to be able to align it in an authentic way with what he recorded at the same moment, simultaneously, from the soma of a neuron. The soma of that neuron somehow acted as an antenna with respect to network activity. And I here… he, I did nothing, with electrodes 1, 2, 3, 4 (actually he had 8, here are 1, 2, 3, 4, they are metallizations on a kind of fork) recorded only fast events, because Local Field Potentials here were not particularly indicative. So he filtered only the high frequency part to show multi unit activity.

And you see that extracellular activity seems to be organized in packets. I don't know if to you or to your second year colleagues a few weeks ago I told about sleep, *slow wave sleep*, in which during sleep or anesthesia the cortex has a mode of oscillation between **UP** activity and **DOWN** activity: up, down, up, down. It depends on the anesthetic but in the case of ketamine-xylazine that is used in this experiment it is maintained as in sleep.

So this activity seems to be synchronous and seems to be in packets and intracellularly the neuron has this subthreshold activity that you don't see, you see only perhaps here the echo of what is the occurrence of activity seen extracellularly. When the neuron fires, this neuron fires, it's not that it fires many spikes in one of these *up states* (I call it up because the potential seems to be stably for some ten or hundreds of milliseconds, seems to be more depolarized, seems to be bistable, as if there is a synaptic bombardment that here is off, here is on, here is off and here is on). But here for example the neuron does not fire, it doesn't fire but someone else evidently nearby fired.

So, in the end — and now we take the 10-minute break — it would be nice to be able to correlate intracellular activity (this was the soma), even intracellular activity in the whole dendritic tree, and give sense to what is extracellular activity. In this case probably they are four other neurons that were in the periphery that more or less did the same thing because all of them had spiking activity roughly at the occurrence of these *upstates*. I stop for ten minutes.

Derivative Relationship between Intra and Extracellular Signal

Ok. Ok. Let's resume slowly. Ok so here is another example in which again in a heroic way, but a little less heroic than what was done in vivo by Joao Couto, two researchers of the laboratory when I was in Belgium and before that in Bern did however *in vitro*, so not *in vivo* but *in vitro* on a culture of dissociated neurons growing on a matrix of planar microelectrodes (**MEA**). Like your chairs, maybe I mentioned it and showed you a recording already, as your chairs where you rest your bottom, so neurons lean on a surface and lean in some points on an electrode. Electrode that was microfabricated with the same principles and technologies of microfabrication via microphotolithography. If you know how they are [made], what are printed circuits or even PCBs, *Printed Circuit Boards*, or chips, microprocessors or anyway very high integration systems are in fact with the same method. Here somehow, despite being small, they are however an order of magnitude larger. Each of these electrodes is of the order of 50 or 30 micrometers and the distance between two electrodes is 200 micrometers.

And here you see a big mess in the sense that there are… what is said for other types of cells is called confluence, cells are packed so much that they touch, their soma touches, neuritic processes are not seen, axons and dendrites are not visible, they are immersed. After a while that they stay in culture, after about two or three weeks, these cultures, these two-dimensional networks, are very interesting because they allow studying emergent behavior due to connections. They have every now and then this activity that if you want is a zoom of what *in vivo* I described to you very briefly, *up state*, so this is probably… damn there is no time scale… This must be around 200 milliseconds.

Again here you see the extracellular recording. You see that the signal is slightly different from before. Before somehow it seemed that the extracellular signal was a totally negative deflection. Now it seems to be something that is made like this. Or something that could be made like this. Excuse me, this goes straight down with these little humps following or preceding. This is a non-simultaneous recording, in this case, made by Dr. Anastasia Moskaliuk, who insisted saying "no, in my opinion this is not the behavior of a single neuron, this is the network". Then, to convince me that we were wrong, she did the intracellular recording and rediscovered that actually even single cells, single neurons that are around this electrode, have the same *up state* and *down state* behavior.

The interesting thing is that behavior seems to be not only an irregular emission of action potential, so very different compared to what we saw in simulations where applying a current the neuron behaves like a kind of pacemaker; here it seems to be a disordered, disorganized behavior, and then the sub-threshold membrane potential seems to be a **random walk**, a kind of stochastic process, a realization of a stochastic process that makes one think it is the integration of many synaptic inputs which however do not arrive in synchronous instants, instants at the same moment, they arrive in an asynchronous way, in a disordered way.

To make it brief, obviously, again, there are enormous differences. I here see only supra threshold activity, in the sense that I see only in correspondence of spikes. Here are 100 millivolts of signal, here are some tens of microvolts unfortunately.

What is seen here is that the amplitude of these extracellular signals seems to change, particularly during this initial phase. So here it could be that it is something that has to do with frequency-current adaptation. And then there are these two little dots that I highlighted for you where clearly there are signals that are completely different in amplitude. It could have been a neuron that was slightly more to the right, farther from the electrode and that fired on its own during this epoch, it is called epoch of synchronous activity. But it was more distant, I see it also here.

So this signal, then I should call it a **multi-unit** signal, is simply the part filtered at high frequency, of which Local Field Potential do not make particularly sense, do not have a particular content. Probably because it is a cell monolayer; in the case of the brain a block of tissue, whatever Local Field Potential is, probably is the effect of summation in 3D. Here in 2D the Local Field Potential doesn't contain details, you don't have particular events, you have it in the high frequency part and in this case it is multi unit activity because certainly they are more than one unit. So again here is filtered from 100 Hz or 100 cycles per second up to 5.000 cycles per second or 5 kHz, while this, the intracellular part, is not filtered if not at 5 kHz. And in both cases sampling frequency is of the order of 20 kHz, 20.000 samples per second, which is information that has to do and doesn't have to do with frequency content, I am thinking of Nyquist sampling theorem.

This is another example, a zoom of the shape of the single event, which here is — since the trace is zoomed to show, I repeat, here it will be $200-300$ milliseconds, the event is too fast — here is a zoom of single traces. This electrode records these events, this electrode here instead you see records another one whose shape has a subsequent little hump as I was saying. So the question is why one has a little hump and the other doesn't? What does it mean? These are 10 microvolts, again these are 100 millivolts.

Extracellular Electrodes and Derivative of Intracellular Potential
And this is a further example in the literature of an article that we resume now in a couple of slides in which intracellular potential was measured and the same neuron had an extracellular electrode simultaneously in its proximity. And it would seem therefore to be a temporal relationship between when intracellular potential stops growing, so the point where the first derivative becomes zero, and the extracellular signal hasn't exactly gone to zero, but it became very small. Before it was negative — aside there must be a minus — this was a growth and then a decrease, here the signal becomes negative and then positive. If there is this thing that extracellular signals are the **derivative** of intracellular signals… again I don't understand why they should be the derivative. The only thing that does the derivative is the capacitor, the capacitance, so it is possible that here this capacitor is responsible somehow for the fact that from outside I hear a signal that is not direct coupling DC but is an AC coupling. In fact in electronics when one couples a system with a capacitor in fact cuts the continuous, is doing a derivative operation.

So the idea is: how do I make sense of this behavior? The key to everything is that dendrites, it is not a big discovery, probably I repeated it other times during previous weeks, **dendrites are not isopotential**, they are not isopotential

portions of the same neuron. The fact that neurons evolved to be spatially distributed can be read as having a meaning, an evolutionary reason why it is necessary that electric potential here, at the Soma, be different from up there where I have dendrites, where they fish inputs, and maybe also different from my axon that is projecting somewhere. It might make sense that there is the need to have signals propagating in time. Delays? Maybe it is necessary to do information processing that there be delay lines coupled as in some computation paradigms? Another possibility is that this is simply an epiphenomenon, no one yet knows with certainty, the fact that a network can be wired effectively.

Keep in mind that in a little piece of cortex of a centimeter on the side there are, I believe, millions of kilometers of cables, of wires. If neurons were point-like it would could have been very very complicated for all connections, simply from the point of view of volume, to attach, attach one neuron to another. If I have instead a structure morphologically, geometrically, spatially distributed, I have many different points to accept inputs. Again, this makes me think… here there are no trees outside… trees also have this behavior, in fact they are arborized, they are called dendritic arborizations (for this also dendrite means from Greek tree), they are probably like this because they have to compete with neighboring trees to catch light, they have leaves and branches extending high to take, maximize surface, take light. Here maybe they have to maximize and take synaptic inputs, or both things. It may be that this is yes a *leftover*, something that evolution left as a side effect, but since the neuron is there it might be important that it has an electric surface, electrically active, that can integrate information.

Imagine, and we will see it, if synaptic inputs arrive in a distant way or in a close way. Maybe they have a different effect in recruiting the neuron. Some could be the famous tiger entering the lecture, sensory neurons might want to project close to the soma, because maybe their effect could be integrated much more rapidly in a much more effective way. If they are far, in the end if they behave like cables, cables with distance attenuate, in the end they are pieces of resistance, it may be that they have perhaps less sense, there is information that is worth having immediately and other that can be neglected.

The Paradox of the Point Neuron and the Need for Distributed Models Anyway the fundamental point is that they are not isopotential. So this is an animation, a stupid *sketch* that I showed you already in which the intracellular part, the extracellular part was described as a resistive continuum and we still keep this that is a resistive continuum. Now if you give me the possibility to make an exception: in the extracellular part, which is also a resistive continuum, is not all at the same electric potential, however for the moment here I will put short circuits, that is zero resistances, later I fish them out these resistances. Here there are capacitive properties of the membrane, of the lipid bilayer and every now and then there are channels. Here, ok, there would be Nernst reversal potential, but now it is not so crucial. What I did last time is say, since the resistance of this pore is much greater than cytosolic and extracellular resistance, I ignore the others, I put them to zero.

This time I cannot do it. Outside I do it, I repeat, then I remedy this, but for the moment it presses me to say that it is not such a good idea, since these

cables have a rather small passage section, perhaps with a lumen of a fraction of micrometer or a micrometer (depends on dendritic tree and various branches, it may be that with various branches diameter becomes smaller and smaller, up to a considerable fraction of micrometer). And so it might be that at least in this type of **longitudinal resistance**, which is exactly that, is not transmembrane but is longitudinal, it counts if I have a piece of dendrite that is long some ten or hundreds of micrometers.

So if I leave these resistances, you see well that if there is a resistor by Ohm's law, probably if a current passes the potential here is different from potential here. So if these are two points of the membrane and I indicate, touch the capacitor because the capacitor is what gives the state variable, gives memory, they are a kind of reserve, of charge, of *reservoir*, not reserve, of container, of accumulator, here. And since potential at this point and at this point might not be the same, here it is that here and here are two pieces of the same neuron that have a different transmembrane potential. This is not a problem, it is a complication, and it is fundamental to take this complication to understand this thing of extracellular potentials.

Buzsáki's Experiment: Relationship between Intra and Extracellular Potential

This is another example, again in a heroic series of works published by **György Buzsáki**, researcher of international fame, Hungarian working in New York for very many years. In particular in the hippocampus, already more than twenty years ago, twenty-five years ago, he had tried to approach the same problem. Here, you see it, this very thin line is the tip, is the lateral section of a cone: it is a pipette, a so-called *sharp* electrode. I always indicate it to you like this, but actually it is quite long, it is stuff that can be several centimeters, whose final part passes from a millimeter of diameter to a micrometer, even smaller in diameter.

If you put it in a tissue — I believe this is a *sketch* to scale however appropriate — here you see a little cell, which is a pyramidal cell of the hippocampus, of CA1 of the hippocampus (it is called an anatomical zone that is called *Cornu Ammonis* 1). In this case the tip of this very thin pipette — which is that pipette there, but put to scale, to scale on something that is a few millimeters, 2-3 mm (these are 250 micrometers, so four of these make a millimeter) — is practically invisible, but it was inside the soma of this cell. And in its vicinity, heroically, these put a **tetrode**.

A tetrode is an extracellular electrode composed of four wires (here it says three, but *tetra* stands for four) that are more or less as when you braid hair: they are intercalated and wrapped around each other until having the final part without insulation. So the tip of this tetrode — which I should have shown you but I don't show you, which here is compared to a little cylinder — in fact has one or more metallizations close. They behave like, again, extracellular electrodes attached to an amplifier and allow measuring electrostatic potential at that point.

Which is again this very rapid event. These are two milliseconds, so this is faster than a second… excuse me, than a millisecond. This is 2 milliseconds. Here is… ok, this perhaps I should define when I take width: one possibility is taking **half width**, which means *half amplitude width*, that is I take maximum, take this distance here, divide it by 2 and at this distance I measure spike width. And roughly if this is 2, it will be a little less than a millisecond.

Beyond this thing here of temporal scale which is useful, again, this is here spatial calibration… excuse me, vertical: it is 15 millivolts for this graph here, while it becomes 30 microvolts for the graph below. But this we knew, these signals are very, very, very small. And it is so clean because the neuron, since it had a pipette inside the belly, was made to fire several times in such a way that they could do an arithmetic mean of multiple repetitions; for this it is so clean.

The Derivative of Intracellular Potential The thing these did, that Buzsáki did for the first time, he said: what happens if I take this trace here [intracellular] and I take the **first derivative**? Taking the first derivative of a trace like this is very easy, it is not a mathematical function for which you have to apply rules. If you have a vector on Python or on whatever is, and you have many numbers in many little boxes (all these are samples, for example: $-65, -65, -64, -64, -63, -5, +20$… I am dramatizing), you have these numbers placed here.

So if this is v and it is a vector, in some programming language like Python it is indicated like this, `v[i]`, and one puts here an index and this index is an integer variable that says where you are. To do the derivative it is enough that you do:

$$\frac{v[i+1] - v[i]}{\Delta t}$$

And if you want you can divide by Δt, by time, by sampling interval. I told you that roughly these signals are for example sampled at $10, 15, 20$ kHz, so you could divide also to have millivolts per millisecond, but if also you don't divide in the end it is only a scale factor.

It seems to me that in MATLAB there is a command called `diff`, so if you do `diff(v)` you automatically have this operation, point by point. And another way to do it is then doing `v[2:n] - v[1:n-1]` (with MATLAB, in which basically you use this that is called **slicing** in jargon, that is allows you to take a subset of the vector). Here from point 2, imagining they start from 1 as in MATLAB (even if it is not a great practice), from 2 to n minus the same vector however *shifted*, which in fact starts from 1 to $n-1$, so that I have this difference always, difference between neighboring points.

I obtain a signal, the derivative. The derivative is this dashed one, this *dotted*, which is not so distant [from measured extracellular trace]. Here is a little… seems a little anticipated, but it seems it gets it quite a lot. So this is one of the first times in the 2000s in which people said: let's go understand the origin of extracellular potential, focusing on the spike, because it is a large signal, because it is easy (easy relatively, but certainly it is easier to have a pipette in the soma, which is relatively large, even if here it was placed randomly and it is easy to record in proximity).

Physical Relationship: Currents and Kirchhoff's Law So there would seem to be a relationship with the minus sign between extracellular potential (V_{ext}) and intracellular potential (V_{soma}), with minus. Again this makes intuitive sense because, I repeat, during a spike sodium from outside enters rapidly inside leaving a negativity outside, leaving therefore… disappearing positive ions inside [of outside].

Some of you might feel a bit resentful to say: "But how, darn, I am an engineer and if you love circuits say: ok, but here this current enters inside, but somewhere it has to exit, it's not that circuits are hanging". So the circuit is called circuit precisely because it is closed. So if here I have a sodium current entering, somewhere [it must exit], because otherwise it is not a circuit, I don't close the circuit, a current cannot flow remaining hanging. By definition of **Kirchhoff's law**, if I have a wire like this, current is zero, because if I take a surface around this wire, summation of currents is null; the only current entering or exiting is one, but it is that one equal to zero.

This is also the reason why little birds don't die on a high voltage cable. This is a little bird and if I do Kirchhoff, sum of currents, I equal to zero. Yes, it is true, they have two legs, but this we see another time.

Variations of Spike Shape in the Train This is the same trace in which they show that, what I was saying, if by chance the shape of action potential by chance changes in a train of multiple spikes… You see that here are intracellular potentials: the first is this one which is the most hyperpolarized, the most polarized and the fastest; the others sitting on this *plateau* of depolarization are a little different, are in particular different both in rise phase and in descent phase. So if there is something that has to do with rise phase and descent phase, maybe it makes sense that in previous graph true trace of extracellular potential results a bit *shifted*, since here it seems that this part here is also *shifted* in time.

To make it brief, here they continued to support the fact that even not only in an isolated spike, but in a train of spikes in which it is known that membrane potential changes, action potential changes a little shape… First spike, second spike, third and fourth seem to be very correlated — here is amplitude, pardon, this is yet another thing — seem to be correlated with what is the **derivative** of intracellular potential.

The derivative, if I write it like this, obviously this is a quantity that grows the more ample is this somatic action potential. This I remind: if I have a function I call $g(t)$ and its derivative I call dg/dt, if I multiply this by 35 or by 63 or by 121, derivative of this new function that has this constant before is the one you had before times 35 or times 63 or times 121.

So the fact that these researchers see that when intracellular spike is more or less ample, also extracellular spike is more or less ample… so there is, when every dot is an action potential, and if I put it in a *plot* like this: this is measure of action potential recorded extracellularly and take this as amplitude (clearly microvolts… ok here is millivolts because 0.04, they are tens of microvolts actually), here instead intracellular amplitude (in fact are tens of millivolts), here points align in a cloud. For which it is not necessarily that there is a determinism, a perfect and not noisy relationship, but it seems there is a correlation, just as I

would expect if it were a derivative. So derivative of a constant times a function is constant times derivative of function.

Then obviously there is a question of **slope** (inclination), because derivative… this is seen well for example if you remember graph of a line that grows. For example if I have this function of time $g(t)$ that is $\alpha \cdot t$. If I… so this α is angular coefficient: the more it is large (if positive), the more this line passing through origin tends to be steeper. I know that when I take derivative of g with respect to time I obtain α. So it is not surprising this thing that when these objects, these waveforms, are steeper — there steepness I simplified it, I extracted it to max, and it is even angular coefficient of that line — but steeper lines have larger derivatives. Here again, spikes having action potential, having steeper rise phase like the first, have wider extracellular amplitude. So it would seem that this thing of derivative adds up.

Limits of Point Neuron and Introduction to Cable Theory

However this discourse, if one wants to make it slightly more quantitative, does not stand if we have, if we consider neurons described with a point formalism. This is the Thévenin equivalent circuit type model we saw, in which for simplicity, to show and start insisting on the role of what is synaptic input, I put a further arc due for example to AMPA synapses. So here they have again their Nernstian equilibrium potential, here they have conductance, permeability of that post-synaptic receptor and when this opens it lets pass a current in agreement with what is electrochemical potential of ionic species to which post-synaptic receptor is permeable.

However, if I consider also this structure and say: "Ok, here I see it this thing I described to you before", I imagine neuron surface as a membrane with many pores and these pores can behave as sources or wells. They "sputter" ions to me, I see it more as a flow of ions — we called it flow, a current, an ionic current — but if I do so for electrotechnical reasons, in fact here in extracellular space output and input of these currents is at the same identical point: circuit is closed here in extracellular space.

A **point neuron** has pores, wells and sources practically coinciding; I wouldn't have here… I between these two points don't have any potential drop, I have zero. I would like it to be extracellular potential. I think it must have to do with this current exiting and maybe then re-entering, because it has to re-enter at a certain point, but in *point neuron* there is no way, no possibility, because these currents cancel, yes, but at same point in space, so no way.

If I start however to consider a spatially elongated structure, distributed, I am thinking of a model that is a kind of what is called **Ball and Stick**. So obviously it is extremely crude stuff, and actually not only is it crude. Now we will see that here this dendrite I can describe it accurately.

Cable Theory: From Lord Kelvin to Wilfrid Rall Maybe I mentioned it during introductory lecture: we will do it with an equation that is same equation of electric cables or transmission lines. And it strikes me because

it is same mathematical formalism that in Nineteenth century **Lord Kelvin** used for oceanic cables, transoceanic for telegraphic communications. And it is surprising: but how, we are in 2025, it is 200 years later and nonetheless that math works. Here you don't see any cable, I am simply imagining there is a compartment, like graph I showed you before, in which I asked you… forgive me, for moment let me simply say there exists an axial resistance, cytosolic, between a piece and another of membrane.

Here this is soma and this is dendrite. If you want it is very similar to what we did when we saw electrical synapses. There were two different cells, here is same cell and there is no connexin, here there is simply a hole: that is this soma is in direct electrical contact, ions can move and can enter and exit from this other compartment. And this other compartment is made of membrane and therefore from here to outside — inside, outside — there are capacitive properties, there are resistive properties (because evidently there are membrane channels), only I repeat that here is sufficiently long to not be able to assume anymore that it is isopotential.

Here if ions move they encounter a resistance; distance is sufficiently large not to be able to be ignored with a fact of proportion: "this as function of this, this is a much larger [resistance] and so that one I neglect". No, I cannot neglect it anymore because object is large, is large and long some ten micrometers, some hundreds of micrometers. There must be some property that tells me if a thing is long or is not long and we will see it later: comes out of cable equation.

Distributed Models and Axial Resistance And doing so I can write… beyond fact that here I should write in theory a differential equation writing $C\frac{dV}{dt} = ...$, and then so I would write a differential equation probably with two derivatives, or a second derivative, so I would write a system of differential equations. It is not anymore as trivial as $C\frac{dV}{dt} = ...$, it is not anymore a simple charge balance equation. But for simplicity, in this extremely simplified scheme — and simplified, I repeat, because actually here I should describe it with a cable, not with another what is called compartment — I simply write charge conservation, that is **Kirchhoff's Law** of currents, for this node, assuming that extracellular space has its own resistance. But this is not enough for me alone, there must be also this [internal resistance] to have this diversity between soma and dendrite.

For continuation allow me to put aside for a moment this second component of theory of volume conduction, because we must surely develop first an *insight*, an intuition, or quantitative considerations on this dependence on space, on spatial coordinate, on shape, on position in dendritic tree in 3D, of what are electrical phenomena linked to excitability — actually in general electrical phenomena in case of neurons — and in fact we approach what is called **Cable Theory**.

Surprisingly it is not such an ancient thing, it is not so ancient the use of this formalism, of this similarity of cable equation in a neuroscientific ambit. Now, this **Wilfrid Rall** [transcribed as Rohl], who is father of this formalism, worked on this theory during '50s and '60s (I believe he passed away very few years ago and he is a giant, you will guess why), and his motivations to use description of cable theory — in general to describe mathematically neurites, dendrites

and axons as objects that are spatially long — was not to understand origin of extracellular potentials, understand why they have a hump up, understand a hump down or in general why… this we won't get to, but why if EEG does like this or if does like this has to do with synchronized or non-synchronized population activity (so *slow wave high amplitude* or *high frequency low amplitude*, but anyway doesn't matter).

His motivations were that actually most of synaptic input currents are not at SOMA. Biologically neurons do not receive all synaptic inputs at soma; they receive a part certainly, but not a considerable part, unless exceptions. So somehow possibility to understand how change currents generated by a synapse that *impinges*, that insists in a distal point and somehow evidently propagates electrically up to soma (me where I have an electrode and see an excitatory post-synaptic potential, inhibitory post-synaptic), is worth understanding how it works.

And another motivation is that — this is more similar, more realistic, more linked to aspect of generation of extracellular potentials — is that anyway currents, I repeat, must be… to be able to be a circuit, must be closed, so these currents must anyway flow outside membrane of a neuron to be able to close circuit. So somehow, despite being extremely convenient, useful, particularly precious in some contexts (in which you will see it those who will do neuro track in a course with me next year, population behaviors have some type of very simple description when however complexity of space is thrown out the window), however in current considerations, and I showed you simply with generation of extracellular spike, a *point neuron* leads nowhere.

Attenuation and Propagation in Dendritic Cable Intuitively this Rall and colleagues had idea that if synapse could be conceived as a current source (you know that actually it is a conductance change, that changing conductance lets flow in accordance with an electrochemical potential for ionic species to which post-synaptic receptor is permeable, a current, but it's not that it is a current generator), but imagining also that it is in simple way a current source: so synapse activates somehow and here by magic flows an electric current. You could think that, always for this reason of circuit closure, it's not that this membrane is impermeable: there are lots of ion channels, maybe most are passive (that is to say are not voltage-dependent or ligand-dependent).

And so if electric current is very intense here, as it moves, as it flows along lateral section of a cable, of a dendrite, it becomes smaller and smaller because in distinct points (here I dramatized it and put it in discrete points), for charge conservation, for Kirchhoff, if a little bit of current escapes out, this that remains must be original one minus the one that escaped out. You can think of it as conservation of mass in case of flow of a torrent that has effluents, so has losses, or a pipe that has losses. If pipe has losses it is clear that downstream you will see a quantity, a flow — I must say pressure or velocity, I don't know, I have to think about it — anyway a quantity that is reduced because part of mass was lost before.

So here intuitively if current changes it means that this structure spatially behaves differently. Note: current escaping here could be a fraction of current

passing and if this current here is smaller (little arrow is smaller compared to little arrow here that is nice thicker), it may be that from electrical point of view, Ohm's law through these channels, potential might have a gradation, might not be same in different points, precisely because current is not same in different points.

So armed with this intuition, let's look at spatial properties and start reasoning about fact that intracellular medium, cytoplasm, must have some **axial resistance**. So not only a transmembrane resistance or conductivity, but also inside axis of neuritic structure there is a certain resistance or conductance (it is same thing), and it is important that, as said in first models, the membrane not be clumped in a single point, but be a distributed quantity. So I don't have a membrane capacitance: I have a membrane capacitance here, then I have a resistor, then I have another membrane capacitance here, a resistor, a membrane capacitance. This is the cable equation. We see it.

In particular this made sense above all in the 60s and even more so today because the shape of dendrites is not so uniform in the nervous system. There are dendrites like those of **Purkinje cells**, the most important inhibitory cell of the cerebellum, that have a frightening complexity, not only because it is a tangle of arborization of the same cell, but because if you could put yourself in the plane of the slide, you would see that this arborization stays only in one plane: it is like a hand, my hand here you see it extended, but from the side you see it thin; it is not a bush, which is an intricate crochet work that is only two-dimensional, so it is as if really I put myself like this, from the side, and I see it thin. Other types of neurons have different morphologies, so somehow I expect that the spatial properties of a Purkinje cell here, here and here, are very different from this cell that probably is a stellate cell of the cortex which instead has a very different dendritic tree.

So both the fact of the impact of remote dendritic activation, is important to understand what is the impact on the soma. I repeat, it could be that in this big mess a synapse that is here or is here or is here behaves very differently from a synapse that is instead proximal, a somatic synapse, close to the soma. And in particular, what does a synaptic input do here? Maybe it doesn't make a neuron fire, maybe intuitively being distant it attenuates a lot… but does it attenuate or does it not attenuate? Does it attenuate and change shape? What hopes do I have to understand something of the nervous system if I record always from the soma and see events that maybe have a different shape? It is the echo of a filter: in the end we are engineers, so as soon as I start talking about cable, as soon as I start talking about capacitor, resistor, capacitor, maybe in someone's mind says: "Ok, I don't know, it will be a filter, it will be a low-pass filter, maybe distributed". But every time I have a capacitor with a resistor somewhere it is a filter, it is a low-pass filter, maybe elongated.

What is seen is that — so the result is — not only dendrites are not isopotential, electric potential attenuates from synapse to soma. Actually it is also interesting that if there is an action potential in the soma this can **back-propagate**. I am not talking about active propagation that happens in the axon and of which we don't speak, but of the fact that a potential in the soma can propagate towards dendrites attenuating. In the end a cable is a bidirectional thing: I can talk from one side or I can talk from the other side, it would seem that it can be

symmetric. Electrical engineers in the room would call this duality principle? No, **reciprocity**, it is a property of reciprocity. If I inject in one point and record from the other I should, exchanging generator and observer, have the same phenomenon. Actually it is not perfectly reciprocal, we see why.

Moreover there exists a kind of propagation time so that a distal post-synaptic potential reaches the soma. Careful, I call it propagation time, but it is not a propagative phenomenon like electromagnetic waves are or like the propagation of an action potential along an axon. In that case, when one says propagation, one has in mind the wave equation, which I won't talk to you about, but you have a waveform, a signal, that is always in time, is always identical to itself; if recorded in another point at a certain interval later, it is still another point, it is always the same waveform. In the case of the axon it is effectively a propagation, because there exist ion channels, voltage-dependent, sodium and potassium, along the entire extension of the axon cable. This has as result the fact that potential continues to self-regenerate. But we don't talk about it because the most basic part, most elementary, most to be understood in a way not necessarily only numerical, are passive dendrites in which there are no active conductances, there are no voltage-dependent conductances. But there is nevertheless a kind of propagation time, so not only activity attenuates, a distal synaptic potential attenuates, but it takes time to interest, to invade the soma. And the shape of this signal, so from the site where it was initiated up to the soma, changes and changes with distance. So in theory if I see it at the soma I could understand from how far away it happened depending on how much its shape is deformed. All things that are of unheard-of power, very interesting, if one knows the mechanism with which this change of amplitude, time and shape are generated.

Spatial Discretization and Specific Parameters

So, this is the first impact with the cable equation which in fact represents a spatial discretization of what are properties of a continuum. I call it continuum in this case because I am thinking simply of capacitive characteristics. The membrane of this cable, of this dendrite, besides the soma if you want, is a collection of a phospholipid bilayer. Yes it is true phospholipids are molecules that are discrete but I can think that they are so small and so numerous that between two points… in two points taken randomly I can continue to have that comparison, that parallel with the parallel plate capacitor. However, thing that for example I cannot do with transmembrane conductances (because there I already spoiled you, we already talked abundantly of Neher, Sakmann, of the discovery of the discrete phenomenon, there it is a discrete phenomenon, channels are not continuous, are here, here, here, maybe there are lots of them), to make it brief, the approach that is done — and is done also with the cable — is that first to pass into discrete, that is to say to think, as I did a little ago for a dendritic compartment and a somatic compartment, I say that membrane is not a single capacitor with in parallel a resistor (yes there should go Nernstian reversal potential), but is a combination of blocks, of these cells.

Excuse me, here intracellular part is below, *inside*, intracellular cytosol. In fact I was saying: "but why aren't there resistances here?". Ok, whatever, there is resistance outside, it is thought that the medium is isopotential. Then we will

see that it is not so, otherwise extracellular potentials that I showed you before, in one point were such, in another point were another value, another waveform. But at this level what I need is to emphasize the fact that axial intracellular resistance is not null. And you see that this little block repeats.

Here it took Lord Kelvin to establish something like that for an electrical conductor, in which electrical conductor had… actually had a conductor and had a *shield*, a shielding, and so there were capacitive properties between conductor and insulator. As now if you cut a piece of wire, you see most insulated wires have a metal mesh around, ground, with respect to which signals are referenced (for example I am thinking of a USB cable; actually inside USB cable you have 5, 6, depends on USB cable, anyway you have multiple conductors). So between conductor and mesh around you have a capacitive effect and also potentially a conductive effect at a certain frequency. And inside you have a trifle [negligible resistance]. Here it is only in biology that you have a resistance between inside and outside, otherwise you would have a short-circuited electric cable. Kelvin had capacitor and resistor alternating like this.

Specific Membrane and Axial Properties This structure we define with accuracy, in particular we talk about properties of capacitance, of transmembrane and axial resistance, referring to **specific** characteristics, because it is convenient, now you will see in what sense. In general, I for example told you that a value that you have to remember, otherwise you fail the exam, is that **specific membrane capacitance** (C_m) is 1 $\mu F/cm^2$. So I am interested, because I don't want for the moment to make a commitment on what geometry is, I want to have magnitudes that are specific, which means they are independent of choice of geometry, for the moment. Shortly, clearly I instantiate them, because once I did discretization I have to go down: "ok, but how big is this, how long was this little piece and how much is lateral surface?". So, first element is capacitance, one microfarad per square centimeter.

This is another value, **membrane resistance** (R_m), that I sold you always as membrane conductance, but it is the same thing. The only thing to pay attention to is that if conductance like capacitance tended to increase the more surface was large (surface in this case is a cylinder, so the more lateral surface of this cylinder is large, the more pores there are, so the greater conductivity, conductance), if I — and now you will realize, simply for mathematical convenience it is better to reason in terms of resistivity… pardon, specific resistance — you see that this is not divided by square centimeter, it is **times square centimeter** ($\Omega \cdot cm^2$). Thanks: conductance was… tended to increase, was divided by square centimeter [Siemens/cm^2]; since resistance is inverse of conductance, also specific resistance is inverse of specific conductance. Here millisiemens becomes kOhm and what was divided by square centimeter goes to numerator becomes times square centimeter. This is the only thing that requires attention.

Instead this number I never told you, it is **axial resistance** (R_i or r_a) and has as value, again, 200 $\Omega \cdot cm$ [Ohm times centimeter]. Note, this is a transmembrane resistance, the more surface is large, the more this resistance is small; while here the more cable is long, the more resistance is large. This does not have to do with currents crossing the cable, but those flowing in cytosol inside. For this you see that it depends linearly… has as unit of measurement… so linearly

in length, not in surface. But still centimeter is unit of measurement, but here square centimeter is because surface is lateral, here is section, longitudinal length [volume resistivity].

I point out to you however that the famous RC continues because specific characteristics per unit of surface simplify, and it is right that it be so. If I do $C_m \cdot R_m$, square centimeter and square centimeter cancel and numerically I obtain what is time constant of a membrane that I told you to be around $20 - 50$ milliseconds for most neurons. So these numbers are not up in the air, they are experimental measures that somehow I give you to fix ideas.

What we do is that morphology even complex of a neuron can be approximated to a kind of combination of pieces of cable, pieces of cable that have a different length and a different diameter. So this part here for example is relatively short but has a large diameter; where there are bifurcations, ok, electrically it will mean that the piece, the start point of a cable — there will be boundary conditions, now we see what they are — coincides for example in terms of current or in terms of potential with end of previous cable. So a structure morphologically even complicated — ok this is not particularly complicated — translates into a sequence of cables. So in the end this is interesting because here is a two-dimensional stuff. Instead in case of cable and of a combination of cables, ok, I have to say on which branch I am, but I have only one linear coordinate, one, a single value. This means that equations I pull out are one-dimensional equations. Yes, it is an ugly partial differential equation, actually it is not so ugly because it is same equation of diffusion and of heat propagation. So perhaps some of you have already seen it: particularly electronic engineers do in semiconductors equation of charge carrier diffusion and in heat dissipation in electronic devices do heat equation, of heat propagation. So here it is convenient for me because it becomes one-dimensional case.

So I know what are specific properties: capacitance, transmembrane resistance and axial resistance. If one gives me: "take this little block with this surface, with this length", it is not a problem. Capacitance of that piece I obtain multiplying this by surface. Here I obtain resistance dividing by surface and here I obtain resistance dividing by length… so you have to give me diameter from which I derive lateral surface. So suppose one gives me radius, $2\pi r$ times length L is external surface of cylinder and L is length; so I divide here, divide here, multiply there. I can pass in this way trivially from specific magnitudes to total magnitudes, total capacitance, total resistance, etc.

I take therefore a little piece, I call it… for moment it is not infinitesimal, I make it become infinitesimal because otherwise one doesn't have fun, derivatives don't appear, there are no limits and difference quotients. Because there we go to parry always; for some reason, maybe I was saying it at beginning of this course, nature has always… physical laws have a structure for which it is easier — at least in classical case, I don't know quantum mechanics — description is through laws that say how things change in time and space. See Newton, see electromagnetism, etc. Here I do same thing. I take a little piece that is long Δx, has a diameter a (so I area know how to calculate it, pardon, radius is a, yes), is $2\pi a \cdot \Delta x$, is lateral surface, because it is there that is piece of membrane with phospholipid bilayer; instead of being made as a sphere it is made as a cylinder. Inside is cable. We will see what happens at sides, caps, there will be

caps, but for moment it is cable, so capacitive properties are of lateral surface. You could also think that a is very small compared to Δx, so effectively *aspect ratio* — I don't know how one says in Italian, ratio between length and height — favors length; in fact neurons are objects that seem long, don't seem squat and fat cylinders. Another thing I need is passage section, which is simply πr^2, πa^2. And at a certain point I say, since it changes with position, it is not enough for me anymore to call it membrane potential at time t, I have to call it at point x and at time t, in most general case possible: $V(x,t)$. Why? Because it is a function of x that potentially changes in space.

So here I… excuse me I said a bullshit, for part of axial resistance I don't divide by L, by length. It has unit of measurement ohm times centimeter because if resistance is resistivity times length divided by surface or divided by area, ρ becomes ohm times… this brings it to numerator, becomes, suppose, square centimeters divided by centimeters. So there continues to be both a dependence on length and on surface, on passage section, but dimensionally it is for this that it depends on ohm times centimeter ($Ohm \cdot cm$), because so it is not only how long you are but how wide is passage section obviously. So I apologize, here "ohm per centimeter" had made me think that counted only length, no counts also… this is correct formula. Yes, since these here have same unit of measurement, one squared and other no, square disappeared for me, so it seemed to me that there was no more a dependence on surface, but actually there is. In fact it comes here when total axial resistance is given by exactly that formula there, where R_i was resistivity times length divided by passage section. I remember this thing here because intuitively the more a thing is long, the more it resists, while vice versa the more it is wide, resistance is lower, because I increase possibility of current to pass. I think always of a hydraulic analogy, for example, or pneumatic. For capacitance there is no problem, I multiply by surface and for transmembrane resistance I divide by surface. Ok, here is simply remembering how one calculates lateral surface of a cylinder and how one calculates area of a circle of given radius, πr^2. Ok, here are numerical values.

Chunk 6 of 6

Discretization of the Cable and Kirchhoff's Law

What I have to do now is consider that I don't have only a small little piece, but I have a little piece preceding and a little piece following. I know that at a certain point there will be start and there will be end, but let me imagine for simplicity to start in middle. If I start in middle it is easier.

So $V(x)$ describes transmembrane tension of a small little piece of cable, but then there will be $V(x+\Delta x)$ on right and there will be $V(x-\Delta x)$ on left. Since electrical properties are dependent on position, this little block here surely has a capacitive current and a resistive current passing inside this little cylinder. However, since also preceding little block and following one have same thing, it will be presumably a balance between an axial current entering here and exiting from other side. It is not so different from electrodiffusion equation, if we did it

(at this moment I don't remember if we did it), in which one imagines in that case charge conservation.

Here I am in a pure electrical context, which I show you in next slide, and start looking at that electrical circuit of a little ago: intracellular part is high and here is extracellular part. In fact here there are… it is isopotential outside for moment, for this chapter is outside. And here at end I am in effect considering a so-called structure, a ladder electrical network (**ladder network**, I believe one says ladder), in which in theory this object here is infinite in this direction and infinite in other direction: these blocks repeat. So in theory I could be rightly frightened and say: "I don't have theoretical tools to be able to treat a circuit that has no end, a circuit that has distributed parameters and is somehow here implicitly an infinite cable in all two directions".

But I show you that it is possible to reason taking a piece in middle, preceding piece and following piece. These three are enough for me. These three are enough for me because somehow there is some symmetry, things repeat. For this piece that is preceding, there will be a piece still preceding that supplies it an axial current, which in turn for Kirchhoff's Law a bit goes in here and branches into a capacitive piece and into a resistive piece, and here continues in another axial component. This axial component passes through a resistor which is axial resistance of cable and again then bifurcates into a current, redistributes into a capacitive, resistive current and another piece of what survives.

Already here you can imagine that intuitive discourse I made you — a current flowing, then is working — at a certain point I imagine that, since this redistributes in this plus this plus this, this here will be lower than that. So as spatial coordinate increases, current will become smaller and smaller. Just to tell you that current is changing: I cannot use same current; current here entering is not same current entering here (aside that there is a resistor, but then there is this branch here in which part of current went away).

Anyway, here in theory you can "plug" yourselves, you can turn off brain and say: "Ok, he is giving me to solve a circuit, so an exercise of electrical engineering". Clearly I do it, I am emphasizing it, dramatizing it to try to simplify it, because I know that not all of you have [studied] electrical engineering like this or some of you haven't even studied it.

So, since I am in phase of calling with a spatial coordinate quantities, I say that this — I define it — is axial current $I_a(x,t)$, because in theory it can change also in time. This I call $x + \Delta x$, so it is the one exiting. This I call $V(x,t)$, transmembrane potential in that point; this I call $V(x + \Delta x)$ and this $V(x - \Delta x)$. I don't need anything else. I don't need anything else because these C, R_m and E I assume are same. If they were space-dependent, ok, I should take it into account. Maybe they are numbers, so they are not dynamic magnitudes changing in time, but they could change in space: it could be something that is not uniform. It means "is not same in all points of space". Why? I don't know, because simply it could be that cable thins. If cable thins radius changes, capacitance changes, resistance changes, axial resistance also changes. But in this case we are thinking of a uniform cable, simplest case.

What I do is I write for this node charge conservation, again Kirchhoff, law of currents (algebraic sum, so take signs as hell you want, provided then you

are consistent with constitutive equations), and given this node or "shell" — so this closed surface that does not cross any electrical component but crosses only wires — and by convention give a sign to what enters and to what exits. I did it a thousand times, so intuitively I see that here enters this thing here and exit three other terms. So yes, I should have put everything at first member, I_a with plus and all others with minus, but also as charge conservation I like to think that balance is that I_a enters and is equal to what exits: I_c, I_m and other I_a. It is only annoying because now we have to write x, $x - \Delta x$ (ok here there is not $x - \Delta x$), we have to write $x + \Delta x$ and all quantities in theory can change with time.

I mark aside what is I_m. I_m I know what it is because that is usual, it is story of current with ohmic model: $(V - E)/R$. $V(x)$, because this is $I_m(x)$, transmembrane current. And transmembrane capacitance, I don't write it yet, but transmembrane current is $I_c = C \cdot dV(x)/dt$. But I write it in a moment.

Here I wrote myself I_a and I wrote it as, for Ohm's law, current flowing in this resistor given a particular potential difference between this point and this point, and known resistance. Resistance I know because it is total resistance R_i, that trans… excuse me, that axial. And since I took current like this, going from left to right, is ΔV divided by R. And this ΔV is V here, which is $V(x-\Delta x)-V(x)$. Ok, and this "mess" here I write it. I would like in other terms to try to write a relation having only V at end of ends, so I move forward, since I know what is constitutive equation of a resistor.

Only it is not finished, because I have also this $I_a(x + \Delta x)$, and it is exactly same thing for constitutive equation, Ohm's law. If I take arrow like this, it means that this current is a ΔV divided by R: R is same, is always this axial resistance, and is difference between potential being here minus potential being here. And I read it: here is $V(x) - V(x + \Delta x)$.

So you can anticipate that I won't get away smoothly with story of difference quotients, because I have both $V - \Delta x$ and $V + \Delta x$, and difference quotient wanted only to have $(V(x + \Delta x) - V(x))/\Delta x$. Here I have also middle, in short it is a bit more complicated, and in fact first derivative is not enough, in end it will be only that… but we do it step by step.

In meantime capacitive current appeared, which I hoped arrived later, this one: capacitive current is one I recited before, $I_c = C \cdot dV/dt$, with dependence on position. And I can simply rewrite this equation substituting terms. It is ugly, but it is not difficult, it is algebra, at this point it is algebra, I did nothing else. So I can see, I can note that here $C \cdot dV/dt$ appears, so appears already as a total derivative. What evidently does not appear yet as a derivative — and I expect it must appear as a derivative — is dependence on space, what I would call a spatial gradient. This is a change in time, a change in space. I here see change in space, but it is discrete: here is V, so difference quotient before, then there is difference quotient after, and then this is again transmembrane current.

Derivation of the Cable Equation and Taylor

Maybe I would like to massage this equation, I rewrite it there. Again, like all other derivations, try to do them yourselves. I repeat, the only thing for

example to remember here is the little circuit. If you remember the little circuit you can write everything else. If you try to remember this expression here by heart it is more complicated, in my opinion you won't manage to remember it. I don't remember it, I have to re-derive it every time.

The thing I do, if you allow me, is change the variables because it annoys me that I keep carrying around $V - E$. If you do this change of variable, small v equal to large V minus E ($v = V - E$), you discover that when you do the derivative in time, the derivative in time of v is equal to the derivative in time of large V, minus the derivative of E. But the derivative of E in time is zero because it is constant, so here basically it comes out exactly the same with the small v. This is by definition small v at the numerator, because I did this change of variable.

Here and here you have anyway the difference of two quantities, each of which carries a minus E, an offset. When you have the difference — I think of the famous differential amplifiers… no, they aren't called differential, they are called? Are they called differential amplifiers? — in which common terms are cancelled. In the end it is a similar thing: each of these, each of these terms has small v minus E, then there is a minus, small v, minus minus plus E; the E and the E cancel out. It happens both here and there. So this expression becomes a little simpler, simply because I removed this part here and it is only an idiosyncrasy of mine, it disturbs me a lot to have that $V - E$. I don't want to have constant terms, I want to have terms that are referenced to the resting potential. But it is simply a change of variables, it is simply changing the labels on axes.

The other thing I did is bring this term here to the first member and you see that it appears… but I didn't do anything else. You see that $V(x - \Delta x)$, $V(x + \Delta x)$ appears and then appeared with the minus sign here, minus $V(x)$ and also here there is minus $V(x)$ because I brought it to the other side, for this reason it is written $2 \cdot V(x)$. And this thing here, although it is a little strange, seems to be a kind of symmetry. The tension on the left, the tension on the right, minus two times… it looks like a kind of, exactly, the discrete description of the second derivative.

But now we see it in a simpler way, while on the right things remain unchanged, apart from having done the change of variable, so small v. Here I multiplied both members by R_m, that is to say I brought this thing here, I made the common denominator, but it is easier for me to say that I multiplied both members by R_m. You see that here it became R_m, here it is $R_m C$ and here it simplified. I wanted to do it because by eye things must add up from the dimensional point of view. Here there is nothing, it is millivolts for this $v(x, t)$. This $R_m C \cdot dV/dt$, is a millivolt with respect to a time, times time, times milliseconds, but RC is a time, so time and time simplifies. Again this is millivolts — yes it is a delta, it is a difference, ok fine but it is always millivolts — and in this at the first member I have resistance and resistance that cancel, dimensionally at least, and again I have millivolts. So I can say: "ok, at least from the dimensional point of view there are no big problems, I haven't done, I shouldn't have made errors".

And so I invoke Taylor. Taylor and Taylor for a function that I can expand, that I think of being in the vicinity of a specific point x; I can calculate it both in $x - \Delta x$, and in $x + \Delta x$. Now I tell you why. In both cases Taylor tells me

that the value of the function I can approximate with a polynomial. In this case I stop it at order 2. You will see why I don't go further (because I am not a masochist) but why I didn't stop at order 1. It means having the value of the function when this delta is 0, so it is $v(x)$, minus the increment Δx times the first derivative (excuse me, it is minus because it is minus and the delta is negative, for this there is the minus), first derivative. Then there is $1/n!$ times the n-th derivative; since n is equal to 2, 2 factorial is 2, 2 times 1 is 2, one half remains the increment squared. So even if it was minus, I don't care, it became Δx squared, plus Δx squared, and obviously I have to write the derivative n, n equal to 2, second derivative.

I do the exact same thing in another point, so I calculate… approximating the function calculated $v(x+\Delta x)$ in the neighborhood of x: again the function that has at that point there, the increment times the first derivative, one half the increment squared times the second derivative.

If I write them like this, it comes to me, let's say, I am tempted to sum them. I could also divide them, but in this case it is convenient to sum them. If I sum them, this term of first derivative goes away, disappears, and in this case of $v(x,t)$ it becomes 2, that is a 2 times $v(x,t)$, which is exactly what we had a little ago. So at the first member I have $v(x-\Delta x)+v(x+\Delta x)$… roughly equal because it is an approximation, I am not considering the infinitesimal terms of higher order, I stop at the second order and I stay there… two times $v(x)$ plus Δx squared (because there was a half and a half) second derivative of $v(x)$.

Here I am reading that I can write the second derivative with this strange discrete behavior. If Euler's numerical method told me "do you have a differential equation of the first order? Do you have first derivatives? Write the first derivative as the difference quotient", this is in fact the same thing but saying "by chance do you have the second derivative to write?". If you want to do it obviously Δx must be very small because otherwise the approximation you make of Taylor is off, you cannot move away so much. But since Δx shortly will become practically infinitesimal, it becomes an equality, it doesn't become an approximation anymore. And I can do it if I have the value of the function on the left, the value of the function further on the right, minus two times the function in the middle point, because this formula here tells me so.

So in the equation where I have this "mess" $v(x)$ on the left, $v(x)$ in the center and $v(x)$ on the right, with the minus and with the 2, it is exactly the quantity I have before. So I can take, if you want, this $2v$ I bring it to the left and here remains second derivative of v. So I have this parenthesis here, I write it as a second derivative. It is true, I have to carry along Δx squared.

And I am embarrassed because I always said: "No, wait, Δx tends to zero", and so here I don't have the limit anymore because I wrote abruptly what is the expression of the second derivative, with this discretization. It is for this that I wanted at the beginning to write R_m, R_i and C as in terms of the specific magnitudes, because there I still have the radius, I still have the length which was precisely Δx of this infinitesimal cylinder, and I still have… *that's it*, I don't have anything else. So I have the surface, the length, all the quantities that now I go to abstract, because this equation here holds for the little piece of length Δx.

And ok, this Δx squared remains. Very well. But I would like to have a thing that is not only for that little piece, where that is I can vary V with x from minus 200 micrometers to plus 300 micrometers, assuming that zero is in the middle. You see that it comes out in a moment, remembering that large R_m is small r_m divided by $2\pi a \cdot \Delta x$, aha, I have Δx here; R_i is r_i times Δx divided by πa^2, I have another Δx here. I feel like these Δx however simplify… no, because I am not multiplying them. If I multiplied them yes, because here for R_m the Δx is at the denominator and here for R_i it is at the denominator [numerator?], but I am not multiplying them, so I continue to remain. And it cancels. And R_m and C, which therefore both have the same… depend on the lateral surface in a way one inverse to the other, so multiplying R_m times C, we did it before, came to 20 milliseconds (it didn't come to 20 milliseconds per square centimeter or multiplied per square centimeter), but you see it from here: in this case the numerator, in this case the denominator times the numerator cancel.

So I manage to write an expression that doesn't have Δx anymore, which is remarkable, it is powerful, because the only complicated thing was understanding this thing of the second derivative but it was only Taylor, it wasn't a complex thing of complex numbers, line integration, triple integrals… no, it was a stuff of understanding what is the discrete expression of the second derivative and recognizing it. This equation here, which is second derivative, I have to use the symbol of partial derivatives because these are not total derivatives being a function of two variables. This is a mathematically correct thing, ok, it is important, it is important, I don't want to trivialize it, but in the end you saw where it came out from. I obviously doing this operation was keeping t fixed, because I didn't want t to change, I wanted it to be exactly the same t in all terms of these two equations that I summed, one to the other, in such a way to be able to write that total second derivative with respect to… excuse me… this second derivative with respect to x, also there I should have written partial derivative. Whatever, here I write it and I reconcile with the mathematicians among you. This equation here is the same equation of heat propagation, in which obviously it is not v, it would be T, temperature in time and space depends both on spatial gradient and temporal gradient.

I show you before further massaging… no, I have to take the break, forgive me. So we stop and see what happens when it is not only the cable but there is also a synaptic input, an external input. We stop for 10 minutes.

The Space Constant Lambda (λ)

Good. Good. So, before proceeding, I would like to check things from the dimensional point of view to be completely calm. So we did it before, but I do it again now, because maybe something interesting might come out.

This quantity at the second member is millivolts, it is a potential $v(x,t)$. This quantity is a partial derivative of the function $v(x,t)$ with respect to time and is multiplied by r_m times c_m. We already did it before, that beyond the fact that they are specific magnitudes, given that they have the dependence on space one at the numerator and the other at the denominator, independently of the choice of space, of geometry, they have as unit of measurement that of a time. Among other things it is RC, by ear it is always the usual thing, it is a time. In the end

it is a time constant (τ_m), it is the time constant that I recognize when there are not these spatially distributed phenomena. It is the same usual equation. Remember that v is large V minus E, so if I that was zero and I brought this to the other side, I would have the usual first order equation, constant coefficients, etc. So anyway time and RC for the Δt simplify, so this too is millivolts.

This thing here is a bit more interesting because at the numerator continues to be millivolts. Do not be deceived by the fact that here is the second derivative. You have here the writing of the second derivative. So this is always millivolts. At the denominator there is a length squared. This quantity here, that if you want we try to look at it, is r_m over r_i (r_m/r_i). So r_m was ohm times square centimeter ($\Omega \cdot cm^2$), r_i was ohm times centimeter ($\Omega \cdot cm$), which was what had deceived me, I had initially given a different physical interpretation.

So r_m over r_i — excuse me, they are small, r_m over r_i, they are specific quantities — becomes… r_m over r_i should become centimeter, because ohm and ohm simplify and centimeter remains at the numerator. And so it would not be sufficient, but fortunately it is pre-multiplied, it is multiplied by the radius a, so a times r_m over r_i becomes… is a length squared and so it adds up, simplifies somehow with the denominator of this second partial derivative.

So this quantity here is the square of a length. If you don't mind, I would be pleased to call it λ^2 (lambda squared). By analogy with what r_m times c_m, small r c, I would like to call it τ, time constant. I anticipate that, so not by direct inspection as we are doing, not by identification of magnitudes on the basis only of physical dimension (it might not be possible, or I should be a mathematical genius that I look at an equation and understand things), but this thing here, beyond the fact of the square… so if you allow me to remove the square and take the square root… So this quantity here — excuse me there is also a 2 divided by 2 [in the radius], the 2 however has no dimensions, for this I had forgotten it before — so the root of the radius divided by 2 and of the ratio between the transmembrane resistivity (or specific resistance) and the axial specific resistance is called **Space Constant** (λ).

Perhaps it is the one that tells me if a cable is short or long, depends on parameters, in the same way with which this τ tells me if a time is short or long. Short or long in the sense that in previous lectures, when I had quantities, for example, linked to membrane potential — so in a case with a *point neuron*, a point neuron not extended in space — if I had some phenomenon to my great joy at a certain point maybe membrane potential would have finally dissipated, would have relaxed to the potential for example of rest exponentially. Here, if I have to tell you is a lot or little time passed, I do it on the basis of what is the time constant. If I say that 10 τ passed it means that here the transient has been exhausted.

So I like to be able to normalize the time scale and the space scale with a kind of quantity τ of membrane and space constant, that tell me what is the order of magnitude. Could have in other terms a cable of very few micrometers of length, but due to parameters, their ratio or radius, if radius was very very small, space constant could be even very very very small, so even a length of some micrometer could be electrotonically long, justifying existence of cable equation. You could tell me: "But the few micrometers… anyway, depends on

that lambda, if it is small or is long, if it is short or is long".

External and Synaptic Currents in the Cable

The thing I want to do now is add… because here now from the point of view, since the goal is to understand what happens in different points when there is for example a distal synaptic input, I here do not have simply the endogenous, autonomous behavior, I don't have external inputs. So what I want to do is take a step back, return to this point, to this point of derivation and say: look that here at this node I am putting in the balance of currents — so I start exactly from the same balance of currents, from Kirchhoff to that node — and I would like to be able to (then maybe I put it to zero when it is not there) I would like to be able to inject an external current, a pipette. I want to put a pipette in a central point, maybe of a cable that is infinite, or a cable that is semi-infinite (so has a start and has no end), I want to put the pipette there, in a point, and I want to inject a total current, large I_{ext}.

Or, this I do in a generic way here, in such a way to say: "Ok, if there are no synapses, whatever, if there are no synapses you put to zero the synaptic conductance". So here I put a further branch that describes to me synaptic currents due to synaptic receptors of some chemical synapse, AMPA, NMDA mediated or GABA-A, GABA-B, what you want. So this node here enriches: it is not only entering I_a equal exiting I_c, I_m, I_a; there is also I_{syn}, synaptic, and there is also another quantity, I_{ext}. Note, these here were all exiting, this is the only one that is entering, like also this one, so it will have a minus sign.

So, ok, synaptic I I write it as I have to write it; external I anticipating the fact that at a certain point, as I said before, there are crazy Δx that I want to simplify. So allow me to say that if total current coming out from my amplifier, from my current generator, is large I_{ext}, I write, define — and I can always do it because I am changing symbol, so small i_{ext}, which in fact is a current density — as I_{ext} referenced to lateral surface $2\pi r \cdot \Delta x$. I write it like this because at a certain point this Δx simplifies, so I can carry it along. I can also not do it, but if I don't do it then I have terms left over. In this way I identify it, identify quantity in a single shot.

Sum of entering currents $I_a(x,t)$ plus external current large $I_{ext}(x,t)$ is equal to sum of all exiting currents: I_c, I_m, this blessed new synaptic current, and other term $I_a(x+\Delta x)$. Enough, I finished, I have to redo same little game. Note, only different thing compared to before is that here at second member I have this synaptic current (and whatever this is expression). And then I have this external current that I bring to right, to second member of equation and will have a minus sign. Which is interesting because I thought that at a certain point you inject a current, you inject it positive… ok, for how this equation is written, when goes into right member must have a minus sign. So this was that of before, I can already at a glance say that here I will have a term plus synaptic term minus external term. Do I have it right? Here it is.

So you see that here instead of using small v I went back because due to synaptic term… and here its synaptic here is wrong, because this E must be E_{syn} (E-synaptic). It is not said that it is… pardon, this is an error of mine, so this E here in this equation is E_{syn}. Was right here, was right here, but obviously is

wrong here. In general, if you find then, since maybe many of you will surely have a study even more in-depth during next weeks, if you catch some error in slides, speak, actually, I am grateful to you.

So and… yes but here then ok then error is on this slide here… no here is still large V everything is because here small v still is not there and I don't want to do it, but wanted to show you how equation came without that offset. Because fact of having an equation of partial derivatives, second derivative in time… equal first derivative in time… excuse me, second derivative in space equal first derivative in time plus something, this is cable equation. Here for moment there is not large V and this is its inversion potential that could be zero in case of AMPA and NMDA. So here I have not yet done that operation of offset, because these can be different. Problem is, this was normalizing small v equal large V minus E, this I told you before and I wrote it, maybe I emphasize it still a bit.

So this was that of before, but here this is another, so doesn't descend from this, descends from an expression in which small v never was there and unfortunately this is synaptic E. I think about it but I ponder but should be written as E_{syn}. And here is minus small i because large I multiplied… excuse me divided this $2\pi a \cdot \Delta x$, which is what remains to me after identifying term of second derivative, brings me to say: "Wait, don't write I_{ext} divided, write small i_{ext}, which is a current density", and you are again in game with an equation that in theory by hand we will never solve (now we try, maybe next time, in some specific cases), but numerically yes.

Boundary Conditions for the Cable

And I show you a notebook on Google Colab in which I show you how to use a neuronal simulator that is called, with great fantasy, **NEURON**. And it is a simulator that exists from Eighties and surpassed different generations of obsolete languages and now has a Python interface and is quite easy to use. Enough to write `pip install neuron` and you have simulator installed. There are other things, but you see it with Google Colab.

I want to underline, first, that there is this identification of time constant and space constant. For moment has no sense of why one is called space constant. Is analogous to time constant, so here is something exponential in time, evidently there will be something exponential in space, clearly in some simplified condition, in some simplified regime. And, again, here allows me to say if a cable is long or short.

Now, differential equations of partial derivatives, like this — these are partial derivatives, and V, unknown, is a function both of point and of time — are not really very easy, even from numerical point of view, for this I use NEURON, an ad hoc simulator. I cannot use Euler method. I would be tempted to say: "You know what? This you know how to write with difference quotient term, with increment in time (so in second variable t); this quantity, second derivative with respect to space, you have discrete formula, was simply a passage before, so in theory you can use it". But choice of Δx and Δt is *tricky*, is complicated and there are other numerical methods much more accurate because system risks becoming unstable. Unstable means that is not anymore accurate, solution you

pull out no… what you pull out from algebraic iteration of this that became an algebraic equation has nothing to do with true solution.

So you do it numerically, but every time you have an ordinary differential equation — this who of you has mathematical reminiscences knows that **Cauchy problem**, given a differential equation of type at total derivatives, for example of first order, you know that exists solution and is unique, existence and uniqueness theorem — when you have specified initial condition, initial in time. Here there is also second derivative in space, so you have to specify other initial conditions. Initial conditions in space are called **boundary conditions**, boundary because it is thought they are at frontier, but are initial conditions in X, but are called *boundary conditions*. You have to specify two, because here degree of derivation of spatial derivative is order of second, and one initial condition in time, because here degree of derivation of this temporal derivative term is 1. Without this you don't have a solution, you have a family of solutions.

And is a bit *tricky*, now we see some types of initial conditions, of *boundary condition* and initial conditions.

Sealed End First boundary condition we see is that in which, that is called **sealed extremity**, *sealed end*. In jargon of an electronic engineer, this is an **open circuit**. Means that here current is null, I don't have a membrane, simply current has no termination. If current has no termination I should go… for in that circuit of which I showed you only a little piece and circuit extended to infinity, you had a piece on left, a piece in center, a piece on left… I have to instantiate it to final piece and final piece will have an $I_a(x,t)$ with x equal to L, for example length of L. With this diction I am saying that $I_a(x,t)$ is a generic quantity and I am saying how varies in that point at this frontier, at this value of X, L, length of cable, extremity.

Could be a cable that on other side is infinite, but in this point is finite, ends, at coordinate L (here X_0 could be where you want, doesn't matter). And fact that here current… there is no closure of circuit, if is an open circuit, for Kirchhoff's law — again for little bird standing on electricity wire and has a single little leg on high voltage wire, summer of currents equal to zero — here only current existing is this lateral current: I_a in that point equal to zero. This is boundary condition for *sealed* extremity.

So if I do things correctly I should go look at expression of I_a in that point and instantiate it for x equal to L. If that current is zero means that… excuse me, if at that value current is zero means that current… this has no sense saying current at zero, ok, I have to think because this is not boundary condition… this is same thing here, instead here I am writing that exiting current is first derivative of potential in that point. Excuse me, I have to review equivalent circuit. Is not an open circuit, is a sealed extremity in which here is a membrane and this membrane is such for which there is nothing anymore downstream and current term for this definition is derivative — making Δx tend to 0 — is total derivative of first order calculated in x equal to L.

Next time I have to think about it and tell you in intuitive way, that here is not I equal to 0 by definition. This is a type of boundary condition. I believe who of you has reminiscences of mathematics maybe calls this a **Von Neumann**

boundary condition instead of **Dirichlet**. Vaguely I have this reminiscence: when boundary condition has a derivative term, inserts in a generic class of boundary conditions, of equations that mathematicians solved and studied for millennia, in which this is called Von Neumann.

Excuse me, this I have to review because I didn't tell you well. No, I made a big mess, pardon, pardon. It is current equal to zero! This is current equal to zero, so engineering jargon is correct, engineering jargon is correct: here current is zero because is an empty extremity, circuit is not closed. So I got wrapped up here because basically I wrote what is current in that point; to put it to zero, current in that point I wanted to write according to definition. And definition traces me back however with a minus sign… so this is definition (apart R_i that I wrote as function of small r_i, for this there is this Δx that appeared, a squared pi, Δx, r_i). And this is not $V(x + \Delta x) - V(x)$, but is $V(x) - V(x + \Delta x)$, so this is minus difference quotient.

So from this that is definition I substituted R_i with specific quantity because I am considering an infinitesimal compartment and doing this I realize that once Δx appears to me this is difference quotient apart minus sign. So I can write it as a quantity that is derivative of V, total derivative of V with respect to space, apart this multiplicative term and minus that is important. But since boundary condition tells me that here current is zero, what is current? This, that is this. Means that derivative of solution is zero. Again is a Von Neumann condition. I apologize for mess.

So, here I am saying that is a condition… is a bit different compared to an initial condition in time. Initial condition in time tells me that at time t function is equal to 0, or equal to minus 60 millivolts. This could be an initial condition in time: along all x at time zero, all cable is at *steady state*, is at rest. $V(x)$ for whatever x, t_0 at time zero is equal to a number. Here instead I don't say what is V at that point, I say what is derivative of V at that point and I say that for example that derivative in that point is zero. Ask well.

Killed End There is another condition called **killed or open end**, in which electrotechnical jargon would mean that there is a **short circuit**, so here is as if tension was exactly tension of *bulk*, of extracellular bath. And in this case — this I say at a glance because I know — so this is a **Dirichlet condition** because is a boundary condition of type "in that point you have that value" and that value is zero because extracellular potential is zero. So V in that point L for whatever t is equal to zero.

Another condition (we won't have to use them in large measure and numerically are in part implemented for free, even if examples I will show you are of semi-infinite cables, so somehow is easier compared to this context). Another example, who has a background of transmission lines recognizes many of same conditions. Formerly also Ethernet network cables had to have a 50 ohm termination to function, or if one left them open or short-circuited had other types of behavior, had in particular reflected wave phenomena. Here is a bit different, but context is similar, in sense that case of terminated boundary condition means that here is a *patch* of membrane with particular Ohm's laws, with particular conditions. In this case is simply a resistive load, so in electrotechnical jargon here means I

attached a load, and so for Ohm's law $V = R \cdot I$ at frontier at $x = L$, $I = V/R_0$. And for same discourse of before this current at frontier I can write as a minus difference quotient, apart sign, and this quantity, that which had been put to zero in first boundary condition we saw, is put exactly at value of L. Again, this is a further boundary condition a bit complicated.

Ideal Voltage and Current Generators There are other two boundary conditions, simple. First is that exists an extremity in which there is — see, Hodgkin and Huxley who treated cables, in fact were them in end to formalize these discourses on basis of cable equation of Lord Kelvin — potential at extremity is **clamped** with some electronic amplifier imposing a certain tension, whatever is current. This means that tension at that extremity is given, is known, is what I called as a command function. I have a command on amplifier saying "now put at minus 30 millivolts" and he keeps it, or "now make it change in sinusoidal way in time 30 times a second" and he imposes me that potential. Is an ideal voltage generator applied here and is for this I wrote it like this.

Dual case is that I here have a pipette behaving as an **ideal current generator**: whatever is potential at that extremity I inject a current. This in particular is more interesting. Again, as before we wrote, so here current is not zero, current is given, known, fixed, an assigned value. And since $I_a(x,t)$ at extremity I can write it as that ratio, minus that difference quotient, and that difference quotient I translate into dx/dt, dV/dx [excuse me], I can write that that derivative in that point at x equal to L and value (apart r_i times a squared over pi, that pre-multiplied this derivative) is equal to current you inject. This makes sense because in a moment we inject… take a cable that is semi-infinite and inject from an extremity a constant current. So to do it we will have to use this formula.

Steady State Solution of Infinite Cable

It is only case, or a couple of cases, that we see because a partial differential equation, as said, is a bit complicated to handle, in particular is complicated to handle in case of transient regime. So in transient is complicated (not impossible, as is complicated writing solution of diffusion equation in transient regime).

First thing we try to do is look at **steady state** regimes. Steady state means that magnitudes don't change anymore in time and if don't change anymore in time that partial differential equation becomes a total differential equation. Yes, there is a derivative term at second order, maybe you don't remember how one solves a linear differential equation but of second order (now we review it), but becomes possible, becomes easy writing solution. And writing solution is very instructive because, for example, makes come out story of **space constant**.

So what we do is consider a cable that is **semi-infinite**. I don't put any synapses for moment, I am simply saying is a cable *nature*, without anything. So this is cable equation, with small v, in such a way I liquidate also problem of $V - E$. There are no synapses, only thing existing is me with a current generator injecting at an extremity a known current, that I call I_0, a constant current. So implicitly I am applying a boundary condition and for rest simply I ask myself: by chance, this equation seeming complicated simplifies if I delete derivative with respect to time? Obviously I can do it when solution loses its dependence

in time, that is doesn't change anymore in time, that is is constant, so derivative in time with respect to time is zero.

So this I am imposing. So I have to think that passed transients, if there are any, if I continue to keep here current always constant and turned on at I_0 (suppose 200 pA), so I am continuing at 200 pA here, at a certain point I will have some distribution of membrane potential. And intuitively you can think that for Ohm's law, maybe here, since this is a passive structure and you remember story that when you inject a current here, part gets lost, a part continues, a part gets lost… in end nothing remains, so is conceivable that potential tends to become smaller and smaller. Maybe changes with an exponential, given that exponentials are omnipresent, but need to see, here I don't see yet that comes out an exponential.

Now maybe yes, in sense that this term I put to zero, derivatives became from partial to total and this, compared to all second order differential equations with constant coefficients that can happen to you, is one of simplest. You remember vaguely that every time you had an equation of order n, you had a so-called **characteristic polynomial**. Famous eigenvalues of matrix (you haven't seen it in matrix way), you have a polynomial taking… that is not differential, is an algebraic polynomial, having an unknown for example call s (how did I call it? s), takes coefficients those that are and terms at exponent, terms being in order of derivative. So here would be $\lambda^2 \cdot s^2$. If there was a first derivative with respect to x, you would have s with some coefficient; if here was written plus 1 $dV(x)/dx$, you would have a characteristic equation that not only has $\lambda^2 \cdot s^2$, would have plus 1 times s. And here, at right of equal, you have term having a zeroth derivative, that for convention means (is called zeroth derivative but is function itself) that in case of algebraic is s^0, so order of derivation extended in this case, here is 0, is mapped in this algebraic case. You should have done it in analysis because is one of fundamental things, but here is very easy because abruptly I can write this equation. Is a second order equation, ok, but I don't have to write usual story minus b plus or minus root of 2b minus 4ac… no, here I see it "between quotes" by eye that solutions of this algebraic equation are $1/\lambda$ and $-1/\lambda$. I am not a genius, I divided both members by λ^2, I can do it because is a non-zero quantity (quantity that is not 0) and remains $s^2 = 1/\lambda^2$. Apply square root on both members, pay attention to fact that every time I do square root I have in theory to take plus or minus. If you are not convinced you can try substituting here: also 1 divided… also excuse me, also $-1/\lambda$ works and satisfies that equation.

And once you have these eigenvalues, are modes, are called **modes** of equation, are in fact exponents going to exponentials. So solution of this equation is, apart constants to identify, is:

$$V(x) = k_1 e^{x/\lambda} + k_2 e^{-x/\lambda}$$

One of two has minus sign and makes me happy, other has plus sign and I worry a bit because this thing here means that, ok, here I don't have time, but means that in space I have something exploding, I have potential exploding. But calm, because I have still to identify constants, that is I have to use boundary conditions, initial conditions or… excuse me here only boundary conditions because time is not there anymore. And here reasoning is following, might not

like you but is a hypothesis of physical consistency in which I say that when x is very large potential cannot explode. Here you could be unhappy because you say: "Thanks, you make it fit, is you imposing that doesn't explode, but would explode". Yes, but I search among all these solutions those remaining finite because biologically wouldn't make sense, physically makes no sense if I here inject a current and this cable has resistances, a transmembrane resistance per unit of surface and per unit of length, and so at a certain point quantities must dissipate, there is nothing accumulating.

So if when x is very large, V must remain finite, only possibility is that k_1 is 0, because if k_1 is not 0 this doesn't happen. Meanwhile I identified one of two constants. k_2 I use with this boundary condition: this boundary condition says "do what you want, you have to take solution, have to differentiate it and this is equal to minus I_0, I_0 given". So I remembering what is λ, derivative with respect to x is minus k_2 divided λ times e to minus x over lambda… written like this, calculated in x equal to 0, so e to 0 makes 1 is not there anymore, equal to I_0. k_2 I write in this way, because I wrote λ as square root of radius divided 2 times r_m divided r_i, and to make it brief, whatever disgusting or antipathetic thing can be written there, in end this is **Ohm's Law**, but for a cable. In sense that V continues to be equal to R times I, I is current I am injecting.

Only problem is that at a certain point if I take x equal to 0, if I put myself at this point of cable and look at it, I stamp my eye on it, x equal to 0, $V(0)$ is equal to this quantity, that is a resistance, I call it R_∞, times I_0 (because e to 0 makes 1). So somehow continues to hold Ohm's law, in that point looking like in a binocular value of potential in that point. Clearly resistance has a strange characteristic that is not familiar one, depends on physical, biophysical characteristics of cable. Nevertheless, when I am not myopic and want only to look V of zero (but experimentally I can do it, I can, with a pipette, can inject a current and read membrane potential in that point).

When instead I search generic case and say: "Ok, tell me what happens at 10 micrometers of distance from where is injected current, 100 micrometers, 1000 micrometers, tell me what is value of V", I read it here: is e to minus x divided λ. Is exactly similar to behavior that in time you have with time constants, is to minus t over τ, here is to minus x over λ. For this I called it **Space Constant**.

I show you solution and then we finish, apart this input resistance. Ok, here at cable I see an equivalent resistor, is not so important. In end what I see varying x… note here I wrote, instead of writing x, I wrote x normalized to space constant, so that I can use it for whatever value of λ. Means 1, means I am at one λ, a bit like what geek engineers do saying time in this case… here means if I am in this point, means passed τ, I measure time in units of τ, so I can say one τ, two τ, here is same thing. And I do it to be able to make this graph holding always whatever is λ.

So V, so trivially is decreasing. Here I normalized amplitude to 1, so I wrote… I divided by value of R_∞ and I_0 (that I call R_∞ because is apparent resistance of a long cable) and function is a decreasing exponential in space. Is a… let's say, is very interesting because if you think about it I have a simple dissipative behavior with a decreasing exponential of first order, a simple thing, telling me if you inject, are somehow pumping energy in a point and is a DC regime, a

constant regime, *steady state*, you are continuing to pump, progressively echo of this input is heard but at a certain point is heard no more. Again, and if this wasn't me with an electrode and was a synapse, if soma was particularly distant, if I activate or don't activate, membrane potential at that distance would be negligible.

Obviously cables are not infinite. Here was to say that if you take values indicated for R_i (here C counts no more, in space constant counts only R_m and R_i), with those values I gave you in one of first slides a was missing, radius (here excuse me I wrote d, diameter, ok what is 2 micrometers of radius), space constant becomes 500 micrometers. This sets… says if a cable is long or short with that diameter. If diameter was very small this quantity would be very different, this quantity would become smaller because is at numerator.

To finish I want only to show you what happens and we see it next time in a **short cable**. A short cable doesn't function in same way. Here I "stretched" it in such a way to compare with same distance between 0 and 2, what in reality is a more complicated graph. Here depends on length, since is a finite cable, depends on length of cable how rapidly V decreases. If cable is very long, that is for example is two times λ, then behavior is similar, not too dissimilar from infinite case. But if cable is only half λ, practically is as if not… yes there is a bit of attenuation, but as if didn't count much. Ergo space constant tells me if in case of a finite cable length is short (which means I can not care about cable equation, everything is isopotential, almost), or if no, actually cable is very very long. For example if is two times λ can be long and if is long has a dramatic attenuation in its length, attenuation going from 100% up to 20%, so cable you have to keep, you cannot throw it.

Introduction: Analysis of the Cable Equation

The underlying idea is to approach the problem of understanding the origin of extracellular signals. And the only way to do this, as I tried to tell you intuitively last time, is to have the distribution in space, as well as in time, of the transmembrane potential. In fact, one also gets the transmembrane current "for free," which, however, changes from point to point depending on the morphology of the neuron, depending on the distribution of membrane channels in the neuron's morphology, and depending on the state of the neuron.

To characterize this, which is a fundamental ingredient that tells us how signal characterization works from an extracellular point of view, we must face this apparent "monster": the **Cable Equation**. This cable equation is a partial differential equation, differential with partial derivatives, and it has two fundamental characteristics, two parameters that depend on the geometry and the biophysical parameters: one is the **space constant** (λ), and one is the **time constant** (τ). In this case, which I have rewritten on the blackboard, I am also considering at a generic point — so effectively here I should write x and t — the additional contribution of a synaptic current and an external current, for example, injected by an experimenter, as we did last time and as we are doing again in this case today. It goes without saying that if there is no synapse at that point, this value here is zero, and therefore this term is not present. If there is no one with a pipette, or with an electrode, or with some opsin activated by

light, or with some other "contraption" injecting an external current at that same point, this term is not present at that point.

This is a partial differential equation and it is a "tough nut to crack" from an analytical point of view, and therefore the first way we started to approach it to try to extract some useful information is to study its **steady state**. When one studies the steady state, it means that one looks for solutions in which the dependence on time is no longer there; therefore, instead of the solution being a function of space, of the point in the cable, and of time, in fact, this becomes solely a function of the point because in time it is constant. If this is so, the derivative with respect to time is zero and that transforms into an ordinary differential equation. Okay, it is of the second order, however, it is always linear; it should be something that you have seen and should "swallow easily" based on your mathematical reminiscences.

What we did last time was to consider the solution in the infinite case. One of you basically told me at the end of the last lecture: given that the degree of derivation in space is two and here in time it is one, well, two initial conditions are needed in space — which are called boundary conditions — and one initial condition. I made you a list of all the possible boundary conditions that can occur; it is not that there are always two. In this case, in the case we saw last time, the cable was infinite, so in that case, it was that problem, that geometry, that generated a particular boundary condition for me.

In the case we are doing today, in which the cable is not semi-infinite but is finite on both sides, I think there is a condition, I assume that given the geometry there is some boundary condition here at the extremity where, for example, the current is zero here, because there is an interruption of the cable. So the whole phenomenology of boundary conditions is a catalog from which to choose from time to time, just as the initial condition is what it is; it is not that *one* initial condition exists. The initial condition is somehow, even if we do not consider the time-variant solution for the moment, it could be that at a certain instant t_0 for all values of x, this is some function of the point. For example, it could be -65 mV for every x, so it could be that the cable is at steady state and at rest along its entire geometry, and this would not be an unrealistic condition; it would be a frequent case.

What I wanted to tell you is that there are a couple of ways to try to examine these equations simply. The first is to remove the time derivative, the derivative with respect to time, thus studying the **steady state**. Another one is — and we will see it later — to approach the so-called **sinusoidal steady-state regime** (permanent sinusoidal regime), in which by means of transform operations in the frequency domain (whichever you like best, Laplace or Fourier; we will do it with Fourier in about half an hour), in fact, again the derivative in time disappears. It disappears because in the transformed domain, temporal derivatives become algebraic operations, they become multiplications by — particularly in Fourier — $j\omega$, so even there in that regime, I lead myself back to the case where I know how to solve it.

I will show you the equation of the transient in which I do not make any, let's say, there is no simplification, there is no hypothesis and the solution changes both in space and in time, but I will give it to you "from above," we will not

derive it.

The first thing we do compared to last time, of which I remind you the result was this, is that in the case of a semi-infinite cable — so here at one extremity there is an end and it has as an initial condition me with a pipette, with an ideal current generator, injecting a given current into it — as it was from a certain point of view trivial to expect, if I look at this semi-infinite cable, which therefore extends to infinity over here, at this point here I see a resistor. This should not surprise those of you who have a reminiscence about so-called transmission lines, or electromagnetism, or coaxial cables: in the end, when one measures the impedance at a point one measures 50Ω. Here at $x = 0$ I measure, if I inject I, I measure $V = R_\infty \cdot I$, a particular R_∞ which is an expression that depends on the parameters. The obviously interesting thing is what happens when I consider not V at this point, so at the access port, but along the entire geometry of the cable. We saw that this simple expression of a decaying exponential, where the scale of this *decaying*, of this exponential decay, is in space; it is not in time as we have been accustomed to looking at until now with the solution of the usual differential equation, etc. Here it is an exponential decay dictated by the space constant, λ, and in fact, it is saying that even normalizing for example to 100% what V is for $x = 0$, let's say it decreases very rapidly. Again, it is on the scale of λ, so it depends on how much λ is with respect to the length to say whether it is rapid in space or not, but in fact, it indicates that a morphologically extended structure like a dendrite attenuates. And it was taken for granted in the end that it was so, just like a distributed multi-compartmental dissipative system, made of dissipative elements. There are no transistors, there are no current generators, here it is stuff that is a combination of resistors and capacitors.

In the **steady state** case, the capacitors are as if they are no longer there, actually, they are no longer there, and the effect that remains is that of the behavior of many resistors in series and in parallel; what I mean is that they are transmembrane and axial.

What happens when the cable is not infinite, semi-infinite, but is short, has a length L, so at a certain point here it ends with its boundary condition? The graph changes considerably compared to this. This graph is "stretched" graphically because I took it from another book but I wanted to put it exactly in the… since the x-axis is represented with a variable normalized to λ, such that it can apply for any choice of λ, I wanted to "stretch" it so I could compare it. And what you see for the semi-infinite cable is this curve marked by $L = \infty$, and you see how different it is compared to L being half a space constant, one space constant, one and a half space constants, etc. When the cable is already two space constants long, it means quite long. Long, short, and based on the… you notice it if compared with the infinite case. If it is infinite it means it is very long; very long means in the case of a finite cable that its length is large compared to the space constant. So if there is something either in the geometry, in the diameter — I don't remember wrongly, in here there is the diameter, it varies with the square root because that is λ^2, but λ^2 is proportional to the diameter, pardon, to the radius it is inversely proportional, directly proportional, it has axial and transmembrane resistivities — but for example to the radius, the geometry… so if a little piece of the dendrite of a neuron becomes particularly thin, its space constant changes and a piece could become short or long. Short

or long, in the sense that if it is short basically it is as if there is no particular, dramatic attenuation effect; 0.5λ is there anyway, but it is very different than at the same distance in the infinite case, in the case of 2λ. If conversely λ is very small, that… that could become long… so the value of the space constant determines for me if a piece of dendrite is long or short.

Why does it have this shape? How does one find the solution? Is it particularly difficult? No. It is slightly annoying and boring because to avoid doing intricate calculations — intricate calculations mean boring from an algebraic point of view, not complicated substantially — there is a choice that now bothers you.

So, the choice is the following: this is the solution, I am therefore in the same case where I do not have this term, here I am using the change of variable name, so I am using small v which is not… let's say it is offset, it is referred to E, the resting potential. This part is not there and it is the solution of a second-order differential equation with constant coefficients, whose solutions are, apart from the constant term to be identified, the two solutions of the characteristic polynomial of that equation, that is, which is obtained from that, from comparing that differential equation to an algebraic equation.

However, I start from here, only that instead of writing K_1 and K_2, I write them in a different way. I repeat: by doing so you have no way to understand the reason for this except at a certain point to say "oh well, okay, it is equivalent", it is only to simplify the calculations. I write it like this, so K_1 and K_2 are two terms, now, completely arbitrary, they are two degrees of freedom. I can always define two other values, two numbers, $\hat{A}$ and $\hat{B}$ — they are others — and I define K_1 as the semi-sum and K_2 as the semi-difference.

You might say: "Oh well, do it if you must, but I don't understand why. Couldn't you stay in this world?" Yes. I hope you agree that if I instead stay in this, let's say, I come into this completely equivalent and arbitrary description… because I can always find, if you tell me "look, however K_1 and K_2 have these values", fine, I can write $K_1 = K_1$, I can write a system of two algebraic equations in two unknowns, the linear system certainly has a solution, so I can find $\hat{A}$ and $\hat{B}$ that make these two things exactly identical.

I do it because in the finite case, what comes out in the solution are not only decaying exponentials but their sum and difference. Why didn't I, for example, see them so much in the courses I took of analysis 1, 2 etc… the hyperbolic sine and cosine functions come out, which seem to instill particular fear, but I will show you why they are called hyperbolic sine and cosine.

Expression of the solution with hyperbolic trigonometric functions

So, if I write this thing in this way, as $\hat{A}$ plus… again in a completely arbitrary way, I can note that $\hat{A}$ appears here, appears here, $\hat{B}$ appears in both the first and the second term and so I can factorize them. Because I like to see that here there is $e^x + e^{-x}$ (beyond the λ) and here is $e^x - e^{-x}$, and then there is a divided by 2.

Have you ever seen these differences of these exponentials? They are customary because mathematicians define, by pure convention — but now you will

understand why — the **hyperbolic sine** as the function given by:

$$\sinh(x) = \frac{e^x - e^{-x}}{2}$$

I always remember that the sine is a little more annoying than the cosine, it is "meaner" (*più cattivo*). When you derive it no… when you derive it the derivative of sine is cosine, but sine I don't know, I dislike it; it is an odd function, it is not even, so for some reason, I associate that there is the minus here. The exponential with the plus and the exponential with the minus are always there; the "divided by two" is there because otherwise it would be too simple to remember if it were normal, and so there is this annoying minus. So this is the definition of the hyperbolic sine. The **hyperbolic cosine** is a bit "gentler" (*più gentile*). Again, the cosine is even, it is an even function, and so the only difference is that here there is the plus instead of the minus:

$$\cosh(x) = \frac{e^x + e^{-x}}{2}$$

And they are called hyperbolic functions, hyperbolic trigonometric functions, because if you try to derive, just like trigonometric functions, if you take the derivative of the hyperbolic sine, you obtain something that is a relative of the hyperbolic cosine.

It doesn't take a scientist because if you derive, apart from one half which is a constant, this exponential remains itself and this other one gets the plus. The derivative of the cosine is not minus sine, as it would be in the case of trigonometric functions; the derivative of hyperbolic cosine is hyperbolic sine. But the fact that they are one… not the dual of the other, but they are one that is obtained by deriving the other somehow, also for reasons that I don't know, of analytical nature, of mathematical nature, they have been called hyperbolic sine and cosine.

They are clearly tabulated, so people in the decades or 50 years ago, 100 years ago found them tabulated, so they didn't necessarily need to have a numerical resolution that insisted on exponentials. Today it is not particularly problematic, even if you know that a computer does not actually calculate the exponential; it does it through a series expansion, it has a certain precision. And so on. Somehow, having these functions that evidently with this type of solutions of partial differential equations that are omnipresent in physics, biophysics, and mathematics — again: the heat transmission equation, diffusion, wave propagation with some change — evidently people said: "Okay, you know what? I'm tired of writing e^x and e^{-x}, I work directly with hyperbolic sine and cosine".

So at this point, I haven't done absolutely anything, I'm not even trying to identify the constants, but I realized that these solutions can also be written like this:

$$V(x) = \hat{A}\cosh(x/\lambda) + \hat{B}\sinh(x/\lambda)$$

And like this, I like it a little more. Or maybe not, I was totally fine here, I liked it just fine here with the exponentials: one pleased me, the other exploded, but I could live with it. But no. No, let's use this form, again because if you don't do it you obtain exactly the same result, but it is a little more laborious

and the expressions grow, they become a bit more annoying, they become in the end always sums and subtractions of exponentials.

So having said this I say: okay, this is equivalent, again K_1 and K_2 arbitrary like $\hat{A}$ and $\hat{B}$ arbitrary.

Given that, as in the semi-infinite case, the boundary condition on the left is that it is me with the pipette injecting a current into it and it had this type of form, that is the derivative of V calculated at $x = 0$, the derivative — so it should be the gradient of V — the derivative in space of V at $x = 0$ is given, it is assigned. For example, a function that changes arbitrarily in time or a constant function in time, since I am studying constant functions. So I need at a certain point to write, to know how to write the derivative. However, beyond the fact that one sees that hyperbolic sine and cosine are simple to derive, here it is a composite function: inside there is x/λ at the exponent. So it is obvious that when I do the derivative, by the chain rule (theorem of derivation of composite functions), I must remember to put $1/\lambda$ both here at hyperbolic sine of x/λ — I pre-multiply $1/\lambda$ — and also in the other term, $\hat{B}/\lambda$ for the hyperbolic cosine of x/λ.

This is the expression of the derivative of V with respect to x. I can if I want calculate it at $x = 0$. For example, the hyperbolic sine becomes 0, while the hyperbolic cosine becomes 1. Ah, they seem like the normal trigonometric functions too: sine at 0 was 0 while cosine at 0 was 1, so a further reason for similarity.

Starting from this, I try to see if I can identify these $\hat{A}$ and $\hat{B}$ with this boundary condition. The second boundary equation is, as mentioned, that here the *boundary* type is **sealed** (sigillato), the outgoing current is 0; which means that the derivative of V with respect to x for $x = L$ here is 0. Since I have the expression of the derivative, I seem to have two conditions, two constants to identify, I should be "on horseback" (in a good position), I shouldn't have problems.

The first expression links me — since one of the two disappears for $x = 0$, this derivative at $x = 0$, this disappears — it allows me to identify $\hat{B}$. Now I write it bigger for you, it is not that important, I would, however, like to encourage you to do it once, to do all these calculations once in your life. You would understand that, okay, it is boring, you must in this case remember cosine and hyperbolic sine, which could be useful in the future, but in general it is just the whole point… and this equation is how one solves in the case where one studies the **steady state**.

In the other case, this boundary equation, I have to put $x = L$, and it is what it is, in the sense that it is quite ugly. You can continue to verify that from a dimensional point of view things add up, because here I have L/λ. λ dimensionally has the dimensions of a length, or L has the dimensions of a length, so if they are micrometers, they are micrometers divided by micrometers or whatever, so the argument is dimensionless as it should be, but I don't feel like simplifying it much. Oh well, at most I can write here that $\hat{A}$ is minus this term here that I bring to the right:

$$\hat{A} = -\hat{B}\frac{\sinh(L/\lambda)}{\cosh(L/\lambda)}$$

or similar terms. It is a number; beyond the fact that it is ugly to look at, it is a number because I know L, I know λ, my calculator does hyperbolic sine and cosine, or Python, or Julia, Matlab, whoever for him.

Here I have the expression of $\hat{B}$, which is given by the current I am injecting. I am leaving it indicated because I would like to arrive at an expression like the previous one, previous in which I had that $V(x)$ at steady state was an $R_\infty \cdot I_0 \cdot e^{-x/\lambda}$. Here obviously it will be more complicated, it will be in terms of this sine and cosine. In fact, a "mess" like this comes out and even when I change $\hat{B}$ with this expression it doesn't improve particularly. So it doesn't improve particularly, but nevertheless, this quantity here, R_∞ divided by… [algebraic expression] and at the denominator it continues to multiply hyperbolic sine of L, it is a number. It is a number, so it makes me think that also in this case this quantity that is at the denominator for I has the dimensions of a resistance and so, again from my point of view that I am injecting a current here, I see a resistor. I see a large resistor if I inject a current and I want to measure the voltage at that point, which is the thing that most people do.

Obviously, there is a term that again, I repeat, is a number, but it is a number that depends on L, so the longer L is, this denominator changes. And the function, let's say, the functional dependence is given by this hyperbolic sine, which in the end is a relative of those two exponentials. If you plot it, you find this expression, this is the right graph, "stretched", but no longer compressed as it was before.

So in fact if you want, if you think that this whole discourse is particularly annoying or difficult, take Python and try to plot, to use it to plot a function, plotting a function with those values and you would see that by varying the values — now I'll show you a notebook that doesn't do this, it does it for the semi-infinite case — you would see how the solution changes. Again, dendrites are clearly not isopotential structures, that is, that have the same membrane potential along all values of x, and the potential, in this case, the potential distribution is induced by a current injection.

Remember that V in this solution, as also in the other one, this is small v and it is obviously to be considered… if you want big V, you simply have to use the change of variables: small v equals big V minus E, so big V equals small v plus E. But it doesn't take a scientist because you can imagine that, since v is referred to 0, which was the resting point that you conventionally called 0, if you referred it to -65 you must add E here, -65. So the potential attenuates. And if by chance this was not my current injection, but there was another neuron establishing a synapse, so a synaptic bouton, in the periphery of a dendrite and the soma of this neuron was for example here… at the periphery, obviously the effect of that synapse would be extremely attenuated.

Steady-state solution of an infinite cable

Let's quickly see another case. I changed the slides yesterday because I think this other way to explain it to you is more approachable, is easier. The solution in the end is this, and it is the only thing you have to notice — and that I forgot,

I_0 here, darn me, but let it be there compared to the semi-infinite case — this is an **infinite cable**, which means it is semi-infinite in all directions: from here going to the right and from here going to the left. So if I, again, am obsessed with this thing whereby, for example, I have a pipette, I inject a current and there I want to see what the membrane potential is (because experimentally people do this), I can think that compared to the previous case which was semi-infinite on the right, here if I inject a current, the current has a way to distribute itself towards the piece on the left and towards the piece on the right. So it is plausible to me that the depolarization at $x = 0$, exactly where I am injecting a current, is **half** compared to before. This R_∞ is exactly the same R_∞ as the semi-infinite case.

The other different thing — apart from me forgetting to write I_0 here, otherwise it wouldn't make sense — you see that here there is the absolute value, which means that there is a symmetry, this is an even function. The modulus of x means that when x is positive here it is e^{-x}, when x is negative it is $e^{-(-x)}$. It basically means that you have, as intuition could tell you, if in the semi-infinite case on the right you had a decrease proceeding from the current injection point towards the infinite periphery, also on the other side it must be symmetric and mathematically I can express it with this modulus:

$$V(x) = \frac{R_\infty I_0}{2} e^{-|x|/\lambda}$$

I'll tell you generally how I do to get, to reason in this case. The **first boundary condition** or hypothesis I must make is that, as I did in the semi-infinite case, is that V remains finite anyway. Here I have the problem that it must do so on both sides (at positive and negative infinity). The **second** is that the solution, if I study it separately from here to the right and from here to the left, must be continuous; there is no reason why there should be a discontinuity of the first kind at the point where I inject a current. I will have a value anyway, maybe the derivative can be different, maybe yes, maybe no, but it doesn't matter: there I inject a current, I will have a potential drop, but physically I have no reasons to think there is a discontinuity.

The **third hypothesis**, which is slightly more annoying and maybe in this way is simpler, is a slightly different boundary condition, because it is me here injecting a current into it. If I am studying the **steady state** anyway and I have a point pipette, when I say I am injecting a current at a point, mathematically I could translate it — even if you surely don't like it — with not small i but with big I. And now let's see what small i is and what big I is: a current that potentially could change in time but will not change in time, which is given by I_0, a constant. Then to say that it is exactly at that point and not elsewhere, I put a **Dirac Delta** ($\delta(x)$). Which means basically that at that point where the Dirac delta is, which means $x = 0$, at that value… it's true, infinite, you are right, but things work because it represents the fact that the current is concentrated only at one point.

And the only thing I have to reason about is if dimensionally, if this is, suppose, picoamperes, I have to be careful if here, in effect, when I say "1 times" (which is the area of the Dirac delta), is not by chance the Dirac delta a quantity that

has as area something that has… or pardon, the amplitude, doesn't have the dimensions of an inverse of a length.

I remind you quickly that one of the possible definitions of the Dirac delta is with a limit process of a rectangle function. So I call this x, I call this big P, delta. This is a function of x that is 0 everywhere, except from $-\delta/2$ to $+\delta/2$. I am inventing it, actually I am remembering it, but I remember it because it is easy. And here, this is a definition, that is, I am inventing this thing here. Here I say that the amplitude is $1/\delta$. If I try to calculate the area, it is the area of a rectangle: the base is δ, the height is $1/\delta$, the area is unitary, dimensionless. Note however: the amplitude is not dimensionless, it is $1/\delta$ which is an inverse of a length. This thing here stays the same (today I have colors, I just trip out with the little colors, but I won't avoid it): if you make the base, change the delta value, make delta smaller, $1/\delta$ inversely proportional tends to grow. At the limit of delta tending to zero, it is demonstrated that this rectangle function P tends to become the Dirac Delta; it has the same properties, but anyway it has amplitude $1/\delta$. When I use $\delta(x)$ here, it is as if I were using this function here: the amplitude, I must remember, which is $1/\delta$, dimensionally is not dimensionless. So you will see it in a moment, I will have to write Δx here, or I have to change my interpretation of I_0 as somehow a linear density. Anyway.

Let's see the solution. This is the solution in the generic case with constants K_1 and K_2 to be identified. This is basically having implemented, even if in a fairly intuitive way, the first hypothesis; that is to say, imagining studying this solution, the first and second hypothesis: this solution in two parts, $x > 0$ and $x < 0$. In each of the two scenarios you have me starting to get alarmed because there is one of the two exponential terms that explodes (either explodes to the right or explodes to the left), so I have to get it out of the way. And the other, continuity, I obtain by saying that for $x = 0$ the solution for $x > 0$ and that for $x < 0$ must be equal. You can verify that when you put the modulus you have "for free" this situation in which in both cases only one of the exponentials survived: the good one, the dissipative one, the gentle one, not the one that explodes. And so you only need now to identify a single constant.

And obviously, you need this **third boundary condition**. The third boundary condition, in the next slide I tell you how it comes out, and has exactly the same form — again I ask you please, here there is I_0 involved which is missing — it has the same form as the semi-infinite case, however there is **1 divided by 2**. "1 divided by 2" I didn't put it there arbitrarily, it is not put in the current I am injecting. I will show you shortly a simulation that, under equal conditions, a semi-infinite case or approximately semi-infinite (it is infinite like this) in which there is this horizontal symmetry, the depolarization in this case is half of what you would have had before. Why? Because, as said, the current escapes both to one side and to the other.

How did I do it? I took what was still written on the blackboard at the top, the cable equation more complete than this I cannot, in which obviously I neglected this term because there are no synapses; in the middle there is only me with my current density. Here I have to put a current density to make things add up, a small i, and this small i we defined last week as the quantity I inject divided by $2\pi a$ (radius) times Δx. But here, if I use the Dirac Delta, it is not a solution, it

is not just any function; it is a function that in itself carries an amplitude that is not dimensionless and so I must necessarily take it into account and normalize, if you want, from this point of view.

After that I do the following thing. Okay, I remember because I always forget it… the only thing I remember is the space constant and that there is, surely there is a half, there is obviously the root because up there it appears as λ^2, being the degree of derivation with respect to x two times; I only remember that it is proportional to the radius through the root, but I can't remember r_m and r_i if they go above or one below. So imagine if I can ask you in the exam what the expression is without speaking; maybe I help you derive it because Archangel Gabriel didn't come to bring it to Earth: we identified it starting from a lumped parameter circuit model representing the distributed parameter one, and slowly writing Kirchhoff we pulled out the partial differential equation and this term came out. End of aside.

This is the equation I have to study, or rather that in fact I have already studied, but I don't want to study it. I want to see if from here I can capture the condition I need to identify that K. In the previous slide I gave you the value of K directly. I want to show you this way, which in my opinion is the simplest of all, of how it comes out. Here I no longer have the dependence on time, I have all the dependence on x. And also this Dirac Delta is a function of x, it is not a function of time. What I can do… you see that since there was this Δx, here there is no longer the Δx that would have remained.

What I can do is apply the integral to both members. If I remember correctly, maybe two weeks ago, when we were in the other classroom at the Policlinico, I had done exactly the same operation talking about my bank account, in which I wanted to tell you how treating synaptic release like a Dirac Delta it became easy then to understand what happened to a variable that was for example… represented for me the fraction of post-synaptic receptor channels in an open state immediately before the arrival of a pre-synaptic spike and immediately after. I had a stupid little rule that was "after equals what I had before plus a jump"; it came out from a similar equation (it wasn't in time and it wasn't in x and the derivative appeared with the first order and not the second order, ok), but I had made the same speech in which I said: if I apply the integral, here I have an exact differential. Okay, the differential… let's say this kills only one derivative; the exact differential, the primitive, is the first derivative of V with respect to x. Then I had said — and this is basically exactly the same term that was there — this term is a term that I am integrating a continuous function on a zero-measure term, on a zero-measure integration interval, so it is zero. And I had also told you that this was partly linked to a heuristic where when equations had an "ugly" input (ugly from the point of view of discontinuities like this), the output by definition of differential equation — which means that the solution is somehow obtained by integrating, and the integral is a thing that "doesn't caress", that smooths, not like the derivative that favors differences, that amplifies, that is a high-pass filtering, that makes a mess — so the output is certainly always more continuous than the input. And also in this case I can use the same reasoning to say: this is a continuous and differentiable function integrated on an interval of zero measure. Here, apart from these which are known constants, given — also I_0, it is me who says it is 20 picoamperes or

50 picoamperes — remains the integral of the Dirac Delta. Note: I am doing this integral between 0^- and 0^+, like last time. Last time it was around, maybe we called it T_0, T_0^- and T_0^+, around the moment when a pre-synaptic release occurred; in this case, it is in space. I am for simplicity saying that the pipette is placed at $x = 0$, so the Dirac Delta is not translated, it is positioned at 0; that is when $x = 0$ this is… takes infinite values. The integral between 0^- and 0^+ of the Dirac Delta is 1. It is the area. This is… I killed the Dirac Delta with its definition: it is a function such that, a distribution such that when one does the integral in a range where it is defined (in the limit case even 0^- and 0^+, because it stays in there), the area is unitary.

$$\int_{0^-}^{0^+} \delta(x)\, dx = 1$$

And so I find this which in fact follows the boundary condition that I would have had in part… that I would have had let's say remembering that I was injecting current, so there was an additional current term in the Kirchhoff current balance at that node: one for the solution going to the right and one going for the solution going to the left.

It is easy, once one writes this quantity, to calculate it in 0^+ and then minus calculate it in 0^-. One discovers that this thing does not vanish and K takes the value you obtain precisely from… putting this here becomes minus $2K/\lambda$, so the 2 comes out from here; what then becomes at the denominator one half (I told you the fact that the current escapes both to the right and to the left). And the other terms (r_m over $2\pi a$) are at the end and λ which is due to the derivative, are found here. So this slide here tells you how I managed to pull out this solution in the previous slide. Clearly here there is "times I_0", I have to modify it and I will do it at the end of the lesson, excuse me. I thought and continue to think that it is this way… despite it being "oh my God, I am integrating the Dirac Delta", which is exactly what we did together two weeks ago, if you didn't die (some of you didn't die at the time). Also in this case you could say: "Okay, here it has a different meaning, totally different; it is me with the pipette and the fact that there is the Dirac Delta is because I am exactly in a microscopic point [without] any spatial extension"; there it was in time. *That's it*, ok.

So here with V, instead of small v, big V, is more something… is more one half, and here too I forgot I_0. I_0 I don't like, probably because it was… because partly the area of the Dirac Delta. In the infinite case, infinite in two directions, again: in each branch you have, as was intuitive to expect, an exponential decay.

It is as if it were exactly the same distribution you would have in terms of temperature if you had a metal bar and you had a heater, a fire at a point. It is clear that to the right and to the left you have gradually a reduction in temperature which is maximum at the peak. *Idem*, the same thing.

Asymmetry of Attenuation in Space

Here I show you, just to liven things up, that when the cable happens to have a bifurcation point — of which I will simply mention quickly but we won't spend

many words on — you basically have this symmetric trend. Here there is a further change at the bifurcation point and I put this figure because intuitively, if you understand here that the current entering this point goes to the left and to the right, so the shape changes around this point… when there is a bifurcation point you could think that the current here again bifurcates to one side and the other in a way dependent on what the possible diameter is. Because in the mix, inside that expression of R_∞, there is λ and therefore there is a, or the square root of a.

So the contribution when you have an infinite cable or when the injection point of your current is not… or the arrival of a synaptic input — you see that in the end the current, apart from the minus sign, or the synaptic input are equivalent, roughly speaking; they are always pieces put as input into that differential equation. In this case, precisely, one has a spatial distribution that represents the point where the current injection occurs as the peak.

This is interesting because this very steep attenuation — again, very steep depends on the choice of λ, or rather on the choice… on what λ is for those parameters — has a very interesting characteristic when speaking of **arborized structures** that are not by definition symmetric. In our case infinite in both directions, yes, it was symmetric. But if you have the current injection, as in this case, at the extremity of this dendrite, which is one of a branch, then there is a further branching, a further branching, of a very complicated structure… here there is no symmetry with respect to other points of this cable. If you want, it is a kind of generalized cable having more branches.

This branch marked as S is a branch that, with its own, its own rectilinear, linear coordinate, has nothing to do with, does not share the potential distribution of the other cable except for a continuity connection at the junction point. But anyway, beyond this possible complexity which was understood only by starting to handle even just the **steady state** — as we are doing — the cable equation… and ah, ok, partly brilliant minds see it directly in the analytical solution, others have to do the numerical simulation. Numerical simulation which is required anyway when dendrites happen to have active conductances. Remember that the cable equation, as it is written, holds only in the case of membrane *leakage* properties; there are no — you can put them, but forget about doing the analytical solution — sodium, potassium currents in that equation. You can certainly put them, they would go to the second member, but you wouldn't write "pen and paper" as we are doing.

What is seen is that if you consider zero in this coordinate here as the point of the Soma, which on these — I repeat — this sort of rectilinear coordinate composed of many cables… you consider it in response to a distal current injection — distal means far, distant with respect to the soma — you would see that from here a current input having a certain amplitude would have enormous attenuation at the soma. This dashed line is the distribution of the membrane potential in space, at **steady state**, when the exact same current was injected at the Soma. It is a little bit more, but it is not so dramatically larger.

So, nobody obviously knows, nobody can state it with certainty anyway: perhaps the dendritic tree of such an arborized neuron, exploiting "for free" the consequences of cable theory, has evolved in such a way as not only to increase

the spatial extension like trees with leaves to catch as much light as possible — in this case to take as much input as possible — but the mathematics causes an injection of current in a particular cable, which you see is also thin (so it has a particular smaller λ, has a smaller radius compared to the diameter of this parent trunk), here the depolarization is very high. And at the Soma, roughly speaking, it has the same effect one would have if the current injection were not over there but at the Soma. Clearly, the attenuation is remarkable.

The interesting thing is that you see that while along this linear coordinate — curvilinear I should say — from the injection point up to this bifurcation point the attenuation is very very high (it goes down practically by 50%… here it is normalized, but it is normalized by the… I don't know what it is normalized by, it should be normalized but I don't understand why this is 3 point something; anyway from 3 point something to 1.8, whatever it is, is a remarkable attenuation), look however at what happens to the distribution of the membrane potential due to this distal current injection in this *branch*, in this arborization S: practically almost insignificant. And so it happens also in the other *branches*. So the attenuation is **asymmetric** and it is not a nonlinear equation, it is a very linear equation; what becomes nonlinear, or rather what breaks the symmetry in this case, is the geometry.

And the second thing we can note is that, since each of these domains can have a different radius and length — ergo a different space constant — and can have boundary conditions where they connect here and then here, it can give rise to what is called **synaptic territories**. That is to say: the fact that a synapse is here could make it function largely independently from a synapse that is instead on the other *branch*, due to a kind of electrical isolation due to attenuation. The attenuation is asymmetric, so the electrical behavior in different *branches* is too.

Note that this graph here, this analysis here and these considerations are from '73, so **Rall** [Wilfrid Rall], the father of this cable theory, contributed dramatically in the '60s, '70s and '80s to the understanding even before particularly performant computing resources.

Idan Segev was a student of Wilfrid Rall and is one of the world experts in this type of multi-compartmental description — one says of the excitable properties of neurons — and in particular he has applied this concept to the specific morphology and geometry of some pyramidal cells, human pyramidal cells. And where you see here the part of this dendritic morphology etc. in red — here, here, or here, or here, or here — and the mathematical description represented in colored form of what I showed you before, that is that the depolarization can remain… this quantified in terms of attenuation, relatively localized thanks to morphology. But this depends on where the inputs are applied.

If they are applied at the Soma — and I will show you a demonstration shortly — this is somehow… the attenuation is relatively… it makes itself felt in the dendrites, but it is relatively low. So an input applied at the Soma has an electrical potential effect on the basal dendrites and also on a part of the apical dendrite. Conversely, if the input is distal, at the soma it is practically attenuated almost 100%, while it remains confined in this portion of the dendritic tree. *Idem* in this case; in this case instead it is an input in the basal dendrites which again behaves as a kind of compartment in its own right.

I will show you now one of the simulations. This is a notebook that is at your disposal on Google Colab. If you have problems speak up. So, this other notebook compared to the previous ones continues to be written in Python, but since the problem is to demonstrate easily — I am giving you two, one I added this morning — to study the cable equation, there are two roads: either I use a simulator, therefore a program, a library if you want, whose job is to simulate cables, or I have to implement the numerical method myself or invite you to do the same. As long as it is Euler, even if Euler is the crudest method of numerical integration, things go well. I told you that for that type of partial differential equation things are more complicated.

So what I do in this notebook is use a program called **NEURON**, with much imagination; it has existed since roughly the '80s (from the 80's) and initially had — and still has, however almost no one uses it — an interface based on an obsolete language called HOC (it was a version of the C language with the initial of the creator, **Michael Hines**). He is an authority in the field because after… maybe it could be the mid-80s, anyway after 20-30 years this continues to be still the reference for the numerical simulation of cables, of networks, of neurons etc. Currently for more than 15 years, it has more than 10 years, it has a Python interface and is relatively easy to install: `pip install neuron`. So just as you install a Python library — matplotlib, numpy, scipy or whatever, pandas or whatever you use — you can install this and you have it in the code. You can do it on this Google Colab that you see; I do `!pip install neuron` and I do it with the exclamation mark because I am doing a so-called *escape*: it is a *shell* command, this is not a NEURON command, but it is an operating system command, however Google Colab allows me to do these things. I am in fact downloading from a *repository*, with this command, I am downloading the Neuron *repository* and installing it in the cloud. A few years ago it was unthinkable to do a demonstration or put you in the condition to — without going too crazy — be able to access a simulation with Neuron without having to install 25 things on your laptop, then it doesn't work… so you are lucky to live in a time of ease from the numerical point of view.

What I do, as usual — if you are brave you can see the code, but it is not the object of this course, if not to stimulate some of you to play with it — in which I fix the space constant because I fix the radius parameter (the radius in this case is 0.5 micrometers and with the other parameters has as space constant value 500 micrometers) and what I can do is change the length of this cable.

If the cable is long, you see that the solution with the red dots, which is the numerical solution, seems to describe well — or rather conversely, seems to align well — with what is the black trace. The black trace is the analytical expression $R_\infty \cdot I_0 \cdot e^{-x/\lambda}$, therefore the analytical expression in the infinite case. While the red case is the finite case. So much so that with the *slider* I am changing… how to make this go away but I think not, not being able to do it… I am changing the length. Now the cable is 4 mm, so 4 times lambda… sorry, 8 times lambda. If I make it closer to… now it is 2007… if I make it 900, this is two times lambda, you see that the theoretical expression in the infinite case deviates from the finite case.

You could help me and maybe help me make another box in which not only do we put the expression of the infinite cable, but we put the expression of the

finite cable, the one with the hyperbolic sine that I believe I interpreted that you liked particularly. The expression is ugly but it is not complicated and it is extremely interesting because in the end neurons are structures that can have both long or compact compartments.

Here you have the same thing if you want to use it, in which this is the situation where I have a semi-infinite cable. In the numerical simulation I cannot make a semi-infinite cable, I have to make it long, but the current injection is in the middle and again you see that if the cable is particularly long, the agreement between the red and the black is beautiful: $e^{-|x|/\lambda}$ with that quantity, remember, $1/(2R_\infty)$. If the cable is short, the solution is not… it is also profoundly different; this is about one lambda, the solution deviates significantly.

There is a further simulation here, which basically you have and in theory you can use. This is in time. This graph here represents, varying time, how in different points of a cable the membrane potential changes in time. Let's say, they look like transients that have a different attenuation. Here they are for different values of… I remember what, they are different positions, different positions for different lengths. When the cable is short… in the end it is not seen particularly in this one, so I should basically say that when the cable is very long compared to lambda, the attenuation is very evident and the thing you notice is that the shape seems to be different. Again, to do a simulation like this I cannot numerically in a simple way do the numerical method, I used Neuron again.

Sinusoidal Permanent Regime and "Effective" Space Constant

Let's see then the case of the transient. However, before doing the case of the transient, I consider the transient in the **sinusoidal permanent regime**, in which by means of transform operations in the frequency domain… whichever you like best, Laplace or Fourier; we will do it with Fourier in about half an hour… in fact again the derivative in time disappears. It disappears because in the transformed domain temporal derivatives become algebraic operations, they become multiplications by — particularly in Fourier — $j\omega$. So even there in that regime I lead myself back to the case where I know how to solve it.

I will show you the equation of the transient in which I do not make any… let's say there is no simplification, there is no hypothesis and the solution changes both in space and in time, but I will give it to you "from above", we will not derive it.

This last graph of the Google Colab comes later. Here is the method I was suggesting to you in which to kill the temporal derivative term, instead of setting it to zero — so saying "ok, let's try to understand something", and something we have learned: symmetry, synaptic territories, attenuation, the fact indeed that there exists a difference between a finite, semi-infinite or finite geometry — the other case is that of studying a solution in the **sinusoidal permanent regime** (AC regime).

I do this because the equation is linear and in principle, if I have a signal, a current, a synaptic input that I can decompose into the fundamental Fourier components, I could suppose to study the individual solutions frequency by

frequency and then recombine them; which is the principle of linear systems theory, recombining them knowing the solution, the response of the system to individual frequencies. But at least one generic frequency I have to find.

It is done with the method of transforms where this quantity here becomes, in the domain of transforms, an operation that is no longer a derivative but becomes a product, an algebraic operation. I want to use Fourier because I find it nicer and because in the end I am considering a sinusoidal regime, so Laplace is no longer indicated when I am dealing with transients. And I remind you that the Fourier transform of this function $v(x,t)$ — I am considering it a function of time, it is also a function of x but this is the Fourier transform in time; I could do it also in space but here I do it only in time — I indicate it as W. It continues to be a function of x, because x is not the independent variable I am considering, but it changes with ω, the so-called angular frequency (that is to say $\omega = 2\pi f$, the frequency, in an equivalent way).

I like W because I easily remember that the transform, if I want to transform small v, I have to put it under an integral, minus infinity plus infinity because it must be stuff on the whole domain of definition of the function, there is $1/2\pi$ and then inside there is $e^{-j\omega t}$ in dt. You see that t is saturated being the *dummy* variable of the integral, so this quantity here is a number, it is no longer a function of time; time is no longer there, the integral is the subtended area. Obviously, time is weighted by ω, so changing ω changes this integral. j is the square root of minus one and I use j because engineers usually use j and do not use i, where i would be the current. In fact J is also often used as current density, anyway j is the imaginary number, $\sqrt{-1}$.

If I do this it can be demonstrated — and you can do it calmly if you haven't done it for years, you could try it again — what is the Fourier transform of the derivative of v with respect to t. And you would find that the transform sign and derivative sign can be exchanged because they are linear operators and you would find that what survives is $j\omega$, which is the derivative of this term… is $e^{-j\omega t}$.

I have already done the little calculation because I am sure it is not so crucial, so here this derivative, so where I have v and there is no time I replace it with W, because for this linearity the derivative twice in space of small v is equivalent to the derivative with respect to space twice of W. Here is the different thing and here it becomes $\tau_m j\omega$ times W plus W. I have already factorized and written it in this way, because this is exactly the same form that the cable equation had in the **steady state** regime, when however there wasn't this "pizza" (mess) here, so for $\omega = 0$.

So on one hand I am pleased to find the DC case when ω is zero; when ω is zero this is exactly λ^2 times the derivative now total — it is no longer v, but it is W, because obviously ω counts — this was the steady state case. When it is not the steady state but it is a sinusoidal regime, what engineers call AC regime, obviously it changes. And I write it in this way to be able to suggest to you that it is as if it were exactly the same solution we saw before, the same equation, and it will have the same solution we saw before.

But here I would like to write not λ^2 divided by blah blah… I would like to write **effective** λ^2, that is a kind of space constant that evidently depends on

frequency, which I can put here and I can say: "Ah, ok, the solution is like in the steady state case, but the space constant changes". That is intuitively I think that at different frequencies the space constant is changing. So for slow signals the space constant could be large, while for fast signals the space constant could be small. It is as if a dendrite, or a portion of it, had an **accordion** behavior depending on how the signals occupying it vary. Which is very interesting, but this way I cannot do it because here I have this damned j. So this is a complex quantity. I would like it to be a real quantity.

A slightly boring calculation follows. Again you will see it is not particularly complicated. I apologize, this should have appeared earlier… ok:

$$\lambda^2 \frac{d^2 W(x,\omega)}{dx^2} = (\tau_m j\omega + 1)W$$

Factoring W I can see that there is this term τ_m and blah blah going to the denominator. If I took this quantity here and called it λ^* **squared** (λ_{star}), this effective lambda, I could write this expression straight away. And actually, I write it because it is exactly the expression, the solution of this AC equation, but I am not yet satisfied because this quantity here is a complex quantity. As indeed is the Fourier transform of a function W: it is a function of variables, a complex function of $j\omega$, it will have a real part and an imaginary part, it will have a modulus and a phase.

So if I really wanted to, beyond the fact of the phase which might perhaps not… I would like to at least understand if it is the modulus. I would like to rewrite something like this as modulus times $e^{j\cdot\text{phase}}$, because if I can do it, then at least the modulus I can say: "Ah, ok, this accordion thing depending on ω I can do it". For the moment I still cannot do it, it is the same comment as a moment ago.

So λ^* I define as what I would like to put here to make this denominator disappear. I would call it λ^*. And since this was λ^2, here I put λ and here below I have to do the square root however. And again here it bothers me further because yes, I understood that λ^* is a complex quantity, but if you put the square root at the denominator too it bothers me further. I would have liked to write λ^* as $A + jB$ (real part and imaginary part) and then see if maybe… or rather, I would like more to do modulus and phase, because the modulus gives me an indication of the amplitude. And if I am talking about attenuation, about an amplitude, maybe that is enough for me. Yes, maybe there will be a phase shift that depends on every frequency, the concept of attenuation that could selectively change some frequencies and not others, I could see it with the *magnitude*, the amplitude, the modulus.

So the goal is to write something like this, to transform this exponential with a piece that is a complex number into $M \cdot e^{j\omega}$, where this M evidently is also a function of space, a function of x, and also the phase will be a function of x. But I want to write it like this because I want to look at this M, because "you appeared to me, this M". I want to understand M, even the phase if it were the case. And this M, this modulus, is obviously a function of the point and the frequency.

How do I do it? First of all I note that this is e^{-x/λ^*}, so it appears at the

denominator. To avoid suffering too much I already start studying $1/\lambda^*$, because you see that here at the exponent it is "1 divided". This brings to the numerator $\sqrt{1+\tau_m j\omega}$. If there wasn't the root, could you tell me what is the part… imagining that there is no root… what is the real part of this complex number $1+\tau_m j\omega$? And the imaginary part, whatever it is. It would be easy. The fact that there is the square root is a little annoying, however I could imagine writing that if I square this, I could in other terms say: if I have C, which is some complex number, let's forget the denominator… $1+\tau_m j\omega$, and that's it. I could think not of writing C, but of writing $C^2 = 1+\tau_m j\omega$. Of this I know how to write the real part and the imaginary part, as you did before.

The only rip-off is that then I have to go back. But I can remember that complex numbers also have another form, but not only this one where you have real part plus j imaginary part, but you have… so C^2, would be $A^2 e^{j\Phi^2}$. With this trick, with which I am not going to stress you more than that, you manage to write both the modulus — and again I am probably more interested in writing modulus, at least the modulus — both the modulus… and this is no longer a complex number, thank you, it is the modulus. And the phase, if I needed to find the phase, the phase is half an arc having as tangent τ_m/ω no, $\omega\tau_m$ (imaginary part over real part). The fact that there is the fourth root there and the fact that here there is a half, comes from the fact that here we did this trick: we squared and then we went back, but not with the same form real part plus j imaginary part, but using the vector notation, so modulus and phase.

Here I invoke, I believe it is the only time… no, not the only time, but anyway it is relatively rare in neuroscience to invoke Euler's formula… ah no, I did it also… no, it is not rare. I invoke **Euler's formula**, which if you see YouTube is full, full of videos where the beauty of this equation is explored, etc.:

$$e^{j\phi} = \cos\phi + j\sin\phi$$

Again I am doing "back and forth" (*avanti e indré*) between the two ways of writing a complex number, because I wouldn't want $e^{j\phi}$… I wouldn't want to miss a part of what… repeat, my goal is to write modulus and phase of this equation here. Of this equation here. So I have to suffer, I have to stress this $1/\lambda^*$ a bit to be able to arrive at least to concentrate here everything that is not… that has no complex terms and here the imaginary part, the phase part.

As said I have to do it in stages. The first is to understand this $1/\lambda^*$ how I write it in terms of modulus and phase. Ok, but the phase I can write further like this, so I can go back and write that e^{-x/λ^*} I can write as an exponential $e^{-xA\cos\phi}$ times $e^{-jxA\sin\phi}$. So this piece here is the phase. I, repeat, was interested in understanding this damned modulus part because I would like again to compare it with the analytical solution in the case where there is not… where ω is zero, where I have no complex numbers and where the solution was simple, it was a decaying exponential, with a λ.

Here what λ must I put? I imagine it is some… you see, beyond the fact that A is this clamorous pizza (mess) here, if there wasn't this clamorous pizza it would be $1/\lambda$. So here it would be $1/\lambda$, $e^{-x/\lambda}$, it is the same thing. But in the AC case, sinusoidal, you have this additional term at the numerator, so inside here, and you also have $\cos\phi$, and here you have the cosine of a half arctangent

blah blah. I do it because ϕ also depends on ω, so not only A depends on ω, but also ϕ depends on ω. This part here maybe I don't care, repeat: I am attracted to understand how to do in an equivalent way this blessed sinusoidal permanent case.

There are relations that I wrote simply to explain to the most adventurous… this let's say I will never ask you in the exam to… not that it is complicated, it is not complicated but it is boring and I don't think you can easily remember the expression of the cosine of the arctangent. I didn't even know this formula existed. This one I remembered… cosine, so a bisection formula, this one I think I remembered, and it is important because at a certain point this cosine of the arctangent is a cosine of half arctangent. Anyway, if I were so brave as to do this cosine of ϕ and write it properly, in the end, I would obtain a quantity that depends on τ_m and ω, besides A which is also a quantity that depends on τ_m and ω.

To make it short, this pizza here I can actually write as this $A\cos\phi$. I can write it with an expression… here I am representing it as "1 over", because here I represent it as an inverse of a… this is an equivalent, effective space constant, AC. It is a horrible expression, but I can plot it and I see that when the frequency is very low… this in semi-logarithmic coordinates… when the frequency is low… low compared to what? Low compared to τ_m.

τ_m, membrane constant, tells me how fast is "fast". If the membrane time constant is of the order of 20-50 milliseconds, if I have something that varies very slowly of the order of 1-2 Hz, comparing 1-2 Hz with the inverse of τ, which should be 20 Hz, makes this frequency negligible. If instead I have an oscillation that is higher than a frequency component that is higher than 20, 30, 50, 100 cycles per second, then wherever I have τ_m times the frequency ($\tau_m \cdot \omega$), here I wrote it as f to be able to say cycles per second and not radians per second, then there it is a fast frequency.

When the frequency is slow, practically λ is one of the terms… it is not this term here under the root, practically it is uninfluential. So if I go slow, for slow signals… if I have an IPSP, just to be clear, and this IPSP arrives… this is a neuron and this is its dendrite, if I have a synapse here and this IPSP let's forget that it jumps very rapidly, but when it decays slowly this *decay* behavior is very very slow and there this graph is telling me: "Look, the attenuation solution of that phase is indistinguishable from the case where it was a cable at steady state".

The problem is here, this instantaneous rise. In reality, it is not instantaneous, but if you remember the value of α for the concentration of a neurotransmitter was a fraction of a millisecond, particularly I am thinking of AMPAs, they go up rapidly: a millisecond could mean a kilohertz. I am literally doing one divided by the time constant; the inverse of a millisecond is a kilohertz. Suppose there are various… ok, 500 Hz, 100 Hz, whatever it is. We are anyway here, where this effect of this term here makes itself felt: the faster you go, the more this term that "kills", lowers the space constant kills it more and more.

So I expect that an IPSP or EPSP or even the inhibitory equivalent has certainly a deformation, cannot pass, despite the attenuations, cannot let the very rapid transients get away with it. Because in itself it is telling me that the cable

becomes with a small space constant. If the cable becomes with a small space constant, even if it is a little dendrite, a small dendrite… if λ is very small, that cable there is as if it became infinite. I am taking it to the extreme to make you understand, so at high frequencies λ becomes small, it is as if the cables became long, so good luck attenuating! Nothing arrives at the soma anymore.

And if you think about it, it is not so impossible to think, this is the same thing I said before, it is not so unheard of to anticipate intuitively because you know that if you have a structure with capacitors, resistors, you have a **low-pass filter**, so it tries to disfavor very rapid changes in time. So if I have really long stuff — I think of Lord Kelvin, telegraph cables — it is stuff… a rapid transient, no surprise if it didn't arrive on the other side of the ocean. Not only did the telegraph note not arrive at a certain amplitude — thanks, you killed it, you attenuated it a lot — but also the spectral content is very different, the rapid transient is completely altered. A filter, if you think you understand what a low-pass filter with an RC in parallel is… if you put many of them, it is like putting many filtering blocks one after the other. If the first one deformed and changed the frequency content of the output, if you put a further filter and then a further filter, so imagining decomposing a cable with a series of filters, at the destination you have something that is strongly *low passed*, strongly passed-low, strongly deformed. Rapid signals are no longer there.

This is a further graph that shows you at different frequencies, this is the solution, the solution in the AC case. You see that it resembles very much the case of a semi-infinite cable, as also this is a semi-infinite case, in the DC case, in the **steady state** case. The steady state case is represented by this blue curve, it means frequency zero. If the frequency changes — increases: 100 cycles, 500, 1000, 1500 cycles per second — it is as if you were changing λ, you are making it much smaller.

This graph is further normalized to λ, again so that it can be used always. So what counts is the comparison: the attenuation is very very abrupt if you go fast and is practically a little less abrupt if you go very slow. Paradoxically if you don't move at all, in the DC case, the blue case.

So this quantity here and how we arrived at deriving it may require some annoyance from the point of view of manipulation of complex numbers, which I do not ask you in the exam, but I would like at least, more or less — particularly from here, from this point here — simply writing Fourier, an intuition to say: "Ah shit, here is λ divided by ω". Yes, it is true, there is j, so this discourse is very intuitive and you cannot do it rigorously. But when ω changes, the effective λ here becomes smaller and smaller. This is an intuition I ask you to develop. And for those who are not particularly annoyed by complex numbers… I am not talking about doing the integral in the complex field of complex variable functions, only massaging expressions to make them return with modulus and phase, to be able to write modulus and to the j phase. The phase would also be interesting and important to examine, but I am not interested; for the moment I am only interested in the modulus. This modulus has an expression that depends somehow inversely on frequency: the more the frequency, the more this term at the denominator kills the λ.

Interpretation of Experimental Data: Somato-Dendritic Recordings

So, now I tell you what we do with this knowledge. The goal is that of extracellular signals, but while we are at it I tell you what this entails from the point of view of the effect of synapses, of dendrites, from the point of view of the soma.

And I simply show you two examples where, not so in the past — maybe you were born in '94, it is not in the nineteenth century — this is a thing that has always struck me and I met the guys who did this experiment and this impresses me: the fact that it is a recent evidence, that it is not an evidence that has always been known. So it is a relatively young field, that of neuroscience, that of neuroengineering.

Here you see an experiment where a pipette was placed to record or stimulate both in the Soma and in the apical dendrite several tens or hundreds of micrometers away. I told you last time that this is absolutely not a simple thing, because while the Soma on a computer screen, despite the infrared microscopy technique, you see it well, the dendrite you see as a thread and it is difficult with a pipette to know if you have only an image, if you are in front, if you are behind or if you are pinching it. If instead I did the same thing with a soccer ball it would be a little easier. The Soma is a soccer ball, the dendrite is really very very thin stuff: the diameter here could be one micrometer, two micrometers; the Soma instead is about ten, 15-20 micrometers, so it is nice and massive.

What was done is injecting a current and making the neuron fire. What you see here, this trace here, is a somatic action potential and this other electrode, which at a distance of several hundreds of micrometers, caught the echo of it. This is a strange thing because it is called **backpropagating** action potential — anterograde perhaps — and it is an unexpected thing. Neurophysiology from the textbook predicted that when a spike, an action potential was generated at the Soma, it would propagate along the axon. What do dendrites have to do with it?

The dendritic structure as I sold it to you, despite there being evidently a remarkable attenuation and a remarkable filtering, is such that with a certain delay — the delay you see apart in the peak but you see the rise of the potential trace after… note that it might not be, no ok, I think here the calibration bar is the same for both… it is a much more attenuated activity, about half, and it is slower: it goes up more slowly and also goes down more slowly, so it is evidently a filtered signal. It is interesting because it seems to be the means by which a synapse that sits here… if you remember two weeks ago, we talked about synaptic plasticity dependent on the arrival time of a pre-synaptic action potential and the arrival of the post-synaptic action potential. I had told you it was pre-post, pre-post reinforced the synapse; if the post occurred first and then the pre, the synapse would depress, unless the time window was too large. This is a cellular mechanism based simply on the cable equation, on the fact that the dendrite is a passive cable — it is an active cable even better, we won't talk about it though — in which at the synapse, here after a few milliseconds of delay, the information arrives: "This neuron is firing and I see the echo of it". So this is the attenuation of the action potential that backpropagates.

Instead, the orthodromic propagation, so directed in one direction from the

apical dendrite to the soma, was done… here this again, the calibration bar is for both traces, one millivolt, so it is extremely smaller. What was done was here injecting a current that simulated, that emulated an excitatory potential, an excitatory post-synaptic current. In other experiments, if you don't like this, the experimenters didn't put the pipette inside the dendrite, they put it around there and then they did, inside the pipette there was a little glutamate and they did a so-called *puff*, they did a release of glutamate. And this glutamate bound to the synaptic receptors that were here in the surroundings, giving rise to an almost identical waveform.

What you see is again what we discussed before: a distal input to the Soma propagates with a remarkable attenuation, as well as with a filtering, a deformation of the… you see that this very steep rising stage does not pass, it is filtered.

Transient Solution and Conduction Velocity

Let's see the case of the **transient**. The solution of the transient I won't tell you how it is derived; if you are interested you can look at or search for the same derivation for the diffusion equation or the heat transmission equation. It is exactly the same mathematics, the same form, only that there it is not V but it is a concentration or a temperature, and they are not transmembrane resistances or capacitances, they are thermal capacities or thermal resistances of thermal coupling, for example of conduction, of heat exchange by conduction or convection; not phenomena linked to Kirchhoff and in the case of diffusion they are conservation of mass, not of charge and current.

To make it short, the solution in time and space, when you have an infinite cable in both directions and you are injecting a current at a specific point — so a kind of Dirac delta with x, but this is also in time, in the sense that it is kept constant — has this form and apparently it tells you nothing. I would like to rewrite it in this way. If I rewrite it in this way I hope that this exponential of $-x^2$ resembles, reminds you of, what is the expression of a Gaussian, of a bell curve. Actually, it is exactly a Gaussian because Gaussians have $e^{-x^2/2\sigma^2}$ (something you call σ^2, the variance) and if it is a Gaussian it means that first the term multiplying the exponential must be $1/\sqrt{2\pi \cdot \text{variance}}$.

So, then this is an interesting observation for those who know or have by chance studied the diffusion equation because there the Gaussian pops up. As in that case also here however… so it is a Gaussian with zero mean, it is centered exactly for $x = 0$, it is this one here. But you see that the variance, this σ that I identified — that is I am calling it variance, but it is not a variance, it is a mathematical term that here only analogously by analogy has the meaning of variance — you see that it depends on the space constant and on time.

[Image of Gaussian distribution spreading over time]

That is, you have to imagine that if I have this cable and I have an electrode that I start stimulating at a point, initially the Gaussian is very very narrow and as time passes it starts to "belly out" (flatten/spread). Not only does it belly out, but its amplitude, this $A(t)$ — you saw it from here, from this exponential

term, I rewrote it here simply for convenience — its amplitude, fixing the point, is an exponential in time, with the time constant you are used to seeing.

So in space there is something with this e^{-x^2}, but it is a simultaneous solution, both in space and in time. In space, like the bell curve, in space it would tell you, taking a flash at a particular instant… so this instant is practically immediately, 0.1 times τ_m (the τ_m is the membrane time constant, suppose it is 20 milliseconds, this means after 2 milliseconds it is like this), after 20 milliseconds it is like this, after 40 milliseconds it is like this: it bellies out and flattens.

This is quite ok to understand because what I am doing is: I gave a "flick" (*schicchera*) here — I have to give a flick with a laser, but I can't — after which, let's say, if it were ink in a tube full of water, this would diffuse laterally and the concentration would be initially denser, the ink would be denser here and then it would tend to belly out. The same thing you have with the electric potential and you can also study it not in space but in time at different points.

It was what I showed you in the Google Colab code at the beginning, but it doesn't make much… it makes sense only if some of you were particularly curious and wanted to get your hands dirty and see how this thing changes here. Here you have in time; in time means that you must have more pipettes, or from the point of view of a simulation have "record here" (so record from $x = 0$), then "record from $x = 0.5$", "record from $x = 1$", $x = 2$, maybe 1, 2, 3 as a function of λ (so of the space constants), and every time you record. Sorry, not every time: you start the simulation and you simply record from distinct points.

You see that for $x = 0$, at time 0 basically the amplitude is infinite and then it starts to decay exponentially. I am looking at this curve here. Something that surely you would have expected thinking of the usual RC, of the usual single-compartment cell. You give it a flick, the potential shoots up and then decreases, decays in time, like with the time constant τ. Again here the t axis, of time, is normalized to τ, so that it is universal. So you see that after one τ the amplitude has reduced by 66%… I will never remember this engineers' thing, whereby after one τ the exponential is at minus 30%, I don't know.

If you move to 0.5, to 1, to 2 lambda, you no longer see the peak; you see it very attenuated and shifted, displaced. In the end also the potential that back-propagated in the simulation I showed you before seemed not only to shorten but to shift. At one lambda or two lambda it seems to be profoundly shifted to the right, as well as attenuated.

What one can do, and Rall did it for the first time in the '70s-'80s, is to try for example to understand what is the position in time… at what time the peak occurs. Note: I am not dealing with the wave equation. The wave equation, which in fact was the one I simulated when I showed you the axon (the all-excitable axon), had by definition a waveform that was always the same and propagated in space. This is the wave equation, the one called D'Alembert's equation. It would have, if I remember correctly, a second derivative term of time, but I could be wrong.

Here there is not a true propagation, because the waveform there is not a primitive that propagates, as in electromagnetic fields, in Maxwell's equation, it

propagates always identical. Here it is something that looks like a perturbation moving both in time (fixing the point) but also in space (fixing the time). So what one can do is plot the position of the peak and say: where is this peak as time varies?

You write this relation and find that in fact it is an almost linear relation, except the beginning. At the beginning precisely there is, for very small times, there is a small exception: this curve seems not to start straight but start with a greater derivative. And you can write that the slope of this curve — in the end this is a space-time curve — so the *slope*, the steepness of this curve is a velocity. Repeat, it is not a wave propagation phenomenon, it is a propagation phenomenon of a perturbation that changes shape. But if I take the peak as good, I can think that this peak could be representative of the peak of an IPSP, of an excitatory potential, a distal inhibitory post-synaptic potential. If I know what the distance is and I know what the velocity of — let's call it — propagation, of conduction (let's call it better conduction velocity) is, I could predict how much time a remote synaptic potential takes to arrive at the Soma. Not only the attenuation which, ok, is there, etc., but also the time.

It turns out that this conduction velocity is given, perhaps trivially, perhaps not… not trivially because there is this 2… space constant divided by time constant:

$$v_{conduction} \approx \frac{2\lambda}{\tau_m}$$

Ok, twice space constant divided by time constant. In the end it was absolutely not intuitive to think that that term put to multiply the spatial derivative and the other to multiply the temporal derivative had to do in such a simple way — and this obviously is an approximation — to describe what seems to be a kind of conduction velocity.

And this thing of conduction velocity, in the '80s or '70s, was compared by Rall with experimental traces. What you see here are experimental recordings made by someone who in the motor neurons of the cat spinal cord recorded the soma and saw different waveforms. Rall normalized them, that is he put their amplitude… he made sure that dividing by the amplitude of each peak, that it was always the same, and so he realized two things. The first is that the shape changes and quite a lot, remarkably; it seems not to be simply a scale factor, it seems to be a filtering. So dendrites filter (we saw it before, we know even that they are low-pass filterings, ok, of a slightly strange type because we also have a spatial component and we saw it with the AC analysis).

And he was able to compare the so-called *half width*, the width, and the time to peak and plot — these are the little triangles — the measurement in an experiment and compare them with the dashed line which is the consideration we made before. He had the equation, the solution in the transient, and he could say: "Well, do I have these experimental results? Do they add up?". And the answer is yes: these points align perfectly on this theoretical curve.

And the *take-home message* is this here: that distal synapses not only attenuate but you also have, let's say, they belly out, you have a filtering. And this is particularly important for attenuation and bellying out, for what is the effect on the Soma. If I am bellied out and I am for example a current traveling

in the dendrite and I enter inside the Soma, maybe I will be lower, but the subtended area (current times time) is the charge, so I could have a different effect depending on my shape.

Obviously, the problem of when, of *timing*, remains. If I have a synaptic input very close to the Soma, the possible *recruitment*, the possible evocation of the action potential can occur within a short time. If I have it distal the peak occurs anyway with a delay. Again, also from the point of view of time-dependent synaptic plasticity, these considerations are in the same order of magnitude as for the potential backpropagating in dendrites; also these which are synaptic inputs propagating directly from dendrites to soma have a delay that is perhaps comparable with those time scales of plasticity.

Dendritic Democracy in CA1 and Spatial and Temporal Summation

There is an exception to this rule, at least from the point of view of attenuation (bellying out, filtering is inevitable), and it was discovered a few years ago in a population of hippocampal pyramidal neurons. This exception is called **dendritic democracy**.

So the normal case is that if you have a far input and a closer input, distal or proximal, at the soma the distal input results very attenuated as well as bellied out, while the proximal input results a little attenuated and a little bellied out, but less. So somehow the more distant you are, the less attention I pay to what you say, because at the Soma, a site close, near to where the action potential is generated, I am little depolarized by a far input. However, in this class of cells, it was found that in the periphery the distal input was proportionally much higher, so that despite the attenuation is called electrotonic — which is the one we described with the cable equation, has to do with the electric tone of what was thought to be a mechanical phenomenon, but in reality is a purely electrical phenomenon and has to do with the cable — at equal electrotonic attenuation, distal or proximal inputs in fact have as peak, as amplitude, practically the same quantity. You see however that here the red is bellied out anyway, but it started wider, so in the end the amplitude is equal.

This is an important graph that summarized this phenomenon in which if you look in the dendrite at different points… so each of these, then this is space in which the distance from the soma, 0, 100 micrometers, 200 micrometers, 300, 500 micrometers, where you have a synapse. If you have a synapse here and you are lucky enough to put an electrode here, you would see that its activation causes a much larger depolarization than it causes here when you have a synapse here. Instead, the electrode placed at the Soma, which you never change, should show an attenuation, you should see an exponential curve $e^{-x/\lambda}$ because this is space, if it is distant I attenuate more; actually you see flat, so it is like what has been called democracy because everyone has the same voice, an equivalent voice to make the Soma fire presumably.

It is not clear if this thing here is a specific thing of some… well it seems it is like this in CA1 cells, pyramidal cells of the CA1 zone of the hippocampus and it is not like this in the cortex. Is there a reason why this is so? Is it a kind of evolutionary effect to compensate for what is a bug, an electrotonic attenuation? "I would have wanted the neuron to be a point where all synaptic inputs had

equal weight, instead I had to for genetic, molecular reasons, express these ramifications which however do not represent me, I don't feel like an arborized dendrite". Ergo I ensure that when a synapse is established, the number of post-synaptic receptors inserted distally or proximally is different. I put many more far away and few close by, so that if there is for example the same amount of neurotransmitter, these make much more distal depolarization and compensate for the attenuation.

Another concept I want to tell you before concluding this part is to conclude the part of extracellular potentials, which will be again relatively qualitative, will be based on the presentation of computer simulation, of simulation studies, is the concept of **temporal and spatial summation**.

Temporal summation you should have overheard with the story of the university paying my salary, of the famous "tails"; some of you in the interruptions continued to use the term I used of the fact that when an event — in this case an IPSP, an excitatory synaptic potential, could be inhibitory — when it arrives before the tail is not completely exhausted, it sums, there is a summation in time. It is not a surprise, this has to do with the fact that the charge balance equation, $CdV/dt = ...$, in the end is an accumulator, it is the equation of a condenser, capacitor, that accumulates. If I integrate both members CdV/dt or that V equals the integral of the current… integral means sum. So if I have a neuron receiving four afferents, four axons with each a synaptic terminal, independent… this is an ugly one, maybe with your help I can make a nicer graph, because they are not IPSP, I could have made it EPSP… instead of being a single pre-synaptic activation event, they are four events very close together, given that each event sums to the previous tail that was not yet exhausted, there is a progressive depolarization.

The same thing happens when you have synaptic inputs occurring at close points of the same dendrite, in a first approximation, so it is not a summation in time, but it is a **spatial summation**. And here too it is not particularly complicated to develop an intuition, instead of having a pipette at a point, or more pipettes if you want, or more synapses, and by pure linearity of that equation superposition of effects holds. That is the response to the sum of inputs to these is the superposition of the individual responses obtained when the individual inputs are administered independently. So there too there is a decomposition due to, if you want, a spatial summation, but there spatial summation continues to hold because the equation is linear.

This is the graph that probably could enlighten you the most. These graphs, these purple, cyan and green curves, are the responses in an infinite cable to a current injection at different points. You see: here the injection is at zero, here the injection was at minus three… I am not a genius, I see where the peak is… in this dendrite condition is only passive, that is the only explanation, I had an electrode or an input there. A current that in space was a Dirac Delta for example centered there, this was centered at 3 and had a smaller amplitude, so these single curves are those I obtain by injecting into the cable one of these currents at a time, independently, I put the others to zero. When I inject them all together, the response — either numerically, or analytically, or simply with this heuristic which is correct, because the cable equation is a linear equation — you obtain it as a superposition, so a sum point by point of the three curves.

So this is the graph one obtains from the response to the three simultaneous currents.

I wanted to show you the last of these simulations in which I have… this is only in time, but there is the spatial component. These synapses are in a remote position, they are many synapses in a remote position and I demonstrate with this the fact of attenuation and the fact of spatial summation. So at the distal dendrite, 414 micrometers, I am activating these synapses and I see these EPSPs which are very large; over there they are large and have these tails. While in black I see the depolarization of the Soma. If I increase the frequency of these events — instead of 30 Hz, 50 Hz, 70 Hz, 90 Hz — I see that progressively the Soma is also rising, is maintaining a depolarization. Why? Because each new event arrives when the tail of the previous one is not yet exhausted.

There is a small *catch* compared to the single compartment thing of my salary, of the university paying my salary, and which you see that if this holds very well in dendrites, in the Soma, that there is a cable in between, things are a little different: there is an attenuation, it could also be that it fails to make the neuron fire. Ok, here in this case I make it fire. So here is an example of spatial summation put also with attenuation.

Again, I hope some of you can be curious and say: "But if I have a lower frequency, but I start to increase in a very remarkable way the amplitude of the synaptic input remotely, so I make these pulses become much larger… how come the neuron doesn't fire?". Sorry, I made a mistake. It is this slider. Here they fire. Ok, here it always fires. So, at a certain frequency, a low amplitude, ok… for example like this, I don't know if it can or cannot add up for you, that despite me increasing the amplitude of synapses… ok, here in the end it is working, so ok, I cannot do it. What I wanted to verify, but I am not managing to find the combination of parameters, is that a phenomenon of **synaptic saturation** exists, whereby even if you increase the synaptic amplitude a lot, not necessarily on the Soma do you have a proportionally high effect. But this is another thing, it is not crucial.

Shunting Inhibition and Dendritic Spines

This is the last aspect, which concludes this chapter, and it is a slightly counter-intuitive aspect. If spatial summation holds, perhaps there are still a couple of slides later… if spatial summation holds, then if you imagine having excitatory and inhibitory synaptic inputs on the same arborization, on the same branch or on different branches, you could imagine that at the Soma a kind of algebraic sum of the effects could occur. So it is no longer in time and space; the neuron has evolved with these arborizations to maximize the surface area, but in fact, it is as if it behaved like a sphere.

The interesting thing is that in some cases there is not necessarily a linear spatial summation, but a **sublinear** (*sublinear*) summation. This happens due to the fact that synapses have a particular characteristic of being — perhaps I have already repeated it *ad nauseam* here — **conductance inputs**. So here it was

written:

$$I_{syn} = g_{syn}(t)(V - E_{syn})$$

and then there was also $1/R_m$ ... But it is this thing here that I dwell on. It is not that when a synapse activates there is an injection of current; the conductance also changes.

You can imagine it — and this is quite easy to imagine — that the opening of channels leads, in a cable-like structure, to leakage, thus changing the total transmembrane resistance. So this transmembrane resistance somehow here is multiplied by the synaptic conductance. If the synaptic conductance increases, somehow it changes or disrupts the transmembrane electrical properties: that is, it makes the membrane more *leaky*, less resistive.

This means that if you apply only, turn on only these three excitatory stimulus synapses (this is a *cartoon*, it is not an accurate simulation), or activate them when there is both excitation and inhibition… in this case you have killed the effect, you have remarkably reduced the effect of excitation. Which seems instead not to happen here, where you have two distinct *branches*. Here, if it were simply an algebraic sum of currents, here too you should have the same effect. When you turn on excitation and inhibition, the inhibition should subtract from the excitation, but the excitation is not subtractive. Excitation and inhibition are multiplicative (or rather divisive) inputs; here they multiply by the state variable, it is no longer current. If it is me injecting a current, then yes, it is a current that is algebraic, which sums if the current is positive or negative; but synapses are different, they have an effect of changing the conductance.

This for example is seen here, it comes from a very important article from a few years ago, in which in particular inputs coming from the auditory cortex, or rather from the periphery of the auditory system, the researchers speculated that it was dependent on their simultaneous activation. If you have only inputs from the left ear and you have the activation of these two inputs, since this is a cable, you paradoxically have an opposite effect, there is no summation. If you want, the activation of this other synapse… imagine that this synapse really makes a current flow. The current going here, if this synapse activates, there are ion channels, post-synaptic receptors that are ion channels (for example ionotropic receptors) that open, so the current here finds a passage and exits as well as continuing, so it is much more attenuated. This is a strange, counterintuitive effect. If instead I activate a synapse only from the left side and only from the right side, so like here, which are on different *branches*, I obtain for example an integration, a summation. I do not obtain this effect which is also called **shunting** (*shunting effect*). I believe that in electronics *shunting* or *shunt* means putting to ground, it means a connection… I believe cardiac shunt, help me out, means doing a kind of bypass. So *shunting* means that in the end it is as if I am putting to ground, I liquidate myself, I dissipate those electrical signals that otherwise would have invaded this part of this branch in a normal way, as expected.

There are two other things I want to tell you. The first is that normally, especially — or rather almost the totality — of excitatory synapses do not arrive in the dendrites on the cylindrical lateral part of the dendrite, but are established on a dedicated structure called **dendritic spine**. This is still a post-synaptic

part and the synapse embraces this sort of knob, so it is more a grasping by the synaptic bouton of the dendritic spine rather than the synaptic bouton touching the naked body of the dendrite. There are also that type of synapses (inhibitory synapses are like that, some excitatory synapses are also like that, in the Soma there are synapses like that, there are also synapses that are on the axon, insist on the axon), but in the dendrites of pyramidal cells, of excitatory cells mainly, there is this structure.

[Image of dendritic spine structure diagram]

And this structure, even if we do not go into detail, has a characteristic of being a kind of small cable and having a bottleneck here, even if I don't remember anything about the history of the cable equation… I can think that here from the electrical point of view having a bottleneck, a very very small diameter, you can think of the space constant becoming very small, you can simply think that from the point of view of a remarkable narrowing I have a very high axial resistance here, because I have difficulty if I am an ion entering inside.

This object therefore functions as a kind of amplifier. If I inject a current here, since here the resistance is very high ($V = R \cdot I$, R is high), V will be very large compared to injecting a current on the naked part of the dendrite.

It has another effect: the fact that if there are calcium currents here, having made a compartment that is almost isolated (because this is a bottleneck where few ions can cross), when you go to measure this with optical methods — so the electrical potential comes… so this is the electrical potential, you see that it is around 1 mV, an EPSP of 1 mV — when you go to measure in time the calcium concentration, you see that inside the dendritic spine this goes up remarkably. Here it is a differential measurement, so it is not absolute, I don't know how many micromolar the calcium went up, but you see that in the dendrite, not far away, the calcium has absolutely not altered. Again, if I have synaptic plasticity mechanisms, a structure like this allows me to localize only at this synapse some phenomenon that could be calcium-dependent. STDP plasticity, *spike-timing dependent*, has a remarkable dependence on calcium that is brought by the backpropagating potential (maybe), is brought by the activation of calcium, of calcium channels here (maybe), however in both cases the increase due to the activation of this dendritic spine is local for ionic concentrations. And what happens is that the depolarization is also very amplified here compared to what it would be if the synapse were on the same *shaft*.

Active Dendrites, Calcium Spikes, BAC Firing and Rall's Model

Anyway, this is not only a *glimpse*, a look at this type of description, which again somehow uses tools that are linked to electrotonic conduction and cable theory. There is one last phenomenon that I tell you quickly before taking a break, it is linked to the presence of voltage-dependent conductances in dendrites, at a particular point of the apical dendrites of a particular type of cortical pyramidal neurons. So in this case the dendrite is not passive; if I wanted to simulate it I would have to modify the cable equation and do it numerically, and I can do it, you can do it in 5 minutes with NEURON and Python.

What has been seen experimentally is that, since at this point, far from the

Soma, there exist voltage-dependent currents — in particular they are voltage-dependent calcium currents, and if you remember calcium currents are also like sodium: they activate… so first of all extracellular calcium is more abundant than intracellular calcium, so if there are channels that activate, they depolarize the membrane locally, like sodium (you lick the salty sweat), also here the calcium outside is very concentrated. When an action potential backpropagates, somehow it interacts with this part and can generate what is called a **Calcium Spike**, a spike to calcium, because here there are conductances that create an additional depolarization. So it is as if in these cells there exist two sites of integration or spike emission: one is the somatic one — actually in the initial part of the axon — where they are sodium spikes, and another dendritic one where they are calcium spikes.

And you see in the blue trace, this blue one, the spikes are almost like cardiac action potentials: there is no inactivation, they are a thing, a "blob". This blob however is sufficient when it is evoked — so it is evoked by an action potential that backpropagates — and once there is this excess of this non-linear depolarization, if you want (because there is an opening of channels and an influx, an electrogenesis, that is a generation of an ionic current), this current obviously propagates both up and down and invades the Soma. And in some conditions this "ping pong" between Soma (when it fires it invades the dendrites) and dendrites (they activate and further by reflection generate a further cascade of depolarization at the Soma), well basically it could decide to fire further.

What is called *Backpropagating Action Potential activated Calcium spike firing*, **BAC Firing**. Now I gloss over the fact of the interpretation as a coincidence detection mechanism between somatic and dendritic inputs. It suffices for me just to leave in your head — and we take a break in a few seconds — that if I have active conductances in dendrites, things can be much more interesting and complicated. In this direction there is a lot to do, to create a ping pong in which if by chance this manages to fire, so imagine that a certain threshold must be reached in the dendrites, and this is sufficiently… somatic firing is sufficiently capable, as repeated frequency (the famous tails), of making the potentials backpropagating in the dendrites sufficiently big — and one that sums in the tails of the previous one — to create this depolarization, here the soma activity by reflection persists longer.

So what normally would have been a *burst*, an emission of a single action potential, becomes a *burst* of 3, 4, 5 spikes. It is represented here, and here in fact there are no additional spikes, there is this little hump which is called *after depolarization*, a depolarization after. If this little hump were even greater there would be an extra spike. I have "for free", repeat, on the dendrite a mechanism that allows me to amplify — and on a single compartment I wouldn't do it — the firing frequency of a neuron. If I fire a sequence of 4-5 action potentials I will have 2 or 3 more, and these 2 or 3 more are due to the fact that I have this dendritic mechanism. I stop for 10 minutes. Thank you.

(Break… Brief summary of technical interactions… Resumption of the lecture).

This thing of electrogenesis due to calcium currents in the apical dendrites of layer 5 pyramidal cells causes conduction here, or integration, not to be linear. While before with the cable equation (which I continue to indicate there on the

blackboard) in fact it is a little different, but it continues to be a capacitor, it is a capacitor both in time and in space. Where I see a differential equation with a derivative and at the second member… in the end it is the charge balance equation, but distributed in space: I think of an accumulator.

An accumulator, if I have an input — exactly the story of the tails of the university paying my salary — if the salary payment frequency were increased, maybe I would expect the response to be linear at a certain point. I don't remember if two weeks ago I showed you — I think so — also that in the dynamic **steady state** case, the amplitude of that rise and fall (if you take the peak, the bottom part, the middle part), anyway tends to grow proportionally with frequency, as one would expect. So the more frequent the activity is, the more I have these tails that… so a kind of *build up* on the previous tail is a behavior that in amplitude is proportional to frequency.

However, the fact that here, when you have a particular depolarization, here you have in fact… it is as if you had a generation of a spike, so from the point of view of amplitude, the fact of having this distal electrogenesis — so in points far from the Soma — causes the behavior that would be linear to become **supralinear**, explosive and even steeper. Because at a certain point, at a certain frequency, the action potentials backpropagating from the Soma to the dendrite count and count additionally, because I have here, only here, voltage-dependent calcium currents.

I show you this curve which was obtained also experimentally by **Matthew Larkum**, who is now a professor in Berlin and is — maybe I mentioned him before — is one of the first who was able in the '90s and 2000s to put multiple pipettes in the same dendrite and is a musician, he is a violinist, so the manual dexterity is obviously reasonable and it is understandable that it is from a musician. He showed that in fact in remote positions, this is the calcium concentration, integration seems to be not only supralinear but seems to be **sigmoidal**.

This thing of sigmoids… so it means that there is some, even if calcium… forget that it is calcium concentration, there is a sigmoid here and there is surely a sigmoid where there is the point where sodium-dependent action potentials are generated. I call it sigmoid here because I told you about the frequency-current curve. The larger the current, the more frequent it is. So there is a minimum value of the input current below which the output is flat, after which it starts to rise and then somehow saturates (see the absolute refractory period); it doesn't continue to go to the stars, at a certain point it saturates.

In the context of *machine learning*, the more biological primitives you have similar to sigmoids, the more you have what *machine learners* call representability power (*representability*) for a series expansion of kernels, of elementary functions that are non-linear, sigmoids. So this thing here has aroused much interest because the fact that there is a supralinear summation could indicate that this object behaves as two units, not one. It is not a perceptron where things sum here and emission occurs here. Dendrites sum, here there is an integration, so a threshold, and the result of this threshold operation — so in the end the perceptron is only in the active dendrites — this is fed to the soma and for example to the integration it does on proximal dendrites and somatic inputs.

So all this could make one think: "Wait, if you thought the brain worked like… not a *Transformer*… but like a collection of sigmoidal units like *deep learning*, *deep architecture* due to the hierarchical characteristic of the various *layers*, the single units are neurons… it could also be that a neuron has as equivalent model, as equivalent concept, two units, not one". Also I mention that there is a very interesting study from several years ago, picked up several times and expanded over the years, by a very smart researcher who is in Crete (it is not the University of Crete, it is the FORTH institute), her name is **Panayiota Poirazi**, who demonstrated that if you take a neuron with a cable, so spatially extended, this does the same thing as a network of perceptrons with a hidden *layer*. So one neuron alone, with many inputs, is a neural network, a neuron. In recent times, **Idan Segev** — the same one I mentioned to you — has published a very interesting article in which, again, he says a neuron is equivalent to a *deep* network. Then what this means for cognition, memory, for emotions, for consciousness, this is another discourse; at least it seems to suggest that from a biophysical point of view there is a substrate that "resonates" computationally with something we are starting to — if nothing else — exploit (understand is a big word in the case of machine learning, we don't always understand why it works). Anyway, we do not treat this topic.

I just want to make you think that when there is an arborized structure with *branching* points, ramifications (I don't know how to say it in Italian, "i branching", ramifications), the possible solution of the cable equation is absolutely possible provided to connect… in this case you see that here the radius of these two children is different from the radius of the father dendrite, you must connect the boundary conditions saying that here there must be a conservation of current, so the outgoing or incoming current here must be the sum of the other two. This thing works very well and is very simple to implement in a numerical simulator like NEURON's. It is demonstrated that there exists some type of relationship, some type of coefficient that depends on the relationships of the radii, the radii of the child *branches* compared to the radius of the father *branch*. And if they are in a particular relationship, which I am not going to comment on… if the father is equal to the sum of the children regarding the radius… actually it is the cube root of the radius must be equal to the sum of the cube roots of the radii of the children, because it is so:

$$d_{father}^{3/2} = \sum d_{children}^{3/2}$$

Rall demonstrated that a model in which you have a single Soma and a single Dendrite is equivalent from the point of view of sitting at the Soma — attention: only from the point of view of sitting at the Soma — to a model instead arborized as much as you want, provided the ratios of father and son radii (even multiples with many ramifications, it doesn't matter) provided this law works, with a complicated arborization. So if I have a synaptic input on this *branch*, in this model that looks complicated from the geometric point of view… if this holds (and more or less in the biological case this holds), I can calculate at the soma the same effect that at equal linear distance the same synaptic input would have in a model where I have a *ball and stick*, I have a single dendrite. Clearly, the neuron is real, there are a lot of dendrites, there are a lot of arborizations, but I do this almost with my eyes closed. While the arborization I have to think about the connection etc. If geometry supports it, then there is an equivalence.

This is what is called **Rall's Model**. So when we talk about Rall's model we also talk about the *ball and stick* model. Ball and stick.

Ok, out of the way this part of the spatial description of the properties of the single neuron.

Volume Conduction and Current Sources

Note: this was essential because I need to know in every point of the neuron's morphology what the currents and potential are, or at least the currents, because intuitively I told you that I can understand what happens to the extracellular space treating it as a **volume conductor** only if I manage to formalize and understand current sinks and sources. So I need, given a neuron messy at will, to have its description. It is true, intracellular, but after I have done this intracellular description, what remains is essential to me with these currents to say to an extracellular point, in this point here, what the extracellular potential is.

And obviously, it is evident that it is affected from all parts of a neuron's morphology. If I change that point, move it, move it from inside the cortex, put it on the skin or even maybe a few centimeters from the head, ok, something must change: the distance between sources and sinks and the point where I measure the extracellular potential must change.

So now I return and conclude, I hope, the story of extracellular signals. This part, as said, is essential; this other one allows me to characterize in every point of space… in this case you see the case where I have a neuron, but you can think — and people have done it in recent years, this textbook is a nice example of the culmination, of the success of these studies — not a single neuron, but a network of neurons, even 100,000 neurons, and in points of space far away go to characterize what is… to say what the extracellular potential is.

Why does it make sense to do it? Because most of the time, if I have an electroencephalogram (EEG) electrode, I put it even on the skin, very far away. If I have an electrode measuring **Local Field Potentials** (LFP), I put it maybe a bit closer, but I certainly don't put it in the belly, inside, not in the intracellular electrode. If I have a metal electrode near the Soma and I measure spikes, I measure the extracellular correlate of action potentials, it might make sense to try to say: "But what am I measuring? Am I actually measuring spikes, am I measuring synaptic activity, what am I measuring?". Given that it might not be so trivial to infer the potential at a point given a distribution of currents.

Now I tell you why I am talking about current distribution and where this 4π factor comes from and where this σ_T comes from which is the conductivity of the extracellular medium, the transmission current. So this modulus of $R - R_n$ is the distance in modulus referred to the same system, a Cartesian reference system of the single sink or current whose value is this I_n.

However at the beginning of the course, now several weeks (time flies when one is having fun), I told you that at a certain point P the potential is given by a weighted sum of charges where the trend is 1 over the distance. And then there was in the middle a term that was $1/4\pi\varepsilon_r\varepsilon_0$, because it came out from the definition of potential, so this had to do with the definition of Coulomb force,

of electric potential and of potential that was… whose gradient, minus whose gradient, would have given me, would have led me back to the expression of the electric field.

Well, I lied to you. Or rather, I lied to you for spatial scales like these. At the beginning of the course I wasn't lying to you: we were considering microscopic electrical behaviors across the membrane and there yes this holds. But at a particular distance it is not that these cells are in a vacuum; they are in an aqueous solution and these objects which are water molecules have a charge distribution that is not with the same center of gravity. The fact that there are two hydrogen atoms and one oxygen atom and they are placed in this way (I think the angle here is 105 degrees, they are reminiscences, things I should remove from my head) causes there to exist a center of mass of the positive charge different from the negative charge.

So I can think — as it is, as it happens — that these **dipoles**, which are called dipoles, these water molecules, go to screen… suppose so H is positive, these blue spheres are negative… go to create a hydration layer neutralizing the charge. So this formula, although correct, is not easy to use for macroscopic distances. Macroscopic means not nanometers, but micrometers, tens or hundreds of micrometers, a few millimeters if I go outside the cortex. Not that it is wrong per se. I have another slide where there are three sketches that should further clarify this point. It is not that I lied to you: in that context, it was appropriate. So in the case where we move to macroscopic distances I should consider the effect of these… shielding, of **shielding**, water dipoles. And this modifies this expression in a known way with exponentials for each term, so it is not only the dependence 1 divided by the distance of the charge. Here is the point where I want to calculate the potential, this is the distance, 1 divided by the distance is not enough: at a certain distance a term e^{-r/λ_D} also holds, a certain, if you want, space constant that is called **Debye radius** (Debye length), a *shielding* distance. And it is of the order of a nanometer.

You can imagine therefore that if I am under the nanometer, ok the exponential stays a little, but if I am at some ten, hundred micrometers, this is already gone, it is at zero, so there is nothing left, I don't see anything anymore. I am so distant that the water dipoles have neutralized any effect. So: one, if I am at distances greater than the Debye radius, I forget about using this treatment. Furthermore, I must forget a treatment that has to do with charges if I am considering times, temporal scales, that are of a few milliseconds. Because I remind you that when we considered, defined the mobility of an ion in an aqueous medium, we wrote the law of dynamics in which friction, let's say, combined with the mass of an ion, generated a transient solution that we said: "Look, basically this is always at **steady state**". But what was this particular time? It was of the order of a nanosecond. So a nanometer (this Debye *shielding* distance) and a nanosecond. Basically this description, beyond this description, is no longer adequate and I must convince myself, I must accept the fact that in these conditions, at this time scale, at this scale of times, the extracellular medium is isopotential but is neutral, **electroneutral**, and therefore isopotential.

This is not that I lied to you: at the nanoscale of the membrane and channels this held, because I was potentially interested in channel currents that… so the crossing time of an ion inside a membrane channel is not so much larger than

a nanosecond, it could be lower, and from the spatial point of view I am not above nanometers, if you remember that the membrane is a few nanometers thick. But at a microscopic and mesoscopic scale (here he calls it macro, but in my opinion, it would be better to call it meso, but this is a fact of philosophy) this description no longer holds. So much so that at the microscopic scale we started talking about concentration, density fluxes or concentration, we started using electrostatic formulations, Nernst, etc., ionic fluxes, ohmic and non-ohmic, equation of… I don't remember anymore what the guy is called who replaces the ohmic formulation of a current inside a membrane channel… Goldman, Goldman equation. If I go even further up on an extended scale — so where I am seeing not the detail of the membrane channel, not the detail of a space very close to the membrane, but I am seeing several neurons, I am at a few micrometers — what counts are the current sources, sources and sinks of current.

In the previous lecture I introduced you intuitively, saying: if there is an excitatory synapse here that opens, sodium or calcium ions enter inside, empty the extracellular medium of positive ions. So this suction current is probably remarkable, important for me to understand, simply because I think of Ohm's law. I have this idea that $V = R \cdot I$, so if I have some current somewhere and I have an electrode and I see a potential, maybe I am seeing the echo of that current, where R is the resistance of… which will vary with distance, linked to the ohmic properties of conductivity of the extracellular medium.

So it comes in the end one of the results of relatively recent activity, of studies relatively recent over the last 15 years, 20 years, is that extracellular potentials can be understood, in very simple terms, in the distribution of currents, because in fact I am assuming that there is charge conservation and therefore Kirchhoff, current conservation. This is what we have already highlighted. In the case of a morphology, at this point, it must be extended, because you remember with a *Point Neuron*, a point neuron, I had no way to see this closure of currents that, if somehow occur extracellularly or intracellularly, must close the circuit. So it was fundamental to be able to discuss and have now a tool, even if numerical (because maybe given the complex morphology and given the distribution of even active conductances in the dendritic tree, leaving aside even just the axon, which is a cable with active electrical properties), I need to have this description telling me how currents evolve and how they exchange. If these currents so far away, when for example there is an action potential here, there is an echo… but this echo for reasons we have now dissected and are all in there in that cable equation… but intuitively it continues to be a thing whereby here the membrane continues to be *leaky* and the current disperses, so the membrane potential changes and so the current changes. If I could know how I have, how I know (because once I have solved the cable equation I have the currents: if in particular they are *leak* currents it suffices to know what g_{leak} is and the *reversal* potential, if they are active currents I will need also the state variables but I have them inside, at least inside a computer simulation), the theory of volume conduction allows describing even on the scalp the distribution of the extracellular electric potential.

And it has assumptions. The assumptions are: 1. That all currents and potentials are **known**, or are measured (they are known in the case of a synthetic, modeling approach like the one I sold you up to now, or at least they can be

measured). Clearly, it is unthinkable to think of being able to measure every single neuron with many pipettes (not even if Matthew Larkum were here could he do it with lots of pipettes in all neurons of a piece of cortex where there are millions of nerve cells). But this is the assumption: I know them and I know them through cable theory, cellular excitability, synapse localization, everything we have done so far in this course. 2. This is another very important hypothesis: the fact that there is a certain extracellular potential at a certain point **does not influence**, does not change the intracellular potential of a neuron that is nearby. What in literature is called interactions, would otherwise be called **ephaptic interactions**; in other terms, if I am a pyramidal neuron, I am "lanky", I have my dendrites etc., and I have one next to me, the fact that I am perturbing — because for example I have a synapse that activated here, so here there was some current — I perturbed the extracellular space, here there is a particular value of extracellular potential. The other is a neuron standing next to it, in theory, it should a little, should feel it. I can only invoke the fact that the neuron behaves a bit like a Faraday cage and so probably the inside is shielded, but if I were to talk to one of you on a particularly "no" day, maybe you would tell me: "But what the heck are you saying? When you have channel currents, you have ΔV: one is the intracellular part, minus the external reference, which I cannot think is isopotential, because you yourself tell me that in different points it has a different extracellular potential". The only thing I could say in my justification is that probably this effect is negligible. It is not during — it is thought — during pathological synchronized activity like some **epileptic seizures**, in which you have a block of cerebral cortex that starts to be synchronized and so there is from the extracellular point of view — simply by a sum, a resolution of effects — a huge quantity of a *build up* of the external electric field and it is thought that that too contributes to trigger further crises or to the persistence of this crisis. But for this theory, there are no ephaptic interactions. This in the end is an interesting thing because it says: "Did you do the cable simulation? Sure? Just give me the data you recorded. I don't want to do calculations again, you give me data, then I deal with putting these currents, doing the *playback* of these currents in different points of the morphology, but it is not that then I come inside and say: wait look you have to change the potential, there is an extra spike". No, what you did, what is called a **forward** description, because it is invoking the linearity of Maxwell's laws, of electromagnetism: if I have sources I can predict V, but I don't go back, it is only *forward*, it is only one direction, I don't go back. So, since it is $0V$, does it influence the cable equation dynamics? No, it is only in one direction and works well enough, the reason why I tell you about it in class. 3. Another hypothesis is this: the brain tissue is a **continuous medium**, ok, it is what is in the title being a volume conductor, conductor of volume. And in particular, the conductivity of the tissue is **uniform**, that is it does not change in space, it is constant in time and is **isotropic**, that is in all directions if I speak of currents it is the same. And you could be dissatisfied and say: "But how? You broke our boxes (bothered us) that pyramidal neurons are all aligned, you sold it to us during the first two or three lessons, of the fact that when yes, in particular as also in this context (there it was qualitative), potentials are measured from the scalp, EEG or Local Field Potential, it is affected by this geometric organization". So here, just like in magnetic resonance (which perhaps some of you will have overheard), the fact of having… here they are not fibers, they are

not axons, they are not nerves, they are not projections from one hemisphere to the other… however in this direction there are pieces of conductor that are placed aligned, so this thing of isotropy might not be so verified. And also the fact that it is a continuous medium, I don't know exactly what it means. That is I know what it means here in the extracellular space, because I imagine it as a kind of bathtub where there is me who am a neuron but next to me there is only salty water. Here there is a mess of other types of cells, fibers passing, so currents — as indeed Rall had also intuited — currents pass also inside the tissue. So what probably happens (and people are starting to characterize and study it) is that this medium is continuous but the conductivity value is not the value you would have taking a little extracellular cerebral fluid and measuring it: it is an **effective** value. With the fact that there is a particular tortuosity, with the fact that for example an ion does not have an easy life passing, it is not a *bulk*, it is not a bath in which I move in all directions easily. But this goes beyond; these are considerations to try to keep a critical spirit in you, not to make you drink everything, or at least be very *aware*, very conscious of the hypotheses behind it.

And for all these considerations at the macroscopic scale something similar to **Ohm's Law** holds. The medium is ohmic, here too, but signals are perhaps… and if they change in time, dielectric properties — that is Maxwell works beyond the fact of electromagnetic propagation, so radiofrequency — whereby maybe signals are not so fast as to generate electronic fields. It is difficult that we can read each other's mind because there is a propagation of radio waves between me and you, because signals vary slowly. But anyway they vary slowly and have to do with dielectric properties, of capacitance. These for the moment I do not take into account and I will not take into account in the continuation.

I want to explain to you why this expression comes out, where it comes from, why I started reasoning about something that is inversely proportional to the distance at a certain point. Clearly I can do it for different points; it will be interesting to do it for points near the Soma during spiking activity. Do I see a spike? And if I move away do I see the spike attenuated? Repeat, this is in the extracellular ambit, so I expect that if one of you speaks and I move away a lot, if the medium is ohmic, the resistance is greater, quantities will be attenuated. But does the shape change? So this I will move, the point where I measure the extracellular potential I will move abundantly. But why does this depend on $1/r$ in the end, 1 divided by the distance from these sources or sinks? And why is there this 4π at the denominator? And why is there the σ_T which is the conductivity of the medium for transmission currents?

To do this I remind you, because probably you have seen something like this in an electromagnetism course (if you took it), what Ohm's Law is in a conductor, not in a wire, not in a one-dimensional structure. So not in a lumped structure and I consider this thing of point sources. Here I am taking a small cube and anyway I treat for simplicity the one-dimensional case. I point out to you that normally the constitutive equation for a resistor is written in this way: the potential drop between this point and this point is $V_a - V_b$. So when you take it like this, then the current I is equal to this $V_a - V_b$ divided by R.

In this case here — and it will be clear why, because I am obsessed with the incremental ratio, because nature intrinsically comes to be described by differen-

tial relations (the reason for this, as said other times, I don't know, it is deeper) — if I take a small cube in space and think of taking this as point x and this as point $x + \Delta x$, if the cube has a certain side of measure Δx, I can instead… instead means I simply have to pay attention to a minus sign, but for the rest nothing, only I warn you that you might be confused now for a millisecond… I write ΔV as V here minus V before. Repeat, I want to do it because this sounds to me like it already prepares me for some differential expression. Here, shortly, I am sure, I am confident, I will start writing dV/dx or dV/dr, the gradient in space. And parenthetically the gradient, so the derivative in space, came handy because I knew how the electric field was written. So if Ohm's law is in a volume conductor, maybe I will have something that is not exactly $V = R \cdot I$; maybe instead of V there will be the electric field. Now we see it.

So written like this, taking this ΔV like this, the current must be taken with the opposite sign, because the current is positive when it flows in this direction, taking $V_a - V_b$ (before versus after). It would have been before versus after, but I took exactly the opposite, I must be consistent; I can do what I want, but I must adapt the constitutive equation if I changed the convention. So instead of writing $I = \Delta V/R$, I describe it in terms of conductance which I like more, but with the minus sign. So this is the first thing: ok, $I = -\Delta V$ is proportional to the current through conductance. And I in the end (I am thinking, dealing with a volume, it is not a lumped conductance, but it is the result of a property of space, which I call conductivity, σ_T of transmission) multiplied by the passage surface divided by Δx. I remember it because it is the inverse of the story of resistivity: resistance is typically resistivity times length divided by the passage section. This I don't know why I remember it better than that… because I understand that the longer a wire is the more resistive it is, and the wider it is the less… sorry, the more resistance is high at equal resistivity, and the larger the passage section, since it is at the denominator, means resistance is low. Here it is exactly the inverse of that, but I don't know why, I don't remember, I should but I don't remember.

With this geometry this is the value of conductance and you see: finally I am happy because I have this Δx, ΔV and Δx. I make Δx tend to zero. Before doing it, if you want, I can write the current I, which here would have been a total current, as a flux, so a current density if you want. That is this is the total current and if I know the current density I have to multiply it by the passage section. I do it because I put myself in conditions where this S goes away. In the end I assumed, because I remembered the capacitor formula, that a total current existed, but it is easier that I can describe in the medium, as also in the case of a neuron, current densities, currents per unit surface. So writing big I as $J \cdot S$ (here $I_t \cdot S$), I cancel S from both members, I can do it as much as it is not null, and what remains is that the current is equal to minus sigma t, the derivative or the gradient of V with respect to x, with the minus:

$$J = -\sigma_T \nabla V$$

So this thing of the minus comes necessarily from this choice linked to the definition of the incremental ratio. But since there is a minus for the gradient, this thing of minus gradient is the **electric field**, it is exactly the definition for a conservative force field of potential. Potential is a function such that if I take

the gradient with the minus I get the field. So, ok, written in an elegant way in a three-dimensional case where writing the gradient, but what I wanted to say is that in the end this minus gradient is the electric field. This is Ohm's law in the case of a three-dimensional volume.

Simply to show this and continue reasoning in the end with this, in the end with this expression, this is enough for me. So, what I do now is take a point current and calculate a point current density and say that the total current, taking a sphere of given radius, so at a certain distance, is the effect one has multiplying the transmission current density, the passage current, by the surface, the surface of a sphere $4\pi r^2$, the external surface of a sphere. So the current I_0 due to a generic current density J... again I here unfortunately reason with a current density, but if I have in the case of a cable, of a neuron, or the (I pull it down), these sinks or these sources might need total currents, because there they are total currents. So I basically substitute J (I_t), in this equation here, $I_0/(4\pi r^2)$. Another way to see it is: if I have at this point a total current I_0, for example due to this sodium channel opening or closing or doing what it wants, to put it in relation with density, with a density, I must divide by what is the passage surface, since this current presumably expands in every direction (since the medium is isotropic) at a certain distance r. Here is where this sphere comes from, here is where this 4π comes from.

Written like this, I am writing $J = -\sigma_T \nabla V$ (derivative of V with respect to r). I integrate both members. This is easy because the primitive of $1/r^2$ is $-1/r$; if I take the derivative of $1/r$ it is $-1/r^2$, so this part here is very easy and immediately the term $1/r$ comes out, it is no longer r^2. How did it work in the end for charges? Potential seems not to vary as $1/r^2$, with the inverse square, but with the inverse of distance. And from this part here instead it is easy because it is an exact differential, so it is all how I take the integration extremes. I choose integration extremes between infinity, a point at infinity and a point at r. Why do I take at infinity? Because at infinity I can say in the *bulk* that I have the reference electrode where the potential is by convention zero. Making this choice comes very handy, but any other choice would have been possible, because the primitive here, $1/r$ or $-1/r$, when I calculate it in r is ok, whatever it is. When I calculate it at infinity, it means actually a limit process, I have 1 divided by something going to infinity and 0, so this term also simplifies. And I have the expression of the potential, apart from dividing by σ_T which goes to the denominator, and it becomes plus and plus on both members.

$$V(r) = \frac{I_0}{4\pi\sigma_T r}$$

So this thing of the minus in the end was necessary to rediscover that expression where somehow it told me that at this point V testifies to me how intense the current is there. If a current is presumably a source, so it is spitting something spitting positively in my direction, I become more positive. So if I hadn't done this, if I had made a mess there, I would have probably had a minus and this thing would have been strange. But how? That current is spitting positive ions at me, it is a positive current, in the electrotechnical convention of positive currents potential must increase and varies with the inverse of distance.

By linearity of the medium, if I don't have a single source, but I have a set of

sources, and in this case I am thinking finally of discretizing a piece of neuron morphology complicated as you want, I discretize it as a sequence of very small point current sources. You could object to me that in fact… and here it is all a cable, why did they become point-like? Is it not by chance there is a slightly more accurate description? In the end they are small cables, they are small cylinders: is it not that there is a description where instead of a description on point currents it holds with line currents? You would be right with this intuition, but we see it probably next week. In this case, every single element has the effect at a certain distance, an effect that superimposes, so the overall effect (I wanted to say by superposition of effects) the overall effect is the superposition of effects that single elements taken individually would have at that point. So given that each element is the current divided by $4\pi\sigma_T$ divided by distance, for distance, you have that the potential due to a series of… for example to a cable, a series of point sources or sinks, is the weighted average of currents weighted by distances $1/r$.

So, we see it now and it is the last thing I tell you, before the end. A better approximation is that of **Line Sources**. To understand this thing here you should or will have to spend 15 minutes looking at how I chose these axes. They are slightly different from how the choice is in the textbook I recommended to you and in fact represent the contribution of a small cylinder (in the end a cylinder that will become infinitesimal) and on a point P putting however this cylinder in such a way that its end coincides with the zero point of this system of Cartesian axes. Don't ask me why in the book they didn't do like this, for me this way is easy, more or less easy to understand.

[Image of Line Source Approximation Diagram]

What I do is: I have a cable of length ΔL (for the moment ΔL is finite, not infinitesimal) and I call this coordinate x, I call this point L and I ask what is the effect of this… this is a point current because it is a little ring on what is a whole cylinder full of currents. With all these little arrows I am assuming that each of these rings, in a process then of limit integration, of integral, each will contribute in its own way at this point. This one here contributes on this point given its distance. For how I chose the axes this is easy. This distance is $x - L$, because this is x and this component is L, squared is Pythagoras, this, plus y squared under root. This is this hypotenuse, this distance here. And once you have done this, in the end it is a walk in the park, so to speak, because the contribution of this current ring on this point, this current ring I call ΔI and it is basically proportionally the part that depends on ΔL on dL on ΔL. If this is a tenth of the length, the current term will scale as a tenth of the total, there isn't much else to understand.

And at this point, again I apply the definition: I have I_0 times dL divided by ΔL, which is the part of current, divided by $4\pi\sigma_T$ times the distance. It is ugly as an expression, but I am pleased that there is dL at the numerator, because it tempts me to say: "Ok, this is the contribution of this dL. If I want to have the sum of you, so the collective effect, the superposition, and therefore have the total extracellular potential, I have to do a summation, or in the continuous case an integral". That is I have to take this quantity here, integrate it from $-\Delta L$ to 0, so making these little rings move in such a way as to cover all this length. And in fact it is… so $I_0/\Delta L$ I can bring outside, this $4\pi\sigma$ here I bring

outside; what remains is 1 divided by this square root of $(x-L)^2+y^2$.

The integration variable is only L, so in theory if I remembered integrals I could say: "Ah, this is a known integral, 1 divided by the square root of variable t, t^2+a^2". I honestly didn't remember this, I went to look at it and after that I verified that if one takes the logarithm of the absolute value and takes the derivative one obtains this quantity. Remember: logarithm is "1 over" and then this is a term, a composite function, so you have to do the derivative of the sum and the derivative of the root with the rule for polynomials. But beyond this thing here — which honestly, given this thing here, particularly with Wikipedia, ChatGPT, trust or don't trust, try to do it yourselves pen and paper to see if this is truly the solution of the integral, just do a derivative — given an expression even ugly like this, somehow you could at least say: "Ok, somewhere there is some mathematician who 500 years ago already did this integral". You can write in this way a new expression, which I am not going to comment on much, which is ugly compared to the other. The other was simple, it was for a point current term, it was $I_0/4\pi$ ... it was only this, where here there was the distance. When you consider a current line, a line source of current, you have dependence on space that is linked to the logarithm of this ratio with these roots. It is a mess (*schifezza*).

I close by making the comparison. If you have the description of the field of a source — so in the case of a real neuron, so in the end it is an integration on many points of the membrane — a point current or this line current, you see that the difference is noted particularly at short distances. Here I am writing with this *shading* of color the description of the extracellular potential at a distance of the order of 10-50 micrometers. I just show you the slide after: here you see that it is a bit different, here it is more circular, here it is more elongated. So in theory one says: "Then there is a benefit because it is different on larger distances". So distances of the order of 500 micrometers… I challenge anyone to compare these isoclines, these extracellular isopotential curves: practically there is no difference in having used one way, the simple one of point currents, or this other way of line currents. And so the group of **Gaut** and **Einevoll** [Gaute Einevoll] made this comparison and said: "I show you what the difference is, the error you commit if you use point sources instead of line sources". The error you make is of the order of a tenth, it is already small, when you move — so maybe it is not negligible a tenth — when you move is of the order of 20-25 micrometers. But as soon as you are around 50 micrometers, the error is of the order of a hundredth. So in theory one, depending on cases, could say: "But if I am measuring a signal that is 100 microvolts, ok, I make an error that is a hundredth. And amen".

I stop here. See you next week which should be the last lecture. Thank you all.

Volume Conductor Theory and Current Source Density (CSD)

The last lecture, last week, continued the exposition of **Volume Conductor Theory**, in which we established that the electrostatic potential at a certain point is not the result of a distribution of charges, but rather of a distribution of current sources. We saw that these current sources can also be described in a slightly more sophisticated way through an approach that, instead of considering

them point-like, considers them as **line currents**. Since, in the end, all objects within a network of neurons are "pieces of cable," it comes naturally to consider the approximation in which they are linear segments.

There exists yet another description that perhaps you have heard mentioned or will hear in the future: it is the one called **Current Source Density** (or *CSD*) . In this approach, the distribution in space and time – but let's focus on space – of the current sources and sinks is not point-like as I presented it to you, but is generally given by an arbitrary function, as complex as you wish.

Just to be clear, it is possible to carry out the exact same mathematical treatment and write an equation that assumes a more complex form, becoming an equation that involves the **Laplacian** operator (∇^2), or the Del Squared operator. This happens when substituting point currents or line currents with this more general description of the current source density. This density represents the fact that at any point in the volume there is, indeed, a density of sources. Therefore, the sources are not point-like.

I am emphasizing the word "point-like" because perhaps some of you might think: "But doesn't saying point-like mean that there is a function of the point where the form of this function is, in this specific case, a Dirac Delta (δ) in 3D in space?". If you suppose that this point has coordinates (x_0, y_0, z_0), I hope it occurs to someone that this is exactly what we did: in fact, starting from (or ignoring) this much more generic and abstract treatment, we went straight to the point. From the point of view of the description of neuronal sources, we have channel currents and, after having examined the **cable equation** together and intuited the fact that one can numerically simulate an arbitrarily complex and accurate arborized structure from a morphological point of view, there we obtain point currents.

In the more general case, I might not have this detailed morphological knowledge. In the end, one thinks of a current source density that can have any shape; in our case, it would have been a collection of Dirac Deltas or something slightly more complex in the case of line sources.

The Inverse Problem and Dipoles I am telling you this simply with this slide because the concept of CSD comes up in the so-called **inverse problem**. What we tackled is called the **forward problem** (or *forward modeling*): it is a modeling, a direct quantitative description in which, given the sources, one calculates what the extracellular potential is at every point.

In reality, very often one measures the potential – think of the Electroencephalogram (EEG) with various electrodes on the scalp – and asks: "What are the sources?". This is an inverse problem. Obviously, you know that in general, in mathematics and physics, the resolution of inverse problems is quite "tough"; finding what this function is such that the equation is satisfied can be very complicated, ambiguous, and ill-posed. **Regularization** considerations are necessary, that is, other hypotheses or conditions that make it possible to solve the problem.

Often, in fact, it is necessary or useful to be able to infer the presence and positioning of some sources, even if in typical descriptions in literature and

in clinical practice (from electrostatic potential to the identification of currents) one hardly ever speaks of single cellular sources. One speaks rather of "effective" sources, which do not necessarily have a direct biophysical correspondence.

At the end of this section, I believe in the last slide, I will show you that we talk about **dipoles**. Why a dipole? Why should there be a dipolar configuration of currents, that is, a current acting as a sink and one acting as a source? In some way we said it: if a structure is similar to these pyramidal cells that typically have (even if we know it is not always like this, but it is "antiquated textbook" physiology) distal synaptic inputs while the generation of action potentials is somatic, then a dipole is created . A distribution is created, a separation of sources and sinks. So we will see that the concept of dipoles can eventually be recovered.

I am telling you this only for general knowledge; we are not even trying to approach the CSD equation. In our case, having this density of sources in the form of a superposition of Dirac Deltas, integrating both members would make the second order disappear, returning to the equation I told you about. The integral over the volume of a function that is a superposition of Dirac Deltas ultimately allows the superposition of the currents, that is, the areas of the Deltas, to survive. So you find the same equation again, but the more general case is this one.

Forward Modeling: Shape and Amplitude of Extracellular Spikes

Now and for the next few slides, we will likely finish within the span of this hour, I would like, on the basis of this forward modeling of the extracellular electrostatic potential, to address two questions.

1. **What is the shape or amplitude of extracellular action potentials?** Those that I have often called *spikes*. In the end, I call the intracellular action potential a spike as well out of habit, but technically the first ones to be measured experimentally were extracellular potentials. On the oscilloscope, one saw these very rapid deflections, like "pins" or "quills" (*spike* in English), and people continue to call them that.
2. **What happens to the shape of the extracellular action potential measured at distinct points, even far from where they were generated?**

This is not a trivial question. Two weeks ago I gave you a sort of approximation, a rough formulation which, if you remember, was:

$$V_{extra} \propto -\frac{dV_{intra}}{dt}$$

That is, minus the time derivative of the intracellular potential, trying to derive that expression from multi-compartmental considerations.

In this case, there exists the possibility (which obviously is only theoretical, since it is not that for all the neurons in my cortex right now, or that of a patient, I have the three-dimensional reconstruction and the exact geometry) to simulate a simple case. I consider a single neuron about which I know everything and I simulate it. To simulate means to solve, for all instants of time in a certain

interval, all the cable equations (even nonlinear ones, in the sense that there are voltage-dependent ionic permeability properties) and I manage, point by point, to characterize what the sources and sinks are throughout the morphology of the neuron.

If I do this for a time of 10, 20, or 50 milliseconds (I am not doing an extended simulation), I observe a condition in which the neuron has been initialized in a certain way such that, after a few milliseconds (e.g., 20-30 ms), it fires an intracellular action potential. Then I stop recording; I am interested only in comparing the shape of the intracellular potential to the extracellular potential .

Considerations on Simulation and the Electrode Interface Another thing I must mention to you is that, if it were me with a simulated electrode injecting current into the soma, that injection would be a source or a sink. In this specific case, I am not considering it because, for example, I assume I have initialized the simulation in such a way as to make the system unstable: the neuron must first fire a spike and then go to rest. I say this because rightly you could ask me: "How is it that in this extracellular recording I don't see the echo of that current injected with a pipette?". From the extracellular electrostatic point of view, if I had injected something inside, my reference electrode would have been outside, so I would have contributed to the extracellular potential as well.

Furthermore, we are not considering the **metal-electrolyte interface**. You might ask me during the break if my colleagues Gibaldi and Gibertoni treat this topic. If they do, you know that it is not exactly the same thing – and we pretend that it is – to take a volume in which there is an electrostatic potential and an electric field, put a piece of metal in a point, and think that the electrostatic potential of the metal is identical to the potential of the *bulk* (of the volume) at that point. There are considerations regarding the material of the electrode (some polarize), the depletion zone, the Helmholtz double layers at the interface, etc. We are not treating this aspect: the signals I am simulating here are considered as if there were an ideal extracellular electrode.

Waveform Variation in Space What I see is that, near the soma, effectively the potential looks like the first time derivative of the intracellular potential. I point out to you that someone among you, last week or two weeks ago, under my prompting correctly answered saying: "Yes, more or less the intracellular potential has a duration of about one millisecond."

What you see extracellularly has a smaller amplitude and is the derivative. Being the derivative, the fact that it goes down and then up corresponds to the rising phase of the intracellular potential and then to its arrest. Having arrived at the maximum point (where the derivative is zero), the derivative was positive, then it accelerated and then decelerated. In fact, with the minus sign in front, the extracellular potential decreased, reached an absolute maximum value (negative), and then returned to zero. Obviously, this rising phase is shorter compared to the total duration of the event. For this reason, intracellularly I see an event of about one millisecond, while extracellularly I see it even narrower.

The interesting thing is that if you move in a **distal** direction, always close to the geometry of the neuron, you see that the shape of the action potential changes. It becomes almost biphasic. You could tell me: "But don't you call this biphasic? There is a negative part and a positive part." Yes, but near the soma, the positive part is very small. Look instead at what happens moving along the dendritic tree: I am always outside and I have a strong transition. Then I don't call it "repolarization" (you should lynch me if I did, because here "positive" and "negative" have no historical meaning linked to membrane polarization). Here it is simply an extracellular signal, and you see that it can change quite a lot, even inverting polarity.

Besides the shape becoming different, the general polarity, which at the soma is mainly negative, inverts and becomes positive. In this distal case, the shape almost resembles the intracellular waveform. This is due to the fact that, when an action potential is "fired" (initiated), the somatic potential tends to be more positive. Therefore, in the extracellular part, evidently a "void" is created or, if you will, a compensation to close the circuit.

Now you have the correct language to describe this thing that otherwise would be only intuitive: outside, near the soma, a negative quantity remained, but at other points of the morphology, during the first depolarization, the circuit must be closed. This is why, during the same phase, at distant points you have a compensation. Extracellularly there is a negative part in the first fraction of milliseconds near the source, but somewhere else there must be a positive phenomenon. And here you have it "for free": by free I mean by doing the simulation of the multi-compartmental neuron with correct morphology and biophysics, voltage-dependent conductances, without synapses in this case, and applying volume conduction theory.

This observation is very powerful because it allows one to say: if I take an electrode and stick it into the cortex of a mammal or a tetraplegic patient and start seeing signals, what signals do I see? Do I expect them to be negative? The vast majority of times yes, for reasons we will see later. But not only is it not rare, essentially it is almost standard to see signals with positive polarity as well. Where do they come from? They are presumably the echo of action potentials, but recorded from points near the dendrites and far from the point where they were generated (the soma/axon initial segment).

Distinguishing Neuronal Types: Spectrum and Waveform

Another important consideration, which always goes in this direction but from an experimental or clinical point of view, concerns what to expect when inserting an electrode into the cortex. We talked about polarity and duration, but there is another fundamental component described for years in the literature, often from a purely practical point of view.

***Fast Spiking* Neurons vs. Pyramidal Neurons** Some neurons, specifically GABAergic interneurons (and not even all their subpopulations), have a much "narrower" action potential due to differences in the expression and kinetics of ion channels. In particular, I refer to **fast spiking** neurons , which are defined as such because they possess voltage-activated conductances with kinetics that

allow the neuron to fire much more rapidly. Just to be clear, they have Potassium conductances (of the *delayed rectifier* type) that are not too "delayed," but "sprightly" enough to intervene rapidly to repolarize and hyperpolarize the membrane.

For many years, one of the pioneers of this description, **György Buzsáki** (of whom I have already spoken), insisted on the fact that by observing a certain waveform extracellularly, it is possible to distinguish between excitatory neurons and inhibitory neurons by looking to see if there is a subclass of signals that are much thinner.

Spectral Content and Electrode Impedance Subsequent to these empirical analyses, people started examining the spectral content of the action potential shape, both intracellular and extracellular. Analyzing the **Power Spectral Density** (PSD) – or, approximately, the Fourier transform – one notes that the signal energy drops drastically above 1 kHz.

Here it is important to make a very interesting addition. You will often hear about the properties of electrodes used experimentally or in clinical practice (I will show you some in the next hour) and one of the fundamental parameters provided is **impedance**. You know that impedance is a complex property, effectively resistance in a sinusoidal regime. When you are told that the impedance of an electrode is, for example, a few hundred $k\Omega$, they are giving you a scalar number, not a function. Implicitly, they refer to the impedance measured at a frequency of **1 kHz**.

Why exactly 1 kHz? Because intracellular action potentials have a frequency content that resides effectively below 1 kHz. If you look at the PSD of an action potential of a *fast spiking* interneuron (the one with the fastest and thinnest signal), you will see that its spectrum is wider: for the same frequency, you have a greater energetic content. This makes sense: being a faster signal in time, its spectrum widens in frequency.

The interesting thing is that, to pass from the intracellular to the extracellular description, it is necessary to apply all the theory we built in the last few lectures (cable theory and volume conduction) on a neuron for which morphology and biophysics are known. Even in this case, it is discovered that, although the frequency content is somewhat reduced, the difference between pyramidal neurons and interneurons persists. Interneurons generate faster extracellular spikes and, consequently, the content at 1 kHz is much higher compared to pyramidal neurons (represented by the dashed trace in reference graphs). Furthermore, the peak amplitude seems to be greater for interneurons.

The Problem of Source Separation (*Spike Sorting*)

All these considerations provide us with fundamental tools to tackle a huge practical problem. In clinics and experiments, when I have to record the activity of one or more neurons with an extracellular electrode, I never find myself in the ideal situation of an isolated neuron in a vacuum. I have a notable packing of tens of billions of cells. It therefore becomes complicated to separate the sources and attribute the measured action potentials to distinct units or to the same unit.

This is crucial. If you are in the motor cortex and want to decode whether a tetraplegic patient is thinking of moving their arm to the right or left, you cannot confuse and attribute all the spikes you are recording to the same neuron. If you did, the signals would have a completely different meaning: that "neuron" would seem to fire at a very high frequency, while in reality, you are recording a population of 10-50 superimposed neurons.

Do you remember the graph I showed you, in which a detection area of a few tens of micrometers was highlighted? Within that radius, you can detect signals from many neurons.

Classification Algorithms Knowing that the frequency content and shape are different, I could invent an algorithm that, observing the events, simultaneously looks at:

1. **Peak Amplitude**.
2. **Duration**, often defined as *half-width* (the temporal duration measured at half the maximum amplitude).

Taking a certain *baseline* (which could be zero, since these signals are zero-mean after high-pass filtering), I calculate the maximum amplitude in absolute value, take half of this value, and measure the temporal distance between the two points of intersection with the curve. I could then say: "I want to be able to recognize, order, and separate them" (this is called **spike sorting**). I want to distinguish not only between strong and weak signals, but also between those that are "tall and thin" and those that are "fat and short," because I know they could correspond to different cellular sources (e.g., interneurons vs. pyramidal neurons).

Attenuation and Shape Variation with Distance However, there is an additional problem. If you move, while remaining roughly close to the soma, by a distance of 200-500 micrometers, the shape and amplitude change dramatically. In particular, the peak amplitude attenuates very rapidly. Therefore, if you hear a spike that appears "short and fat," it could be an interneuron, but it could also be a pyramidal neuron that is simply more distant.

The signal amplitude reduces with distance (the extracellular medium attenuates as $1/R$), but the shape also changes. If you normalize the amplitude of curves simulated at different distances (10, 30, 50, 70 micrometers), you will notice an interesting thing:

- The **initial part** of the spike remains almost unaltered.
- What changes is the **recovery phase** (the second part of the wave). At 70 micrometers, the total duration of the event becomes perhaps one and a half times the original.

All this is very useful for designing *spike sorting* algorithms. I can take advantage of the fact that after a hundred micrometers the signal changes spectral characteristics (it becomes more "spread out"). This allows me to attribute a meaning to the distance of the source as well.

The "Cocktail Party Problem" To close the concept of separation, I give you the usual, perhaps stupid, example of the **Cocktail Party Problem** . You are at a cocktail party, talking to an interlocutor in front of you, but there are a lot of people talking loudly around you. Yet, you manage to isolate and understand only the words of your interlocutor. It is true, you probably help yourself by watching their lips, but you could do it even blindfolded. Your brain, at a cognitive level, manages to perform this source separation.

At the cellular level, an algorithm must do the same thing: it must separate sources that overlap. Since Maxwell's equations are linear, the effects of many neighboring neurons simply sum up due to the principle of superposition of effects.

In summary, an accurate modeling description (which includes how the amplitude and spike width change as a function of distance) allows us to understand and answer extremely complicated questions about the nature of the signals we record.

Signal Attenuation Laws in Space

A direct modeling description allows us to answer fundamental questions: is it true that the amplitude attenuates as one moves away from the source? Yes. But how? Does it follow a $1/R$ law? If I move twice as far away, does the amplitude halve, become a quarter ($1/R^2$), or an eighth?

The answer is surprising: it depends on the **morphology of the neuron**.

Comparison between Models: "Ball-and-Stick" vs. Detailed Models If you consider a very simple model, such as **Rall's** model (what we called *ball-and-stick*), the mathematical properties are interesting. Last time we said that this model allows substituting an accurate description of synaptic inputs and complex arborized structures with a single "ball" (the soma) and a single cable (the dendrite). When the geometry is so simple, effectively the amplitude attenuation as a function of distance is more or less linear on a logarithmic scale. It seems like an attenuation proportional to $1/R$.

If instead we use a morphologically detailed model – such as **Hay's** model (developed with Idan Segev) – which represents a human pyramidal neuron with all geometric characteristics, we notice a different behavior. In this case, the attenuation is much more rapid in the first 50-100 micrometers and then tends to saturate or change slope.

Analysis on Log-Log Scale If I based my considerations and algorithm design only on the *ball-and-stick* model, I would make a mistake, attributing an incorrect distance to signals that are attenuated by morphological complexity and not just by geometric distance. Looking at the graphs on a **log-log** scale (where power laws become straight lines whose slope corresponds to the exponent):

- For the *ball-and-stick* model (and perhaps for the complex model at very short distances), the slope suggests a $1/R$ trend.

- However, for Hay's detailed model, at greater distances the slope changes. The trace becomes parallel to reference lines indicating a decay like $1/R^2$ or even almost like $1/R^3$ (dipole or quadrupole).

There is no standard recipe or simple little formula. The potential does not simply go as $1/R$ (like the potential of a point charge) or $1/R^2$ (electric field), because here we are not observing the effect of a single static source, but of a distributed structure with cable properties that influence the generation of the signal itself.

Detection in Noise and "Listening" Volume

These considerations lead to a practical question: what is the volume within which I can detect a spike with an electrode? Every recording system has intrinsic noise. Besides the electronic noise of the amplifier and the bandwidth, there is the thermal noise (**Johnson-Nyquist** noise) of the electrode itself, which is proportional to the resistance (or rather, the real part of the impedance) and the temperature. Imagine an electrode at 37°C with an impedance of a few hundred $k\Omega$: it is a noise generator.

You could establish a minimum detection threshold, for example 30 μV, below which the signal is considered "drowned" in noise. You might think that this threshold defines a sphere of fixed radius (e.g., 80 μm) around the electrode. The answer, once again, is not that simple: it depends on the **cellular type**.

Differences between Cellular Types (Pyramidal vs. Interneurons) Let's compare three morphologies :

1. **Pyramidal Neuron**.
2. **Martinotti Cell** (interneuron).
3. **Neurogliaform Cell** (interneuron).

If we trace the **iso-amplitude** curves (the frontier where the signal peak is exactly 30 μV), we see that the shape and extension of the detection volume are drastically different:

- For a **pyramidal neuron**, I can probably detect signals at a few hundred micrometers distance. Near the soma, the signal is huge, on the order of 800 μV.
- For a **Martinotti Cell**, the detection volume is not spherical but elongated (a kind of ellipsoid) and much smaller. Near the soma, the signal drops to about 130 μV.
- For a **Neurogliaform Cell**, the signal at the soma is barely 50 μV. If I move away even slightly, I drop below 30 μV and lose the signal.

This introduces a notable **sampling bias**. If you put an electrode in layer 5 of the cortex, you might "catch" only the large excitatory pyramidal neurons and completely miss the inhibitory interneurons, simply because their signals are too weak or decay too quickly in space. If you are designing a neural interface, this is critical: cortical computation is not performed only by excitatory neurons!

Role of Active Dendritic Conductances

Finally, one last consideration on dendritic biophysics. We know that the dendritic tree is not passive. There are *calcium spikes* and phenomena of dendritic electrogenesis due to voltage-dependent conductances (Calcium channels, NMDA, etc.) that make dendrites active structures, capable of nonlinear computations and coincidence detection.

But are these active conductances seen from the extracellular point of view? We simulated two conditions:

1. **Active Model:** The complete neuron with all dendritic conductances.
2. **"Active and Passive Replay" Model:** We take the current generated at the soma in the active case and "replay" it in a passive morphological structure, without active dendritic channels.

The result is that the extracellular traces are **practically indistinguishable**. The amplitude difference is minimal, on the order of 10-15 μV on a 300 μV signal (less than 10%).

The conclusion is that, although active dendrites are fundamental for intracellular computation, from the point of view of an extracellular electrode, it is very difficult to understand if that particular neuron is doing something special (like generating dendritic calcium spikes). The extracellular electrode is substantially "blind" to these fine phenomena.

Now let's take a break. Upon returning, I will tell you what happens if you have an axon-only structure and whether it is myelinated or not. We will stop for 10 minutes. Thank you all.

Myelination and Saltatory Conduction

Ok, before the break I mentioned the issue of myelination. Do not confuse it with melanin, which has nothing to do with it and relates to skin pigmentation. **Myelin** is a dark-colored substance that, in particular, characterizes some glial cells called **oligodendrocytes**. These are not present for all axons and not even for all collaterals of the same neuron; they act like hot dog buns wrapping around the axon and effectively creating an electrical insulation structure. This happens for all peripheral nerves, for example.

Just as a camera cable is insulated from the external space (which could be non-conductive air or an aqueous solution rich in electrolytes), myelin insulates the plasma membrane. If an insulator is useful for containing leakage in a long cable, in the case of an axon this has fundamental consequences. If you remember, last week we played with a *Jupyter Notebook* on Google Colab simulating a cable equipped with many active channels (voltage-dependent Sodium and Potassium). That device was able to propagate the action potential with constant amplitude to the end of the cable. In that case, however, there was no myelination: conduction was continuous.

When there are oligodendrocytes, the membrane does not have direct access to the extracellular medium, so all conductance values (leak, maximum Sodium, maximum Potassium) are effectively approximately zero at those points. There

can be no transmembrane currents. This leads biology to a fantastic evolutionary solution: leaving exposed zones that instead have conductances. These points, called **Nodes of Ranvier** , are "hot" points for excitability properties. Between one node and another, there is an insulated interruption of a few tens of micrometers. The resulting conduction is called **saltatory**, because the signal effectively passes from one point to another. This leads to a notable acceleration and a shorter propagation time compared to the case without myelin.

Long-Range vs. Local Projections In the case of cerebral cortex neurons, long-range projections – such as those from one hemisphere to the other or the **pyramidal tract** (named so for anatomy, not only because the cells are pyramidal) which projects to the spinal cord – require signal integrity and maximum speed, just like a transoceanic cable. However, in the same neuron there are the so-called **collaterals** (bifurcations of the axon going sideways). 80% of these remain in relatively close blocks of the cortex (local intracortical connections).

Simulated Comparison: Myelinated vs. Unmyelinated Axon

Using a simulation with accurate morphology and biophysics, we can compare three cases:

1. **Soma** (generation point).
2. **Myelinated Axon** (with interruptions at Nodes of Ranvier).
3. **Unmyelinated Axon** (continuous active cable without interruptions).

Intracellular Point of View From the intracellular point of view, we immediately notice a difference in the **resting potential** (*baseline*).

- The Soma is much more depolarized.
- The Axon stays practically always at rest until a **cytosolic lateral current** arrives.

Lateral propagation of the action potential from immediately preceding points changes the local transmembrane potential, causing the next point to "explode" and generate a spike. It is a cascade: a perturbation by geometric proximity causes, after a while, the next excitable point to explode as well, until invading the synaptic terminals. In the case of the unmyelinated axon, the resting potential is further hyperpolarized.

The Question of the "Kink" in Spike Onset Aside from the *baseline*, the traces seem similar, but the most attentive might notice that spike generation in the axon seems to be very steep. Some years ago (maybe 15-20), an article in *Nature* questioned the **Hodgkin and Huxley** model for cortical neurons. The Hodgkin-Huxley model (originally for the giant squid axon) predicts a smooth charging curve before spike emission. The article argued that action potentials in the cortex were more *kinky* (more "naughty," or rather, with a sharper elbow), starting much more steeply. Different biophysics was hypothesized. It is likely not different biophysics, but an effect of propagation in excitable structures that generates this extreme stiffness in the rising front.

Extracellular Point of View The real surprises come when looking at the **normalized extracellular potential** (to the negative peak).

- **Soma:** Has the classic behavior similar to the first derivative of the intracellular potential (down, then up, with a final small hump representative of the *after-hyperpolarization*).
- **Myelinated Axon:** Has a very stereotyped shape, similar to the soma but is a **narrower** spike with fewer "frills." It goes down and comes back up rapidly.
- **Unmyelinated Axon:** Has a different shape, a bit wider and with a strange predominant initial part.

If you see "strange" signals in an *in vitro* cell culture, you might ask: "Is myelin missing?". The answer would almost certainly be yes. Having an in vitro preparation (dissociated cells) that preserves myelination is almost impossible because glial cells do not reconfigure correctly. The case of an acute brain slice is different, where the original cytoarchitecture is preserved.

Attenuation as a Function of Distance The most interesting thing is how amplitude attenuation changes moving away from the source (10, 100, 1000 μm):

1. **Soma:** Amplitude decays very rapidly.
2. **Unmyelinated Axon:** Decays **less rapidly** compared to the soma.
3. **Myelinated Axon:** Has intermediate behavior.

If you see a huge "slap" of a signal (thousands of microvolts), you are almost certainly over the soma. If you see more attenuated signals with different shapes, you might be close to an unmyelinated axon. These considerations are very useful for interpreting "blind" signals recorded experimentally.

Synthetic Data and Algorithm Validation

To recap, this direct modeling (*forward modeling*) is essential to guide the development of algorithms or create **benchmarks**. In reality, when I insert an electrode into brain tissue, I do not have the **Golden Truth**. I don't know how many neurons there are, I don't know when they are firing. Only by knowing the truth could I validate my separation (*spike sorting*) and detection algorithms.

The Problem of "Colored" Noise Furthermore, there is the problem of noise. In a real recording, noise is not necessarily Gaussian and additive. The "noise" you see in a filtered extracellular trace (the high-frequency part) is often a *ruckus* generated by the periphery: it is the activity of thousands of other distant neurons that are "drowned" in the signal. It is not legitimate to assume that the statistics of this noise are Gaussian or symmetric, because background spikes still have a polarity (they go down or up) and are not distributed in space uniformly.

For this reason, the scientific community has started creating **synthetic data**. One creates a block of fake neuronal tissue *in silico*, where I know exactly where all the virtual electrodes are and when the spikes occur. In this context, I can add "authentic noise" (generated by the background activity of the simulation

itself, not added artificially as white noise) and test algorithms in controlled conditions.

Purists might object: "This is still just a theory, it is not the truth." True, but since experimental truth is inaccessible for large populations (while accessible for a single isolated neuron with patch clamp), simulation remains an indispensable tool.

Local Field Potentials (LFP) and Multi-Unit Activity (MUA)

Let's now make the same argument for **Local Field Potentials** (LFP), complicating the scenario, however: we no longer consider a single neuron, but a mini-population of pyramidal neurons. These neurons are not exactly identical nor perfectly side-by-side; they are dispersed over a few hundred micrometers, just as would happen in real cortical or hippocampal biological tissue. They are not all perfectly aligned.

Population Simulation and Filtering We simulate the extracellular potential at distinct points, corresponding to the sites of a *multisite* electrode (a *shank*). Imagine a real electrode – I will show you a photo shortly – used experimentally or clinically, which possesses several exposed metallizations (contacts). In this simulated case, we have 22, with a distance between one and the other (*pitch*) of about 50 micrometers (so between contact 1 and 4 there are about 150-200 micrometers).

I can generate synthetic activity in this network of neurons (which includes synaptic activity as well) and simulate the trace for a few hundred milliseconds. Subsequently, I filter the 22 simulated traces to separate two components:

1. **Local Field Potentials (LFP):** The low-frequency part, obtained with a low-pass filter (e.g., from 0 to 100 Hz, or up to 500 Hz).
2. **Multi-Unit Activity (MUA):** The high-frequency part, obtained by filtering, for example, from 500-750 Hz up to 5000 Hz.

Note: 5000 Hz is much larger than 1 kHz, which was the concentration point of single action potential energy. I am considering a range that includes very rapid activity.

Spatial Localization of Components Observing the results, one notes that MUA activity – much more jagged and rapid – occurs specifically corresponding to electrodes **13-17**. These electrodes are positioned geometrically close to the **somas** of the neurons in the simulation. Therefore, MUA activity is likely linked to *spiking* activity (action potentials). It appears jagged because these rapid variations were not eliminated by filtering, but also because neurons are not all doing the same thing at the same instant. There is a superposition in time and space of the effects of different sources. Extracellular spikes can be a bit more attenuated, wide or narrow, and do not occur synchronously; perhaps they are distributed in a time window of 10-20 milliseconds, forming what is called a **volley** (a group discharge).

Looking at the MUA trace, it is difficult to say how many spikes there are because waveforms overlap. If I had a trace with two well-spaced units and a

lot of silence in between, I could distinguish them. But not here.

Relationship between MUA Amplitude and Firing Rate However, I could ask myself if the amplitude of this *Multi-Unit Activity* (which, note, is on the order of a few microvolts, e.g., 4 μV, so in reality, it would be tiny and easily overwhelmed by electronic noise) is not representative of global activity. I would like to extract the exact times (T_1, T_2, T_3), but it is impossible. But I can hypothesize that the more activity there is – the higher the **firing rate** of the population – the greater the amplitude of the MUA signal. It is as if the effects summed up. Remember the story of the "tails": even if events have very short tails, if there are many of them and very packed in time, they can sum constructively.

In this simulation (where I can control everything by turning a "crank," which is impossible experimentally), one sees that there is a relationship between the population *firing rate* and the average MUA amplitude:

- **Linear Relationship:** There seems to be direct proportionality for *firing* frequencies on the order of a few tens or hundreds of Hertz.
- **Power Law:** At higher frequencies, the linear relationship is lost. A numerical *fitting* suggests a power trend (e.g., cubic root of the fourth power), but it leaves something to be desired.

The important message is that, by observing MUA amplitude with an electrode in the motor cortex, I can instantly infer the local population *firing rate*, useful for example to decode motor intention (e.g., moving the hand right or left).

Spatial Attenuation: LFP vs. MUA There is a substantial difference in spatial attenuation between LFP and MUA. In a graph showing signal amplitude as a function of radial distance (moving away from the neuronal "column"):

- **MUA:** Attenuates very rapidly. The slope suggests an intermediate decay between $1/R^2$ and $1/R^3$. To see MUA, I must be lucky and have the electrode very close to the somas.
- **LFP:** Persists much longer in space. Signals are on the order of hundreds of microvolts (e.g., 500 μV) and are seen at greater distances. They likely decay as $1/R^2$.

Consequently, it is easy that with an electrode positioned "somewhere" I see LFPs but **do not see any MUA**, because the latter is too attenuated by distance.

Biophysical Origins of Local Field Potentials LFPs are not generated only by slow phenomena like distal synaptic currents. Reality is more complex:

1. **Distal Synaptic Currents:** Currents generated by receptors like NMDA or Calcium currents, which are slow.
2. **Spike Contribution (filtered):** Even spikes have low-frequency components, due to currents not involved in the immediate explosion of the action potential but are slow (e.g., post-hyperpolarization currents, Potassium, or persistent Sodium). These components survive resistive medium attenuation better.

3. **Glial Cells (Astrocytes):** Often neglected (we haven't talked about them much), glial cells are as numerous as neurons (or more, in the human brain). They play a metabolic role and a role in synaptic transmission, and possess their own ionic currents that change slowly over time, contributing to slow signals.
4. **Ion Concentration Variations:** Under normal conditions, current loops exist that close the circuit in the extracellular medium. However, in pathological conditions (e.g., **epileptic focus** or sustained hypersynchronous activity), notable differences in ion concentration (e.g., Calcium depletion or extracellular Potassium accumulation) can be created over macroscopic areas. This contributes significantly to LFPs.

LFP Interpretation and Polarity Ambiguity

To conclude, I would like to sum up and give you elements to remember, with a fundamental *caveat*: interpreting signals is not trivial, and simulations clearly demonstrate this.

Let's analyze **Local Field Potential** polarity in relation to the position of active synapses:

1. **Distal (Apical) Excitatory Synapses:** If you activate an excitatory synapse in the distal part of the dendritic tree (far from the soma), positive ions (Na^+, Ca^{2+}) enter the cell. This creates an extracellular current **sink**. Consequently, in the dendritic zone, you record a **negativity**. To close the circuit, current must exit somewhere else (typically at the soma), creating a **source** and therefore somatic **positivity**.
2. **Proximal (Basal/Somatic) Excitatory Synapses:** If the excitatory input is near the soma, the sink (negativity) is at the soma, while the source (positivity) is found distally in the apical dendrites.

The "Source-Sink" Ambiguity Here arises the interpretative problem. Imagine having an electrode near the soma and recording a **positivity**. You might be tempted to say: "Ah, positivity means local inhibition." Indeed, if you activated inhibitory synapses (e.g., GABA-A, permeable to Chlorine) at the soma, Chlorine would enter (negative charges in), leaving positivity outside. However, as we just saw, somatic positivity can also be generated by the **passive echo** (the return source) of a distal excitatory synapse.

Thus, two functionally opposite situations (distal excitation vs. local inhibition) can produce a similar extracellular signal (local positivity). This ambiguity requires extreme caution: without knowing the full spatial distribution of the potential, it is risky to infer the nature of the synapse solely from the sign of the LFP.

The Case of Distributed Activity Things get even more complicated when considering a network of neurons or a complex distribution of synapses.

- **Distributed Activation:** If excitatory synapses bombard the entire neuron (both apically and basally), sources and sinks may cancel each other out. Extracellularly, the resulting potential could be almost flat or null, making even very intense synaptic activity "invisible."

- **Synchrony:** If spikes or synaptic events are not perfectly synchronous, but slightly out of phase (like in disordered *Multi-Unit Activity*), signals can mix in such a way that distinguishing individual components is not possible.

The Dipole Model: Validity and Limitations

Despite these ambiguities, observing charge separation (dendritic negativity and somatic positivity, or vice versa) has consolidated the concept of the **current dipole** for decades. This model has been fundamental for interpreting the Electroencephalogram (EEG). If we consider pyramidal neurons of the cortex – with their long apical dendrites aligned parallel to each other – as dipole generators, the sum of their fields explains macroscopic signals recorded on the scalp.

Comparison with Mathematical Dipole But how accurate is this approximation? If we compare the field generated by a simulated pyramidal neuron with that of an ideal mathematical dipole (a point source-sink pair):

- **Far Field:** At distances of a few hundred or thousand micrometers, the two descriptions are practically indistinguishable. The dipole approximation (or subsequent terms of the multipole expansion) works well.
- **Near Field:** At short distances (fractions of a millimeter, within cortical thickness), the dipole model fails. The real neuron has an extended structure, not point-like. The dipole tends to overestimate or underestimate the potential depending on position (creating error lobes), failing to capture the complexity generated by dendritic morphology.

Notes on Mathematical Formulation Just for completeness, the dipole moment $\vec{P}$ for a discrete distribution of currents is defined as a position-weighted sum:

$$\vec{P} = \sum_n I_n \vec{r}_n$$

In the simple case of a dipole (two currents I and $-I$ at positions $\vec{r}_1$ and $\vec{r}_2$), due to charge/current conservation, this reduces to:

$$\vec{P} = I(\vec{r}_2 - \vec{r}_1)$$

Where $(\vec{r}_2 - \vec{r}_1)$ is the distance vector between source and sink. The resulting potential at large distance decays as $1/R^2$ (unlike the monopole which decays as $1/R$) and depends on the angle θ (direction cosine) between the dipole vector and the observation point.

This concludes the part on extracellular potentials and the application of biophysical principles to the interpretation of electrophysiological signals.

We stop and take a 10-minute break.

Introduction to the Demo Notebook for Data Analysis

So, I just want to mention the existence of one last *notebook* that you have available on the GitHub repository and that works on Google Colab. It is titled

"Demo Notebook for Electrophysiological Data" and contains datasets of extracellular and intracellular recordings.

In case any of you simply want to play with it – and playing can mean plotting it with different colors, zooming, calculating the mean, variance, seeing what the average frequency is in the case of an intracellular trace, calculating duration, or plotting the derivative over time – you can do whatever you want. I believe that only a small portion of you are curious or motivated to explore. It's not even for your own sake, because you know (you will have understood after about 50 hours that we have been together) that if no one shows up in class I am sorry, but if you ask me questions that light me up, I stand here talking for another 4-5 hours. Know that if you want to play on these things, you can talk about it. University is this in the end: it is not a vocational school and it is not a technical institute where one gets a piece of paper to do the profession of bioengineer who "plugs in ultrasound machines" in the hospital. That is also important, but it is not the content of this course.

Data Management on Google Colab

To bring data into Google Colab there is a slightly complicated balancing act, but it is something I have already solved for you in the code. Inside the GitHub repository, there is a directory called `data` and inside there are zipped files. I zipped them and they are a reduced portion (you don't have a 10-minute recording of 100 neurons) because GitHub imposes space limits and does not allow repositories to be too heavy.

You find different types of data:

- An **electromyography** trace (acquired from my arm last year or two years ago), made available as an ASCII or binary file. There you should have a reference to a notebook that perhaps had to do with "frogs," where I show you how to access data.
- Data on **Microelectrode Arrays (MEA)** coming from slices of cortical tissue.
- An **intracellular** recording from the soma of rat cortex neurons.

Shell Commands in Colab: Download and Decompression The acrobatics consists of using system commands directly in the notebook. Google Colab expects Python instructions, but by prepending the exclamation mark (`!`) we can execute commands of the **shell** (of the underlying operating system, which is a Linux virtual machine). The fundamental command is `curl` (or `wget`), which serves to download the file. It took me several hours to find the correct direct link to the zipped file on GitHub.

The command looks similar to this:

```
!curl [URL_TO_ZIP_FILE] -o dati.zip
```

By doing so, I tell the virtual machine to download it to the cloud. Nothing prevents you from doing it on your laptop, but if you need help installing Python or otherwise, just ask (although I doubt all 50 of you will).

Subsequently, the file needs to be unzipped. I need an automatic procedure (I cannot click with the mouse as on the desktop), so I use:

```
!unzip dati.zip
!rm dati.zip
```

`unzip` unpacks the archive and `rm` (remove) deletes the original zip file to clean the file system, since I now have the extracted data.

If you open the icons on the left of Google Colab, you will see the **file system** of the working directory populated by the new files. You will find files with extension `.bin` (raw binary data) and files `.h5`.

- The `.bin` files are nine in this case (not 4096 as in the real matrix, to save space).
- The `.h5` files follow the **HDF5** (*Hierarchical Data Format*), a very efficient protocol for data acquisition and exchange, supported by libraries in Python, Julia, Matlab, R, etc.

Loading and Visualization in Python

Once these objects are in the local file system, I have to load them into Python memory, that is, inside vectors (arrays). I do this using the **NumPy** library. If you don't know how to do it, there is no magic: just search on Google or Stack Overflow "how to load binary file into numpy array". I managed it in a few seconds, you can do it too.

Iteration and f-strings The only particular thing about the code I provided you is the iterative loading. Instead of writing the instruction eight times to load `data0.bin`, `data1.bin`, etc., I created a generic `for` loop:

```
for i in range(n_channels):
    filename = f"data{i}.bin"
    # data loading...
```

I use Python 3 **f-strings** (those with `f` before the quotes) that allow variables to be inserted directly into the string (e.g., `{i}`). At *runtime*, the loop replaces `{i}` with 0, 1, 2, etc., generating the correct filename.

Plotting with Matplotlib Finally, I use the **Matplotlib** library to visualize the data. It is a library with endless documentation that allows customizing every detail (line thickness, colors, axes), which sometimes gives me fear for its complexity compared to the simplicity of Matlab. However, I use the most basic function:

```
plt.plot(data)
```

I don't even put the X axis, I simply plot the sequence of samples for each of the eight electrodes. This is the raw trace (*raw data*), which probably covers only a few seconds of recording. I invite you to investigate it.

Spectral Analysis and Signal Filtering

At this point, from the raw data, I proceed to calculate the spectrogram or, more specifically, the **Power Spectral Density** (PSD). I didn't program it from scratch, despite having reminiscences of electrical communications and signal theory; I simply used the Python function that calculates PSD by applying the **Welch window** . Those of you who have done something with signal processing know what "Welch's method" means; if you don't know, ask Google. In theory, every time you encounter a word you don't know, you don't necessarily have to spend an hour on it, but roughly you could use Wikipedia or Google wisely (if the site of Covid conspiracy theorists pops up, maybe avoid that information).

Spectrum Observation and Artifacts Calculating the PSD for all eight electrodes, I see that at a few thousand Hertz (or cycles per second) the traces go down, as expected. What I *do not* see, and what I would have expected to see, is a peak at about **50 Hz**. Being the graph on a log-log scale (this is 100, 90, 80, 70, 50…), I would have expected power grid noise. Here there are not enough points to say for sure. What I see instead is a small peak ("little spike") at very high frequency that seems to be there in all electrodes. I don't know what it is: perhaps a numerical or digital noise at a frequency I am usually not used to using.

Digital Filtering: LFP and MUA Separation I can use filtering to extract the different components:

1. **Local Field Potentials (LFP):** I use a low-pass filter.
2. **Multi-Unit Activity (MUA):** I use a band-pass filter.

For LFPs, for example, I use a **Butterworth** filter. Again: don't know what a digital filter is? Don't know what a **FIR** (*Finite Impulse Response*) or **IIR** (*Infinite Impulse Response*) filter is? Take a look. You don't have to study it deeply, but it is useful to know roughly what it is about. This is the transfer function that Python allows me to implement with this beautiful library called **SciPy** (*Scientific Python*), which allows me not to know how to do anything "by hand" from the point of view of filter implementation, providing me directly with a ready-to-use low-pass or band-pass filter.

Software Tools and Didactic Feedback

I wonder if in the course of my colleagues Gibertoni and Gibaldi you do something practical in **Matlab**. You should probably do something in Matlab. If not, talk about it: your comments, positive or negative, are precious for posterity, especially if they are informal and preliminary rather than entrusted only to official questionnaires (there it is difficult for you to freely write "the guy sucks" or "I like it," while verbally it is more direct).

For example, regarding the use of **Python** transversally across all courses: I tried this year, but I believe Gibertoni and Gibaldi continued using Matlab. *(Interaction with students: "For reasons… in my opinion either all Matlab or all Python since Python has a huge quantity of libraries… but not yet or not entirely? Ok. Ah, ok. I didn't want to leave something out… Ok, not good, not good. Thanks for telling me.")*

It is true that this is not a master's in *Data Science*, but "playing" a little with data, removing noise, is important. Otherwise, it ends up that only N of you (maybe 7 or 8 people) develop the curiosity to do it, while the majority of your colleagues lack this skill.

Trace Exploration: LFP, MUA, and Epileptic Seizures

In the *notebook* you have a way to "play," to filter **Local Field Potentials** (low frequency part) and **Multi-Unit Activity** (high frequency part). You can zoom, for example on a window of 500 milliseconds, and see the different traces. Evidently something happens at that specific moment; it was visible even from raw data. In raw data, it seemed that all traces at a certain point started intense activity. This blue trace seems to have started later; perhaps the initial one was this purple trace (number 4).

I don't know exactly who initiates this sort of **miniature epileptic discharge**, but the tissue the data comes from is effectively a piece of epileptic tissue (or made such *in vitro*). From the LFP point of view, you might notice that the onset of the crisis (*seizure*) is not simultaneous on all channels. You could ask me: "Was there a spatial relationship?". Yes, there was. How far apart were these recording sites? I can tell you: a few tens of micrometers. So, if one wanted to play with data, one could extract some characteristic of seizure **propagation**, measuring delays between one electrode and another. Being *in vitro*, it is not a complete clinical epilepsy, but it is **epileptiform** activity (it has the shape of epileptic activity).

Introduction to Peak Detection

Let's pass now to the analysis of **Multi-Unit Activity**. You might want to detect spikes and you will soon have the necessity – we talk about it now quite lightly – to find a way to define a **threshold** whose crossing tells me if that is a peak or not.

In the code I show you how I implement **peak detection**. The idea is that maybe some of you, particularly "pain in the neck" or conversely obsessive-compulsive (OCD), tell me: "Here it is crap, this code is thrown there." It is true, it is thrown there a bit on purpose, not to annoy you but to stimulate someone to say: "No wait, let me comment it well, or let me change colors… here it is ugly that before you didn't use axes and here you used them." Obviously your input would be precious, but it is not mandatory.

So, I detect signals. In some cases I see only negative deflections, in others only positive. What do I do with all these things? I can extract them trace by trace, plot waveforms (and we will talk about this for *spike sorting*) and then visualize results for each of the channels (e.g., `Data1.bin`, `Data2.bin`, etc.).

Even if here I plotted signals as negative (simply to have them all in one graph), you see that not all neurons presumably caught by this electrode do the same thing at the same moment. But, if you squint your eyes, you see that there is a sort of "band" of activity here and another band a few hundred milliseconds later: a sort of first wave and then another wave, like a first crisis and a subsequent second crisis.

Intracellular Data Analysis

As for intracellular data, if you plot the entire trace (not just a 30-second window), you will see that there is a defined structure. I myself was injecting a negative current, a hyperpolarizing step, to study the exponential trend of the membrane charging and discharging curve. The goal was to extract, by performing an exponential *fit* on this response, the value of the **membrane time constant** (τ_m).

So, a person could analyze those data by fitting an exponential. Or, looking at spikes, you might want to analyze the firing frequency. It is not mandatory to do it for the exam, but you can do it for the rest of your life if you save these data (as long as GitHub or Google keep them online). You will also find the reference to the scientific article from which these data were taken, which explains what we wanted to do and why that specific cell (a rat cortical neuron) fired in such an apparently disordered way.

Technological Evolution of Electrodes

I would like to dwell on how, in the last 50-100 years, various technological discoveries have guided discoveries in neuroscience. From the use of the first **tungsten wires** (which we have already seen) to **tetrodes**, up to the so-called **Silicon Probes**.

From Tungsten Wires to "Jennifer Aniston Cells" Every technology enabled specific discoveries:

- With **tungsten wires**, **Place Cells** in the hippocampus (the "GPS of the brain") were discovered, as I mentioned in the first lectures.
- With **tetrodes** (four twisted microwires), researchers like **Rodrigo Quian Quiroga** found in the cortex of awake patients the so-called **"Jennifer Aniston Cells"**.

It is a very interesting result (published in *Nature* or *Science* several years ago): showing a patient different photos of Jennifer Aniston – with different hairstyles, from different angles – that specific cell fired selectively only for her. There was also the "Bill Clinton cell." This discovery reopened the debate on the theory of **"Grandmother Cells"**, which hypothesizes a hyper-specialization of neural coding: a single cell firing only when a specific concept is perceived (the sight, smell, or memory of a specific person, like one's grandmother).

Spatial Integration and Temporal Stability Depending on the application (basic research or clinical), it may be important to have devices that guarantee specific spatial integration or long-term temporal stability. There exist different categories of electrodes:

1. **Tetrodes:** They are handcrafted in the laboratory by twisting four very small wires (on the order of a micrometer), which are then cut at the tip. Despite insulation, only a small part of the tip remains exposed. Being four very close recording points, they allow effective signal triangulation.
2. **Polytrodes:** They are more refined from an industrial point of view. They have a glass or quartz *shank* (a needle) with electrodes "embedded"

inside that emerge on the surface at specific points. They offer reproducible geometry: if I buy ten, they all have the same characteristics.

3. **Utah Arrays:** Developed in the 70s at the University of Utah. They are rigid matrices of needles (e.g., 10x10) that penetrate tissue. They have been widely used and validated.

Silicon Probes (*Michigan Probes*) and Neuropixels

The most technologically advanced class is that of **Silicon Probes**, also called **Michigan Probes** because originally conceived at the University of Michigan. They are built entirely with **microphotolithography** methods, the same used for printed circuit boards (PCBs) and microprocessors: masks, *photoresist*, metal deposition. The *shank* is made of silicon (or flexible polyimide in some models) and has microscopic dimensions: width of a few tens of micrometers and thickness of a few micrometers.

Advantages of Microfabrication The interesting thing is that they allow integrating tracks and contacts with extreme precision.

- If we zoomed on the tip, we would see electrodes and tracks similar to those of a PCB.
- Everything is covered by an insulating layer, except for specific points where metal must be exposed to saline solution to record.
- Electrode dimensions (e.g., 50 micrometers or less) are comparable to those of neurons.
- They can be commercially mass-produced.

Neuropixels: The "Non Plus Ultra" The main problem with traditional probes is the connector: if I have hundreds of contacts, I must have hundreds of output wires. The currently most advanced solution (for now only experimental on animals, not in human clinics) are **Neuropixels** . Developed by an international consortium that includes **IMEC** in Leuven, Belgium (a non-profit silicon *foundry* where I worked too), they use **CMOS** technology.

- They have **thousands of contacts** along the shank.
- They do not have thousands of output wires. They function like **CCD** sensors in cameras: they possess an integrated electronic circuit that **multiplexes** signals.
- They do not read all sites simultaneously in analog, but scan and digitize signals (e.g., row by row) at very high speed, sending out a compact digital data stream.

Clinical Applications: "Utah Array" and Neural Interfaces

The **Utah Array**, despite being technologically less sophisticated than modern Neuropixels, was developed in the 70s and has been so widely used and studied that it obtained approval from certification bodies (like the **FDA**) for implantation in humans.

So, when Elon Musk claims to have been the first, last year or two years ago, to implant a neural interface in a patient, it is not accurate. About 15 years ago

– maybe you remember from the first or second lecture – there was already a patient playing *Pong* with their mind, or a paralyzed patient moving a robotic arm to drink from a straw (*BrainGate* project). These patients had precisely a Utah Array implanted in the motor cortex (or its variations, where needle length is not uniform to record from different cortical layers).

Signal Acquisition Chain

Once extracellular or intracellular signals are obtained, they must be processed. This is a part that will have been widely discussed in other courses, but of which you should have an essential smattering.

1. **Pre-amplification:** Signals are tiny (order of microvolts or tens of microvolts). It is necessary to amplify them, and it must be done **as close as possible to the source** (*headstage* pre-amplification) to prevent noise along cables from overwhelming the signal.
2. **Filtering:** Amplifying the signal amplifies noise too. At a certain point, it is necessary to filter.
3. **Sampling and Digitization:** To use a computer, you must sample the signal in time and digitize it in amplitude (make it discrete).

The Sampling Theorem (Nyquist-Shannon) All acquisition systems, from the most expensive to cheap microcontrollers (like an Arduino for 2-3 euros), possess an Analog-to-Digital Converter (ADC). The fundamental rule is given by the **Nyquist-Shannon Theorem**: to correctly sample a band-limited signal, the sampling frequency f_s must be at least twice the maximum frequency f_{max} contained in the signal:

$$f_s \geq 2f_{max}$$

I remind you of an intuitive way to remember this concept, which they didn't explain to me as a student but I find useful. Sampling a signal means, in the time domain, multiplying it by a **train of Dirac impulses** (equispaced Dirac Deltas). Since a product in the time domain corresponds to a **convolution** in the frequency domain, and since the Fourier transform of a train of impulses is itself a train of impulses, the effect of sampling is creating **replicas** of the original signal spectrum at regular intervals (centered on multiples of sampling frequency). If replicas overlap (aliasing), information is lost.

Analog-to-Digital Conversion (ADC) and Resolution

Besides temporal sampling, there is amplitude **quantization**. Digital systems have a fixed-length "word" (bits) that imposes finite resolution in number representation. I cannot represent arbitrarily small potential differences; I must "settle" for discrete levels.

Bit Depth and Quantization Levels Resolution depends on the number of bits (N) of the ADC converter:

- **12 bit:** $2^{12} = 4096$ discrete levels.
- **16 bit:** $2^{16} = 65,536$ discrete levels.

Obviously, going from 12 to 16 bits increases costs exponentially (from a few euros to thousands of euros for professional boards like those from *National Instruments*).

Dynamic Range and LSB (*Least Significant Bit*) There is another crucial parameter: **dynamic range** (e.g., $\pm 5V$, $\pm 10V$, $\pm 1V$). Often it is selectable. Suppose having a range of $\pm 5V$ (total excursion of $10V$) and a **12 bit** ADC. Minimum resolution (LSB) will be:

$$\text{LSB} = \frac{10\,V}{4096} \approx 2.4\,mV$$

This means every digital step is $2.4\,mV$.

- **Intracellular Case (Amplified):** If I have an action potential of $100\,mV$ and amplify it $100\times$, it becomes $10\,V$. In this case, I fill the whole range and have plenty of levels to describe it. I am happy.
- **Extracellular Case (Small signal):** If I have an extracellular signal of $100\,\mu V$ ($0.1\,mV$) and amplify it little (or if the range is too wide), I might end up with a signal that, even amplified, is on the order of a few millivolts. If my final signal is, for example, $10\,mV$, with a resolution of $2.4\,mV$ I have available only **4 or 5 levels** to describe it. The result would be a "jagged" signal, a very ugly digital staircase with enormous information loss (*quantization noise*).

That is why, when signals are small, either high amplification (gain) or a converter with a much higher number of bits (16 or 24 bits) is necessary to reduce quantization step amplitude. In the computer, when you plot data, you see many points and don't notice quantization, but if you do an extreme vertical zoom, you would discover that intermediate values between discrete ADC levels do not exist.

Signal Reconstruction and Sampling Theorem

There is an image I found on Wikipedia which, although only indirectly related to Nyquist-Shannon, illustrates the concept well. It shows a waveform where, with few samples, one tries to reconstruct the original trace . Remember that the concept of the sampling theorem is that you can express a continuous trace with a discrete set of samples, but you must have a way to "go back." The way to go back mathematically is multiplication (or convolution) with the anti-transform of an ideal low-pass filter (a *box* in frequency), which in time corresponds to the famous **sinc** function:

$$\text{sinc}(x) = \frac{\sin(x)}{x}$$

This function is 1 at zero and then should be 0 everywhere, but in reality, it has these *ripples* (oscillations) that lead to an *overlap*. If you do things right and the sampling frequency is sufficiently high, all other translated *sincs* (used to represent other samples) cancel out at sampling points, allowing perfect reconstruction. If you have few points (or they are too far apart), the approximation is wrong: the reconstructed trace does not correspond to the original one.

Filtering: Causal vs. A-Causal

Now, before taking a break, I wanted to represent a critical aspect of spike analysis: the type of filter used. How many of you know what a **causal filter** and an **a-causal filter** are?

Causal Filters and Phase Delay Causal filters are those having a causal impulse response, i.e., zero for times $t < 0$. They are those physically realizable in real-time. If you remember the story I told you, where you have an input and output that "chases" the input (think of a simple RC low-pass filter):

$$\tau \frac{dX}{dt} = -X + U$$

In this case, output X follows input U with certain attenuation and delay (phase shift).

- If input varies very slowly, X copies it almost perfectly.
- If input varies very rapidly, X tries but remains **delayed**.

This delay is an important feature when doing band-pass or low-pass filtering to extract LFP or MUA. Simply speaking of extracellular spike shape, **the causal filter alters its waveform**. It introduces phase distortions that can shift the peak or change its symmetry.

A causal filter is however necessary if you must work in **real-time** (e.g., interpreting signal for a neural prosthesis *on-the-fly*), because you cannot know the future of the signal.

A-Causal Filtering (Zero-Phase Filtering) If instead you have a pre-recorded trace (*offline* analysis), you are not bound by causality. You can use a-causal filtering that eliminates phase delay. Typically it is done like this (*filtfilt* algorithm):

1. Filter the trace a first time forward (introducing a delay Δt).
2. Take the filtered trace, **reverse it in time** (first sample becomes last).
3. Pass it again through the same filter.

In this way, the second pass introduces the same delay but in the opposite temporal direction, compensating exactly the delay of the first pass. The net effect is filtering with **zero phase delay**.

The Reviewer Anecdote and Carbon Nanotubes Some years ago, describing in an experimental article the effect of an electrode covered with a thin film of **carbon nanotubes**, my demonstration was: "See, the extracellular potential shape is indistinguishable from that of a standard electrode without layer." A *reviewer* "killed" my manuscript saying: "You used causal filtering, so the waveform is altered in both cases, making the comparison unreliable on fine details." He was right (he is a purist), even if I was comparing two quantities altered in the same way. From that moment on, if I can, I always use a-causal filtering to preserve the real spike waveform.

Introduction to Multisite Spike Sorting

Let's pass now to spike train analysis. Starting from raw signal, we pass to MUA signal (high-pass or band-pass > 100-200 Hz). This removes DC component (zero offset) and enhances rapid variations.

Imagine having four traces from a **polytrode** electrode (four close contacts).

1. **Different Amplitudes:** I see that on each electrode there are large signals and small signals. With considerations made in previous hours, I can say: "Probably these are different units. Large ones are neurons close to this contact, small ones are farther."
2. **Triangulation:** The interesting thing happens when contacts are very close (like in **tetrodes**, where distance is minimal). It could be that I see the **same identical neuron** on multiple channels simultaneously.

If I see a signal at the same identical temporal instant (without appreciable delays, given the medium is purely resistive and not capacitive) but with different amplitudes or shapes on different electrodes, I have powerful information. I can do a sort of **triangulation**.

This concept is the basis of modern **Spike Sorting**: if the electrode allows seeing the signal from multiple sites, I can use spatial waveform variability to separate sources . This drastically improves the ability to distinguish two neurons that perhaps, on a single electrode, would have similar and confused waveforms (e.g., one "tall and thin" and one "short and fat"), but which seen from four different angles reveal their distinct identity.

Example of Hippocampal Recording

I show you another example where **Local Field Potentials (LFP)** and **Multi-Unit Activity (MUA)** which can be glimpsed, are seen. The trace is raw and comes from an electrode inserted inside the **hippocampus**. It is a probe with many metallizations (independent electrodes), each represented by a trace with a different color.

You see that only corresponding to the hippocampus zone called **CA1** (where CA1 pyramidal neuron somas reside) does there seem to be this *multi-unit* component too. Corresponding traces show these jagged signals, which are spikes. For the rest, in other layers, almost exclusively very slow components (LFP) are observed.

Spike Detection Algorithms (***Spike Detection***)

Let's return to MUA trace analysis (zero-mean raw). Soon you will need to detect when there are spikes. Typically it is done with a **threshold crossing** algorithm, which is shockingly trivial. Choose a threshold (an amplitude value in microvolts). Then, for every "blessed" sample of your vector (where you have digitized numbers), verify the condition: *"Is amplitude less than threshold?"* (if threshold is negative). If no, continue; if yes, found an event. The first time this happens, you must mark the event. Otherwise, you would record crossing for all consecutive samples remaining beyond threshold during the peak. So, use a boolean variable to remember you are "beyond" threshold; next time, the

only condition interesting you is when you return above (or below) to re-arm the detector.

The Threshold Choice Problem Whatever the intuition behind the algorithm, it clashes with threshold choice. **What threshold do I put?**

- If I put it too high (in absolute value), I lose smaller peaks (the two small spikes in the drawing on the board).
- If I put it too low, I detect noise.

The only rational way is to **estimate noise bandwidth**. If I hypothesize noise has a **Gaussian** (normal) distribution, I can estimate its **standard deviation** (σ). Given mean value is zero, I can define an event as anything exceeding, for example, 3σ or 5σ. Each of these choices has a statistical implication: if noise were purely Gaussian, taking 5σ (on extreme distribution tails), I would have a **false positive** once "in a blue moon." Mathematically Gaussian tends to zero but is never zero, so in theory there could be an extreme fluctuation, but in real cases it is very rare.

The Problem of Non-Stationarity and Outliers However, two problems arise:

1. **Stationarity:** Is noise stationary? If I estimate bandwidth now, will it be equal in a few minutes? (Different electrodes don't worry, but time does).
2. **Signal Presence:** I cannot tell neurons: "Shut up everyone, I listen only to noise." Noise itself is composed of background neuronal activity. Furthermore, I must estimate noise standard deviation **while there is spiking activity**.

Spikes have large amplitude. Even if short-lasting, they are **outliers** compared to background noise distribution. If firing frequency is high, these peaks start weighing on classical standard deviation estimation (based on root mean square), increasing it ("inflating it"). If noise estimation increases, my threshold (5σ) rises, and I risk becoming "deaf" to smaller signals precisely when there is lots of activity.

Robust Estimators: Median and MAD

For some years, the method of choice – particularly for distributions that are **asymmetric** (*skewed*) or contaminated by outliers – does not use classical standard deviation, but an estimate based on **median**.

You know mean and median are different:

- **Mean:** Sum of values divided by number, weighted by probabilities. Very sensitive to tails (spikes).
- **Median:** Value creating a numerical watershed (50% samples above, 50% below).

If I have a distribution with a long tail (spikes), mean is "pulled" towards the tail. Median, instead, remains anchored to background noise center. I want

noise estimation to be **robust** and not affected by neuronal activity I want to detect.

Median Absolute Deviation (MAD) To estimate variability (noise bandwidth), **MAD** (*Median Absolute Deviation*) is used, defined as median of absolute deviations from median:

$$\text{MAD} = \text{median}\left(|x - \text{median}(x)|\right)$$

To make this value comparable to classical standard deviation (σ), a scaling factor is used. It is proven that for a Gaussian distribution:

$$\sigma \approx \frac{\text{MAD}}{0.6745} \approx 1.4826 \cdot \text{MAD}$$

This estimator is **unbiased** and consistent. In literature (including our lab), we use this criterion: calculate MAD, scale it to obtain estimated σ, and fix threshold at **5 times** this value.

Robustness Comparison: Variance vs. MAD Simulating artificial data with increasing firing frequencies:

- **Estimation with Classical Variance:** As soon as spikes increase (5, 10, 30 Hz), noise estimation grows rapidly. Threshold rises and I stop seeing small spikes.
- **Estimation with MAD:** Threshold remains practically constant up to very high firing frequencies (10-15 spikes per second). Since physiologically cortical neurons rarely fire above 20 Hz on average, this method is much more robust. It is "deaf" to spikes and listens only to noise.

Adaptive Threshold and Waveform Extraction

It is possible to make threshold **adaptive**: every 10 seconds I redo MAD calculation and update threshold. This compensates possible *drifts* due to temperature changes, electrode polarization or biological molecule adsorption on surface, which could alter impedance and thus thermal noise.

Once threshold is calculated (e.g., $5 \times \sigma_{MAD}$):

1. **Detection:** Identify crossing instant. I can work on absolute signal value $|V(t)|$ to capture both positive and negative peaks.
2. **Alignment (Cut and Paste):** After marking instant, go take a temporal segment (*chunk*) starting a few milliseconds before and ending a few milliseconds after event.

I want to align them to capture entire waveform. If I have a tetrode (four traces), for each event detected on one channel, I extract corresponding segments on **all four channels** simultaneously. This creates a "family" of curves for each electrode, ready for next phase: *spike sorting*.

Feature Extraction and Feature Space

Once waveforms are aligned, what do we do with them? If we superimpose them all, we see a "tangle." In some cases (like tetrode example), two groups are clearly distinguished: one with large amplitude (maybe fluctuating) and one with small amplitude. Does it make sense to do it by eye? Maybe not. It makes sense to extract quantitative **features**.

Intuitive vs. Data-Driven Features

1. **Intuitive Features:** Peak amplitude, duration (half-width), area under curve.
2. **Spectral Features:** I could take Fourier transform of every single waveform and use amplitude or phase of coefficients at dominant frequencies as features.

Hope is that, projecting every spike in a space defined by these features (e.g., a 2D graph with Amplitude on X and Duration on Y), points do not distribute in a continuum (like height of people in a classroom), but form separated **blobs** (clouds). A **bimodal** distribution allows me to draw a separation line: "Everything below is unit 1, everything above is unit 2."

Principal Component Analysis (PCA) In most general case, I abandon idea of choosing features a priori myself (I could be wrong or choose correlated features, like amplitude and area) and ask data: "What is best feature set to discriminate waveforms?". Here comes **Principal Component Analysis** (PCA) .

If we consider every waveform (composed of N samples) as a point in an N-dimensional space, PCA finds an orthonormal basis maximizing data variance.

- **First Principal Component (PC1)** is direction along which data vary most.
- **Second Component (PC2)** is orthogonal to first and captures maximum residual variance, and so on.

Often, using just first 2 or 3 principal components, one manages to explain 94-95% of signal variance. This is a formidable **compression** and *data-driven* feature extraction algorithm.

Clustering and the Inverse Problem

Projecting spikes in space of first principal components (e.g., PC1 vs. PC2), I can finally see if blobs separate. If I see 5 distinct blobs (red, yellow, green, blue, fuchsia), I could say: "There are 5 neurons." But here inverse problem arises:

- **How many classes are there really?** Are they 5 or 21?
- **Adaptation and Bursting:** What if two close blobs were actually **same neuron** changing waveform over time? As I told you talking about adaptation, when a neuron fires a spike train (*burst*), Sodium channels partially inactivate. Last spike of train is "lower" and has lower rise speed compared to first. A "stupid" algorithm would see two different shapes and classify them as two different neurons (unit overestimation).

- **Overlap:** If two spikes occur almost simultaneously, resulting waveform is sum. Algorithm will see it as strange shape and classify it as "neuron N+1" or discard it as noise.

The "Engulfed Electrode" Experiment To solve "who is who" ambiguity, some years ago in a European project we tried a radical approach: forcing neuron to "eat" electrode. We used mushroom-shaped electrodes (*gold mushroom*), with a stem and head of about 1 micrometer, decorated with peptides stimulating **endocytosis** (or phagocytosis/pinocytosis). Cell recognizes peptides and tries to engulf gold particle. Creates actin ring tightening mushroom neck, sealing junction .

Result? Perfect electrical coupling. From that electrode we recorded **only and exclusively one unit**. No need for *spike sorting* or complicated algorithms: correspondence was 1:1. This is only way (besides patch clamp) to have absolute certainty of source.

Automatic Clustering Algorithms

Returning to standard case (without phagocytosis), algorithms exist to automate blob separation:

1. **K-Means:** You must tell it a priori how many clusters (K) to look for.
2. **Mixture of Gaussians:** Models data as superposition of Gaussian distributions (mean and variance).
3. **Superparamagnetic Clustering (SPC):** Invented by **Rodrigo Quian Quiroga** ("Jennifer Aniston cells guy"). Exploits analogy with magnetic systems physics (phase transitions) to identify clusters even of irregular or non-spherical shape, without having to specify a priori number of classes. It is one of most powerful methods currently.

However, no method is perfect ("There is no free lunch"). *Spike sorting* problem remains an ill-posed problem.

Intracellular Signal Analysis

Finally, let's close with intracellular data analysis (where you are with electrode inside neuron belly). Here analysis is much more powerful because you can **stimulate** cell.

Stimulation Protocols In data you have or in real experiment, you can inject different current waveforms to characterize cell:

1. **Steps:** Constant positive (depolarizing) or negative (hyperpolarizing) currents of variable amplitude.
2. **Ramps:** Current growing linearly to find exact firing threshold.
3. **Sinusoids (Chirp):** Oscillations at increasing frequency (as seen for electrical synapses and resonance).
4. **Noise:** Injection of stochastic fluctuating trace (we will talk about it next year for those who survive, makes sense for theoretical information analysis).

Feature Extraction for Classification From these responses you can extract quantitative parameters (*features*) to classify neuron:

- **Membrane Time Constant (τ_m):** From charge/discharge exponential fit.
- **Rheobase:** Minimum current necessary to make neuron fire.
- **Adaptation:** How much firing frequency slows down during spike train.
- **After-Hyperpolarization (AHP):** Depth and duration of hyperpolarization after spike.
- **Spike Width:** Distinguishes pyramidal (wide) from *fast-spiking* interneurons (narrow).

Putting these features in a classifier, you can say: "You are a layer 5 pyramidal neuron," "You are a Martinotti interneuron," etc.

Course Conclusion

And that's it. You know where to find me. Do not hesitate to contact me if you have questions or problems, I am happy to answer. Let's make an appointment. It is likely that during Christmas holidays I won't answer emails, but from January onwards count on me.

Thank you all and good luck.