TEST2

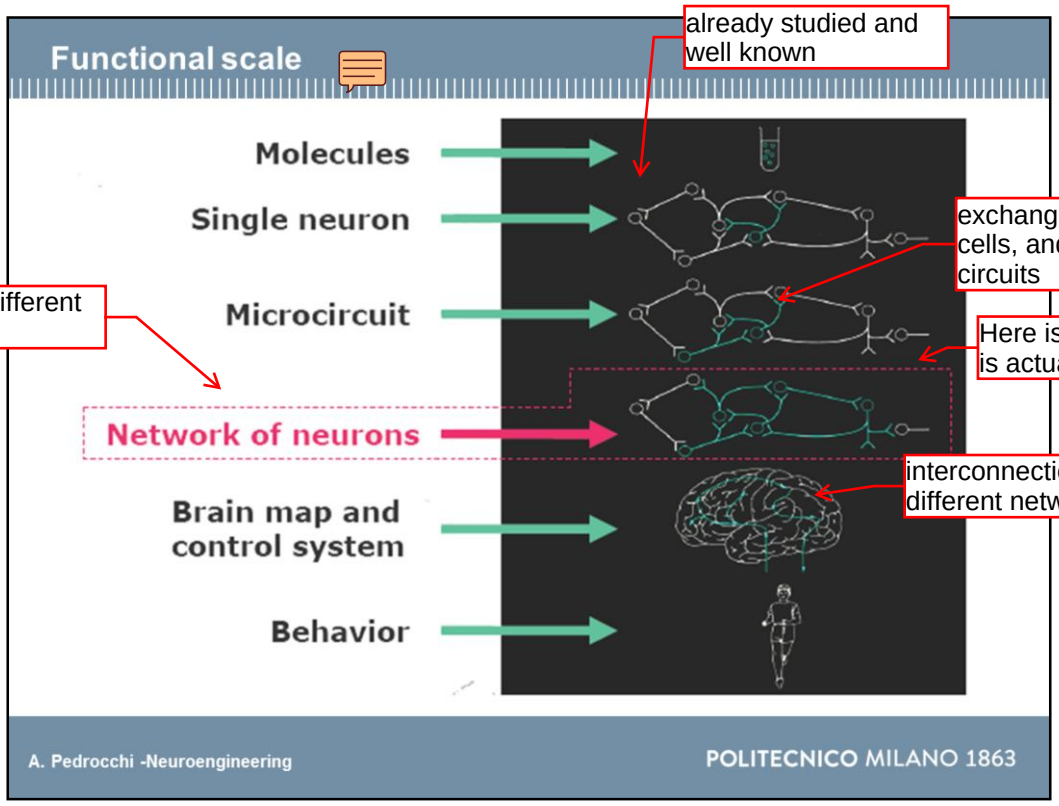
Neuroengineering 2017/18

NEUROENGINEERING FOR BIOLOGY 1-
Electronical tools to interface neuronal networks

Reference: SPIRA AND HAI Multi-electrode array technologies for neuroscience and cardiology NATURE NANOTECHNOLOGY VOL 8 FEBRUARY 2013

http://www.nature.com/nnano/journal/v8/n2/pdf/nnano.2012.265.pdf

The point is to try to figure out what are the current methods to investigate experimentally neuronal circuits, slices, neurons activity what are the technological means available for biologist to do this kind of experiments (Connectivity, signal processing by neurons etc.)



In the present figure, we can see the different "functional layers" concerning the neural system. We stop our investigation at the neural network level. Why do we stop there? Because the microbiology literature has investigated the single neuron level and the interaction between single neurons in details. Those levels are well known and they are something that is firm knowledge. However, the content of information is not given by the single neuron activity, but it is given by a network of connections. It is not easy to interface the natural neural network to study information flow, and therefore we need good instruments to study at the natural neural network level [see next slide].

We need to set the functional scale we want to work with:

N.science=> the whole multiscale approach. going through molecules (synapses are formed, how do they talk) -> single neuron activity, comp. models, -> Microcircuits dynamics (the way the signal is exchanged between different cells, and organized in small circuits)

Where the real info processing occurs is at the level of networks:
if you think about ANN, you can immediately realize how the info processing is not into a subset of the network, or the functioning of one single neuron, but the info processing is really inside the whole network. Because of the kind of structure that ann has, and it's mimicing the natural one, there's not even a clear addressing of where the information is. It's exactly one main difference between the neural approach in respect to the standard approach: you dont know where memory is addressed.

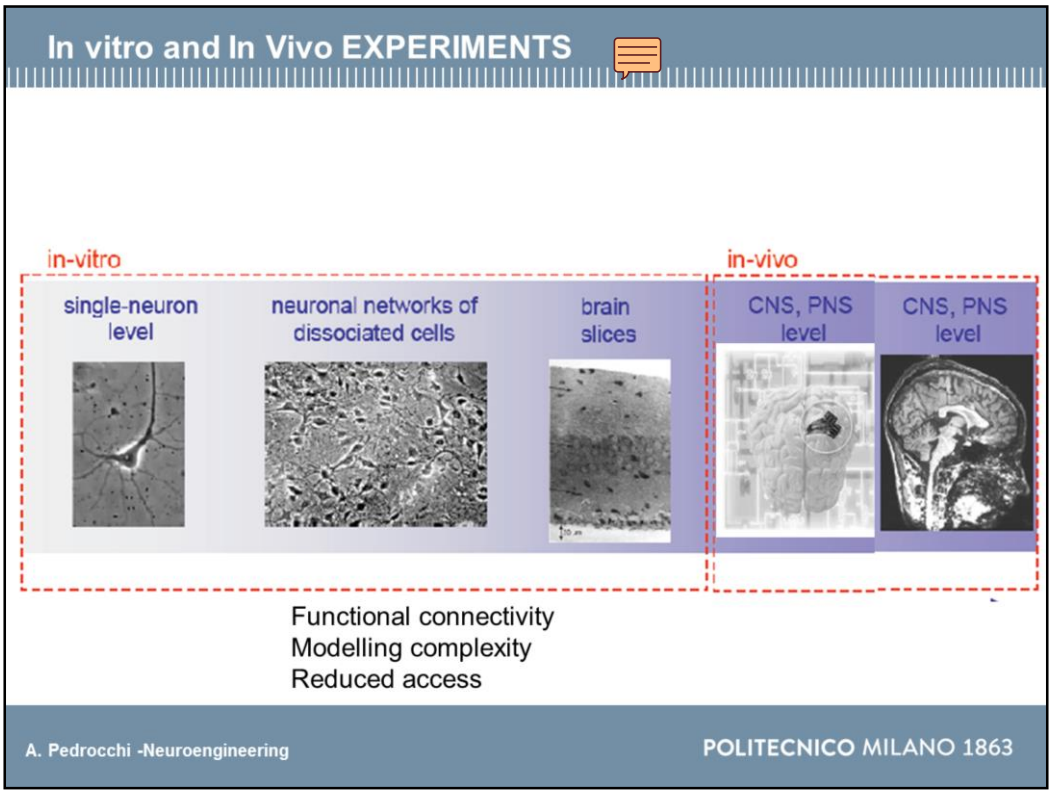
Of course you need to go to HIGHER scale, to make brain maps, to know how the different networks are interconnected, adding up to understand behavior. So each single scale has different methodological and experimental approaches. Now we'd like to focus on microcircuits and network.

GOAL

The point is that

Both the single-neuron level and the CNS-level are well known but the dynamics of neural networks is still far from elucidated. For this reason, studies at this level of analysis are essential to deeply understanding neural pathways.

This question opens some very interesting technological challenges



Because of the huge amount of cells in the brain what we can know by in-vivo experiments is very limited and concerns a subsystem where the boundary conditions are not under control (i.e. the activities of the other connected areas).

Deep brain recording has been done on primates and animal and only very rarely on humans (and anyway only on pathological subjects as epileptic kids ~~in order to know the area of the epileptogenic foci or on very few (a couple) experiments for controlling robot with cortex matrices of electrodes on SCI patients…~~

Or we can have some deep recording from the PD patients who are undergoing DBS implants during the surgery to check for the position of the electrodes, but these are short recordings…

Anyway, deep recording is done by matrices of electrodes in the case of cortical recording or by wires with electrodes in the case of deep brain. To have a rough idea of the size of these data, we can have about 10 contacts on each wire, about a few wires implanted (about 10, max), each electrode records the signal of about a few cells (2-5)… so, at the end, we are recording a few hundreds of cells (10*10*5) in an area, where many thousands of neurons are indeed working together… the undersampling is quite strong!

The other possible source of information is by using in vitro models.

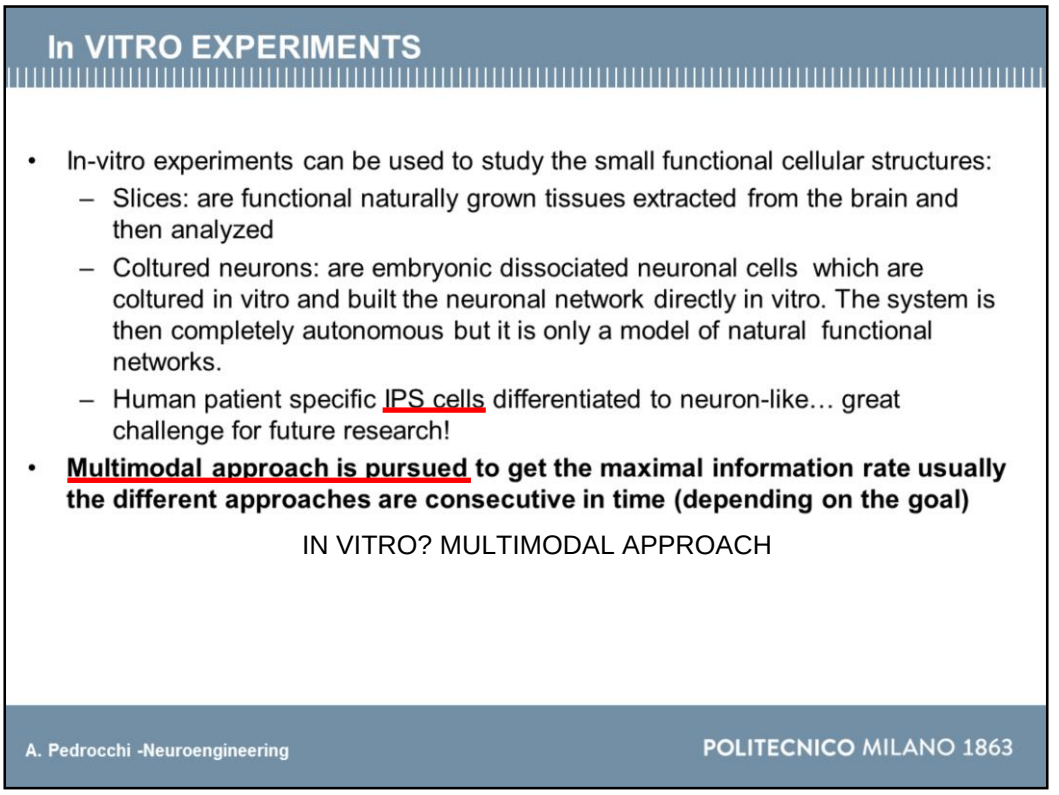
It should be noted that in vitro models require suitable technologies to interface with the model itself. The method has to be respectful and not to perturb what you want to measure.

While from a reading technological point of view, in vitro is simpler that in vivo, in vitro models are to be properly designed and a lot of effort of biologists has been devoted to proper culture solutions able to mimic the physiological environment and to assure that networks developed in vitro are behaving as in vivo ones.

This step can't be taken for granted, preparation of culture needs always to follow detailed validated protocols and often baseline behavior needs to be tested before performing any specific experiment.

Neurons are very delicate cells, and in vitro neuronal culturing is quite challenging, especially for assuring glia cells nutrients and proper perfusion of medium, besides temperature, Ph, Humidity and so forth. Medium change is for example a procedure that perturbs the neurons activity but still it is not fully measured the impact of this perturbation…

Summary



How is it possible to have neural networks in vitro models?

- Neural tissue slice. With this method, the network is developed in vivo and then extracted and treated/cultured so to keep it alive also in vitro (at least for a certain amount of time…). The technical problem here is that there are dead neurons at the boundaries that are sources of noise and that the biological networks have a complexity that often goes beyond the actual biological sample.

From embryonic rats, it can be taken:

-Single neurons, before their growth. If they are placed in suitable environment, they continue to grow and start to form connections. In this case, we obtain networks that have characteristics very similar to those that would have developed in vivo. Note: With this method, the network is formed in vitro because the neurons are taken before that they develop as a network.

All these are animal essays…

Recently, IPS (induced pluripotent stem) cells have been differentiated to neuron-like cells, the paramount advantage of this essay is that you can have patient specific neuron-like culture by a skin biopsy… but still a lot of effort is needed to really prove that these neuron-like cells are "neuron-like"…

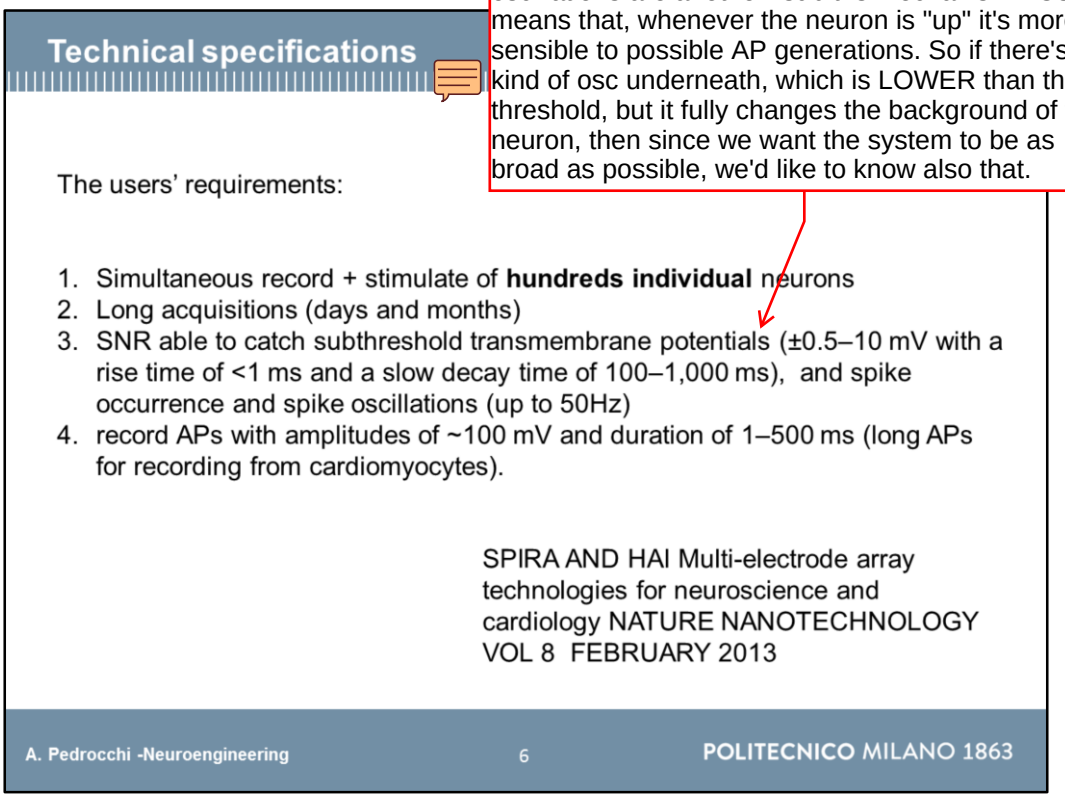
See http://www.hsr.it/research/organization/divisions-centers/division-of-neuroscience/vania-broccoli/

"Stem cells and Neurogenenis group I San Raffaele,

Defects in early developmental processes during embryonic neurogenesis are the biological rationale of several human neuropathologies. In particular, many genes, shown to play a pivotal role in key processes as neural stem cell proliferation, early neural commitment and cell migration, are responsible of human hereditary neural syndromes. We are interested to understand the genetic mechanisms that control the behavior and fate of neural stem cells and the pathways by which different types of neurons arise by a similar pool of neuroblasts. Interestingly, some transcription factors playing a crucial function in GABArgic neural differentiation have been found mutated in neural syndromes leading to epilepsy, mental retardation, West Syndrome and some forms of autism. Understanding how these genes act in directing neural differentiation and how their mutations are causing the disease is a main challenge of the current investigations pursued in the lab. These efforts are instrumental to establish in vitro protocols of neural stem cell differentiation to tightly control their specific neuronal cell type (GABAergic, Glutamatergic or Dopaminergic) and their maturation and activity state. "

le cellule IPS avranno le stesse caratteristiche e difetti derivanti dal soggetto

subth potentials: shorter and with a slow decay because they have the "rc model" underneat. But they also could be oscillating. High frequency oscillations are another "sub ths mechanism". Osc means that, whenever the neuron is "up" it's more sensible to possible AP generations. So if there's this kind of osc underneath, which is LOWER than the threshold, but it fully changes the background of the neuron, then since we want the system to be as broad as possible, we'd like to know also that.



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To objectively evaluate the different approaches, we examine the principal achievements in relation to a list of biophysical parameters that are needed to decipher the functional connectivity map of a neuronal network.

An ideal imaginary device would allow the user to:

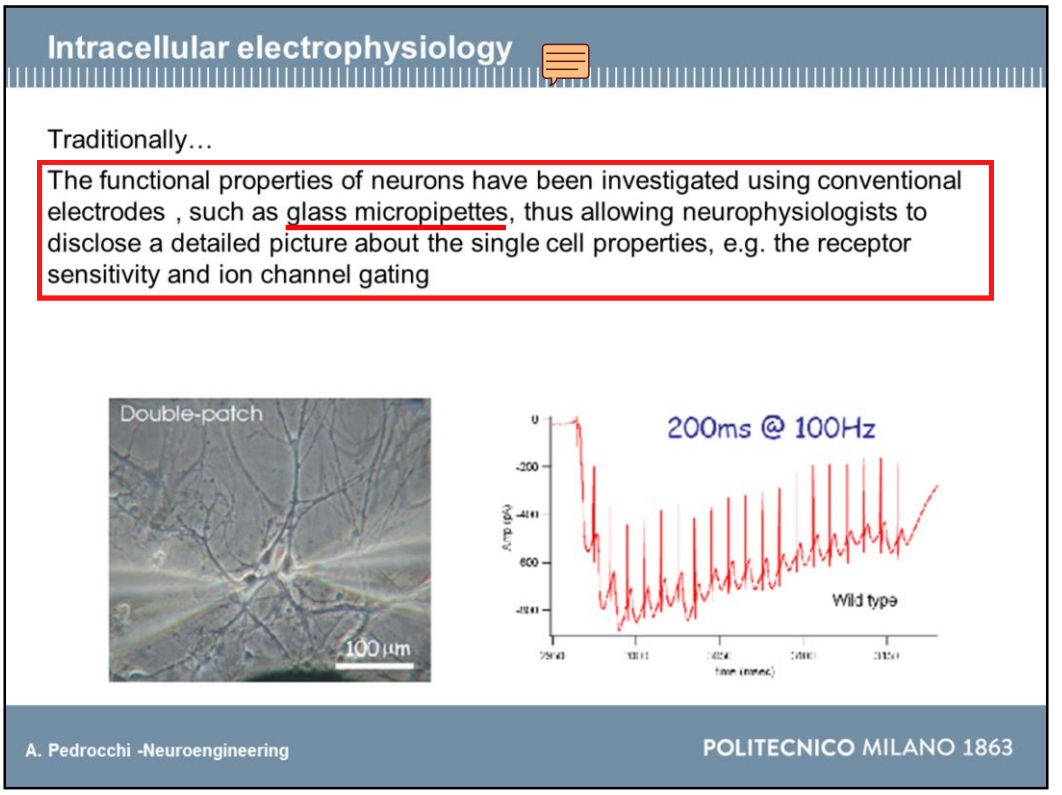
(a) simultaneously record and stimulate hundreds of individual neurons intracellularly,

(b) maintain a stable contact with the neurons for recording and stimulation for days and months,

(c) monitor the transmembrane potential in the relevant cell-physiological range of −80 to +30 mV,

(d) detect subthreshold potentials such as excitatory and inhibitory synaptic potentials with amplitudes in the range of ±0.5–10 mV with a rise time of <1 ms and a slow decay time of 100–1,000 ms, and to record membrane oscillations in the range of ±5 mV at frequencies of 1–50 Hz,

(e) record APs with amplitudes of ~100 mV and duration of 1–500 ms (long APs for recording from cardiomyocytes).

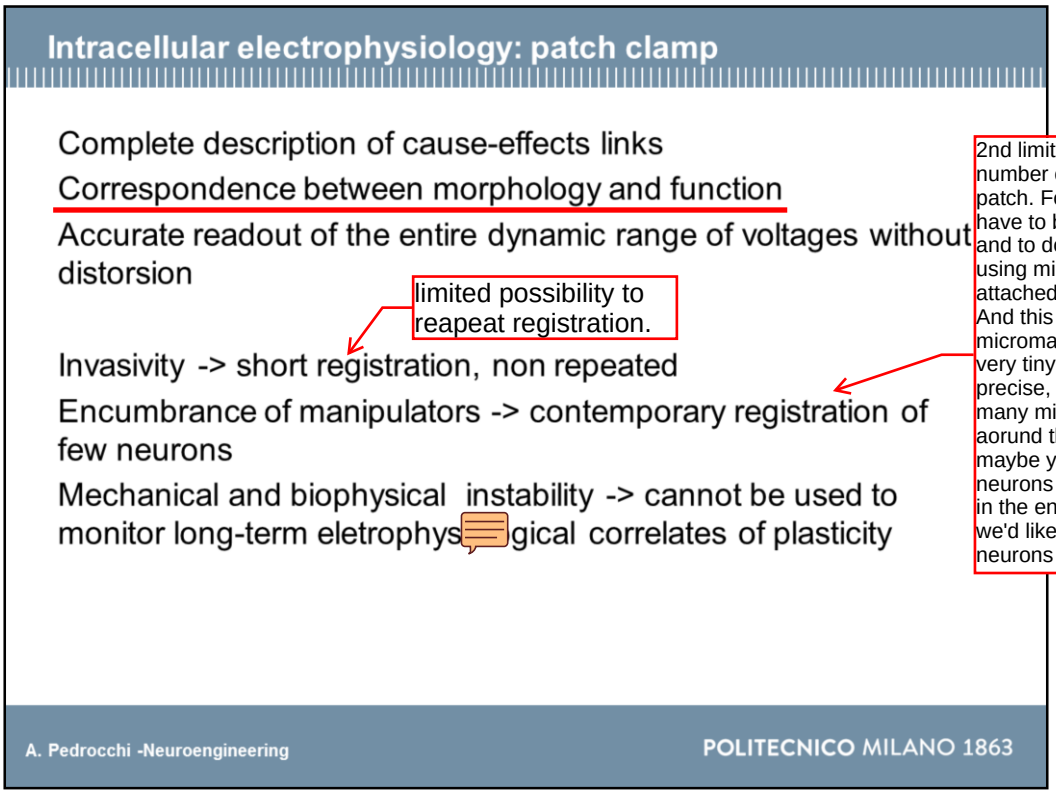


At the single neuron level, the standard procedure is the patch clamp [see slide].

The invention of intracellular recording and stimulation technologies were hallmark developments that enabled the biophysical 'language' by which individual neurons transmit electrical information, communicate and 'compute' subthreshold synaptic information to be deciphered. The power of intracellular recording systems is that they exhibit very good electrical coupling with the cell and provide accurate readout of the entire dynamic range of voltages generated by cells without distorting the readout over time. Yet, the use of sharp or patch microelectrodes is limited to individual neurons as steering of the electrode tips into target cells requires the use of bulky micromanipulators and the duration of intracellular recording sessions is limited by mechanical and biophysical instabilities.

MICROELECTRODES

Patch clamp is the reference experimental setup for deriving for example the data for the compartimental neuron model described in the Computational Neuroscience 2 lecture.



The traditional intracellular electrophysiology has some advantages [see slide]

-The locally, well identified cause-effect link is perfectly known

-There is a correspondence between morphology and function

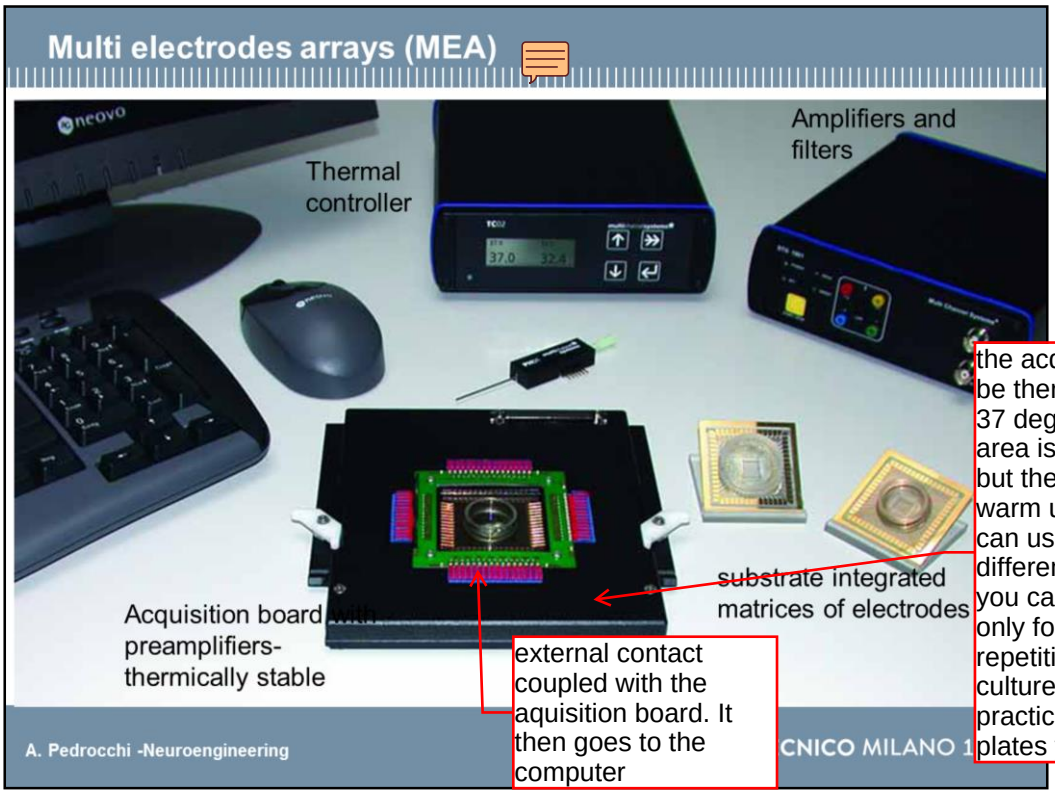
- Good SNR and dynamic response

The traditional intracellular electrophysiology has some limits as well [see slide]:

- It is invasive and therefore it perturbs the neuron which dies just after the measure.

- The spatial resolution is linked to the number of clamps that are placed on the neural culture. The positioning has to be done at the microscope, and it is not possible to place more than 1 or 2 clamps. Investigation at the network level is not possible with this method.

- There is an intrinsic biophysical and mechanical instability that prevents to prolong experiments over few hours



The MEA-Systems record, amplify, and analyze signals from biological samples *in vitro*. The data is analyzed by the included data acquisition software. MEA-Systems are used to record from brain or cardiac slices, neuronal or cardiac cultures, *ex vivo* retina, cell lines or stem cells.

IT'S BIDIRECTIONAL

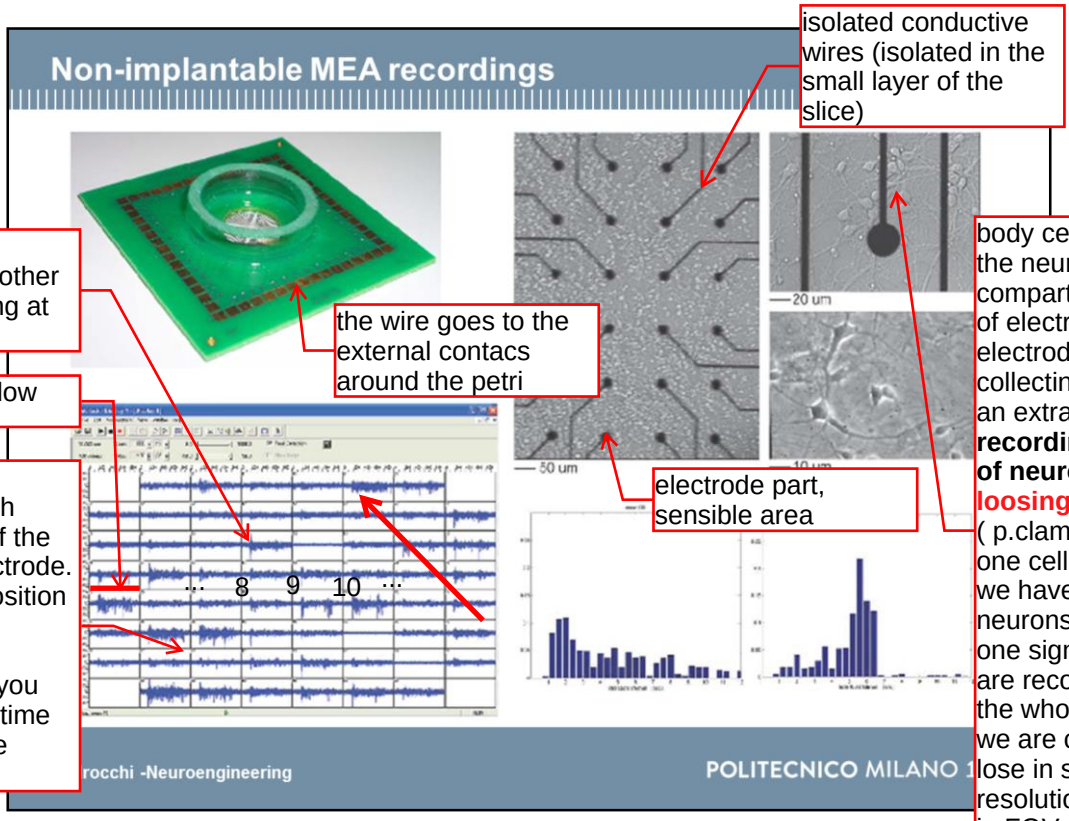
Multielectrode arrays (MEAs) or microelectrode arrays are devices that contain multiple plates ~~or shanks~~ through which neural signals are obtained or delivered, essentially serving as neural interfaces that connect neurons to electronic circuitry. They are based on substrate integrated matrices of electrodes for the recording of extracellular activity. There are two general classes of MEAs: implantable MEAs, used in vivo, and non-implantable MEAs, used in vitro.

MEAs:
- implantable
- non implantable (in-vitro)
EXTRACELLULAR POTENTIAL

Possible alternative to intracellular electrodes

extra cellular electrodes, based on MEA: they are analizing a signal from invitro, but using electrodes which are inserted into the floor of the slice. They contain multiple plates, and on the surface of the plate (the base of the petri) inside the center part of it you'll have conductive patterns isolated in the thin layer of petri, and just opened on some points, so that they are sensible to the potential (extra cellular, you are not entering any cell, but there's an area of sensibility around the electrode).

IMPORTANT! Remember: problem of dark neurons



MEA are microscopic slides where electrodes are inserted. Connections between each electrode and the board for data collection are of course isolated.

The obtained measure is an extracellular measure with respect to a reference electrode, thus a voltage difference is measured. It should be noted that each electrode does NOT correspond to a single neuron. MEA has a spatial resolution that corresponds to a cluster of neurons.

The standard type of in vitro MEA comes in a pattern of 8 x 8 or 6 x 10 electrodes. ~~Electrodes are typically composed of indium tin oxide or titanium and have diameters between 10 and 30 µm.~~ These arrays are normally used for single-cell cultures (i.e. many cells but all of the same type) or acute brain slices.

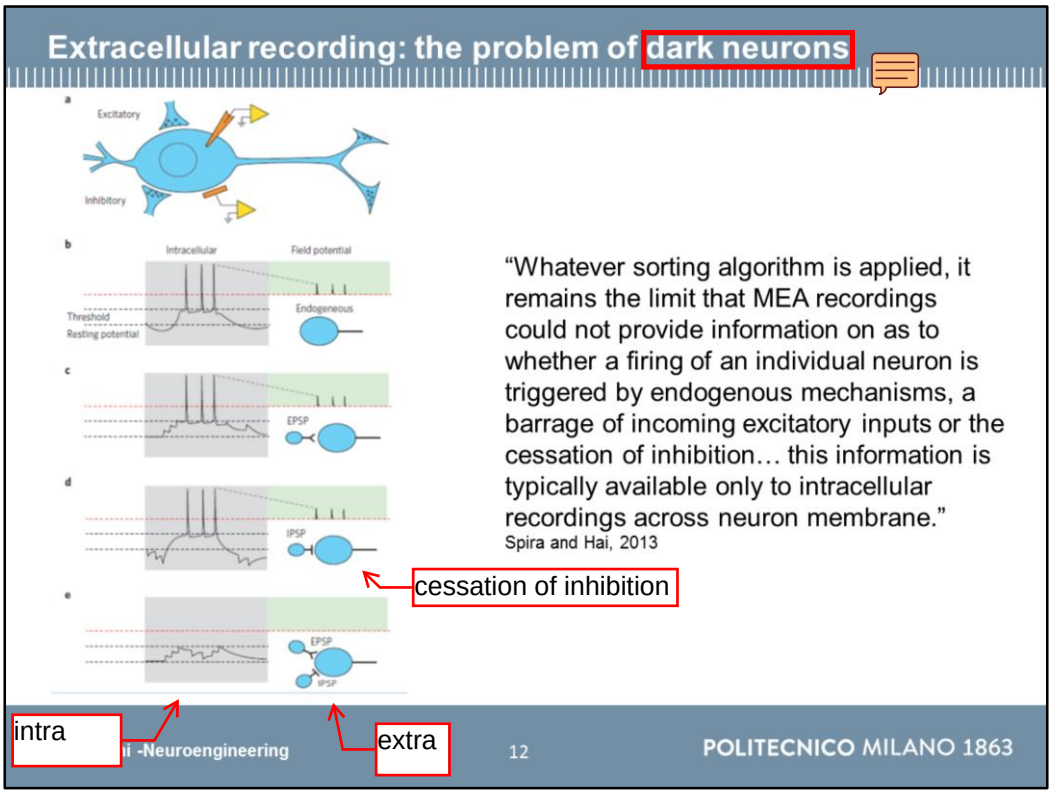
In case of recording in vitro slices, one major issue in order to obtain quality signals concerns that electrodes and tissue must be in close contact with one another. The perforated MEA design applies negative pressure to openings in the substrate so that tissue slices can be positioned on the electrodes to enhance contact and recorded signals.

A different approach to lower the electrode impedance is by modification of the interface material, for example by using carbon nanotubes, or by modification of the structure of the electrodes, with for example gold nanopillars or nanocavities.

MEA

The extracellular space is conductive as well, and although the resistance is very low, it is not zero.

According to Ohm's law (V=R*I), the extracellular current results in a small voltage that can be measured with extracellular electrodes. Extracellular signals are smaller than transmembrane potentials, depending on the distance of the signal source to the electrode.

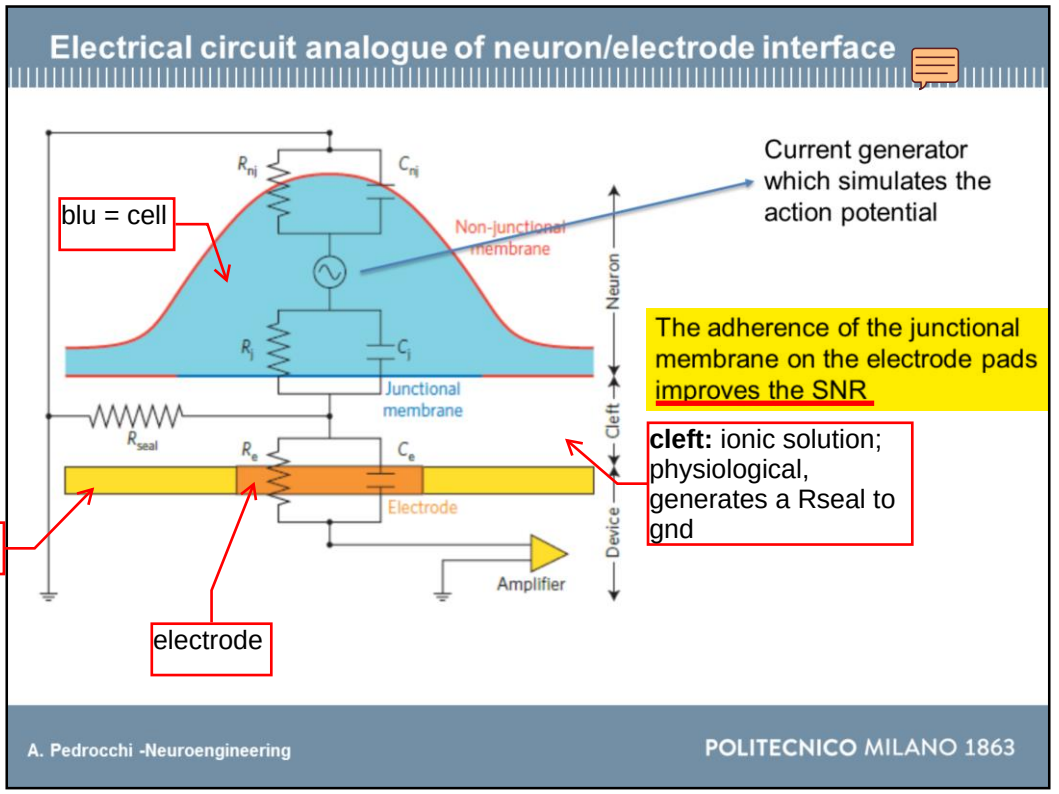


Endogenous membrane properties as well as excitatory and inhibitory synaptic inputs regulate the firing patterns of individual neurons. This is depicted in the schematic of a neuron (blue) that receives an excitatory and an inhibitory synaptic input in a Subthreshold and supra-threshold electrophysiological activity of the neuron is recorded by an intracellular (upper orange electrode) and an extracellular (lower orange electrode) electrode. The amplifiers are depicted in yellow. The intracellular recordings are shown in the left panels of b–e, and the corresponding extracellular recordings are shown in the right panels (green background). In b, a neuron endogenously generates a train of APs (of approximately Δ100 mV) by depolarization of the membrane potential from the resting value of approximately −80 mV (bottom dashed line) reaching a threshold level to fire APs (middle dashed line) at about −50 mV, and then the

membrane potential endogenously repolarizes. The extracellular electrode picks up the FPs generated by the APs (marked by vertical lines and green background). Note that the recorded FP amplitudes range between 0.01 and 1 mV, and are not drawn to scale. The attenuation factor (1/100 to 1/1000) is so large that

subthreshold potentials generated by individual neurons cannot be recorded. Thus, the extracellular electrode is practically 'blind' to the subthreshold events (grey background, below the red dashed line). In c and dthe very same pattern of APs firing is generated by excitatory (c) and inhibitory (d) synaptic inputs. Whereas in c summation of excitatory synaptic potentials depolarizes the neuron to reach the firing level, and the neuron stops firing when the barrage of the excitatory inputs stops (leading to membrane repolarization), in d the train of APs is generated by dis-inhibition (the cessation of the barrage of inhibitory synaptic inputs). The significant differences

in these mechanisms (b–d) cannot be detected by the extracellular electrode. Furthermore, unless an individual neuron is firing APs, synaptic inputs are not 'visible' to the extracellular electrodes at all (e). In this example, the extracellular electrode does not detect the presence of a neuron that receives a barrage of excitatory and inhibitory synaptic inputs. These inputs may be of significant importance to the functioning of the neuronal circuit.



Depending on cell's morphology around the electrode, the electrical signal measured by each electrode is composed by different signal sources. The contribution of each source to the signal recorded by an electrode is a function of the distance from the source to the electrode. The recorded signal is affected by the membrane, the medium, the electrode pad etc. which act as capacitors. The recorded signal is therefore a sum of different contributions [see slide for formula].

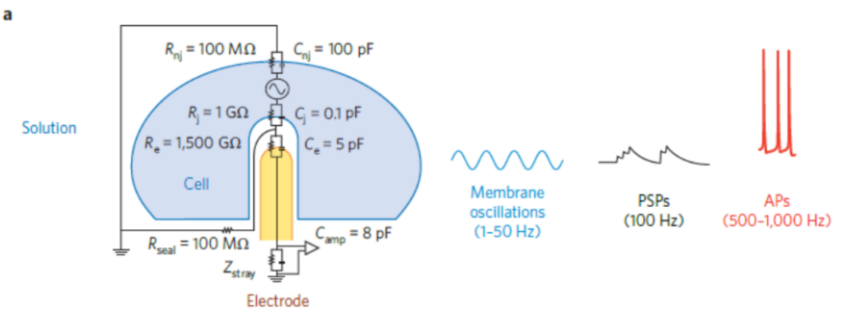
There is a strong attenuation of the signal and also a filtering of high frequency provided by capacitive elements.

Figure 1 |

Schematic layout depicting the spatial relationships between a neuron and a substrate-integrated electrode and the analogue passive electrical circuit. The cell body of a neuron (light blue) resides on a sensing electrode (orange) integrated in the culture substrate (yellow). The electrode is coupled to an amplifier (yellow). A cleft filled by the culturing media (ionic solution) interposes between the cell membrane and the electrode–substrate. The neurons plasma membrane is subdivided into two: the part that faces the electrode (blue) is defined as the junctional membrane and is represented by the junctional membrane resistance (Rj) and the junctional membrane conductance (Cj). The rest of the membrane, defined as the non-junctional membrane (red), faces the bathing solution and the culture substrate. This part of the membrane is represented by the non-junctional resistance (Rnj) and the non-junctional capacitance (Cnj). The physiological solution within the cleft generates the seal resistance (Rseal) to ground. The electrode (orange) impedance is represented by the electrode resistance and capacitance (Re and Ce, respectively). The electrode can be a passive element or a transistor. For simulation purposes of APs or intracellular current injections, current can be injected into the analogue cell-circuit in-between Rnj and Rj. ~~Under physiological conditions current is generated by transient changes in the membrane conductances.~~

~~The colour coding shown here is used in Fig. 4 to depict the different components of the analogue electrical circuit.~~

MEA: SNR and distorsion



COUPLING: ratio between the maximal voltages recorded by the device in response to the maximal voltage generated by an excitable cell.

We want to be able to record the smallest possible signals from each single electrode

The electrical coupling between a neuron and a sensing pad is defined here as the ratio between the maximal voltages recorded by the device in response to the maximal voltage generated by an excitable cell.

The surface area of the junctional membrane can be anywhere between a very small fraction of the cell surface area, up to approximately 50% in cells that flatten while adhering strongly to substrate-integrated sensing pads. This variable depends on the geometry of the sensing pad and the morphology and adhesion characteristics of the specific cell. The junctional membrane can thus be of very high resistance and low capacitance. This implies that only a small fraction of the current generated across the neuron's membrane, flows through the junctional membrane. Reduction of the junctional membrane resistance would be very effective in improving the electrical coupling coefficient between a neuron and an electrode

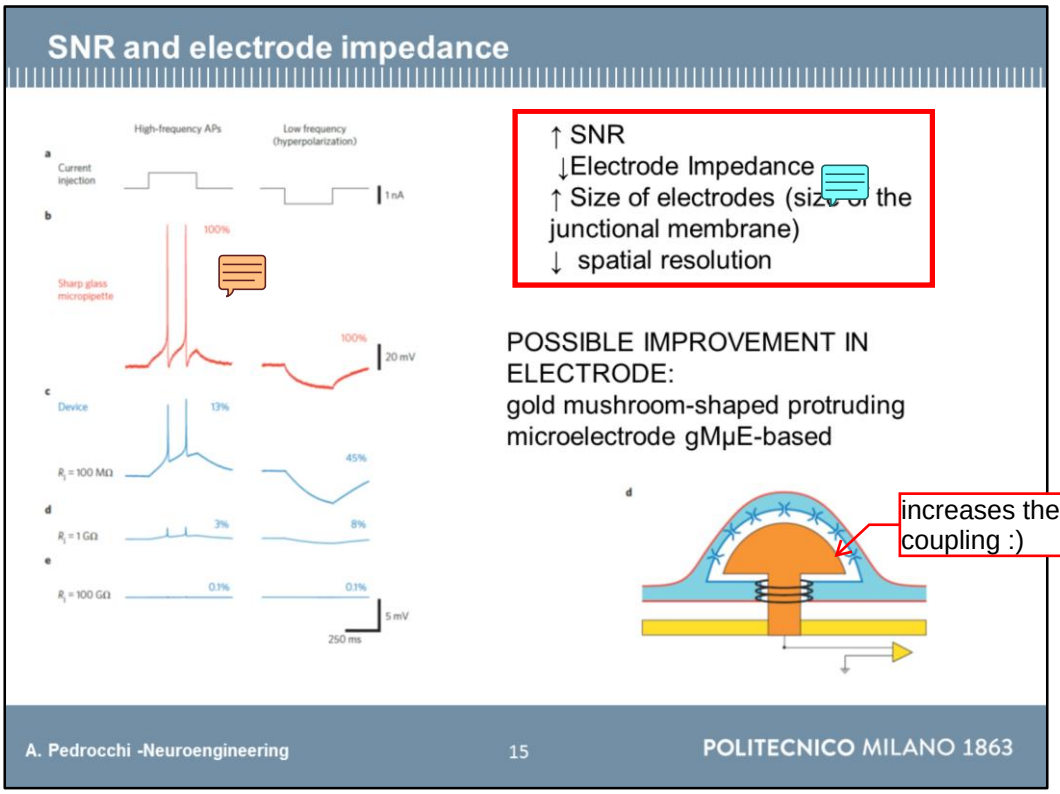


Figure 3 | Dependency of the electrical coupling on the junctional membrane resistance and pulse duration. Shown is a simulation of the cell–device coupling of APs and a long hyperpolarizing pulse at three different values of *R*j (100 M**Ω**, 1 G**Ω** and 100 G**Ω**) **a**, Schematic illustrations of the depolarizing (left) and hyperpolarizing current pulse (right) delivered to generate two APs and membrane hyperpolarization, respectively. **b**, Simulation of the ensuing intracellular potentials recorded by an intracellular electrode (red). **c**–**e**, The recorded potentials by an extracellular-located electrode (as shown in Figs 1 and 2) under different junctional membrane values (blue).

d, A neuron engulfing a gold mushroom-shaped protruding microelectrode gMµE-based. Note actin rings surrounding the mushrooms stalk stabilizing the configuration. They use of a chemically functionalized micrometre-size mushroom-shaped gold protrusion as the sensing electrode providing an increase of the neuron–microelectrode electrical coupling coefficient from approximately 0.1% as recorded by a planar extracellular MEA to approximately 50%

Out of the five criteria to evaluate of the benefits of the approaches, the gMµE-based MEA provided multisite, simultaneous, intracellular recording and stimulation for periods of days (which is for as long as we carried out the recordings). The filtering properties of the gold electrodes and the a.c. amplifier used do not enable the resting potentials of the neurons to be recorded. Nevertheless, the configuration successfully monitored subthreshold synaptic potentials and APs. ~~The filtering nature of the recording system can be deconvoluted and thus unfiltered high-quality recordings of APs and synaptic potentials can be retrieved. A stable electrical coupling between gMµE and a neuron coincided with the formation of cytoskeletal~~

~~actin rings surrounding the stalks of the mushroom-like structure. Individual gMµE enables both voltage recordings and application of current. So far, attempts to obtain in-cell recordings and stimulation from rat hippocampal neurons and primary cardiomyocytes were unsuccessful. It should be noted nevertheless that these attempts were limited to gMµE functionalized with poly-d-lysine rather than by the engulfment promoting peptide.~~

The drawbacks are that the microfabrication of such electrodes is much more complex and consequentely the costs.

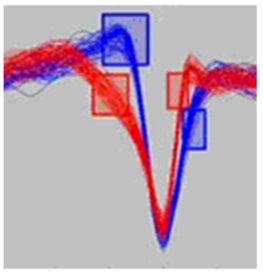
MEA slides are not disposable but anyway they can't be efficiently used for more that 10-15 cultures because cleaning procedures and sterilization riun the surface of the slide and SNR is worsen.

Spike sorting

Two cells afferent to the same electrode will in general have a different covered area.

Even if they cover the electrode in the same way, their spike waveform will be different because in general they have a different nature and ionic channel density (Vj).

Assumption: the shape of the spike of each neuron is stationary



A. Pedrocchi -Neuroengineering POLITECNICO MILANO 1863

However, it can be hypothesized that during registration physical characteristics of the neural culture are kept constant, and therefore each neuron fires with the same spike shape. We can therefore apply algorithms for spike recognition/spike sorting that recognize a template. If the algorithm identifies a template that is repeated in time, then the template can be linked to a source. With good post-processing analysis, it is possible to reconstruct a spatial resolution given by template recognition, but it is not possible to link the templates to the network morphology.

Another possiblity, improving processing from a software point of view:

main problem: **reduction in spatial resolution**.. how do we solve with data processing ?

how do we improve the res. of the system?

best solution: spike sorting. It's absolutely used in deep brain electrodes, mea electrodes, in slicing... etc... it's very used. The hypotesis is that, **the geometry and the characteristics of the cells are stable over the experiment time window**. If the geometry and characteristic of the cell are not changing during this time we can assume that **on each electrode [xxx] [the voltage generated by different neurons] has a different shape.**

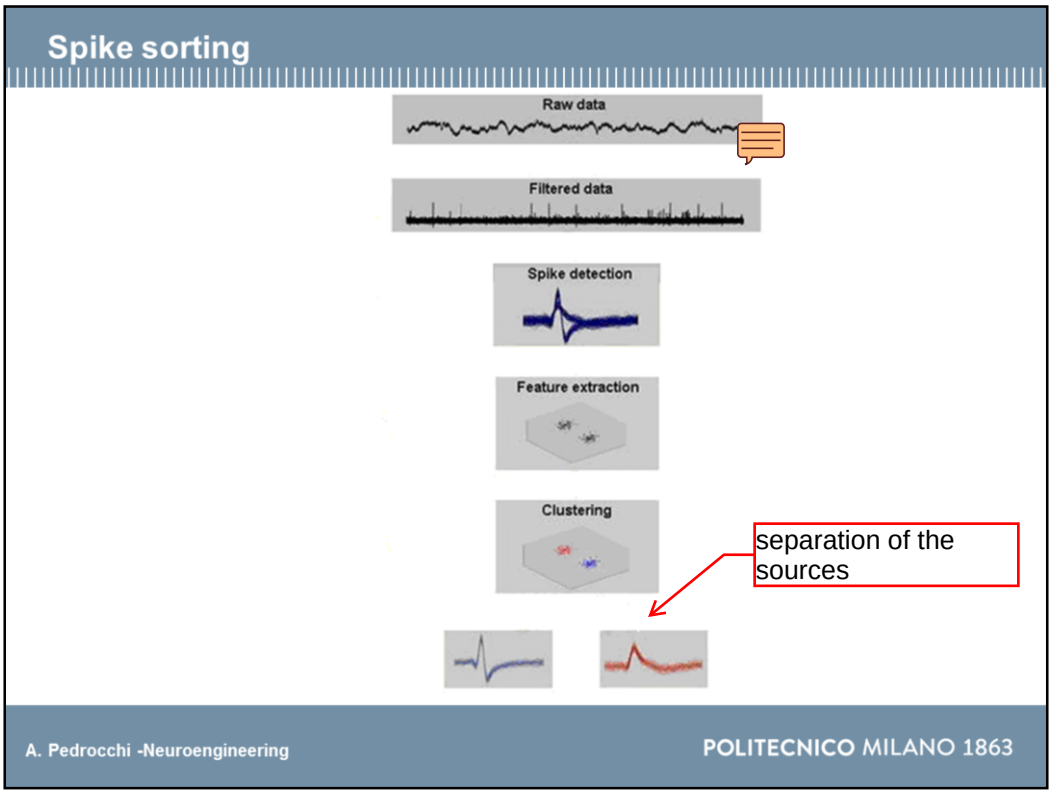
ofc the assumption it's true in 1 hour recording! so geometry is fixed.

The trace is just one single rec. of one electrode, not single neurons. BUT the shape of spikes generated by N1 is always similar to that shape. Why it should be different?!? And we can also assume that the shape of N1 is different from the sape of N2. So what can I do is recognize by threshold were we have spikes (1st step, thresholding)

From thresholding we select just were spikes are. So this info is just 0-1.

Then I can extract around each single spike the SHAPE of the signal. Let's say 3ms, or 10ms.

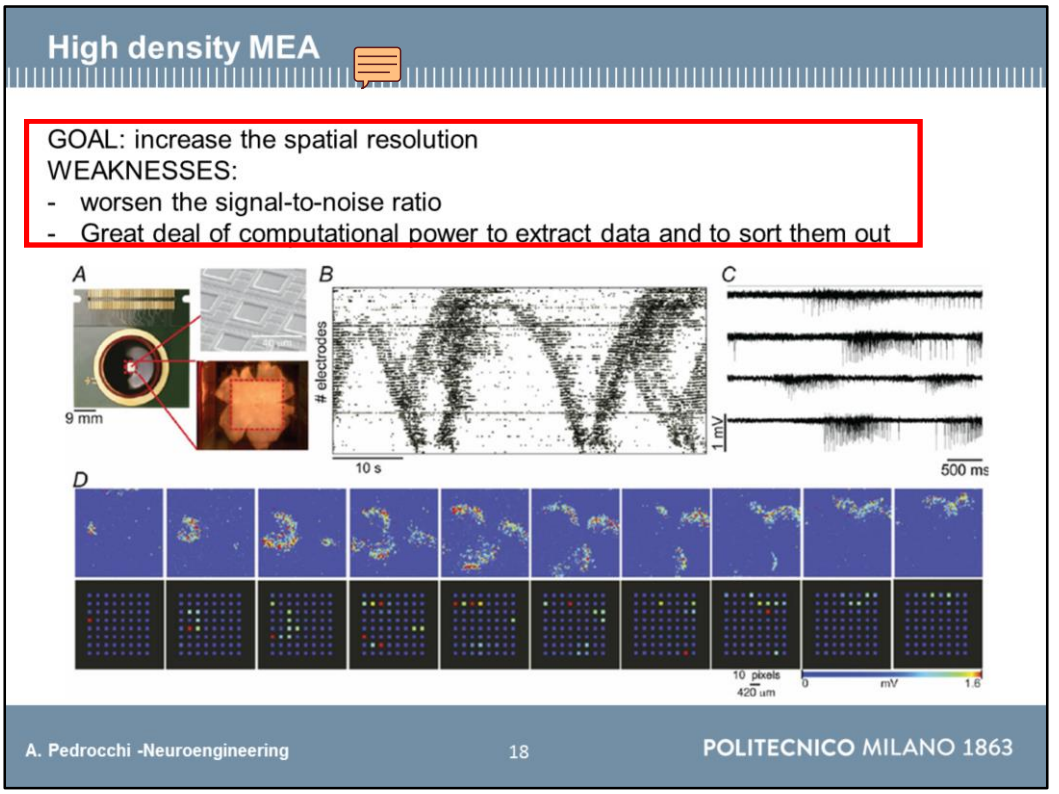
And then I can **CLUSTER** those spikes into different shapes and templates. NOTE I need an **UNSUPERVISED** APPROACH! I dont know before how many spikes, or what's the shape! I can suppose tho' that the signals are coming from diffrent sources (2..3..4..?) And so I make a clustering into those signals so to separate the sources.



From filtered data, it is possible to recognize the event “spike”. From identified spikes, you need to create a template data set. With the template data set, you can identify when each neuron was spiking.

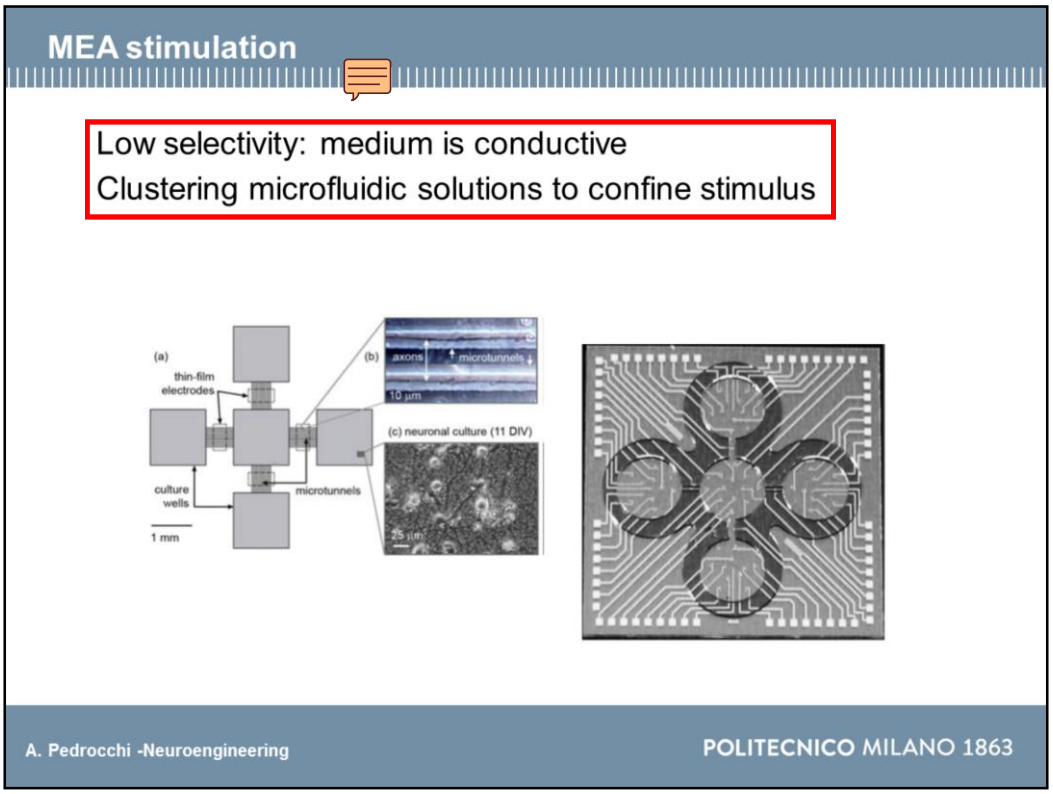
Note – the electrical shape of the spike is not important. The information is when the neuron is spiking. The different electrical shapes are used only to distinguish among different neurons, but do not carry any information content.

By postprocessing the data I have amplified the resolution. Still I dont know if this (the blue) is one neuron, an the red is another one. I know that those are two separate sources of spiking signals of my network. If n1 and n2 always spikes toghether i'll never be able to separate them :(but then I'll have 1 source and the others.



High spatial resolution coupled with wide field of view are one of the key requirement of MEAs and allow signals sent over a long distance to be taken with proper accuracy. When higher spatial resolution is required, high-density MEA are used. These arrays usually have a square grid pattern of 256 electrodes that cover an area of 2.8 by 2.8 mm. Increased spatial resolution is provided also by CMOS-based high-density microelectrode arrays featuring thousands of electrodes along with integrated readout and stimulation circuits on compact chips of the size of about 1 cm^2. Even the resolution of signals propagating along single axons has been demonstrated.

~~Figure 1. High-density recordings with the active pixel sensor (APS) multielectrode array (MEA) A , the APS MEA chip. The red dotted line demarcates the electrode area. Top inset, scanning electron micrograph illustrating the topography of individual electrodes on the chip. Bottom inset, magnification of the active area of the chip with a retina positioned on the electrodes. B , spike raster plot of spontaneous episode of activity in a P11 retina. C , raw signals on four sampled channels from the same recording. D , two-dimensional time-lapse (every 1 s for 10 s) view of the activity. The S . D . of the voltage is estimated in 10-ms bins and plotted using an exponential colour coding scheme to emphasize large deviations and effectively threshold small deviations. Bottom row: same episode after downsampling the resolution to a simulated 8 × 8 array with an electrode pitch of ß 334 µ m.~~



It is possible to stimulate neurons in the culture to investigate the network response to a given stimulus. However, the electrical stimulation has a very low sensitivity [see slide].

In another special design, 60 electrodes are split into 6 x 5 arrays separated by 500 µm. Electrodes within a group are separated by 30 um with diameters of 10 µm. Arrays such as this are used to examine local responses of neurons while also studying functional connectivity of organotypic slices. Separation between the two subcluster can also be achieved by proper microfluidic stimulation

[1h10m]
Another limitation of invitro studies:
you are mainly putting together all the same cells: you are not making a real model of different cells.

For example, if we think about the cerebellum we had different types of neurons, which works in the network, and the information is in the flowing of information in different types of neurons.

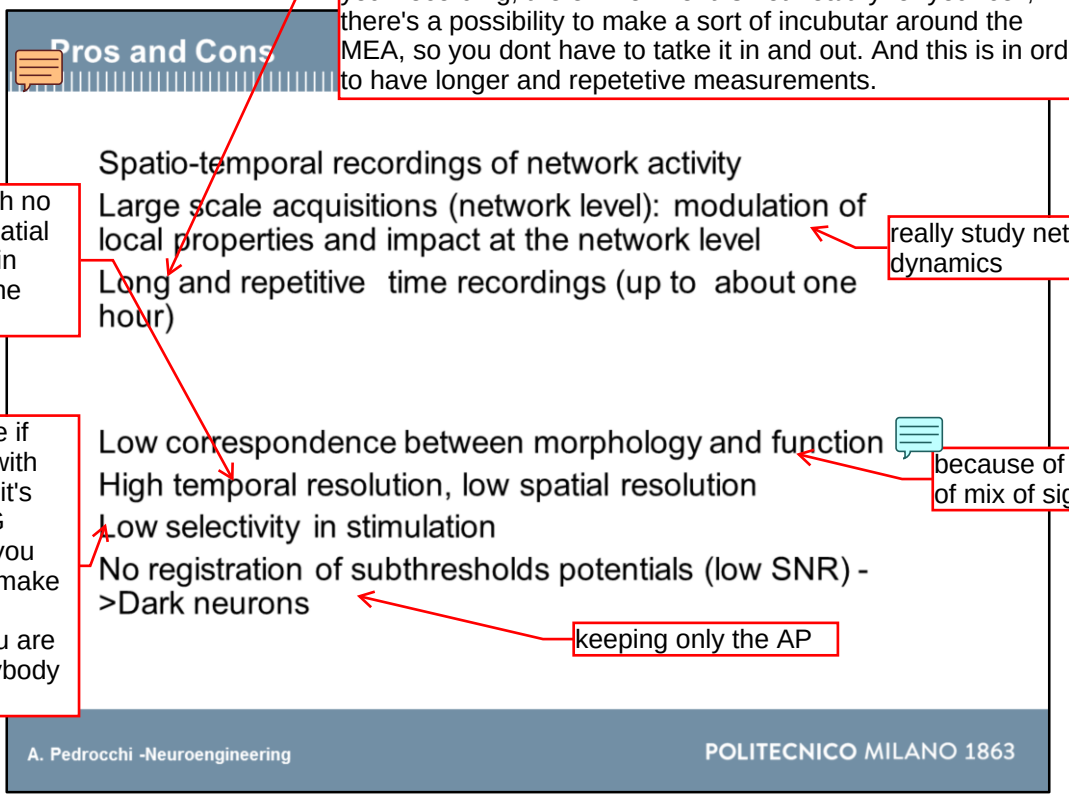
If you do a petri you put only purkinje, and you dont have separation of different cultures (usually).

But these are the advances in MEA technologies in using **PULLED cultures**.

Pulled cultures IS VERY DIFFICULT, YOU NEED THE P.C. TO STAY WITH PC, AND THE GRC TO STAY WIT THE GRC.
"maybe the PC would like to have a different medium to respect the GrC"

The **two medium should separeted** (also because otherwise when you are recording everything spreads out). But you want them to be **interconnected,** to synapse! it's difficult to find a way to phyisically separated but junciton them! So physically separated but functionally connected.
Microfabrications has helped: **"Microtunnels** which allows only axons to be connected and not exchange mediums"

Or what's also done is **"microfluidic",** with a flow of medium different from the areas of cultures, so they don't mix up and you can separate two cultures which are in the same petri.



not exactly true.. neurons are very delicate, so they need to stay in a very controlled environment.. so incubators. You take it out and then you just have the t°C control, and then you put it back... but what happens is that over time you cant go on with your recording, the environment is not healthy for your cell, there's a possibility to make a sort of incubutar around the MEA, so you dont have to tatke it in and out. And this is in order to have longer and repetetive measurements.

up to 10kHz with no problem, but spatial resolution only in some point of the culture

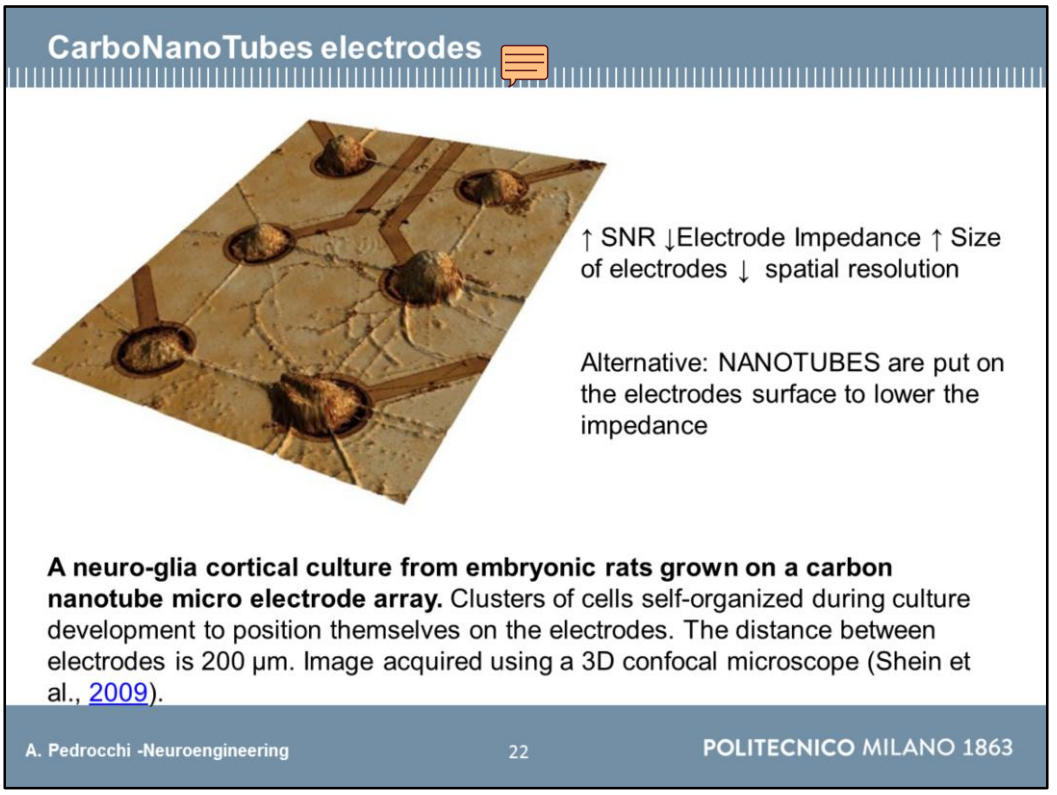
really study networks dynamics

from the outside if you stimulate (with the electrodes) it's like SHOUTING inside a room, you are not able to make a selective stimulation.. you are activating everybody or nothing.

because of the issue of mix of signals

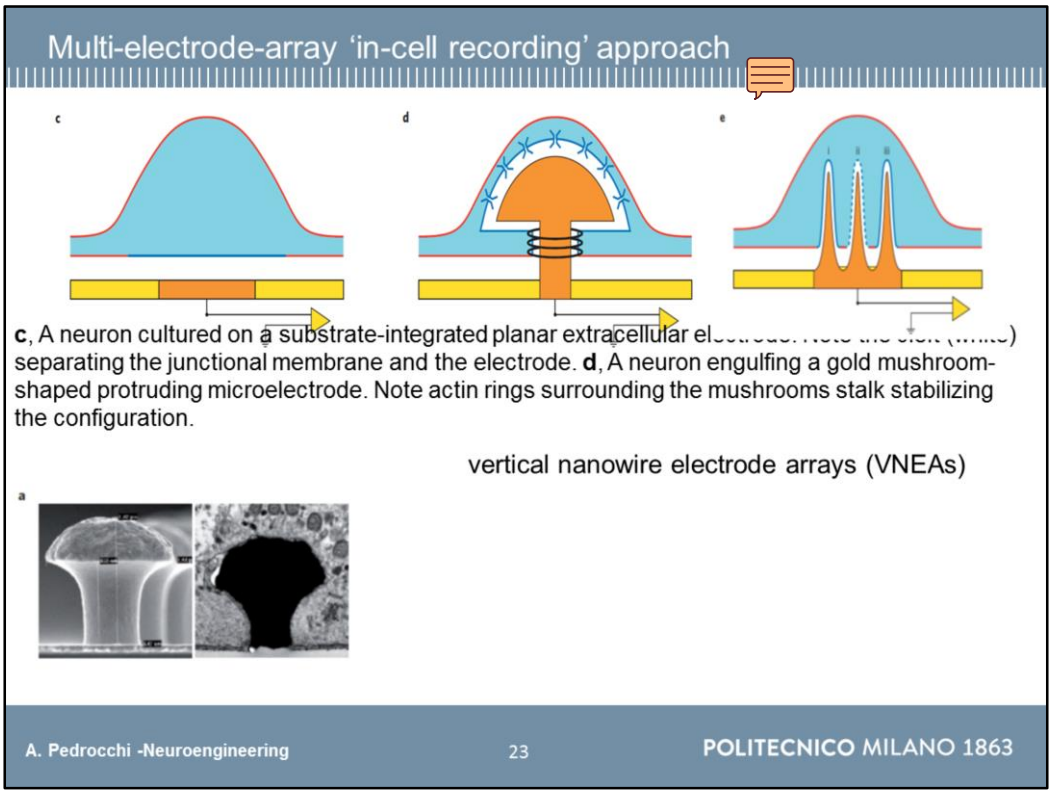
keeping only the AP

Extra slides for those who wants to go deeper…



Reducing the surface area of individual sensing pads to match the dimensions of individual neurons enables the density of the MEA and its spatial resolution to be increased. This however, is reflected by the reduction of the FP amplitudes as a result of the increase in impedance and consequent reduction of the signal-to-noise ratio. Thus, the electrodes geometry and the ensuing impedance both place constraints on reducing the electrode size. Increasing the surface area by using nanostructures such as spongy platinum black or Ti3N4, gold nanoflakes and nanopillars, or carbon nanotubes is used to compensate for the dimensions of the electrode surface 'visible' to the cell.

Although effective in reducing the impedance values up to 95% at approximately 1 KHz, in practice the recorded FPs are still in the range of hundreds of microvolts. This is most likely due to averaging of the complex positive and negative currents concomitantly generated by a number of sources, over fractions of the large surface area of the electrode. This 'averaging' usually results in reduced amplitude of the electrical readout. It should be noted, however, that reduction of the electrode impedance may be very effective in improving the readout signals when applied under conditions in which a single cell 'covers', engulfs or internalizes a single electrode



Recently, a number of laboratories began to merge the advantages of extracellular MEAs and intracellular microelectrodes.

The first series of studies reporting on successful multisite, non-invasive, intracellular recording and stimulation by MEAs were published between 2007 and 2010 (Spira lab). In these studies they increased the neuron–microelectrode electrical coupling coefficient from approximately 0.1% as recorded by a planar extracellular MEA to approximately 50% by the use of a chemically functionalized micrometre-size mushroom-shaped gold protrusion as the sensing electrode (Fig. 4d and Fig. 5a). The increased coupling coefficient was associated with an intracellular recording of a monophasic positive attenuated intracellular AP instead of a typical biphasic FP.

The key to the multi-electrode-array 'in-cell recording' approach developed by us is the outcome of three converging cell biological principals: (a) the activation of endocytotic-like mechanisms in which cultured *Aplysia* neurons are induced to actively engulf gold mushroom-shaped microelectrodes (gMμE) that protrude from a flat substrate, (b) the generation of high *R*seal between the cell's membrane and the engulfed gMμE, and (c) the increased junctional membrane conductance.

The neuron/gMμE interface was generated by chemically 'luring' the neurons to engulf the protruding gMμE by a highly conserved cell biological mechanism — endocytosis (which is a cell biological mechanism that underlies the internalization of particles into the cells). The shape and the dimension of the gMμE were selected to mimic the geometry and dimensions of dendritic spines67. To facilitate the engulfment, the gMμEs were chemically functionalized by an RGD-based peptide.

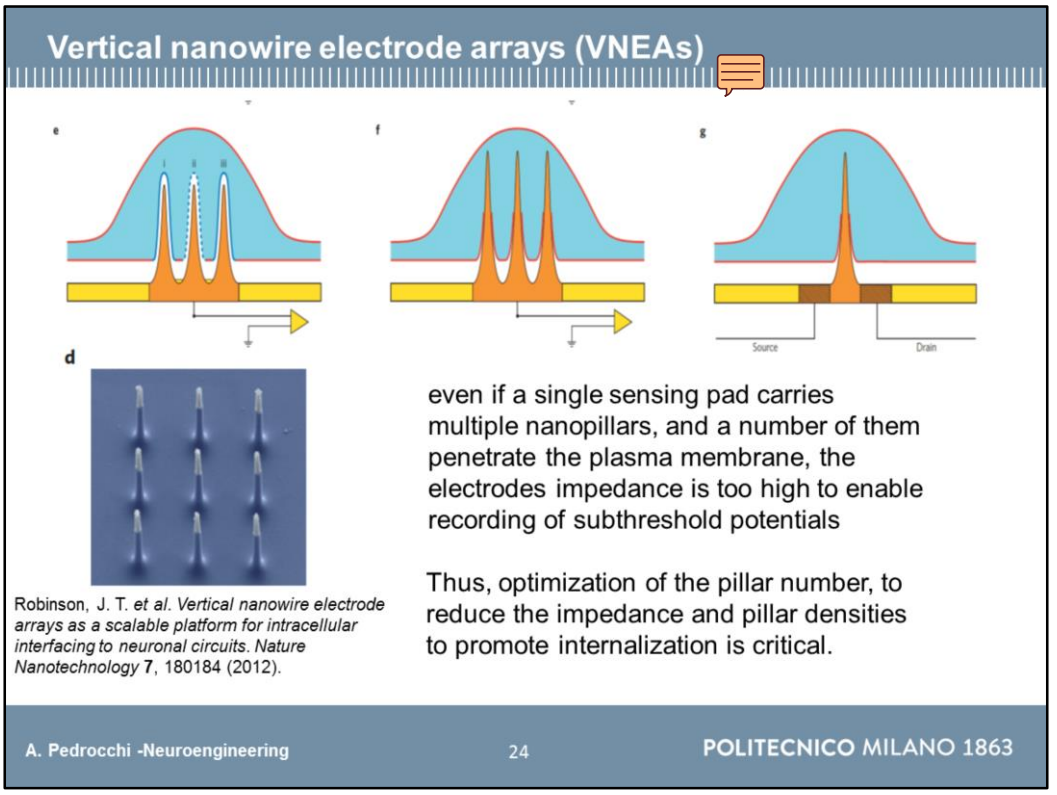
The engulfment of the microelectrodes is generated by molecular cascades that include the restructuring of the cytoskeleton to form an actin ring around the stalk of the 'gold mushroom' .

Out of the five criteria to evaluate of the benefits of the approaches, the gMμE-based MEA provided multisite, simultaneous, intracellular recording and stimulation for periods of days (which is for as long as we carried out the recordings). The filtering properties of the gold electrodes and the a.c. amplifier used do not enable the resting potentials of the neurons to be recorded.

So far, attempts to obtain in-cell recordings and stimulation from rat hippocampal neurons and primary cardiomyocytes were unsuccessful. It should be noted nevertheless that these attempts were limited to gMμE functionalized with poly-d-lysine rather than by the engulfment promoting peptide.

So far, the gMμE-based MEA is the only device that enabled the recordings of both APs and subthreshold synaptic potentials, and that can also be used for effective intracellular stimulation. Nevertheless, this coupling was demonstrated using large *Aplysia* neurons but as of yet has not successfully been applied to rat hippocampal neurons and primary rat cardiomyocytes.

We can also use ACTIVE electrodes: FET



stop qua

e, Nanopillar electrodes extending into a cultured cardiomyocyte but that do not penetrate the plasma membrane (i). After the application of an electroporating pulse (ii) the nanopillar gains access to the cytoplasm. The electroporation is transient and the junctional membrane resistance recovers to control level within minutes (iii). **f**, An array of nanopillars that penetrate the plasma membrane forming direct physical contact with the cytosol. **g**, A nanopillar that serves as the gate for a nano-FET penetrates the cell's membrane.

A recent study from Park's laboratory used vertical nanowire electrode arrays (VNEAs) constructed from a doped silicon core encapsulated by silicon dioxide and tipped by Ti/Au, to generate an identical configuration to sharp intracellular electrodes (Fig. 4f and Fig. 5d). In the study, 3 × 3 arrays of 9 nanopillars, 150 nm in diameter, 3 µm in height at 2 µm pitch were grown on 16 sensing pads. Embryonic rat cortical neurons or HEK293 cells were then cultured on the VNEAs for a number of days. About 50% of the VNEAs spontaneously penetrated through the plasma membrane of the HEK293 cells as demonstrated by the fact that current injection through the VNEAs generated a voltage drop across the plasma membrane. In cases where spontaneous penetration of the membrane was not evident, an electroporating pulse (approximately ±6 V, 100 ms) was applied to penetrate the membrane of the neuron. The effect of the electroporating pulse on the integrity of the membrane was not shown.

Consistent with the intracellular positioning of the VNEA, all recorded APs were positive monophasic. However, the coupling coefficient and signal-to-noise ratio were insufficient to enable recordings of subthreshold synaptic potentials. In fact, in relation to the five examination criteria listed above, the recordings obtained by the VNEA do not provide significant advantages over extracellularly positioned gMµE in cultured hippocampal neurons. **The relatively low coupling coefficient of the VNEA-based sensor is most likely due to the high impedance of the VNEAs. An advantage of recording by VNEAs over recordings by classical substrate-integrated planar electrodes (but not small electrodes) is that a single pad records APs from an individual neuron.**

Four recent studies demonstrated for the first time that localized membrane electroporation may lead to transient intracellular recordings of attenuated APs. In all four studies the intracellular access was transient suggesting that electroporation activates repair mechanisms that seal off the electroporated nanopores58, leaving the protruding nano- or microelectrodes out of the cell (Fig. 6).

It is of interest to note that the nanopillar approach does not provide a significant advantage over planar extracellular electrodes as the recorded potentials are attenuated by at least an order of magnitude by the inherent high electrode impedance and the insufficient *R*seal. It should also be noted that even if a single sensing pad carries multiple nanopillars, and a number of them penetrate the plasma membrane, the electrodes impedance is too high to enable recording of subthreshold potentials. Theoretically, the impedance problem could be solved by increasing the density of the nanopillars over the sensing pad. Nevertheless, when the density of protruding nanostructures exceeds a certain bound, the pillars do not penetrate the cell membrane, analogous to a dense 'bed of nails'.

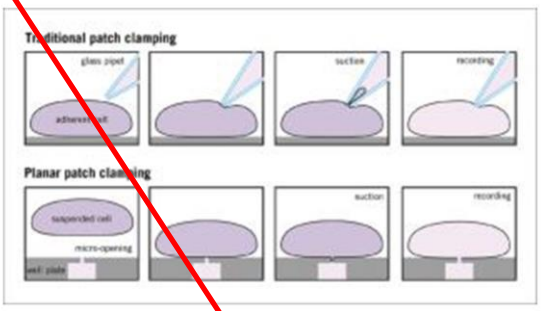
Using advanced semiconductor-based nanotechnology and the classical concepts of mechanically penetrating the cell plasma membrane by sharp glass microelectrodes, Lieber's laboratory demonstrated intracellular recordings of 80–100 mV APs from beating cultured cardiomyocytes. This was done either by the so-called kinked nanowires or pillar-shaped protruding silicon nanowires, nanofabricated as the sensing gate electrode of an FET. To facilitate the penetration of the electrodes into the cells the device's surface was modified by phospholipids Using both types of nanosensors, full blown cardiac APs of 75–100 mV, ~200 ms were recorded.

The high-quality measurements of the APs were made possible by three factors: (a) the nanoscale size of the sensors that enabled its insertion into the cytosol through the plasma membrane of the cells, (b) the formation of GΩ resistance between the plasma membrane and the nanostructures, (c) the fact that the size of the sensing area does not affect its sensitivity. It should be noted that whereas the aspects of nano-dimensions and GΩ seal formation are essential components to enable high-quality recordings, the key to the success is the use of the gate electrode of an FET as the sensing electrode rather than passive metal or silicon-based micro- or nanoelectrodes. Thus, in contrast with passive conducting lines, where the signal is significantly attenuated due to stray capacitance, an FET effectively amplifies the signal *in situ*. It should be noted that FETs are more susceptible to failure due to leakage currents, whereas passive electrodes are not affected as dramatically by device imperfections.

When considering the branched intracellular nanotube-FET or kinked nanoelectrode devices as tools to map functional synaptic connectivity, a major hurdle is the signal-to-noise level of the device. Examination of some recordings reveals noise levels of more than 20 mV. Whereas the noise is attributed to the nano-dimensions of the FETs and thus can be reduced by adjusting the FET size, the present device does not provide the resolution to enable the recording of miniature potentials, synaptic potentials and small membrane oscillations. Another unsolved problem that would need further study is to enable the accurate recordings of the resting potentials.

It is conceivable that merging the cell-biological principles of evoking engulfment of the electrodes on the one hand and the use of FETs on the other may provide both a stable neuron–electrode configuration and intracellular access. Once achieved, such a device may be applied in arrays that make use of the well-established multiplexing capabilities of ultra-large-scale integrated transistor arrays.

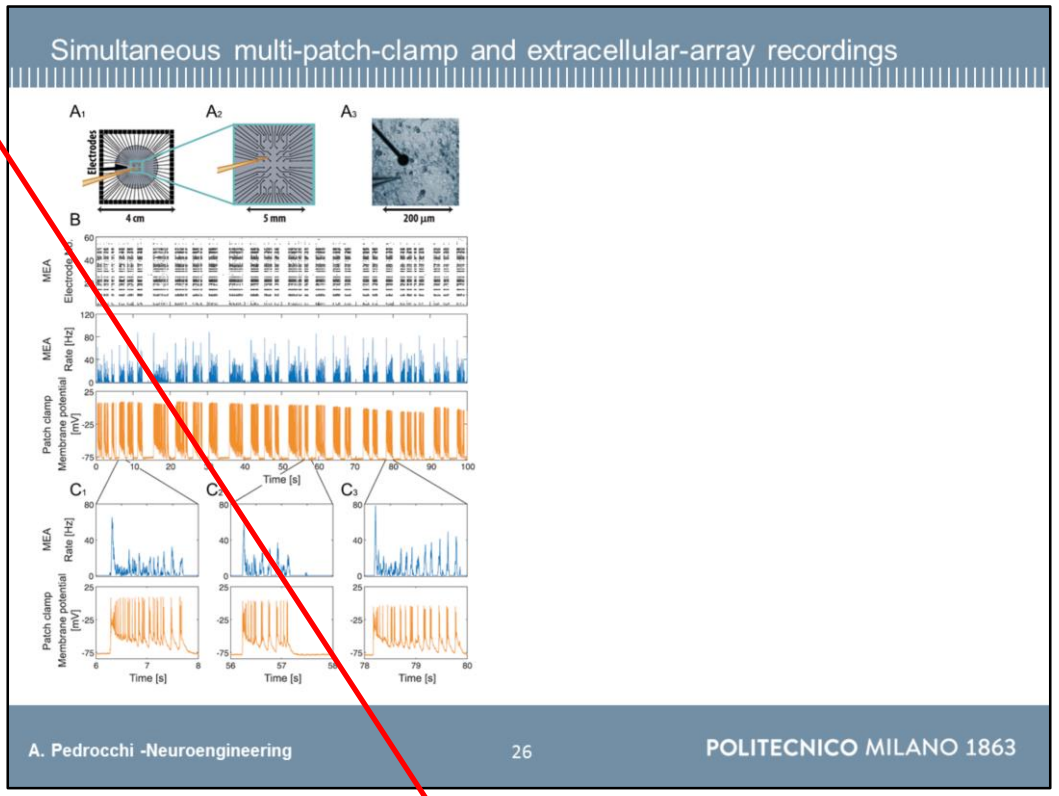
Planar patch-clamp MEA technology



The approach is extensively used to acutely patch cells in suspension relying on suction to draw individual cells to the aperture and to form a giga-seal resistance. Thus far the approach was not suitable for studies of long-term adhering cultured neuronal networks

Each aperture was connected to a microfluidic channel. Negative pressure pulse through the microfluidic system broke the junctional membrane establishing a classical whole-cell patch-clamp configuration. In a fraction of the experiments the patch configuration was stable for a number of hours and the properties of the synapses formed between the two cells could be investigated. The signal-to-noise ratio obtained by the planar patch-clamp device matches that of conventional patch-clamp recording. Although promising, it should be noted that the somata of the isolated neurons adhered to each other but did not extend neurites on the culture substrate. Thus the neuron–device configuration did not simulate the complex growth pattern of cultured mammalian neurons but rather is closer to the cell suspension mode of patch-clamp recordings.

When considering the potential use of planar patch-clamp MEAs as tools to map functional synaptic connectivity among cultured neurons, two major problems have to be dealt with: (a) the recording duration is expected to be limited by the perfusion of the neuron by the microfluidic solutions (Fig. 4h), (b) the sensor's density (apertures) is expected to be limited by the backside fluidic system.



Roni Vardi, Amir Goldental, Shira Sardi, Anton Sheinin & Ido KanterScientific Simultaneous multi-patch-clamp and extracellular-array recordings: Single neuron reflects network activity **Scientific Reports** | 6:36228 | DOI: 10.1038/srep36228

http://www.nature.com/articles/srep36228?WT.ec_id=SREP-639-20161115&spMailingID=52768996&spUserID=ODkwMTM2NjQzMgS2&spJobID=1045291907&spReportId=MTA0NTI5MTkwNwS2

The increasing number of recording electrodes enhances the capability of capturing the network's cooperative activity, however, using too many monitors might alter the properties of the measured neural network and induce noise. Using a technique that merges simultaneous multi-patch-clamp and multi-electrode array recordings of neural networks *in-vitro*, we show that the membrane potential of a single neuron is a reliable and super-sensitive probe for monitoring such cooperative activities and their detailed rhythms. Specifically, the membrane potential and the spiking activity of a single neuron are either highly correlated or highly anti-correlated with the time-dependent macroscopic activity of the entire network. This surprising observation also sheds light on the cooperative origin of neuronal burst in cultured networks. Our findings present an alternative flexible approach to the technique based on a massive tiling of networks by large-scale arrays of electrodes to monitor their activity