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# **Problem description**

Through The NTNU Cyborg initiative, a cybernetic (bio-robotic) organism is currently under development. Using neural tissue, cultured on a microelectrode array (MEA), the goal is to use in-vitro biological neurons to control a robotic platform. The objective for this thesis, is to research and discuss the necessary aspects of developing such a cybernetic system. A literary search into similar studies is suggested, along with getting acquainted with the biological sides of the project. The student is free to explore any fields deemed relevant for the goal of embodying a neuronal culture. The student may contribute to this discussion with own ideas and suggestions. Part of the objective for this assignment, is to lay the ground work for a further Phd project as well as for the future development of the NTNU Cyborg system.

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# Sammendrag

Gjennom NTNU Cyborg, er en kybernetisk organisme (en blanding av biologi og robot) under utvikling. Ved hjelp av nevrale vev, dyrket på toppen av mikroelektroder (MEA), er målet å bruke biologiske nerveceller til å styre en robot. Denne avhandlingen danner grunnlaget for denne utviklingen, og fungerer som et forstudium til en Phd oppgave angående samme tema.

Det har blitt gjennomført en undersøkelse av de nødvendige aspekter ved det å integrere en nervecellekultur i en robot. Med utgangspunkt i lignende forskning, samt kunnskap fra fagområdene nevrovitenskap og informatikk, er de nødvendige komponenter for å bygge et slikt system diskutert.

MEA2100-60 systemet som er grunnlaget for kommunikasjon med nervecellekulturen, har blitt beskrevet. I tillegg har det blitt foreslått en distribuert MEA-Kyborg infrastruktur på tvers av de tre hoveddeltagende instituttene: Institutt for teknisk kybernetikk (ITK), Institutt for nevromedisin (INM), og Institutt for data teknikk og informasjonsvitenskap (IDI). Robot plattformer, som nervecellene kan integreres i, har også blitt introdusert; fra Animats og Hybrots til NTNU Cyborg roboten.

Vi har videre sett på hvordan vi kan kode informasjon til nervecellene gjennom elektrisk stimulering, samt dekode informasjonen fra kulturen gjennom analyseringen av MEA opp-takene. Gjennom stimulering og opptak foreslår vi å 'lukke sløyfen' ved å mate sensorisk data fra roboten plattformen til nervecellekulturen, og bruke opptakene som instruksjoner til roboten.

Treningen av en nervecellekultur er en av de mer utfordrende oppgaver innenfor prosjektet. Med inspirasjon fra biologi og kunstige nevrale nett, har vi sett på mulige metoder for å trenere biologiske nevrale nett gjennom undervist trening, ikke undervist trening og premiert trening.

Det har i tillegg blitt gjennomført en kort diskusjon angående modelleringen av en nervecellekultur. Vi har her sett på hvordan en realistisk modell kan bygges opp, og fordelene ved en slik modell.

Til slutt, har vi oppsummert temaene i avhandlingen, og presenterer en plan fremover, for når MEA'en er oppe og går.

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# Abstract

Through The NTNU Cyborg initiative, a cybernetic (bio-robotic) organism is currently under development. Using neural tissue, cultured on a microelectrode array (MEA), the goal is to use in-vitro biological neurons to control a robotic platform. This thesis forms the basis for this development, and serves as the preliminary for a further Phd involving the same topics.

Investigation into the the necessary aspects of embodying a neuronal culture though a robotic platform has been conducted. Building upon similar embodied research, as well as knowledge from the fields of neuroscience and computer science, the necessary components for building such a system are discussed.

Beginning with the more practical issues, the MEA2100-60-System for communicating with the neuronal culture was presented, along with a proposed infrastructure for distributing the MEA-Cyborg system across the three main participating departments: The Department of Engineering Cybernetics (ITK), the Department of Neuroscience (INM), and the Department of Computer and Information Science (IDI). Robotic bodies, for embodying the culture, have also been introduced; from Animats and Hybrots to The NTNU Cyborg robot platform.

We have further looked at encoding information to the culture through stimulation, and decoding information from the culture through analyzing the MEA recordings. Through stimulation and recording we propose to 'close the loop' by feeding sensory data from the robot platform to the neuronal culture, and using the culture recordings as robotic instructions.

Training a neuronal culture is one of the more challenging tasks within The NTNU Cyborg project. With inspiration from biology and artificial neural networks (ANNs), we have looked at some mechanisms for training neuronal cultures though supervised, unsupervised and reinforcement type training.

For the sake of completion, a brief discussion on some aspects of modeling a neuronal culture has been included. We have discussed how a realistic model may be constructed and the benefits of doing so.

Finally, putting together the topics discussed in this thesis, a plan moving forward has been suggested for when the MEA is up and running.

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# Preface

This report serves as the Master thesis for the author, Martinius Knudsen. It was written in the spring of 2016 at the Department of Engineering Cybernetics (ITK) at the Norwegian University of Science and Technology (NTNU). The work was supervised by associate professor Sverre Hendseth.

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# Abbreviations

AI	=	Artificial intelligence
ANN	=	Artificial neural network
AP	=	Action potential
CNN	=	Cultured neural network
IDI	=	Institutt for Data teknikk og Informasjonsvitenskap
INM	=	Institutt for Nevromedisin
ITK	=	Institutt for Teknisk Kybernetikk
LTD	=	Long-term depression
LTP	=	Long-term potentiation
MCS	=	Multi Channel Systems
MEA	=	Multielectrode/microelectrode array
MQTT	=	MQ Telemetry Transport
NN	=	Neural network
ROS	=	Robot Operating System
SNN	=	Spiking neural network



# Introduction

What is a memory? What is a thought? How do we make up our minds about what to do next? Cognitive scientists and philosophers have been debating such questions for ages. The brain is arguably the most robust computational platform in existence. It is able to process complex information quickly, is fault tolerant, and can adapt to noisy inputs[51]. Understanding how circuits of neurons contribute to the complex properties of the brain and how they break down in disease is one of the biggest scientific challenges of our time.[61].

Studying how the brain processes and encodes information is however, difficult because access to it is limited by skin, skull, and the sheer number of cells[51]. For this reason, more and more studies are conducted in-vitro; in a dish outside the body. By using networks of a few thousand neurons and glia, one has tremendous access to the cells, not feasible in-vivo. This allows for manipulation and recording at the millisecond and micron scales, to determine the cell- and network-level morphological correlates of learning and memory[75]. One particular method for studying these in-vitro networks, is though micro-electrode technology which enables the recording and stimulation of a neuronal culture grown on a microelectrode array (MEA). It is this method that the NTNU Cyborg project will be exploring.

To tie our cell- and network level inquiries to behavior, we reembody our cultured networks by connecting them to artificial animals/robots, either simulated or real. By combining in-vitro biological networks with computer software and robotics into new hybrid systems, we combine the best of both worlds: the adaptive and regenerative properties of living neural networks and the programmability and computational power of electronic chips.[59] This task requires efficient hardware, a distributed infrastructure, protocols for analyzing and stimulating a neuronal culture, methods for training goal behaviour into the culture and more. All these topics will be discussed in this thesis.

Through embodying our neuronal culture we hope to unravel some of the mysteries of the

brain as well as enable development of functioning hybrid cybernetic systems. If we and others are successful with this new approach, we may better understand the substrates of memory, thought, and behavioral control.[75]

## 1.1 The NTNU Cyborg initiative

From The NTNU Cyborg website[29] (Written by the author of this thesis, Jan Onarheim and Øyvind Stavdahl):

Through NTNU biotechnology initiative, coordinated by Professor Stig W. Omholt, a project to develop a Cyborg (cybernetic organism), a combination of machine and living tissue, has been started. In this project, the aim is to enable communication between living nerve tissue and a robot. The social and interactive cyborg will walk around the campus raising awareness for biotechnology and ICT, bringing NTNU in the forefront of research and creating a platform for interdisciplinary collaborations and teaching.

A robot base has been purchased, which will act as the robots transport and navigation platform. Associate Professor Øyvind Stavdahl, at the Institute of Cybernetics (ITK), has supervised the selection of the robot base though engaging several students working with the NTNU Cyborg as master thesis project. Associate Professor Sverre Hendseth (ITK) also has several students engaged in the same way. The neurological part of the project, which involves the cultivation of nerve cells, is under development at the medical faculty (DMF). The project is administrated by project directors Stefano Nichiele (IDI) and Jan Onarheim (IET).

Work on the cyborg is currently being carried out in separate subprojects. The robotics part is under development and the ambition here is to have an autonomous robot walking in Glassgården, Elektrobygget by 2016. The robot should then approach people when they come close, greet them and maybe even invite them to be friends on the robots facebook page. This will be realized through several projects and master's theses, as well as through EiT villages.

In connection with this project, a self-sufficient student organization has been established. This student group cunnrently consists of upto 30 students who use the robot / cyborg platform in conjunction with specialization projects, master thesis, EiT villages, as well as voluntary work. A research assistant, Martinius Knudsen (author of this thesis) is engaged to help establish and develop this activity, as well as assist in organization of The NTNU Cyborg project as a whole. This organization will be closely related to the academic activities of ITK. The ambition is to create an activity that will eventually recruit students from many disciplines, including non-technical ones. The student organization should eventually be able to define its own tasks and issues and stand on its own feet.”

As of 01.05.2016 two PhD projects are ongoing, with direct connection to the cyborg-activity. One in neuroscience supervised by Dr. Ioanna Sanvig (INM) and Dr. Axel Sandvig (INM), and one within nanomedicine supervised by Prof. Øyvind Halaas (IKM). Six student specialization projects, two master thesis and one EiT group project have been completed (all at ITK). Currently, three master thesis are ongoing and four EiT groups have been engaged to work with the robotic aspects of the cyborg. Also, The NTNU Cyborg as an organization is being further expanded over several faculties, and new relevant academic staff are participating with research from their respective studies. More students and Phds are being engaged in the fall.

The NTNU Cyborg connects many different disciplines across departments and faculties. Some examples of relevant research fields are: biotechnology, neuroscience, cybernetics and robotics, IT, design and production, electronics, ethics and psychology.

### **1.1.1 Reasons for the initiative**

There are many possible application and research outcomes that we hope will result from the initiative.

#### **Putting NTNU on the map**

The development of a cyborg is not only interesting, but also really cool. We are hoping that the public will find it cool too. By making a bio-robotic organism in the form of a social and interactive cyborg, NTNU will be making headlines and leading the way towards new types of lifeforms. The initiative aims to promote biotechnology through the cyborg and increase the public interest around NTNU and enabling technologies.

#### **Robotics research**

The Cyborg does not only serve as a publicity stunt, but also as a great platform for robotics research and as a platform for student projects and cooperation. The students are working on developing a social-interactive robot to wander the campus hallways. Accomplishing such a feat, is a research goal in and by itself.

#### **Learning about the brain and nervous system**

Since we are essentially studying neural cells, we hope to better understand the cell- and network-level substrates of memory, thought, and behavioral control. We hope this research will give us greater insight to how our brains operate and that our results facilitate the field of neuroscience.

#### **Medical applications**

There are many possible medical applications that may result from studying biological neural networks. A better understanding of the processes leading to biological cognition can facilitate progress in understanding neural pathologies[9]. E.g. for diseases such as Alzheimer's or patients who have suffered strokes, understanding how one may retrain damaged brain regions could help these patients restore important cognitive functions. For patients suffering paralysis or patients with amputees, it may be possible to design neurally controlled bioelectronic prosthetics. Through a better understanding of neural signaling, one may also possibly aid patients with Parkinson or those suffering epileptical seizures

by supplying electronic help and warning. The research may also help improve current brain-computer interface (BCI) technology.

### **Other applications**

Along with medical applications, the project has the possibility to pioneer future hybrid human-electronic devices, bionic robotics, robust biological computation platforms and bio-silicon neural networks. With hybrid systems, we may also be creating fundamentally different types of artificial intelligence.

In addition, the research may aid in the further development of artificial neural network (ANN) models. ANNs are already heavily inspired by biology and have proved to be quite efficient solving a wide variety of tasks. Through a better understanding of the workings within biological networks, new insights may be applied to next generation ANNs, which may possibly open a new arena for problem solving.

### **Philosophical satisfaction**

Besides its potential applications, research into the workings of the brain is simply fascinating. There is something philosophical satisfying about studying how you and I can find something philosophically satisfying.

## **1.2 Thesis goal**

This thesis aims to explore and outline the current state-of-the-art research in the area of embodying neuronal cultures. By doing so, we hope that we may bridge the current gap between the robotics development conducted at ITK and the culturing of neural cells conducted at INM.

This thesis may deviate somewhat from a standard technical engineering thesis. The questions that are attempted answered, border on that of scientific research rather than solving an engineering problem. Many of the topics covered have yet to be understood in the scientific arena. Without the ability to conduct experiments (as, during the writing of this thesis, the MEA system has not yet been set up) it is difficult to answer some of the questions being asked. What this thesis does however attempt, is to lay the ground work and foundation for developing the cybenetic system as soon as the MEA hardware is available. The focus here is on the investigation of similar studies as well as the relevant scientific fields of research to enable the embodiment of our own neuronal cultures in a closed-loop robot-MEA hybrid system.

### 1.2.1 Research questions

Four main focus areas will be investigated, all of which play an important role for the in-vitro research we are conducting through the NTNU Cyborg:

How to make a cyborg:

- What hardware is necessary for interfacing the neuronal culture?
- How do we efficiently distribute the MEA-robot setup across the participating labs?
- How do we close the MEA-robot sensory-motor loop?
- What should and can the neuronal culture control on the robot?

Communicating with a neuronal culture:

- How do biological networks signal information?
- How do we encode information into the culture?
- How do we decode information from the culture recordings?

Training a neuronal culture:

- How can we manipulate the behaviour of the embodied culture in its environment?
- How do we train the culture through stimulation?
- What mechanisms are needed for a culture to show signs of learning?
- How can we achieve adaptation and learning in a neuronal culture?

How to model a neuronal culture:

- How may we realistically model a biological neural network?
- What models are available?

These questions are some of those we ask ourselves in the NTNU Cyborg project, and that we wish to give some more insight to in this thesis.

## 1.3 Recommended reading

Some scientific background knowledge of biology, robotics and computer science is beneficial, but not strictly necessary. The background chapters attempt to provide the reader with the relevant information and concepts to understand the rest of the thesis.

The book 'Neuroscience' by Purves et al.[129], which is also used in some NTNU neuroscience courses, is a great resource to neuroscientific concepts and workings. The book 'Bio-Inspired Artificial Intelligence' by Dario Floreano and Claudio Mattiussi[59], used in a NTNU AI course, provides a great introduction to artificial neural networks (ANNs),

specifically chapter 3. Chapter 3.12 in the same book, also introduces 'Hybrid Neural Systems', which describes how biological neural networks may be used through MEAs. Also, Chapter 18.7 in the book "Artificial Intelligence A Modern Approach"[137] by Stuart Russel and Peter Norvig, used in AI courses at NTNU, introduces ANNs as well. Similar embodied MEA-robotic papers provide great insight into the process of embodying neuronal cultures into artificial systems[162, 164, 163, 8, 9, 37, 50, 51, 128, 130]. Otherwise, the rest of the papers found in the bibliography, of which are mentioned in the thesis, are all informative.

For more information regarding The NTNU Cyborg robot development, the reader may refer to the authors specialization project[95].

For more information about The NTNU Cyborg initiative, please visit our website:  
<https://www.ntnu.edu/cyborg>

### 1.3.1 Resources in neuroscience

: Here are some great neuroscientific resource-websites which greatly aid the process of gathering information about the field. The resources include: people in the field, conferences, journals, papers, databases, societies, laboratories, online tutorials and other educational resources.

- Neuroscience on the internet<sup>1</sup>
- Neurosciences Resource Guide<sup>2</sup>
- Jim Perleowitz' Computational Neuroscience on the web<sup>3</sup>
- Neurotree<sup>4</sup>

## 1.4 Thesis structure

A quick introduction to the following chapters is presented here. Although this thesis is in large part a literature study of similar embodied studies, it is important to note that it is just as much a discussion. All topics are discussed with regards to their relevance for The NTNU Cyborg project, and the author also contributes to the discussion with own ideas and thoughts.

**Chapter 2** presents a brief overview of the information/literature gathering process that supplied the context within this thesis.

Chapter 3 and 4 are background chapters into neuroscience and artificial neural networks (ANNs) respectively.

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<sup>1</sup><http://www.neuroguide.com/index.html>

<sup>2</sup><http://psychologydegreeguide.org/neuroscience-resource-guide/>

<sup>3</sup><http://home.earthlink.net/~perlewitz/>

<sup>4</sup><http://neurotree.org/neurotree/>

**Chapter 3** provides relevant neuroscientific concepts that the reader may be unfamiliar with. We look at the difference between in-vivo vs in-vitro studies, describes stem cells, neurons, networks and the cultivation process. We also investigate what mechanisms enable biological networks to learn and adapt to incoming information.

**Chapter 4** introduces the concepts of artificial neural networks, with particular focus on biologically realistic spiking neural networks. We also discuss the training of artificial networks.

The chapters 5-7 are the authors open discussion around embodiment, communication and training of the culture, using methods and results from similar projects to guide the discussion.

**Chapter 5** investigates the hardware, interface and robotics aspects of reembodiment a neuronal culture. This part discusses the more practical setup for making the cyborg.

**Chapter 6** looks at methods to analyze and utilize the culture recordings, as well as how to stimulate the culture. We also investigate encoding and decoding of information to and from a neuronal culture.

**Chapter 7** discusses training of a neuronal culture with inspiration from the brain, computer science and similar in-vitro MEA research.

**Chapter 8** is a very brief chapter about how one might go about modelling a biological neural network.

**Chapter 9** summarizes the previous chapters as a plan moving forward,

**Chapter 10** wraps up the thesis.

# Method

A large part of the time used in writing this thesis has been devoted to studying neuroscientific concepts, biologically plausible artificial neural networks and studies involving interfacing, analyzing, embodying and learning in neuronal cultures. Another time consumer has simply been finding appropriate literature in a field that is in its early development. There has been much frustration over long hours trying to find answers to questions that ultimately where found not to have been answered yet. For the vast literature that was available though, extensive filtering of the most relevant material to include has been done. The aspects deemed relevant where deemed so, based on methods from similar studies and other aspects the author found relevant though acquired knowledge in the field of neuroscience and computer science.

As the MEA is not currently up and running, the focus has not been on implementing anything physically, but instead laying down the theoretical ground work for when the MEA is up. During the study, the author investigated setting up a distributed MEA-robot infrastructure. This was however, difficult to begin with as the majority of this coordination was sourced to IDI. Also, we are not quite sure how the software we are getting from MultiChannel systems may be interfaces. Similarly, the author contemplated making a biologically accurate model of a cultured neural network using spiking networks as a starting point. It was eventually reasoned that this would be a big enough task for a thesis in and by itself. Both setting up the infrastructure and modeling would ultimately take time away from the prioritized objective; studying the necessary aspects of embodying a neuronal culture.

## 2.1 Literature search

The literature in this thesis was gathered largely through Google Scholar, NCBI PubMed (biomedical literature) and NTNUs research database along with BIBSYS. The books

Neuroscience by Purves et al.[129] and Bio-Inspired Artificial Intelligence by Floreano and Mattiussi[59] contributed a great deal to the background chapters. Publications from labs and people such as Potter Lab[111] and Warwick[161] were also studied. Some thesis from IDI, regarding biologically plausible neural network models, were also useful as they covered a great deal of neuroscience theory and described the mathematical workings of biological neural system (to the extent we currently understand) quite well. Some research papers referenced in topic-relevant Wikipedia pages were also a helpful sources of information.

To give the reader an idea of how the relevant literature was obtained, here are presented a few (very small percentage of actual) example search terms: cultured neural networks (CNNs), neuronal cultures, embodiment, bio-robotic systems, embodied cultures, learning in CNNs, learning in the brain, reinforcement learning, spiking neural networks (SNNs), learning in SNNs, analyzing neural networks, stimulation of neural networks, dopamine, microelectrode arrays, MEA, neural coding etc.

Papers were evaluated and chosen based on: the author(s) reputation in the field, the amount of publications of the author(s) in the field, the organization the author(s) came from (e.g., which institutions and labs), the number of papers that had cited the current paper, whether the paper referenced to other good papers, and whether the paper had been published in a journal and was peer reviewed. Although some deviations from this list may occur, the author has tried to gather relevant and decent literature with the best intentions.

### **2.1.1 Images and illustrations**

Illustrative images where mostly gathered from research papers, the mentioned books and Wikimedia commons because of their open publicly approved licensing[170]. All images without reference are created by the author of this thesis.

## **2.2 Known in the field**

During the literature search, the author got familiar with certain names, journals and conferences that seem to be relevant in the field of MEA research.

### **2.2.1 People**

Some frequently occurring researcher were observed. Here follows a short list of some of the main contributors in the field of reembodied cultured neuronal networks.

#### **People:**

- Steve Potter (Laboratory for NeuroEngineering, Georgia Institute of Technology)

- Kevin Warwick (Emeritus Professor, Coventry University & University of Reading)
- Thomas DeMarse (Department of Biomedical Engineering, University of Florida)
- Douglas J. Bakkum (Research Scientist, ETH Zurich, Department of Bio Systems Science and Engineering)
- Daniel Wagenaar (Assistant Professor, Department of Biological Sciences, University of Cincinnati, Ohio, USA)
- Karl Dockendorf (Biomedical Engineering Department, University of Florida, Gainesville)

A helpful resource for finding graduate student and postdoctoral connections between most researchers in the field of neuroscience, is through the website Neurotree[46].

## 2.2.2 Conferences and journals

The following conferences and journals have been used for publishing several of the MEA studies.

### Conferences:

- Annual International Conference of the IEEE Engineering in Medicine and Biology
- IEEE International Conference on Neural Networks
- IEEE Transactions on Biomedical Engineering
- International IEEE EMBS Conference on Neural Engineering
- International Joint Conference on Neural Networks

### Journals:

- BMC Neuroscience
- BioSystems
- Defence Science Journal
- Frontiers in Neural Circuits
- Journal of bioscience and bioengineering
- Journal of Biotechnology & Biomaterials
- Journal of neural engineering
- Journal of Neuroscience Methods
- Neurocomputing
- PLoS Computational Biology
- PLoS One

- The Journal of neuroscience

# Background: Biology

Since we are ultimately using a neuronal culture in our bio-robotic/cybernetic system, it is appropriate to introduce some relevant biological concepts, as to allow the reader better understanding of the topics to come. We will here briefly introduce neurological concepts such as stem cells, neurons and neural structures in the brain. We will also discuss the current theories on how neural networks code information and learn. Lastly, we shall summarize the cultivation process of making a neuronal culture.

## 3.1 In-vivo vs in-vitro

**In-vivo** (Latin: 'within the living') studies are those in which the effects of various biological entities are tested on whole, living organisms usually animals (including humans), and plants as opposed to a partial or dead organism.[90] **In-vitro** (Latin: 'within the glass') studies are typically conducted with microorganisms, cells or biological molecules outside their normal biological context, using test tubes and petridishes.[90]

The advantage of using in-vitro methods, is that it permit simpler, more convenient and more detailed analysis than can be done with the whole organism. In terms of a neuronal culture, it allows for non-invasive stimulation and recording of the neuronal network, unhindered by skin, skull and other tissue.[164]

The primary disadvantage of in-vitro experimental studies, is that it is challenging to extrapolate from experimental results back to the biology of the intact organism. Investigators must be careful not to over-interpret their results, which can lead to erroneous conclusions about organism and systems biology.[135] In the case of a neuronal culture, one must consider that this neural network usually operates in a highly organized brain hierarchy (see chapter 3.4) that is likely very important in its correct functioning.

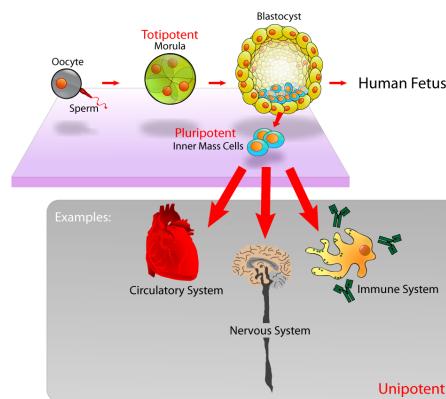
The NTNU Cyborg project will be conducting in-vitro experiments in its studies. A neuronal culture provides a good biological platform for interfacing with the robot platform. For this reason, in-vitro neuronal cultures are the main focus of this thesis.



**Figur 3.1:** In-vitro dish. From huffingtonpost.com

## 3.2 Stem cells

Stem cells are undifferentiated biological cells that can differentiate into specialised cells and divide (through mitosis) to produce more stem cells.[158]. Thus, the stem cells have the ability to grow into the neuronal cells needed for a neuronal culture. Stem cells grow into adult neurons in about one month, developing all the essential properties of functional CNS neurons[122]. Furthermore, stem cells allow the development of several phenotypes (not only neurons but also astrocytes and oligodendrocytes) that ensure the correct contribution of trophic substances and cellular junctions for the better and more physiological functionality of neurons in culture.[158] Figure 3.2 shows the process of a stem cell becoming a specialised cell.



**Figur 3.2:** Process of stem cell becoming specific tissue By Mike Jones via Wikimedia Commons

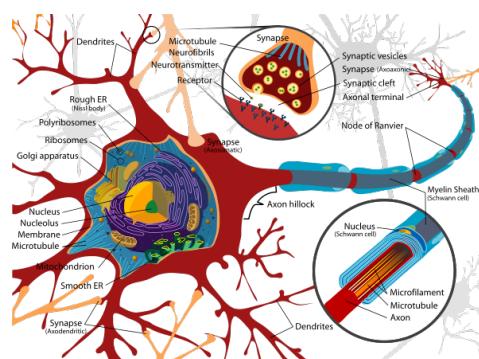
## 3.3 Neurons

A neuron (or nerve cell) is an electrically excitable cell that processes and transmits information through electrical and chemical signals. These signals between neurons occur via synapses: specialized connections with other cells. When neurons connect to each other they form what we call neural networks. Neurons are the core components of the brain and spinal cord of the central nervous system (CNS), and of the ganglia of the peripheral nervous system (PNS).[129, 59]

### 3.3.1 Anatomy

The composition of a neuron is illustrated in figure 3.3. Here follows a short description of its main components[129, 59]:

- **Soma:** the body of the neuron which contains the nucleus.
- **Dendrites:** cellular branched extensions from where the majority of inputs to the neuron, from other neurons, occur.
- **Axon:** a finer, cable-like projection that can extend tens, hundreds, or even tens of thousands of times the diameter of the soma in length. The axon propagates nerve signals, in the form of action potentials, away from the soma (and also carries some types of information back to it). Many neurons have only one axon, which usually has many branches to target cells.
- **Axon hillock:** the part of the axon where it emerges from the soma. The axon hillock has the greatest density of voltage-dependent sodium channels, making it the most easily excited part of the neuron. This contributes to it very often initiating the action potential down the axon.
- **Axon terminals:** the endpoint of the axon which contains synapses, specialized structures where neurotransmitter chemicals are released to communicate with other neurons.



**Figur 3.3:** Illustration of the neuron anatomy by LadyofHats via Wikimedia Commons

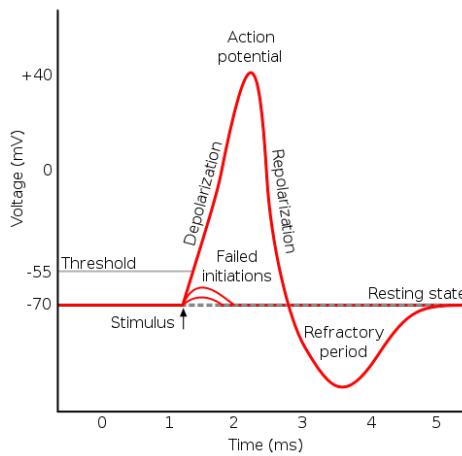
### 3.3.2 Action potentials

An action potential (AP) is a short-lasting event in which the electrical membrane potential of a cell rapidly rises and falls, following a consistent trajectory. The AP in a neuron occurs when the cell potential surpasses a certain threshold, usually around -55mV, and acts as an all-or-nothing event. The AP causes the axon hillock to 'fire' a wave of potential down the axon, causing the release of neurotransmitters, which further effect the potential of the post-synaptic neurons. The membrane potential of a cell rises due to upstream cells firing APs or other extracellular potential changes (e.g., through microelectronic stimulation). The connection strength between two neurons (the amount of branches between the cells, the amount of neurotransmitter released, and the amount of receptors located on the post-synaptic cell) will affect how much of a potential rise will occur. During the AP the cell goes through an absolute and relative refractory period. During the absolute refractory period (from the threshold is reached until after hyperpolarization), the cell is unaffected by stimuli. During the relative refractory period, the cell is harder to excite. In neurons, APs play a central role in cell-to-cell communication. APs are also known as 'spikes', and the temporal sequence of APs generated by a neuron is referred to as its 'spike train'. The whole AP lasts about 1ms.[129, 59]

#### Phases

The action potential consists of five main phases (figure 3.4):

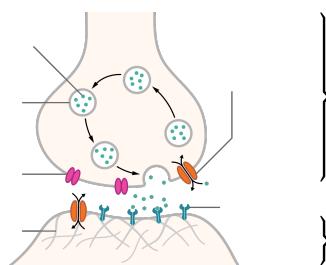
- **Increasing potential:** the potential increases from the 'resting potential' (around -70mV) due to pre-synaptic (upstream) neurons firing or changes in the extracellular environment.
- **Threshold:** once the membrane potential threshold is reached, the axon hillock fires an AP down the axon.
- **Depolarization:** when the neuron fires, the potential increases due to the opening of voltage-gated sodium channels, which allows sodium ions to rush into the cell. The potential increases until it reaches around 40mV.
- **Repolarization with hyperpolarization:** voltage gated potassium channels open when the potential is positive, causing potassium to rush out of the cell, which decreases the potential. The potential drops slightly below the resting potential: hyperpolarization.
- **The relative refractory period:** After hyperpolarization comes a refractory period where the potential is below the resting potential until the sodium-potassium pump is able to pump the sodium back into the cell and the potassium out; restoring the cell's original state. During this period, the cell is more difficult to excite.



**Figur 3.4:** Action potential by Chris 73 via Wikimedia Commons

### 3.3.3 Synapse

A synapse is a structure that permits a neuron to pass an electrical or chemical signal to another neuron. In a *chemical synapse*, electrical activity in the pre-synaptic neuron is converted into the release of a chemical called a neurotransmitter that binds to receptors on the post-synaptic cell. Synaptic communication usually occurs from the axon terminals of the pre-synaptic neuron, to the dendrites of the post-synaptic neuron. The neurotransmitter may initiate an electrical response or a secondary messenger pathway that may either excite or inhibit the post-synaptic neuron. There are a large variety of neurotransmitters, each effecting the receiving neuron differently. In an *electrical synapse*, the pre-synaptic and post-synaptic neurons are connected by special channels called “gap junctions” or “synaptic clefts” that are capable of passing the electric current directly (without the use of neurotransmitters). The main advantage of an electrical synapse is the rapid transfer of signals from one cell to the next. Electric synapses however, do not allow the same control and diversity as chemical synapses.[129, 59]



**Figur 3.5:** Synapse by Thomas Splettstoesser via Wikimedia Commons

## Neurotransmitters

Neurotransmitters, also known as chemical messengers, are chemicals that enable transmission of signals across chemical synapses, from one cell to another. They are received by receptors on the target cells. Neurotransmitters play a major role in shaping everyday life and functions. Their exact numbers are unknown, but more than 100 chemical messengers have been identified.[129]

## Neuromodulation

Neuromodulation is the physiological process by which a given neuron uses one or more neurotransmitters to regulate diverse populations of neurons. This is in contrast to classical synaptic transmission, in which one pre-synaptic neuron directly influences a single post-synaptic partner. Neuromodulators, secreted by a small group of neurons, diffuse through large areas of the nervous system, affecting multiple neurons. Major neuromodulators in the CNS include dopamine, serotonin, acetylcholine, histamine, and norepinephrine.[129] Neuromodulation seems to play a large role in learning. E.g., numerous studies link dopamine secretion to reinforcement learning and motivation[34, 115].

### 3.3.4 Classification

Neurons exist in a number of different shapes and sizes and can be classified by their morphology and function.[31].

Functional classification:

- Afferent neurons: convey information from tissues and organs into the central nervous system and are sometimes also called sensory neurons.
- Efferent neurons: transmit signals from the central nervous system to the effector cells and are sometimes called motor neurons.
- Interneurons: connect neurons within specific regions of the central nervous system.

Within these groups there are again many types of neurons, but we will not go into further detail on these here. In the NTNU Cyborg, we will be experimenting with different cultures built up by varying mixtures of neurons.

### 3.3.5 Excitatory vs inhibitory neurons

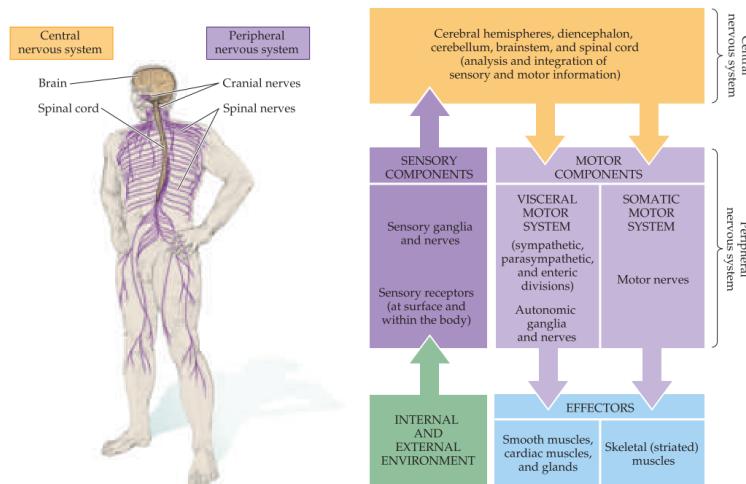
The neocortex contains both excitatory ( 80%) and inhibitory ( 20%) neurons, named for their effect on other neurons.[21] Excitatory neurons are the information processing work-horses of cognition and receive around 10,000 inputs each from other neurons (excitatory and inhibitory). Activated collections of millions of these neurons represent thoughts and perceptions.

## 3.4 Structure of the brain

The nervous system coordinates voluntary and involuntary actions and transmits signals to and from different parts of the animal body. In vertebrate species it consists of two main parts: the central nervous system (CNS) and the peripheral nervous system (PNS). At the cellular level, the nervous system is defined by the presence of neurons.[129]

The **CNS** comprises the brain (cerebral hemispheres, diencephalon, cerebellum, and brain-stem) and the spinal cord. The CNS handles all analysis and integration of sensory and motor information.[129]

The **PNS** consists mainly of nerves that connect the CNS to the rest of the body. The PNS includes sensory and motor components. The motor components are further divided into somatic and visceral systems, also called the autonomic nervous system. Somatic nerves mediate voluntary movement, while the visceral systems controls involuntary motor function through the sympathetic (fight or flight) and the parasympathetic (rest and digest) nervous systems.[129]

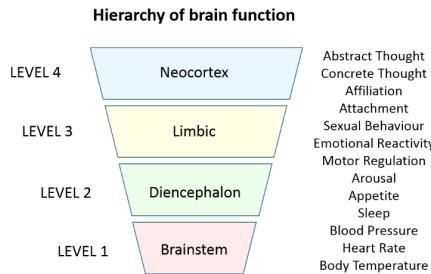


Figur 3.6: CNS and PNS[129]

### 3.4.1 Brain hierarchy

The nervous system is highly structured and complex. Neurons don't just connect randomly with each other, as they may do in a culture neuronal network. The brain is organised in a hierarchical fashion, such that all incoming sensory information first enters the lower parts (the brainstem) going upwards to the higher parts (the neocortex), see figure 3.7.[129, 69, 94]

Description of each layer[6]:



**Figur 3.7:** Brain hierarchy.[6]

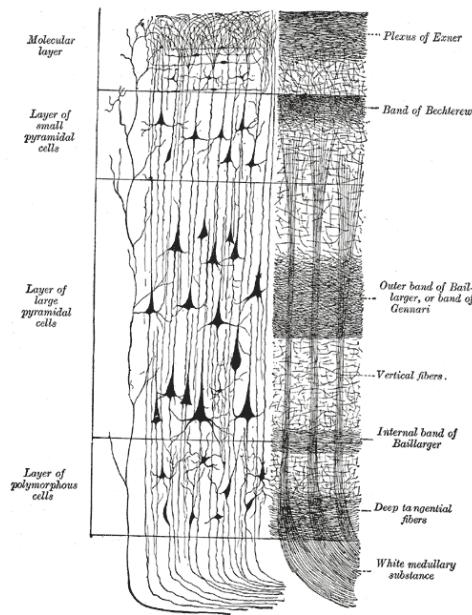
- **The Brainstem:** this primitive brain, essentially common in function in all creatures, processes billions of pieces of incoming information and directs them to the right locations. In humans, it controls basic functions such as body temperature, heart rate and blood pressure. Hard wired into it are the instantaneous ‘fight or flight’ instincts of self-preservation over which we have no control.
- **The Diencephalon:** deals with (still relatively basic) functions such as sleep, appetite, arousal and motor function.
- **The Limbic:** deals with emotional reactivity, sexual behaviour and attachment. The Limbic is part of the bigger ‘limbic system’ controlling hormone activity in the body.
- **The Neocortex:** the most recently evolved part of the brain, which handles affiliation, concrete thought and abstract thought.

### Neocortex

The neocortex is the seat of intelligent thought in the mammalian brain. High level vision, hearing, touch, movement, language, and planning are all performed by the neocortex. In the human brain, it is the largest part, and top layer, of the cerebral cortex, with the allocortex making up the rest. The neocortex is 2-4 mm thick and made up of six layers (as shown in figure 3.8, labelled from the outermost inwards: I to VI).[94]

Given such a diverse suite of cognitive functions, one might expect the neocortex to implement an equally diverse suite of specialized neural structures. This is not the case. Viewed under a microscope, the physical characteristics of the different cognitive regions look remarkably similar, revealing a complex replicated circuit pattern. Similar regions of the neocortex can learn to represent entirely different things in reaction to different inputs.[69] In one study by Roe et al.[134], the visual projections in ferrets were routed to their auditory pathway. They found that the auditory cortex can accept and process visual input in a similar way as the visual cortex. They reasoned that the sensory neocortex is not uniquely specified to process inputs of a single modality.

Another benefit that is believed to come from such a layered and hierarchical organization of the neocortex, is the ability to represent complex patterns through simpler ones.



**Figur 3.8:** The 6 layers, I-VI, seen found in the neocortex. Henry Vandyke Carter via Wikimedia Commons

For illustration, let's consider vision. At the lowest level of the hierarchy, your brain stores information about tiny sections of the visual field such as edges and corners. These low-level patterns are recombined at mid-levels into more complex components such as curves and textures, which are further combined to represent high-level object features, such as heads, cars or houses. To learn a new high level object you don't have to relearn its components.[69] This concept is illustrated in figure 3.9.

## 3.5 Neural communication

The study of *neural coding* involves measuring and characterizing how stimulus attributes, such as light, sound or touch, are represented by neuron APs. Neurons are remarkable in their ability to propagate signals rapidly over large distances and, in this way, communicate with one another. We have already seen how they do this by the use of action potentials. Although APs can vary somewhat in duration, amplitude and shape, they are typically treated as identical all-or-nothing stereotyped events. Since all APs are treated identical, there can't be any information encrypted in the AP itself. Instead, neurons are thought to communicate through the series of spikes they produce and the spike patterns that emerge across neuron populations. There is however, an ongoing debate to just how these coding mechanisms work. These theories include rate coding, temporal coding, population (dense) coding and sparse coding. With the development of large-scale neural recording and

decoding technologies, researchers are beginning to crack the code[82].

### 3.5.1 Encoding and decoding

**Neural encoding** is understanding how neurons respond to a wide variety of stimuli, and to construct models that attempt to predict responses to stimuli. The ultimate measure of success is the ability to make testable predictions.

**Neural decoding** is the reverse of neural encoding: mapping from response to stimulus. The challenge is to reconstruct a stimulus, or certain aspects of that stimulus, from the spike sequences it evokes.

### 3.5.2 Coding schemes

There are in principle two categories of coding schemes; those based on the sequence of spikes (such as rate-based and temporal coding) and those based on the spike patterns emerging from the ensemble of spikes (such as population (dense) and sparse coding).

#### Rate coding

Rate (frequency) coding is a traditional coding scheme assuming that information about the stimulus is contained in the firing rate of the neuron, rather than as specific spike sequences. The rate-based model states that as the intensity of a stimulus increases, the firing rate increases, generally non-linearly[83]. In motor neurons, for example, the strength at which an innervated muscle is flexed depends solely on the 'firing rate'; the average number of spikes per unit time[26]. When analyzing activity using rate coding, precisely calculating firing rate is important.

#### Temporal coding

Until recently, scientists had put the most emphasis on rate encoding as an explanation for post-synaptic potential patterns. However, functions of the brain are more temporally precise than the use of only rate encoding seems to allow[147]. When precise spike timing or high-frequency firing-rate fluctuations are found to carry information, the neural code is often identified as a temporal code. A number of studies have found that the temporal resolution of the neural code indicate that precise spike timing is a significant element in neural coding.[154, 28, 153, 7].

To illustrate the idea of temporal coding, we look at how spike sequences that may appear similar using rate-based coding, contain different information temporally: Say we record the activity of a neuron over 12 ms (remember that one AP lasts 1 ms). We indicate this using though a binary representation where a 1 indicates an AP, and a 0 indicates no

AP. Temporal coding allows the sequence 000111000111 to contain different information from the sequence 001100110011. However, using rate-based coding, they both display 6 spikes/12 ms which means they are equal in information.

Temporal coding may also be rational to assume, due to the time dependency of the neurons membrane potentials. E.g., the spike train 1000100010, may not cause an AP as the membrane potential will have time to sink between spikes and never reach the threshold. However, the spike train 1110000000, may cause an AP as the three consecutive spikes don't allow the membrane potential to sink in between, and thus pushes the membrane potential over the threshold.

### **Population coding**

Population coding communicates information through the joint activities of a number of neurons. In population coding, each neuron has a distribution of responses over some set of inputs. Essentially, if a neuron is connected to several upstream neurons, the different spiking patterns formed by the upstream neurons will effect the neuron differently. Population coding is found to occur in the motor cortex, premotor cortex, and other cortical areas[62, 8].

Population coding has a number of advantages, including reduction of uncertainty due to neuronal variability and the ability to represent a number of different stimulus attributes simultaneously. Population coding is also much faster than rate coding and can reflect changes in the stimulus conditions nearly instantaneously.[73]

### **Sparse coding**

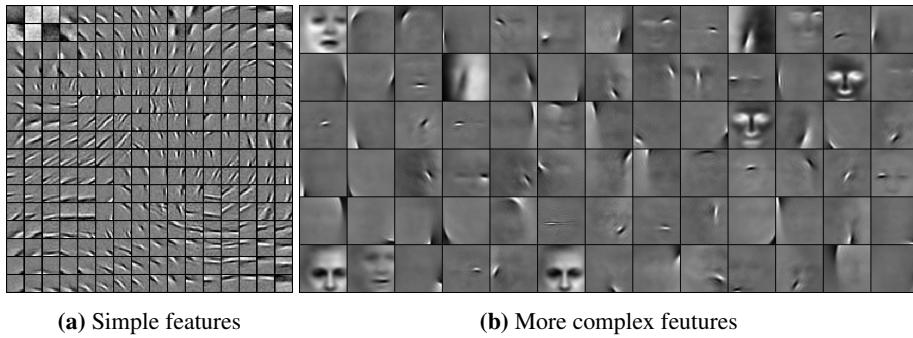
While population coding may be referred to as 'dense' coding sparse coding is well, 'sparse'. Both however, indicate coding by means of an active population of neurons at a given time. We refer to sparse coding when each particular representation is encoded by a relatively small percentage of active neurons.[69]

Sparse coding is easiest explained through an example: the very large set of English sentences may be encoded by a small number of symbols (i.e. letters, numbers, punctuation, and spaces) combined in a particular order for a particular sentence. Therefor, sparse coding for English would be those symbols.

Here is another example to illustrate the difference between dense and sparse coding: In a 1 megapixel image (1000 x 1000 pixels), each pixel is contributing to encoding the image. This is a 'dense' representation. In a sparse representation, we instead have e.g. neurons that become active for horizontal lines and others vertical lines, each at different locations in the image. If there are no horizontal lines, those neurons are silent and do nothing. Thus, what is encoded are the features rather than the space, and only those features that are present activate the neuron. The code becomes 'sparse' since contradictory features cannot be simultaneously present. One can think of this as each neuron looking for a

particular feature. When the feature is seen, the neuron fires. Most will not fire, because there are more features being looked for than there are features in the image.[85]

The observant reader may have noticed the similarity with sparse coding, and how the neocortex works. This is because it is theorized that the neocortex codes features through its layers in a sparse manner.[165, 13] In the Neocortex, inhibitory neurons guarantee that only a small percentage of the neurons are active at one time. The hierarchy, or layers described the previous chapter, will first look for low level features (lines, angles etc) in the first layers and higher level features (faces, cars, animals etc) in the top layers (see figure 3.9). Thus, information in the brain is always represented by a small percentage of active neurons within a large population of neurons.[165, 13, 69] This arguments for the biologically plausibility of sparse coding.



**Figur 3.9:** Sparse coding: Each square represents a feature a neuron may be looking for. (a) simple features such as lines and curves spotted in the earlier layers of the neocortex. (b) more complex features such as faces spotted in later layers. Image form Stanford wiki

### 3.5.3 Combing schemes

Are the different coding schemes mutually exclusive? When we talk about which coding scheme to utilize, it is important to note that one mechanism does not necessarily exclude the other. E.g., temporal coding, in a way, entail rate-based coding (the same does not apply the other way around). Temporal and rate coding can also be used, and likely are, in conjunction with population or sparse coding. Also, dense population coding and sparse coding seem to operate on slightly different mechanisms. Where population coding is a local process, being the pattern of active neurons into a downstream neuron, sparse coding takes advantage of the structure of the network.

## 3.6 Learning

In neuroscience, learning is the process by which new information is acquired by the nervous system and is observable through changes in behaviour.[129] Memory refers to the

encoding, storage, and retrieval of learned information.[129] Memory is essential to all learning, because it lets you store and retrieve the information that you learn. Learning depends on memory, because the knowledge stored in your memory provides the framework to which you link new knowledge, by association.[23]

Evolution has developed some amazing and complex learning mechanisms in the brain, which we gain to learn from. Since a cultured neural network (CNN) replicates networks in the brain, its natural environment, it is natural to look here for clues on how to efficiently train a CNN. The exact mechanisms for how learning comes about is however not fully understood. Here we present some of the basic mechanisms important for learning, and some theories to how learning is enforced.

### **3.6.1 Synaptic plasticity, Hebbian learning and STDP**

We begin first with the lower level mechanisms.

#### **Synaptic plasticity**

Synaptic plasticity is the synapses ability to strengthen or weaken over time in response to increases or decreases in activity.[74] There are several underlying mechanisms that cooperate to achieve synaptic plasticity, including changes in the quantity of neurotransmitters released into a synapse and changes in how effectively cells respond to those neurotransmitters. Cells may increase response to neurotransmitters by alteration of the number of neurotransmitter receptors located on a synapse and/or by neuromodulation. Since memories are postulated to be represented by vastly interconnected networks of synapses in the brain, synaptic plasticity is one of the important neurochemical foundations of learning and memory.[129, 59]

#### **Hebbian learning**

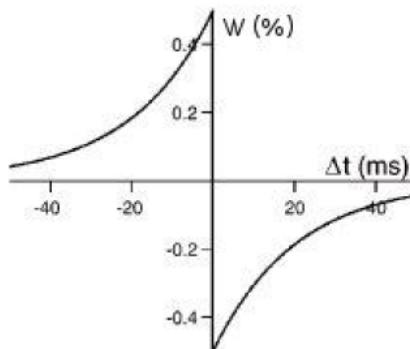
Hebbian theory proposes an explanation for the occurrence of synaptic plasticity. Introduced by Donald Hebb in 1949 in his book 'The Organization of Behavior',[152] Hebb states the 'Hebb's rule' as follows:

When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.[152]

Hebb emphasized that cell A needs to 'take part in firing' cell B, and such causality can only occur if cell A fires just before, not at the same time as, cell B. This important aspect of causation in Hebb's work foreshadowed what is now known about spike-timing-dependent plasticity (STDP), which requires temporal precedence.[32]

### Spike-timing dependent plasticity

Building further upon Hebb's rule, spike-timing-dependent plasticity (STDP) is a biological process that adjusts the synaptic strength between neurons according to the relative firing times of the pre-synaptic and post-synaptic neurons[19]: if an input spike to a neuron occurs immediately before that neuron's output spike, then that particular input is made stronger. If an input spike occurs immediately after an output spike, then that particular input is made weaker. Also, the amount of time in between the pre-synaptic and post-synaptic spike will have an effect on how much the connection strength is adjusted. Closer intervals equal greater adjustment (see figure 3.10).[129, 59]



**Figur 3.10:** Spike-time dependant plasticity (STDP). Cell A spikes before cell B: increased synaptic strength. Cell A spikes after cell B: decreased synaptic strength.[4]

## 3.6.2 Memory

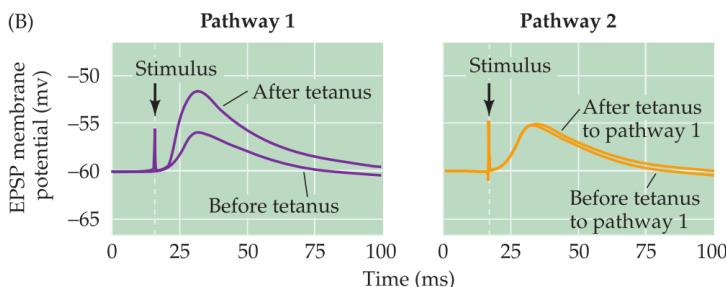
In order to learn, there must be some recollection of previous experience. It is widely accepted that the synaptic connections between neurons and neural pathways form the foundation for memories[20]. Memories that are recollected become further strengthened by the firing of those neural pathways, through Hebbian learning. On the other hand, memories that are not often recollected, may fade away[20]. Memory follows the 'use it or lose it' principle. This is, for example, why when studying for an exam, it helps to repeat the curriculum many times.

### Potentiation and depression

We learned that synapses can be strengthened or weakened, however this was not the whole story. The lasting effect of this strengthening and weakening is also an important factor. When synapses are strengthened we say that they are potentiated, when they are weakened they are depressed. When the lasting effect is long-term (minutes to hours, days etc)

we have long-term potentiation (LTP) or long-term depression (LTD). On the other hand, if the effect is short-term (a few ms to a few minutes) we have short-term potentiation (STP) or short-term depression (STD). Short-term effects are usually due to a short-time increased release on neurotransmitters between the communicating neurons.[129] Long-term effects are usually due to molecular changes like growing of new dendrites or axon terminals between cells which make for a stronger long-term connection.[129] It has been shown that high-frequency (tetanic) electrical stimulation causes LTP in synapses in the hippocampus.[129]. In contrast, low-frequency stimulation and prolonged inactivity of synapses in the hippocampus may weaken them[16].

The mechanism behind LTP may be described as such (courtesy of a video presentation by Carleton University[155]): High-frequency firing causes sodium to rush into the post-synaptic cells, which is followed by more sodium channels being transported to the dendritic wall of the target cell. This in turn, allows for even more sodium uptake from the pre-synaptic cell. If this continues further, neuromodulation comes into play by growth of more dendritic branches between the neurons. This leads to the post-synaptic neuron becoming increasingly sensitive to pre-synaptic APs, which means that the synaptic strength has been increased and we have achieved LTP.



**Figure 3.11:** Demonstrating LTP through tetanic stimulation. After tetanus, the response to a single stimulation is significantly increased.[129]

### 3.6.3 Associative learning (unsupervised)

Associative memory is one of the most common forms of memory used in everyday situations[149]. Through unsupervised learning, sensory such as sound, vision, taste, smell and touch are 'associated' to each other creating a complete 'picture' of the environment. E.g., the sound of a car along with how it smells and looks. This is why the smell of a certain dish can invoke childhood memories, or a song takes you back to a road trip of your past.

Unsupervised learning in the brain stems from the simultaneous firing of neurons which, according to Hebb's theory, makes the connections between them stronger. When one sees a car, neurons in the visual cortex fire the neural code that represents the car. At the same time, the sound of the engine elicits neurons dedicated to hearing to fire according to the

engine rumble. This simultaneous firing at both places serves to make connections, and thereby associations, between them.

### **Classical conditioning**

Classical conditioning, also known as Pavlovian conditioning, is an associative learning process in which a non-rewarding stimuli is learned to be associated with a rewarding stimuli. The basic facts about classical conditioning were discovered by Ivan Pavlov through experiments with dogs. In his classical 'Pavlovian conditioning' experiment, he studied how dogs would respond to a conditioned stimulus (CS). He measured when dogs would produce saliva in anticipation of food. He trained the dogs to associate the ring of a bell (the CS) to be followed by a treat, which is the unconditioned stimulus (US). The food is the reward, but the question was if the dogs would salivate by just hearing the bell go off. What happened was that dogs eventually began to produce saliva when the bell rang. The bell became a predication conditioned signal that food was on its way. This indicated not only associative memory, but also that the brain predicts events to come, which will be discussed later in this chapter.[17]

#### **3.6.4 Concept learning (supervised)**

Concept learning, also known as category learning, is a form of supervised learning mechanism. Concepts are the mental categories that help us classify objects, events, or ideas, building on the understanding that each have a set of common relevant features. Concept learning may refer to a learning task in which a human or machine learner is trained to classify objects by being shown a set of example objects along with their class labels. The model that emerges is then applied to future categorization without known examples. Consequently, the task is known as learning from examples.[89, 172]

One could argue that supervised learning is yet another form of unsupervised learning. When someone tells a child that what they are looking at is a car, their associative memory is associating the sound of the word 'car' with the visual input of that car. The brain simply learns to correlate people's words highly with the objects of attention. The distinction between supervised and unsupervised learning in human examples is thus; supervised learning suggests a teacher or some example-solution correlate being involved.

#### **3.6.5 Operant conditioning and reinforcement**

Operant conditioning, or conditioning through reinforcement, is a type of learning where the consequences of actions, such as reward or punishment, result in a modification of future behaviour.[124, 59] Operant conditioning is distinguished from Pavlovian conditioning in that it uses this reinforcement to alter an action-outcome association in contrast to a stimulus-outcome association (the agent must actually perform an action). The exact mechanism that implements reinforcement learning in biological neural systems is the subject

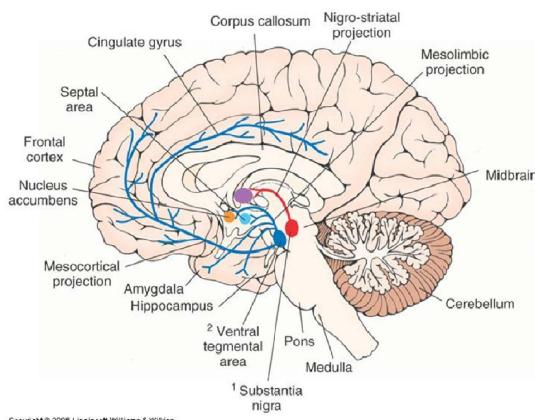
of much research and is not yet completely understood.[59] There are however, some mechanisms that are believed to play an important role.

### The reward system

The reward system consists of a group of neural structures responsible for desire, pleasure and positive reinforcement.[142] Research has shown that brains in humans and animals rely heavily on reward pathways when learning new tasks[142]. Reward pathways are neural pathways that are associated with reinforcement learning. A neural pathway connects one part of the nervous system to another via a bundle of axons[129].

**Dopaminergic pathway:** Some neurotransmitters are particularly associated with reward, such as dopamine. Dopamine participates in motivation and incentive drive by controlling plasticity processes in the brain[119, 11, 66]. Dopamine strengthens the neural activity leading to rewards and tells the memory centers to pay attention to all features of the rewarding experience.[124] In the context of reward-related learning, dopamine also functions as a reward prediction error signal: the degree to which the value of a reward is unexpected.[141]

Dopamine is released in the brain through dopaminergic pathways, which the brain includes several off[40]. The majority of dopaminergic cell bodies are found in the the Ventral Tegmental Area (VTA) in the Basal Ganglia. The VTA neurons project to numerous areas of the brain, from the prefrontal cortex to the caudal brainstem and several regions in between (figure 3.12)[40]. In essence, the VTA releases dopamine though its neural pathways to different regions in the brain, as a reinforcement mechanism.[17, 124]



**Figur 3.12:** Dopaminergic pathways.[]

While dopamine is critical to many learning and behavioral processes in the brain, animals can learn to select actions correctly even in the absence of dopamine[18]. Indicating multiple reinforcement learning systems in the brain[54]. Interestingly, dopamine also does not seem to be involved in the signaling or prediction errors for aversive outcomes.[115] One theory is that the Habenula plays an important role in punishment.[2]

### The distal reward problem

In experiments involving rewards, dopamine is typically released some time after the behavior that caused the reward being obtained. This gives rise to the 'distal reward problem': how does the brain know which neurons and synapses are responsible for bringing about the reward? The firing patterns are obviously long gone, and the neurons and synapses involved might have seen significant activity in the interim period, between action and reward.[4] This process is not yet fully understood, though a study by Nitz et al.[114] suggest that DA signaling through D1 receptors impacts brain processes linking actions and their temporally distal consequences.

### 3.6.6 Prediction and temporal difference learning

The brain is a real time predicting machine[24, 69], and learning has been proposed to stem from prediction errors known as temporal difference (TD)[115]. TD, which is actually a term from machine learning, has received attention from neuroscientists because it has been discovered that the firing rate of dopaminergic neurons in the VTA and Substantia nigra (SNc) seem to play a role in prediction error like TD[157]. Through prediction error based learning, learning means trying to improve future predictions and tends to occur especially when reality doesn't match the prediction.[157]

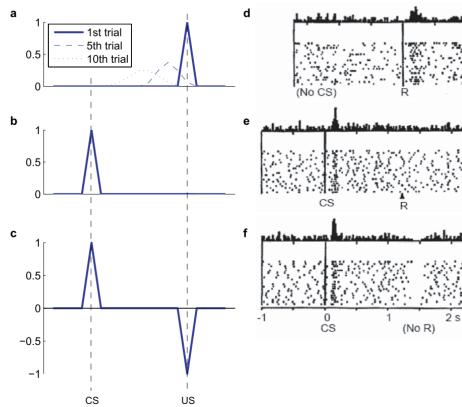
In the Pavlovian conditioning experiment explained above, we saw that the dogs predict food when the bell rang. In a similar experiment, measuring the firing rate of dopaminergic neurons in monkeys[140], it was observed that:[4]

- when an unexpected reward (US) was received the firing rate was high
- when an expected reward (due to CS) was received the firing rate remained unchanged.
- when an expected reward (due to CS) was withheld the firing rate fell below normal.

When a CS appeared, the dopamine fired to the CS but not the US. The dopamine responded to the unexpected and not in the reward itself. This indicates that dopamine is involved in error prediction and motivation maybe more than reward. If we continue this pattern further, we may apply a new CS prior to the former CS, e.g. having a light go off before the bell. Now the light may trigger a dopamine response, and we see how the occurrence of events leading up to a reward may enable prediction of that reward.

### 3.7 Cultivation of nerve cells

Neural cultures propose a non-invasive and efficient way of analyzing living neuronal networks with much of the anatomical complexity and dynamics of real brain circuits[53], but with a manageable size of only a few thousand neurons and glial cells.[75]. A cultured



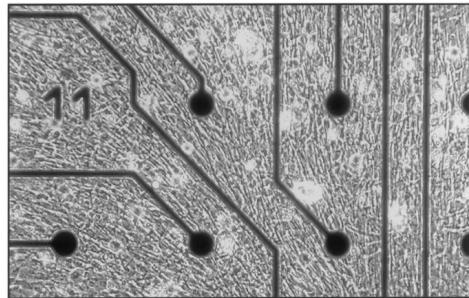
**Figur 3.13:** Pavlovian dopamine neuron firing[115]

neural network will form the biological computational unit for the NTNU Cyborg. It is also, in this culture, that the main scientific research will be conducted.

The exact cultivation process is the responsibility of INM, who will be experimenting with different neurons and networks topologies. Here we summarize the fairly standard methodology described in similar neuronal culture studies[162, 163, 49, 51, 44, 50, 122, 75, 127, 9]:

Cortical tissue, from embryonic rats, is dissociated using enzymes. Neurons from this process are cultured, in-vitro, onto the surface of microelectrode arrays (MEAs) (chapter 5.1). Neurons that are grown on the MEAs are covered with a gas-permeable membrane which permits repeated observations without risk of infection from bacteria. The MEA also needs to be filled with a conventional cell culture medium containing nutrients, growth hormones, and antibiotics. An incubation chamber must also be used to mimic the biological environment (the animal body) in which the cells usually live. This involves regulating temperature, humidity, gas (nitrogen, oxygen, CO<sub>2</sub>) concentrations etc. Dissociated neurons begin forming connections within a few hours in culture (even left to themselves without external input other than nutrients), and within a few days establish an elaborate and spontaneously active living neural network. After one month, the culture matures, and the development of these networks becomes relatively stable and is characterized by spontaneous bursts of activity. A great video summarizing the cultivation process may be found here: [35] courtesy of Chadwick M. Hales, John D. Rolston and Steve M. Potter.

Important variables to consider when growing a neuronal culture are; cell density, number of cells, types of cells and topology. Several studies report 10,000–50,000 cortical neurons on an MEA.[143, 160, 128]. Potter et al.[128] reported a density of 5000-10,000 cells per square millimeter in their cultures.



**Figur 3.14:** Neural cells grown on a MEA. Scale: 200  $\mu\text{m}$  between electrodes.[128]

### 3.7.1 Spontaneous activity

A very general observation about neural networks cultured over MEAs is that, regardless of their origin, they all develop some sort of synchronous and spontaneous bursting activity when they mature.[106] Depending on the cell culture used, a consistent bursting activity is likely to dominate after 18 to 25 days in-vitro.[49] One of the most common patterns of this spontaneous activity takes the form of network-wide bursts. In one study by DeMarse et. al[49], these bursts are reported to be semi-periodic, occurring every 5 to 15 seconds and typically 100 to 1000 ms in duration. The study also showed that stimulation pulses delivered to a network of cultured cortical or hippocampal neurons primarily tend to result in the production of a network wide burst of activity rather than eliciting responses from only a few neurons.

While it is not completely understood why these network-wide bursts appear, it is suggested that the spontaneous firing may be due to not having enough inhibiting neurons in the culture[12]. It may also be that bursting might be due to no more than a pathological manifestation of the fact that the culture conditions in-vitro interfere with biological processes[106].

### 3.7.2 Challenges using neuronal cultures

#### Keeping the neurons alive

The key challenges in using neuronal cultures is that they are very fragile and need very specific conditions to be held alive. The primary cause of death of neuronal cultures is either infection or changes in osmolarity[127]. Potter et al.[127] developed a system for keeping cultures alive for possibly over two years, by sealing them in a gas-permeable MEA culture chamber that keeps insects out and the water in. This setup enables much longer-term experiments to be conducted than before[75].

#### Lack of structure

Compared with the highly organized structure of the brain, such as in the neocortex, randomly grown neural networks may not possess the same abilities when taken out of

this structure. Instead, the cultures topology is highly random, and it's difficult to steer the connections formed by the neurons. In addition, sensory deprivation in culture makes them less good in forming good networks[162]. At INM, there is currently research being conducted attempting to guide some of the neural axons, which would be helpful in enabling a more organized network.

#### **2D vs 3D**

Neuronal cultures are typically grown in a monolayer (2D) fashion.[75] The brain, on the other hand, operates in a 3-dimensional world. We don't know quite how this dimensionality reduction effects the network.



# Background: Artificial neural networks

ANNs are computational models that attempt to capture the behavioral and adaptive features of biological nervous systems. A wide variety of ANNs exist, most of which do not attempt to replicate the full complexity of biological neural networks (BNNs). Doing so, is both computationally expensive and not necessary for many of the AI tasks in which ANNs are employed. Also, scientists have yet to figure out the full complexity of how neural networks operate in the brain. Instead, ANNs replicate the gist of what is observed in the brain. There has however, been a recent rise in more biologically correct ANNs, stemming from advances in neuroscience and more powerful computers enabling the complex modeling.[59]

An ANN can be summarized by its most important parts (and their biological representation):

- Nodes (neurons)
- Weighted connections between nodes (synapses)
- An integration function summing the inputs from upstream nodes x their input weights (4.1) (total depolarization of cell soma caused by each presynaptic (upstream) neuron).

$$a_i = \sum_{j=1}^N w_{ij}x_j \quad (4.1)$$

- An activation function which calculates an output value using the sum from the integration function.
  - 2nd generation models: output represents firing rate of neuron

- 3rd generation models: output represents all-or-none AP of neuron

ANNs have been shown to be able to model complex non-linear mathematical expressions using basic principles found in BNNs[22, 65]. However, the learning algorithms are usually quite simple. The complexity of the trained algorithm comes from the data, not the algorithm. ANNs are often utilized in artificial intelligent agent systems, where hard-coding all state-action pairs may be infeasible. ANNs are in addition great at pattern recognition and may be used in a wide variety of classification problems.[137] One great thing about ANNs and SNNs: is that the models allow us to experiment with different topologies and training algorithms, which we in turn can be compared with biology and see if our hypothesis are correct. In this way, not only does neuroscience inspire computer science, but computer science may also aid neuroscientific research and understanding.

In chapter 6 we will look at how ANNs may be utilized in analyzing the culture recordings. In chapter 7 we will look at how some of these protocols may be applied for training a cultured neural network. In chapter 8 we look at how we may model a neuronal network using biologically plausible neuron models. In the following chapter, we will first introduce the main categories of neuron models, moving on to network organization, before diving into the training of ANNs.

## 4.1 Neuron model generations

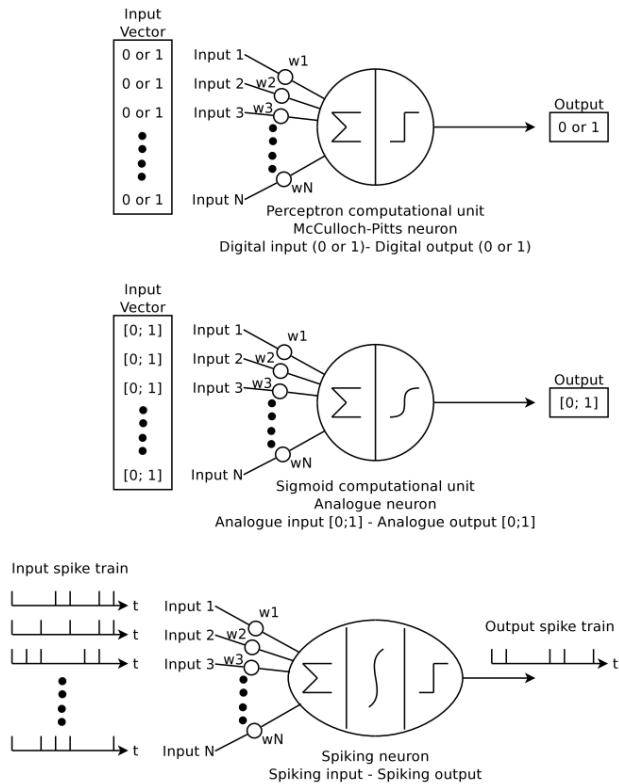
An artificial neuron is characterized by a set of connection strengths, a threshold, and an activation function[59] (figure 4.1). The types of neurons used in a neural network are often categorized into three different generations according to their computational units (i.e. neurons and synapses)[47] and their input/output correlations[96]: binary signals, continuous values and spike events. The models also display varying degrees of biological realism, with spiking networks being the most plausible.

### 4.1.1 1st: Perceptrons

The first generation of ANNs have computational units that are called perceptrons or threshold gates. They are called threshold gates because this class of artificial neurons fire when their total input reaches, or surpasses, some threshold value  $\vartheta$ . These neurons are only capable of digital output (they either fire or they do not). The neurons are composed each of two computations: a sum and an activation function (in this case, the threshold). The sum receives and sums the inputs from a set of weighted synapses while the activation function uses this sum to determine to fire or not. Equations 4.2 shows how this works.

$$\Phi(a_i) = \begin{cases} 1 : & \sum_{j=1}^N w_{ij}x_j > \vartheta_i \\ 0 : & \text{otherwise} \end{cases} \quad (4.2)$$

where  $\Phi$  is the activation function,  $w_{ij}$  the weight of a synapse and  $x_j$  the value of the synapse (the output of the upstream neuron). Both the inputs and the outputs have



**Figur 4.1:** Different generations of neurons used in ANNs.[47]

values that may be equal to either 0 or 1 (discrete values). For this reason, perceptron networks are sometimes called bit-networks. Bit-networks are universal in the sense that any boolean function can be approximated by some multilayer perceptron with a single hidden layer.[47, 4, 59]

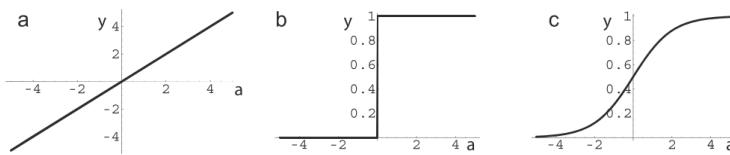
### 4.1.2 2nd: Rate-based

The second class of artificial neural networks also perform two computations: a summation of the weighted input synapses, and an activation function using that synaptic sum. This time the activation function is a continuous function of type  $f : R \rightarrow R$ , mapping the inputs of a neuron to a continuous output value. A common activation function choice is the sigmoidal, or logistic function (equation 4.3).

$$\Phi(a_i) = \frac{1}{1 + e^{-ka_i}} \quad (4.3)$$

where  $a_i$  is the input sum, and  $k$  is a scaling factor that determines the inclination of the slope shown in figure 4.2. Sigmoid functions limit outputs to  $[0;1]$ , whereas the hyperbolic functions produce outputs in the range  $[-1;1]$ . The output is sometimes called the 'spike-rate' or 'firing-rate', which refers to its biological equivalent. The coding scheme used to encode information in these networks are therefore rate-based (chapter 3.5).[47, 4, 47, 4, 59]

Second generation ANNs are more efficient than first generation networks, and are also universal for analog, as well as digital, computations: they can approximate, arbitrarily well, any continuous function with a compact domain and range, using only a single hidden layer.[59]



**Figur 4.2:** a) linear function  $\Phi(a_i) = ka_i$  with  $k=1$ ; b) threshold function with  $\vartheta=0$ ; c= sigmoid function with  $k=1$ [59]

### 4.1.3 3rd: Spiking neurons

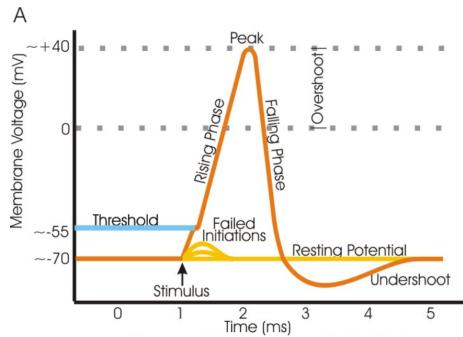
While 2nd generations neurons can be said to output a variable spike-rate, spiking neurons output all-or-nothing spikes (like biological neurons). These spikes, model the action potentials (APs) found in biological neurons. Spiking neural networks (SNNs) also model the biological membrane potential within each neuron (which acts as the internal state of the neuron). This introduces the concept of time into the simulation, while earlier the neural networks were based on abstract steps of simulation. Neurons of the 3rd generation are thus more biologically plausible than those of the previous generations. In addition, they take advantage of temporal coding by utilizing the time dimension; hence, these networks may compute functions using less neurons.[47, 4, 59].

SNNs have shown great promise in the field neuroscience as they are able to model biological neurons to a much greater degree than its predecessors. However, SNNs have been shown to be harder to apply to classical problem solving tasks in which 2nd generation ANNs are widely used. This is due to their increased complexity and because efficient training algorithms have yet to be developed for this newer generation.

#### Spiking neuron models

There have been developed numerous neuron models ranging from the biologically plausible but computationally complex, to the computationally simple but less realistic. These spiking models, model the biological neuron action potential (AP). The models incorporate the resting membrane potential, the threshold potential, depolarization, repolarization,

hyperpolarization and the absolute and relative refractory periods to some degree (figure 4.3). These AP phases were described in chapter 3.3.2.



**Figur 4.3:** Modeled action potential[4]

In [77], Izhikevich does a thorough review of various spiking neuron models. 11 models for spiking neurons are evaluated on their ability to reproduce the 20 most prominent features of biological neurons and the models computational cost in doing so. Some of the most popular models of spiking neurons are (ranging from most complex and realistic to simplistic): Hodge-Huxley, Izhikevich's and leaky integrate and Fire (LIF). For comparison, he found that the Hodgkin-Huxley model (which could model all features) required 1200 FLOPS (floating point operations). The Izhikevich model (which could also model all features) required only 13 FLOPS. Lastly, the LIF model (which was limited in modeling all features) required only 5 FLOPS.[78]

### Leaky integrate and fire

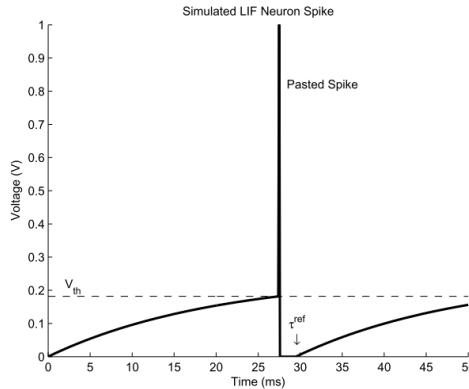
The leaky integrate-and-fire (LIF) model is a bit more limited when it is evaluated on its ability to simulate biological neurons, but updates each neuron efficiently.[78] The model equation is displayed in 4.4.

$$\frac{dV}{dt} = -\frac{1}{RC}(V(t) - J(t)R) \quad (4.4)$$

where  $R$  is the resistance of the membrane,  $C$  is the membrane capacitance, and  $V(t)$  is the voltage at time  $t$ . This equation may be recognized as the linear differential equation for RC circuits, with the bilipid membrane of the cell acting as a capacitor accumulating electric charge from the dendrites, and the ion channels acting as resistors. Because the bilipid membrane is not a perfect insulator, current 'leaks' out of the cell at a speed defined by the membrane resistance and voltage, hence 'leaky'.[17]

### Izhikevich

The model by Izhikevich[77] strikes a good balance between simulation detail and computational costs. Izhikevich's model is able to reproduce all the 20 features evaluated in [78]. Equation 4.7 shows the model.



**Figur 4.4:** Izhikevich model showing effects of the various parameters on the shape of the pulse and recovery of membrane potential.[17]

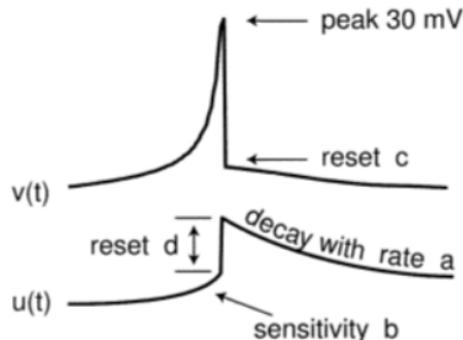
$$\dot{v} = 0.04v^2 + 5v + 140 - u + I \quad (4.5)$$

$$\dot{u} = a(bv - u) \quad (4.6)$$

and the following equation for after-spike resetting:

$$\text{if } v \geq 30 \text{ mV, then } \begin{cases} v \leftarrow c \\ u \leftarrow u + d \end{cases} \quad (4.7)$$

Where a, b, c and d are dimensionless parameters, and  $\dot{v} = dv/dt$  where t is time. The variable v represents the membrane potential of the neuron and u is a variable describing the recovery of the membrane potential with respect to the resting potential. I represents the input to the neuron from other neurons.[17]



**Figur 4.5:** Izhikevich model showing effects of the various parameters on the shape of the pulse and recovery of membrane potential.[77]

In figure 4.5 we can get a sense for what the various parameters does to the shape of the spike and its recovery.

- The parameter  $a$  affects the time scale of the recovery variable  $u$ , lower values yields slower recovery.
- Larger values for  $b$  gives a higher coupling between  $v$  and  $u$  making it possible to have subthreshold oscillations.
- $c$  describes the after-spike reset value.
- $d$  is the after-spike reset value for  $u$ .

### Hodgkin-Huxley

At the more complex end of the spectrum we find the Hodgkin-Huxley model[71]. This model is incredibly detailed, describing the membrane potential and the currents of  $\text{Na}^+$  and  $\text{K}^+$  ions. It is one of the most important models of neuroscience. This level of detail, however, comes at a computational cost. Since the model is computationally inefficient for modeling a large scale neuronal culture, we will not go into further detail on this model here.

### STDP model

We looked at how the synaptic strengths between neurons adjust through STDP in chapter 3.6.1. A model for the STDP process was formulated by Song et al.[144] as shown in equation 4.8 (with slight modification).

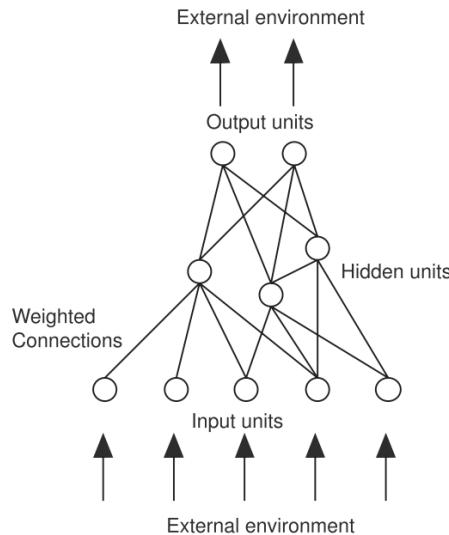
$$\Delta w_{ij} = \begin{cases} Ae^{(x/\Delta t)} & : \text{if } \Delta t > 0 \\ -Ae^{(x/\Delta t)} & : \text{if } \Delta t < 0 \end{cases} \quad \text{where } \Delta t = (t_i - t_j) \quad (4.8)$$

where  $\Delta w_{ij}$  is the synaptic weight change between the pre-synaptic neuron  $i$  and the post-synaptic neuron  $j$ .  $A$  is the magnitude of the weight change.  $\Delta t = (t_i - t_j)$  represents the relative firing times of the pre-synaptic and post-synaptic neurons.

## 4.2 Architectures

A neural network is simply a group of interconnected neurons. An ANN is typically comprised of an input layer, a (optional) hidden computational layer and an output layer (figure 4.6.[59, 137] There are many ways to design ANNs: numbers of neurons to use in each layer, selection of neuron model to use, and how the connections between neurons are made to name a few. Here, we look at the most important model concepts and introduce some relevant models for this thesis.

An artificial neural network is composed of several interconnected units, or neurons. Some of these units receive information directly from the environment (input layer), some have a direct effect on the environment (output layer), and others communicate only with units within the network (internal, or hidden, layer).[59, 137]



**Figur 4.6:** Illustration of an artificial neural network.[59]

### 4.2.1 Network concepts

#### Feed-forward networks

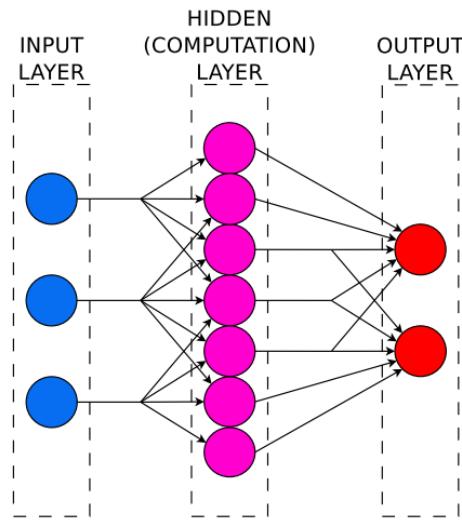
A simple example of a *feed-forward* neural network can be expressed by the three-layer network depicted in figure 4.7. The group of neurons on the left make up the input layer, whose task is to collect stimuli from the external world; similar to the sensory neurons in animals. The middle layer is the computational layer; similar to inter-neurons in the brain. Finally, the output layer on the right, generates the output stimuli of the network; similar to motor neurons.[59, 137]

#### Recurrent networks

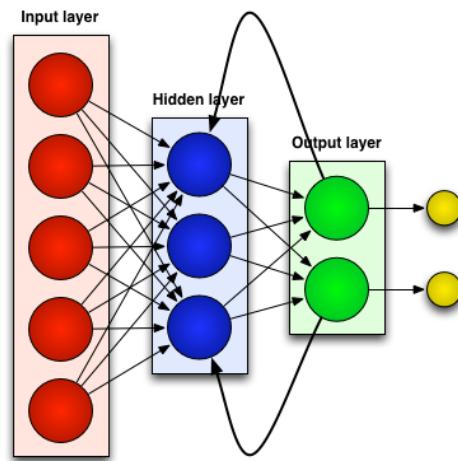
More complex structures of neural networks may include *recurrent* connections: connections between neurons of the same layer or propagating backwards. Figure 4.8 illustrates a recurrent network.[59, 137]

#### Deep neural networks

Deep neural networks are identified by the use of many hidden layers. They are often used in conjunction with 'deep learning', a branch of machine learning, which attempts to model high-level abstractions in data through multiple processing layers composed of multiple non-linear transformations.[64, 52]



**Figure 4.7:** Illustration of a feedforward network.[47]



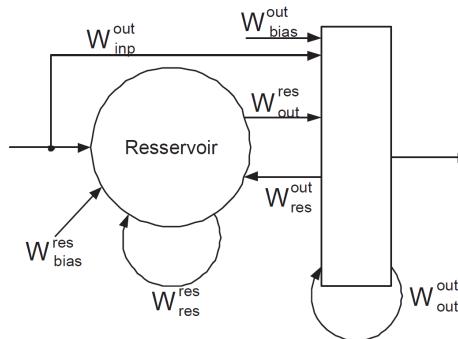
**Figure 4.8:** Illustration of a feedforward network.[47]

## 4.2.2 Reservoir computing

Reservoir computing is a framework for computation using a 'reservoir'; often a type of neural network. Input data is fed to the reservoir in which the feedback weights between nodes inside the reservoir have random values and are not tuned during training. Instead, only the weights from the reservoir to the output layer are trained. The benefit of this is that the non-linear nature of the reservoir is exploited while only needing to tune the linear feed-forward weights from the reservoir to the output layer. Essentially, this allows us

to compute a non-linear function using linear training.[139] Figure 4.9 demonstrates the principle of reservoir computing.

Liquid-state machines and echo state networks are two major types of reservoir computing. Echo state networks use recurrent neural networks, typically using 2nd generation sigmoidal models in their reservoir. Liquid state machines use spiking neural networks. Since SNNs model CNNs better (discussed in chapter 8), we will continue our discussion here on LSMs.



**Figure 4.9:** Reservoir computing: Input is fed into the reservoir, which outputs to the output layer. Only the weights between the reservoir and the output layer are trained.[112, 139]

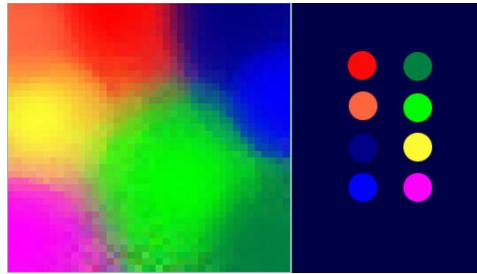
### Liquid state machine

A Liquid state machine (LSM), initially developed by Wolfgang Maass[97], consists of a large collection of spiking neurons/nodes. Each node receives time varying input from external sources (the inputs) as well as from other nodes. Nodes are randomly connected to each other. The recurrent nature of the connections, turns the time varying input into a spatio-temporal pattern of across the nodes. These patterns are read out by linear discriminant units.[97, 27] A STDP learning rule may also be applied to the liquid, as to have the network adapt to the data.

### 4.2.3 Kohonen - self organizing maps

A Kohonen network[86], invented by Teuvo Kohonen, is a type of self-organizing map (SOM) capable of clustering and dimensionality reduction of data. SOMs lie within the category of competitive learning algorithms: a form of unsupervised learning in which nodes compete for the right to respond to a subset of the input data.[86, 5] Kohonen networks provide a way of representing multidimensional data in much lower dimensional spaces: usually one or two dimensions. Kohonen networks have the ability to cluster and adapt to data in real-time.[86, 3, 5]

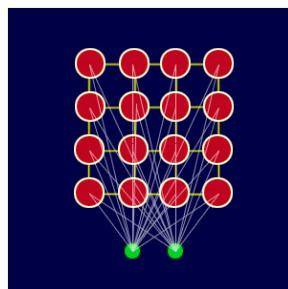
A common example[3] used to help teach the principals behind SOMs is the mapping of colours from their three dimensional components: red, green and blue, into two dimensions. Figure 4.10 shows an example of a SOM trained to recognize eight different colours. The colours have been presented to the network as 3D vectors, one dimension for each of the colour components, and the network has learnt to represent them in the 2D space. Notice that in addition to clustering the colours into distinct regions, regions of similar properties are usually found adjacent to each other.



**Figur 4.10:** 3 vectored colors represented in 2D.[3]

General algorithm for SOMs[3]:

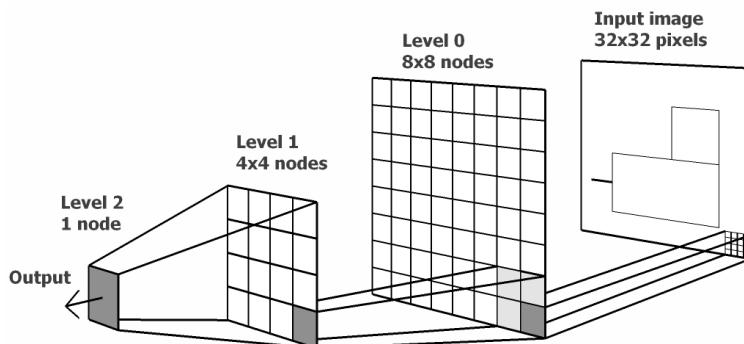
1. Randomize the map's nodes' weight vectors
2. Grab an input vector  $\mathbf{D}(t)$
3. Traverse each node in the map
4. Use the Euclidean distance formula to find the similarity between the input vector and the map's node's weight vector
5. Track the node that produces the smallest distance (this node is the best matching unit (BMU))
6. Update the nodes in the neighborhood of the BMU (including the BMU itself) by pulling them closer to the input vector



**Figur 4.11:** Example of Kohonen network.[3]

#### 4.2.4 Hierarchical temporal memory

Hierarchical temporal memory (HTM), developed by Numenta[117], is a very interesting type of machine learning technology that aims to capture the structural and algorithmic properties of the neocortex (chapter 3.4.1). HTMs model neurons (called cells in HTM), which are arranged in columns, layers, regions and in a neocortical inspired hierarchy (figure 4.12). The full overview of HTM may be found in their own paper[69], but the main idea of the model will be presented here.



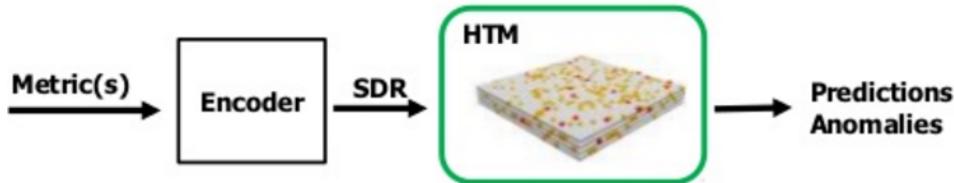
**Figure 4.12:** Hierarchical Temporal Memory (HTM). The hierarchical layered structuring inspired by the neocortex.[69]

**How it works:** HTM builds on the sparse coding principle, as described in chapter 3.5.2. HTMs are organized in a layered fashion, like the neocortex, where the top layers look for simple features that come together to form more complex features in lower layers (figure 4.12). For example, if the HTM is to recognize a face, Features may start in the first as lines, then curves, then ears/nose, then parts of a face and finally whole faces such as demonstrated in figure 3.9. Another property of sparse coding is that it allows incomplete representations, or slightly different representation of the same object to still be recognized.

**The role of time:** The time aspect in biological networks is very important. In 'the real world' there is no pattern recognition without time. Even looking at an image is an operation in time as the visual data, even if static, is still continuous and our eyes are constantly moving. In the same way, HTMs demand a continues stream of data, in contrary with most ANNs which can e.g. classify a static image with no aspect of time.

**Training:** HTMs are trained through exposure to a stream of (sensory) data. Learning involves incrementing or decrementing the synaptic weights on a dendrite segment (the input to a neuron). The rules for doing this follow Hebbian learning. Synapses that are active and contributed to the cell being active, have their weights increased. Synapses that are inactive and did not contribute, have their weights decreased. The exact conditions under which synapses weights are updated differ in the 'spatial and temporal poolers' (which are not necessary to describe here, but may be well described in the paper[69]). Like a biological system, the learning algorithms in an HTM region are capable of 'on-line learning', i.e.

they continually learn from each new input. There isn't a need for a learning phase separate from an inference phase, though inference improves after additional learning. As the patterns in the input change, the HTM region will gradually adapt.[69]



**Figur 4.13:** HTM: from input to prediction. The encoder converts the data stream into the correct input format.[116]

## 4.3 Training protocols

In ANNs, and as we assume in the brain, learning happens by the modification of synaptic weights between neurons/nodes. Basically, the algorithms that concern the training of ANNs, try to modify these weights as to achieve the correct input-output mapping.[59, 137] We categorize learning into three main forms: supervised, unsupervised and reinforcement learning.[59, 137] There are many algorithms developed for training ANNs within these protocols. We shall here primarily look at those training mechanisms that hold some biological plausibility. In chapter 7 we will look at how some of these protocols might be applied for training a cultured neural network.

### 4.3.1 Unsupervised

Unsupervised learning is the machine learning task of inferring a function to describe hidden patterns from unlabeled data.[59, 137] Since the examples given to the algorithm are unlabeled, there is no error or reward signal to evaluate a potential solution. This distinguishes unsupervised learning from supervised learning and reinforcement learning. With unsupervised learning in ANNs, the synaptic weights 'adjust themselves' to the inputs, often through a hebbian/STDP mechanism described earlier, or through some sort of clustering network (such as self-organizing Maps).

### 4.3.2 Supervised

Supervised learning, or learning by 'teacher', is the machine learning task of inferring a function from labeled training data.[76, 59, 137] The training data contains a set of training examples, consisting of an input object and the desired output value. With the given set of

example pairs  $(x, y), x \in X, y \in Y$ , the aim is to find a function  $f : X \rightarrow Y$  that approximates the examples. This function can then be used for mapping new examples.[59, 137] Optimally, the trained ANN is capable of correctly classifying new unseen instances. Tasks that fall within the paradigm of supervised learning are pattern recognition (classification) and regression (function approximation).

A commonly used supervised learning algorithm is error backpropagation. This algorithm attempts to minimize the average mean-square error between the networks output  $f(x)$  and desired target output  $y$  over all example pairs, using gradient descent.[59, 137] The ANN is tuned, through training, by adjusting the weights between nodes as to minimize the error. This is done by propagating from the output of the network backwards, and adjusting the weights according to how much they contribute to the error. While backpropagation has been a successful algorithm in standard ANNs, there is little evidence of its biological plausibility. In the brain learning occurs locally in each synapse; there is only available local information, not any backpropagated measure of global error.[56]

### 4.3.3 Reinforcement

While supervised learning is a problem initially posed and solved in computer science, reinforcement learning (RL) is inspired by animal behaviour. RL differs from supervised learning in that no example input/output pairs are given, nor are sub-optimal actions explicitly corrected. This is typically due to incomplete information (about the environment). Instead, pairs are generated, on-line, by an agent's interactions with the environment. At each point in time  $t$ , the agent performs an action  $y_t$  and the environment generates an observation  $x_t$  and an instantaneous cost  $c_t$ , according to some (usually unknown) dynamics. The aim is to discover a policy for selecting actions that minimizes some measure of a long-term cost, or by maximizing some accumulative reward. This also involves finding a balance between exploration (of uncharted territory) and exploitation (of current knowledge), in order to not get stuck in a local maxima. An example application would be in a AI agent simulation where the agent, operating in some environment, has objectives to complete; e.g. a world full of food and poison where the agents objective is to eat food and steer away from poison.[148, 59, 137]

#### In 2nd generation ANNs

While most RL algorithms are table based; updating an action-state table such as in Q-learning[137]. ANNs are also frequently used in place of this table because the action-value table can become very large.

One method, is to train ANNs though some sort of value function in conjunction with error backpropagation.[137, 59] Another interesting method, that has become increasingly popular, is training the ANN using a genetic algorithm; where the synaptic weights evolve threw genetic evolution. In this method, individuals/agents compete to procreate and pass on there genes to the next generations. Agents are given 'fitness scores' based on there performance and ability to solve tasks in their environment. Efficient agents are more

likely to procreate. Good 'genes' (in ANNs, the genes represent synaptic weights) are then passed to improved child generations. Mutation of genes are also involved to increase the search space. Through evolution, individuals become more and more efficient at solving the task at hand for each generation.[137, 59]

### In spiking neural networks; reward-modulated STDP

Genetic algorithms may also be applied to spiking neural networks (SNNs). Of more biologically inspired methods though, the majority of SNN models use a reward-modulated STDP rule to reinforce more strongly those neurons active in a certain desirable state.[4, 17]

The reward-modulated STDP rule, proposed by Izhikevich[79], sets out to solve the distal reward problem (chapter 3.6.5). This is achieved through there being left a trace whenever a synapse is updated according to the STDP rule (chapter 4.1.3). Using this STDP trace, combined with the release of dopamine in the presence of reward, allows the recently active synapses (leading to the reward) to be further strengthened or weakened depending on their contribution to the reward. This STDP trace, which we call the *eligibility trace*, models the theorized activity of some enzyme important for neuroplasticity.[4, 17]

Izhikevich[79] uses the following model of the extracellular dopamine concentration  $d$  in the network:

$$\dot{d} = -d/\tau_d + DA(t) \quad (4.9)$$

$\tau_d$  is a time constant of dopamine uptake, reducing the total amount of available dopamine.  $DA(t)$  models the production of dopamine by dopaminergic neurons in the midbrain, specifically in the areas VTA and SNc.

The eligibility trace model[79]:

$$\dot{c} = -c/\tau_c + STDP(\tau)\delta(t - t_{pre/post}) \quad (4.10)$$

$c$  is the STDP eligibility trace, which decays to  $c = 0$  in an exponential manner, and  $\tau_c$  is the variable manipulating the rate of decay. The effect of changing  $\tau_c$  is thus to increase or decrease the sensitivity of plasticity to delayed rewards. A typical value for  $\tau_c = 1s$  means that the synaptic plasticity will be negligible about 5 seconds after the STDP event.  $\delta(t)$  is the Dirac delta function which ensures that the value of  $c$  only updates if one of the neurons fired during the current simulation time step.[4]

Finally, we have the *update rule*, which updates the synaptic strength[79]:

$$\dot{s} = cd \quad (4.11)$$

$d$  and  $c$  are the dopamine concentration and eligibility trace, respectively. Thus, the update rule is dependant on the amount of dopamine available, and the time since last STDP update in a given synapse.



# Kapittel 5

## Embodying the neuronal culture

Why bother to create the hardware and software necessary to enable a network of neurons to interact with the real world? Because neural systems evolved to control a body and thereby interact with the world[41]. Nervous systems evolved to aid the survival of motile organisms, by directing their interactions with their environment.[128] In animals, neural output is expressed continuously, while being modulated by a continuous stream of sensory input. This tight sensory-motor loop is likely to be important for learning and functional behavior.[128]

In the attempt to study in-vitro networks closer to their natural environment, several studies have conducted research with embodied in-vitro neuronal cultures:

- Reger et al.[130] connected a lamprey brain bi-directionally to a mobile robot. The robot was presented light stimuli and the brain reacted to this stimuli by telling the robot to follow or escape the light source.
- DeMarse et al.[51] interfaced a neuronal network cultured on a micro-electrode array to a computer emulated animal, coined an 'Animat', moving inside a virtual world.
- DeMarse et al.[50] used a neural culture as a flight controller, controlling a planes pitch and roll.
- Potter et al.[75] used neural cells to control a mobile robot which they coined a 'Hybrot'.
- Bakkum et al.[8] used neural cells to control their robotic drawing arm, MEART.
- Warwick et al.[162] used neural cells to control a small sized mobile robot.

Others have been successful in sending control commands to the nervous system of live animals, such as cockroaches[72] or rats[151] as if they were robots. These studies can

inform us about information processing and encoding in the brains of living animals[39], but do however raise some ethical questions.

Following these approaches, with the aim of establishing general interfacing techniques and computational methods, we shall interface a robot with a population of neurons cultured on a micro-electrode array (MEA). In this chapter, we approach embodiment by looking at the hardware and infrastructure necessary for developing a closed-loop MEA-robot system. We shall also look at some artificial bodies which may be candidates for reembodiment our neuronal cultures; Animats, Hybrots and The NTNU Cyborg.

## 5.1 Hardware

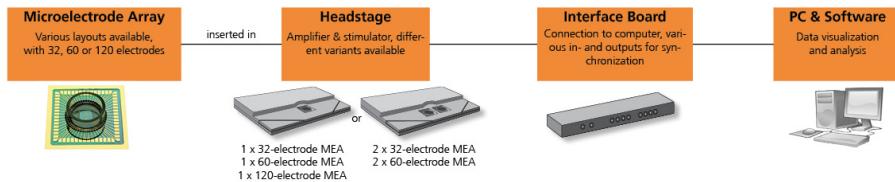
In order to do anything useful with the neural culture, we need to be able to interface it. For this, we need a variety of specialized hardware. First, we need an electrode petri dish to grow the neural network on, such as a microelectrode array (MEA). Second, we need a recording system sensitive to the cultures low intracellular voltage spikes and tolerant to electrical interference. Third, we need a stimulation device in the form of a spike generator that can induce appropriate voltages into the neural culture without damaging the neurons. Finally, computers must be set up to run the analyzing software, send stimulation signals to the culture, to interface with the rest of the hardware, to distribute the MEA-robot system, and to log all data. In addition to this, but not exactly part of the interfacing system, the delicate culture also requires a special incubator regulating temperature, humidity and gas concentrations (oxygen, nitrogen, CO<sub>2</sub>) to mimic the environment in which the neurons normally operate: the animal body.

*As of March 2016, the Department of Computer and Information Science (IDI) at NTNU was approved the funds for purchasing the MEA2100-60-system from Multi Channel Systems[166]. Multi Channel Systems provide a relatively niche product that is used in many in-vitro neuroscience projects similar to the one of The NTNU Cyborg[75, 51, 9, 164, 163, 162]. As of June 2016, the MEA2100-system was ordered and to be shipped June 16.*

### 5.1.1 MEA2100-60-system

The MEA2100-System[168] enables the possibility to record from neuronal or cardiac cultures, stem cells, or brain or cardiac slices. The system is a compact solution with integrated data acquisition for recording from 60 MEA electrodes (upgradeable to 120 electrodes according to MultiChannel Systems[168]) and 8 additional analog channels. It has an integrated filter amplifier and 3-channel current or voltage stimulus generator, as well being complete with headstage, MCS-interface board 3.0 multiboot, 5 MEAs, data acquisition and analysis software, 1-channel temperature controller, power supply, and accessories.[168]

In the following, the hardware components that make up the MEA2100-60-system are



**Figur 5.1:** Overview of the MEA2100-system from MultiChannel Systems[168]

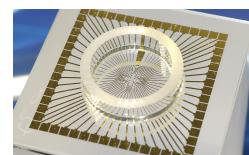
summarized. For the more detailed specification of the MEA2100-60-system, the reader is referred to appendix A.

### Micro-electrode array

Microelectrode arrays (MEAs) are specialized tissue culture dishes, in which living neurons can be grown over multiple bidirectional electrodes for stimulation and recording. These electrodes permit the investigator to measure the activity of the small living neural network as well as manipulate that activity to study how information is processed, encoded, and translated into the network's outputs. Each electrode can detect the extracellular activity (action potentials) of several nearby neurons and can stimulate activity by passing a voltage or current through the electrode and across nearby cell membranes. These extracellular electrodes are not harmful to the cells, and thus allow continuous recording and stimulation for as long as the culture is maintained.[49] MEAs allow relatively high resolution, long term, and continuous studies on the role of embodiment throughout the life of a cultured neural network.[9] NTNU Cyborg has purchased 5 MEAs (60MEA200/30iR-Ti-gr).

#### A note on MEA resolution:

The 60 electrodes is far from single cell resolution when we consider the thousands of cells in a culture (though through spike sorting (chapter 6.2) we achieve higher resolution). Ideally, we would have single cell resolution or close too. Higher resolution enables for a more detailed spatio-temporal network analysis. The MEA2100 is upgradable to 120 electrodes resolution, doubling that of the standard 60. However, there is research being conducted greatly increasing these numbers. Ballini et al.[10] are, for example, experimenting with a 1024-channel CMOS MEA with 26,400 electrodes.



**Figur 5.2:** The MEA from MultiChannel Systems[168]

### Headstage with stimulator

The headstage (MEA2100-HS60) is the core element of the system. It houses and heats the MEA, amplifies and digitizes the signals and has an integrated stimulus generator. The

lid of the headstage can be opened easily and the MEA is placed in the headstage from the top. By closing the lid, the contact pads surrounding the MEA connect to the contact pins. The built-in amplifier makes sure that the recorded signals are amplified close to the signal source, thereby minimizing noise. The data is then sampled at 50 kHz/channel on all channels simultaneously, ensuring excellent data quality.[168]

### Interface board

The interface board (MCS-IFB 3.0 Multiboot) (figure 5.3) receives data from the headstage via a High Speed eSATA cable. In the interface board, there is a programmable digital signal processor, which can be used for real-time signal detection and feedback. The board is also equipped with various analog and digital in- and outputs for synchronization with other instruments. The interface board connects to the computer via USB.[168]



**Figur 5.3:** The interface board[168]

### Real-time signal detection and feedback:

Real-time signal detection and feedback is integrated into the Digital Signal Processor (DSP) within the interface board. This enables fast and predictable reactions related to recorded analog signals with minimum time delay (1ms as opposed to over 100ms if a PC is to perform the same job). The real-time detection loop is illustrated in figure 5.4.[168]

### TC01 temperature controller

In the MEA2100-headstage, there is a heating element right beneath the MEA. It is controlled by the included temperature controller, so the temperature of the sample is observed and kept stable at all times. The desired temperature may be selected on the device itself or using the included control software TCx-Control.[168]

### Stimulation generator

The stimulus generator, integrated in the headstage, offers 3 different stimulation patterns: monophasic, biphasic and bursts. One can choose between current and voltage stimulation and also select the electrode. All configurations (stimulation patterns, output, and electrodes) are defined via the included MC Rack data acquisition software.[168]

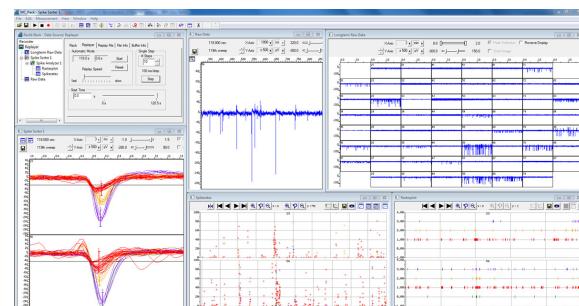


**Figur 5.4:** Real-time spike detection within the interface board.[168]

## Software

The MEA2100-system also includes the software package **MC Rack**. The software allows combining virtual instruments, such as oscilloscope, filter, event detector, spike sorter, sound output, or signal-triggered TTL pulse, with the rest of the hardware. MC Rack controls the stimulation patterns and when to stimulate the culture. The program can also be used for offline analysis of data, which can be further exported and analyzed with Neuroexplorer, Matlab or a custom program.

During the writing of this thesis, MC Rack was downloaded and tested from MultiChannel Systems website[167], as to get a feel of the software though their available demos. For further information on the software, the reader may be interested in the MC Rack manual[108], or the 'MEA Application Note: Neuronal Cell Culture – Cultivation, Recording and Data Analysis'.[150]



**Figur 5.5:** MC Rack software from MultiChannel Systems[168]

In addition to MultiChannel Systems MC Rack, it is worth mentioning the **MEA Bench**[88]

software, as it has shown up in many in-vitro neural culture related papers[163, 160, 75, 8, 128]. MEABench is a set of interacting Linux programs for recording data from MEAs and for real time processing. The software can be used to acquire and visualize data from MultiChannel Systems MEA hardware in real time. The software offers on-line spike detection, as well as suppression of artifacts[88].

### 5.1.2 Optogenetics

While MEAs are great for both the analyzing and stimulation of a neuronal culture, the resolution (60 electrodes vs thousands of neurons) is limited. Also, the recording artifacts which stem from electrical stimulation are a problem. There is however, a very promising technology that seems to solve these issues; optogenetics. Optogenetics is a biological technique which involves the use of light to control cells in living tissue, typically neurons, that have been genetically modified to express light-sensitive ion channels. It is a neuromodulation method employed in neuroscience that uses a combination of techniques from optics and genetics to control and monitor the activities of individual neurons in real-time.[48]

Welkenhuysen et al.[169], developed a silicon-based multi-electrode-optrode array (MEA-OA) for in vitro optogenetics, enabling the best of both worlds. They demonstrated that their device allows for artifact-free electrical recording, as well as reliably eliciting spiking activity in neurons. They achieved an astonishing single cell resolution stimulation capability, enabling them to determine the full spatial and temporal activation patterns and spike latencies of the neuronal network. These results are very impressive and demonstrate the improvement through this technology.

We won't dive further into the full working of this technology here, as it is not something we have plans to implement in the NTNU Cyborg project quite yet. It is however, included in the discussion as a promising and exciting method we may want to look into in the future. The ability to increase the recording resolution to the cell level would give us a much greater insight to the network behaviour. Also, enabling direct neuron stimulation, versus the one by the extracellular multi-cell stimulation performed in MEAs, enables much more sensory information to be encoded into the network.

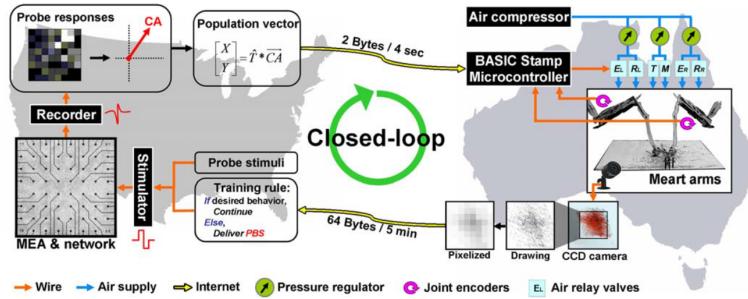
## 5.2 Infrastructure

Now that we've covered the necessary hardware, we can begin to design the infrastructure for our embodied system. Using the recording and stimulation hardware described in the previous section, we wish to build a real-time, closed-loop structure. A key part of this feedback loop, is a system for reembodiment of the in-vitro network through some simulation or robotic platform, as will be discussed in chapter 5.3. Recordings from the neural network can be considered as motor outputs to a robotic system, which act to change the state of the environment in which it operates. Stimulation on the other hand, can be considered sensory input from the robotic environment to the neuronal culture. In this section,

we first let ourselves inspire by similar projects and look at how they organized their experiments. Then, we propose an architectural distributed solution for our own project, The NTNU Cyborg.

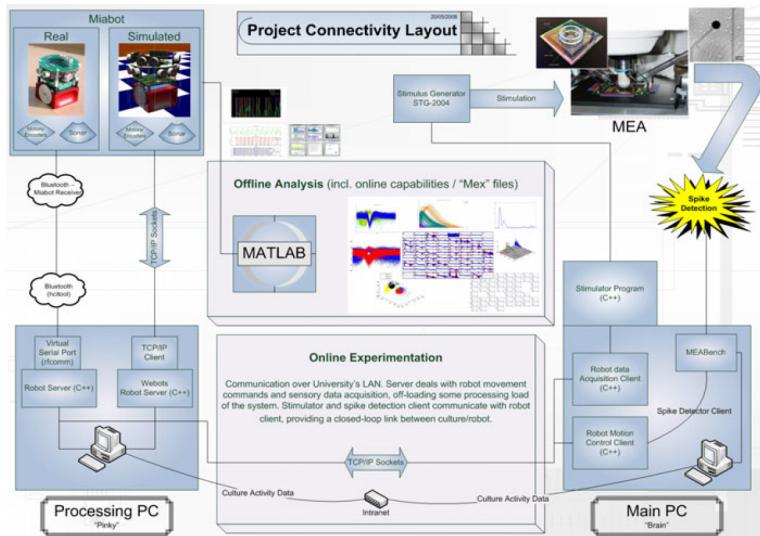
### 5.2.1 Setups from similar experiments

Bakkum et al.[8] used a distributed bi-directional neural interface for controlling a robotic drawing arm as shown in figure 5.6.



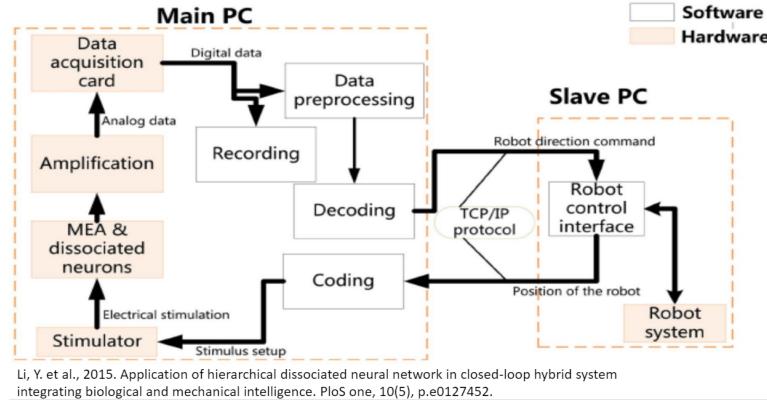
**Figure 5.6:** Closed-loop system for the MEART drawing robot by Bakkum et al.[8]

Warwick et al.[162] also used a distributed setup where computers perform dedicated tasks and communicate via TCP/IP protocols between them. Their setup is depicted in figure 5.7.



**Figure 5.7:** Closed-loop setup for controlling a miniature robot by Warwick et al.[162]

As a last example, introduced by the Department of Neuroscience (INM), NTNU during our NTNU Cyborg workshop (05.02.2016), the closed loop setup from Li et al.[92] is shown in figure 5.8.



**Figur 5.8:** Closed-loop infrastructure by Li et al.[92]

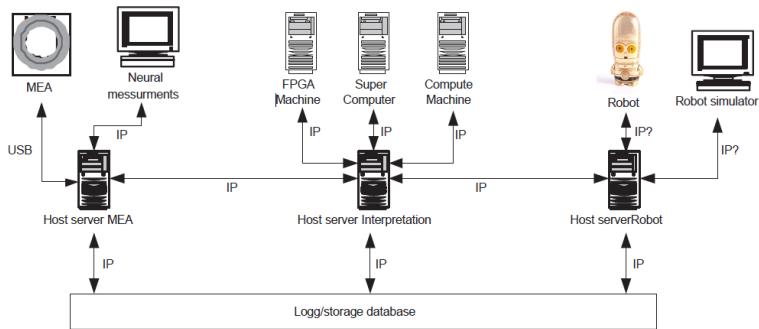
All these setups demonstrate the closed-loop MEA-robot organization of embodied systems, and serve as good inspiration for our own project. We see that there are similarities between different projects, and that there seems to be a pretty standard way of setting up the distributed system infrastructure using specific task computers at each node.

## 5.2.2 Distributing The NTNU Cyborg between 3 institutes

With inspiration from the projects above, we are now ready to design our own setup. The first difference we are to make, compared to the above, is that we will be distributed our system across the three main institutes involved in the project: The Department of Neuroscience (INM), the Department of Computer and Information Science (IDI), and the Department of Engineering Cybernetics (ITK). This calls for some sort of coordinating module, to enable multi-client access to data logs, as well as enable MEA experimentation from external locations.

Figure 5.9 drawn by Tufte and Nichele[112] as discussed with the core NTNU Cyborg team (there along the author of this thesis), drafts a possible setup. The proposal is divided into three main nodes that are placed individually at each of the three main institutes. In this way, all have responsibilities and ownership in the common platform, and also enables all partners to use the infrastructure individually. An IP protocol would serve as communication between nodes. As such, all communication is done over the Internet. The IP protocol also allows for new nodes to easily be incorporated in the future. Here follows a short description of each node:

**Node 1**, placed at the Department of Neuroscience (INM), serves as the host server which connects the MEA2100-60-System[168] to the rest of the distributed system. The MEA



**Figure 5.9:** Proposed infrastructure for the NTNU Cyborg by project members Gunnar Tufte and Stefano Nichele[112]

can be accessed by standard (MEA) software[167] locally, or through the IP protocol externally. This host server will make MEA recordings and stimulation requests available to clients.

**Node 2**, placed at the Department of Computer and Information Science (IDI), serves as the interpreter of the RAW MEA data, which it receives from the Node 1 server. The data can then be used by any interpretation software (artificial neural networks, artificial development, generative system, AI learning, self-organization etc) on any platform. In figure 5.9, an FPGA, super computer or any desktop computer is shown as possible devices handling the RAW data. Node 2 will thus receive the RAW data, process it and forward the result via TCP/IP to Node 3.

**Node 3**, placed at the Department of Engineering Cybernetics (ITK), serves as the node which interacts with the robotic platform. Node 3 receives the processed MEA data from node 2 and uses this data to further control a real world robot (such as the NTNU Cyborg platform) or simulation. The sensor data from the robot is sent back to Node 2, where Node 2 processes and sends this data back as stimulation requests to the MEA driver at Node 1.

**Logging:** While the system is up and running, it is suggested that all data from all nodes should be logged and stored in a database. The database can then be used for offline experiments and gives us possibility to mine and analyze this data. Mining the data could give us insight into unseen network properties.

### 5.2.3 The two-servers setup

Building upon the setup above, we offer a slight modification to enable a little more flexibility from the point of view of ITK and INM. The system mostly relies on two main servers: one for communication with the MEA (at INM), and one for communication with the robotic platform (at ITK). Processing data at IDI could be achieved by using a client instead of a server. This does not make the role of processing data by IDI any less im-

portant, but may make for a more flexible design as it enables INM and ITK to conduct experiments directly with the MEA server. This is beneficial for testing purposes. In addition, one would be able to conduct experiments with either MEA or robot separately, e.g. INM will not likely be interested in using the robot platform in their studies. The parts of this two-server setup may be described as such:

### **MEA server: Recording from and stimulating to the MEA**

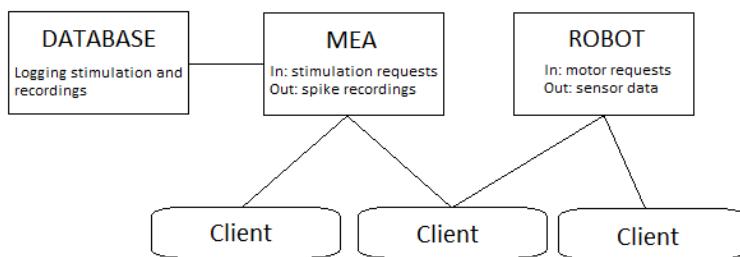
The MEA server represents node 1, described in the above section. It will naturally be located at INM, as it will connect to the MEA hardware. This server will handle spike detection and spike sorting locally, and may share recorded spike data to clients. Clients may set voltage and current stimulation parameters through the server, as well as which electrodes to stimulate and how. It may also be natural to include the logging database as part of the setup at INM.

### **Robot server: Robot motor control and sensory data**

The robot server represents node 3 in [112]. It will communicate with the robot platform. The role of the server is to send sensory data from the robot to clients, and receive motor commands.

### **Clients: Processing data and requesting operations**

A client can be located anywhere. The client requests the RAW data from the MEA server and processes this data using some processing algorithm (see chapter 6.2). The result of this processing may then be used as motor function requests to the robot server. Back from the robot server, the client receives sensory data. The sensory data is converted to some stimulation sequence by the client, which it passes on to the MEA server. The MEA server in turn, uses these instructions to stimulate the neural culture with spikes.

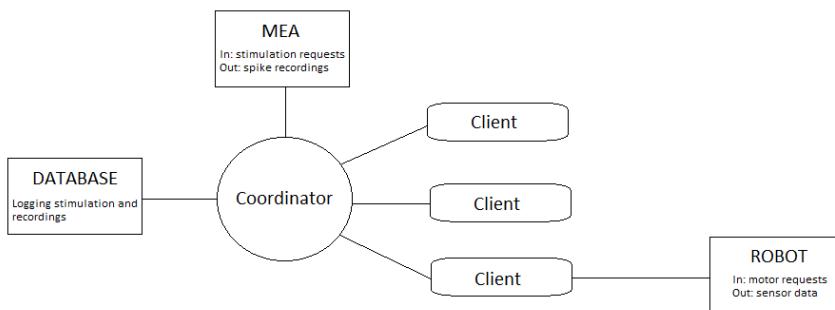


**Figur 5.10:** The proposed two-server setup

### 5.2.4 MQTT coordinator

As a final (and as of June 2016 most recent) proposal, IDI (Tufte and Nichelle) has suggested a setup using a global coordinator. This coordinator is to be developed and maintained at IDI along with the logging database. The argument for this setup, is to enable an efficient messaging system between nodes and to better coordinate activities. Also, as it is primarily IDI that will be maintaining servers and the database, it is natural that these are located at their department. This solution is not very different from the two-servers setup. Here, the coordinator at IDI is simply an extension of the MEA server, and should allow great flexibility and cooperation.

The idea is to implement a MQTT-based coordinator: MQTT (MQ Telemetry Transport) is a lightweight messaging protocol originally developed for small sensors and mobile devices. It is a simple and lightweight publish/subscribe messaging protocol, designed for constrained devices and low-bandwidth, high-latency or unreliable networks[107]. Figure 5.11 shows an idea of the design for this setup. The coordinator node would make logged data available to clients and also allow clients communicate with the MEA.



**Figur 5.11:** Architecture with coordinator node.

### 5.2.5 Challenges using a distributed setup

One challenge with all these configurations is to enable full functionality through the servers and coordinators. The MEA2100-60-System includes the MC Rack software which might only be configured to be used locally. If this is the case, we will either lose a lot of functionality using the system through a server-client setup, or we will have a big job in front of us making an API that enables full functionality externally. Either way, this is a challenge we must solve as only using the MEA locally is not an option if we are to use it to control a The NTNU Cyborg robot platform.

Another issue with all these setups, which we unfortunately can't do anything about, is the fact that the culture cannot, according to INM, be out of its incubation chamber for more than four hours at a time. The headstage is not fitted within the incubation chamber and therefore, the MEA must be placed on the headstage each time we want to conduct

experiments. This means IDI and ITK Have to call INM and have them place the MEA on the headstage every time there is an experiment to be conducted. This is a bit impractical, but solvable through a phone call. There are options for fitting a headstage within an incubation chamber, but this option is beyond our budget for now.

## 5.3 Embodiment: The robotic body

There is a necessity for interaction with the environment[41], something that cultured neurons are virtually incapable of without sensory systems. Therefore, when the hardware is up and running, we want to embody our neuronal culture. In the development of a Cyborg, it will be natural to test our system and training algorithms in different environments before connecting the cultured network directly to the NTNU Cyborg robot platform. These testing environments should allow for gradually increasing complexity, as to make the culture work in simpler settings first, before going on to the more difficult tasks. Here, several environments are proposed, from virtual environments to real world robots. The suggestions are listed in increasing order of complexity. The culture should pass certain tests in the lesser complex environments before moving on to the more complex.

### 5.3.1 Animat

Though their research with bio-robotic systems, DeMarse et al.[51] developed a virtual creature as the body for their CNN which they coined 'Animat'. An Animat operates in a virtual environment. The Animats 'brain' however, is biological and real in the form of a neuronal culture. The culture can be given senses through electrode stimulation and motor functions through reading the culture recordings, which operate the Animats actuators in the virtual environment.

A virtual creature, or simulation, provides an easy and safe setup for testing the distributed bio-robotic system. It also allows much more determinism than operation in the real world. Experiments may include tasks such as wall and obstacle avoidance[163], following of other virtual creatures, locating food etc.

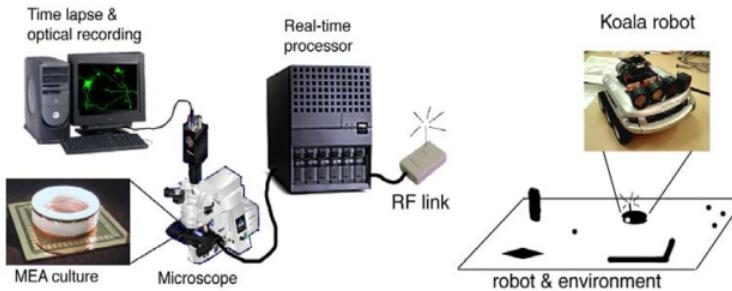
### 5.3.2 Hybrot

As a step up from the Animat, we may start to experiment with a real-world small-scale robot. Such a hybrid-robot organism has been termed a 'Hybrot'[9]. A hybrot is a robot given biological properties, making it part machine part biology. A Hybrot is kind of the opposite of what we traditionally call a cyborg; while a cyborg is usually referred to as something biological becoming more machine (like a human with bionic arms), a Hybrot is a machine becoming more biological (like a robot interfacing with a neuronal culture). Using this definition, The NTNU Cyborg may actually be better called The NTNU Hybrot. However, people tend to be more familiar with the term Cyborg (through movies such as Terminator) rather than the term Hybrot.

Utilizing real sensors and motor actuators, the testing environment is more complex than that of the Animat, and a step closer to the NTNU Cyborg platform. The benefit of using a small scale robot is the safer and more controlled environment than that of a larger system. It is also a fairly cheap solution. Even when the NTNU Cyborg bio-robotic system is up and running, it will probably still be more convenient to perform scientific experiments with a smaller robot.

There are several providers of such small-scale mobile robots. Bakkum et al.[9], used a Koala robot[132] from K-Team Mobile Robotics[133] in their studies. The koala has several sensors and is mobile by wheels. K-Team also provides a slightly smaller Khepera robot[131], which also is a good alternative. In general K-Team delivers several good solutions which we might consider purchasing as test vehicles.

As with the Animat, the same experimental tasks apply for the Hybrot.



**Figure 5.12:** Hybrot setup by [9], using a Koala[133] small-scale mobile robot.

### 5.3.3 The NTNU Cyborg

The biological control of the NTNU Cyborg robot platform is our main goal. Although exactly what the neuronal culture will control is yet to be decided. One option, following the theme of Animats and Hybrots above, is to have the culture control the robots mobile base: the Pioneer LX[105] by MobileRobots[103]. However, in this large system, highly dependent on safe and accurate operation within its environment (the campus hallways), it may be hazardous to have a neuronal culture take full control. In addition, the base includes the necessary navigational abilities for autonomous operation. For these reasons, we may consider other options when it comes to the biological control of the NTNU Cyborg robot. Some ideas are having the culture control the cyborgs mood, likes and dislikes, troll face or arms.

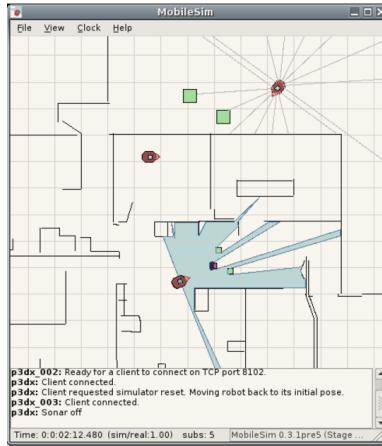
#### Sensory feedback to the neuronal culture

The Pioneer LX includes laser, sonar and bumper sensors and the Cyborg also has a Kinect mounted with included microphone. All this sensory information may be converted and

mapped as sensory input stimulation into the neuronal culture. The data will naturally need to be compressed into a much simpler form. One possibility with the Kinect sensor, is to utilize its ability to recognize gestures, and feedback these gestures to the culture. This way, the culture may be able to respond to a smile or an angry face in an interactive manner.

### MobileSim simulator

MobileSim[104] is a simulator software for the Pioneer LX navigational base, and may be used as a sort of advanced Animat. It may also serve as a testing base for controlling the Pioneer LX, if we choose to have the neuronal culture operate this. MobileSim simulates all sensors and motors as those of the real Pioneer LX.



**Figur 5.13:** The NTNU Cyborg as of May 2016. The base is the Pioneer LX[105] from MobileRobots[103]

**Figur 5.14:** Screenshot of the Pioneer LX simulator: MobileSim[104]

# Communicating with the neuronal culture

Once the necessary hardware and software is set up, and the neural network is cultured on the MEA, it is time to start talking to our newborn organism and listen to its first words. There is a challenge though, this newborn of ours doesn't speak any language we are familiar with in our day to day lives. This newborn only understands neural code, a language we have yet to fully understand. Understanding this language is certainly extremely important in establishing better bi-directional interactions between the brain and external devices. In addition, for neurological disorders, establishing improved knowledge about the fundamental basis of the inherent neuronal activity is critical.[163]

Using our knowledge of neural coding from chapter 3.5, we attempt to communicate with our culture through electrical stimulation. We shall also try to understand the answers we get, through analyzing the culture recordings. We shall further look at how we may go about using stimulation and recording for closing the loop in our closed-loop system.

## 6.1 Stimulating the neural network

The stimulation of an embodied neuronal culture is essentially 'sensory' information from the robotic environment. Stimulation is important as it is the only information the culture receives from the outside world. Speaking in terms of embodied networks, the input side of MEA technology is not as technically well-developed as the output or 'motor' side. But it is equally important in our closed-loop paradigm.[128] There are two main aspects to consider when it comes to stimulation: the stimulation parameters used (voltage, current, frequency etc.) and the coding mechanism (rate-based, temporal, population coding etc.)

We need to understand which of these speak the neural language most efficiently, and also take care not to harm the culture by incorrect stimulation.

### 6.1.1 Stimulation parameters

There are several stimulation parameters to consider:

- Frequency: the stimulation spike frequency used, i.e. the amount of spikes over some time interval.
- Voltage amplitude: the pulse voltage used.
- Pulse current: the stimulation current (one can choose between voltage or current stimulation)
- Pulse duration: the duration of the input voltage pulse.
- Pulse type: monopolar, bipolar, burst
- Interstimulus interval (ISI): the time between successive inputs.
- Duration of the whole stimuli: how long the successive stimulation is continued.

A general observation is that cells respond differently to different stimulation protocols[57, 159]. For voltage controlled stimulation paradigms, for example, parameters such as the amplitude, polarity, waveform and duration of the voltage pulse(s) affect the number of cells responding to the stimulation and the possible generation of bursts.[106] Care must be taken to use voltages and/or current densities that do not harm the surrounding cells or damage the electrodes themselves, especially when very long-term stimulation is envisioned[106]

#### Voltage vs current stimulation

Researchers traditionally preferred current controlled stimuli due to simpler calculation of the electric field and potentials in the medium surrounding the electrode resulting from stimulation. These are directly proportional to the current passing through an electrode.[25, 128]

Potter et al.[128] however, argue that there are significant advantages to using voltage controlled stimulation: voltage control avoids electrochemical reactions. Current controlled stimuli can easily exceed voltages that can damage electrodes and harm neurons (such as voltages exceeding one volt). They further argue that; the key advantage of current control, the ability to calculate the electric field and potential, is compromised in MEAs due to leakage currents through the insulation layer. This, they claim, may reduce the current passing through the electrode by as much as 30% (depending on the insulation). Due to these arguments, they use voltage-controlled positive-first biphasic pulses of less than 1 volt. We will see in the next section that most studies have adopted this voltage-controlled paradigm.

### 6.1.2 Single pulses and their network response

Single electrode stimulation pulses can consist of several parameters. We wish to know how to set these parameters as to obtain responsive and non-harming stimulation. We have here investigated the stimulation procedures of several studies employing MEAs:

DeMarse et al.[50], in their study using a neural culture to set the proportionate weights of an autopilot, used  $200\ \mu s/600\text{ mV}$  bipolar pulses. In another study by DeMarse et al.[51], where they controlled an Animat, they used  $200\ \mu s / \pm 400\text{ mV}$  pulses. Pizzi et al.[122] used pulses composed by 10% negative voltage (-35mV), 90% positive voltage (35mV). In order to experiment the cells reactivity to electrical stimulation, they stimulated the cells with frequency bursts varying from 40 to 800 Hz. Warwick et al.[162, 163] used an electrical stimulus consisting of a  $\pm 400\ \mu s/600\text{ mV}$  bipolar pulses that were delivered at varying interstimulus intervals (ISI). Bakkum et al.[8] used  $400\ \mu s/500\text{ mV}$  bipolar pulses and Dockendorf et al.[55] used bipolar  $200\ \mu s/500\text{ mV}$  pulses. Wegenaar et al.[159] found that the pulse amplitude is the main determinant of stimulus efficacy. Potter et al.[128] found that positive-first bipolar voltage-controlled pulses were the most effective stimuli in their repertoire. They further found that the width (time) of voltage pulses are less important. They concluded: the voltage effects the number of cells directly stimulated, and this number grows linearly with the amplitude of that pulse. The width of the pulse, however, only needs to be wide enough to allow the cell membrane and all the parasitic capacitances in the system time to charge. Potter et al. further found that there was no benefit in stimulation times above  $400\ \mu s$ .

Looking at the network response of these stimulation pulses, it was found by DeMarse et al.[50, 49] that there was an increase in bursts for approximately 100 to 200 ms following the pulse. They also showed that high-frequency stimulation of pulses reduced the number of APs following stimulation, while low-frequency increased the number of APs. Similarly Dockendorf et al.[55] concluded that stimulation of most channels consistently induced population bursts lasting longer than 100ms. Shahaf et al.[143] also registered 'a rich repertoire of reverberating electrical activities, lasting 100 ms or more'. Warwick et al.[163] stated that the typical behaviour of their cultures was generally a period of low-frequency activity prior to stimulus, followed by heightened network activity induced within few ms after stimulus. This activity decayed typically after 100 ms, to baseline pre-stimulus activity. Because of this network wide response to stimuli, most studies choose to wait 200 ms after stimulation before analyzing the responding recordings as to let the network settle.[50, 49, 55, 163] DeMarse et al.[49] stated that 'it is difficult to elicit responses from these networks within a few seconds due to an inherent refractory period following each burst'. This they said, created an upper limit on the rate at which information can be input or read out from the network, which is problematic during experiments requiring real-time control.

From these studies, it can be summarized that the common single stimulation protocol is to use bipolar pulses consisting of  $200/400\ \mu s$  width and  $500/600\text{ mV}$  amplitude. These pulses propagate through the network and evoke a responsive burst of activity for approximately 100 to 200 ms. This information is a great starting point for our own study. We will however, need to conduct our own tests verifying the effects of these parameters in our

neuronal cultures.

### 6.1.3 The effect of stimulation frequency

While the parameters used for a single pulse are important, the frequency effect of successive pulses is also very interesting:

The study “Adaptive Flight Control With Living Neuronal Networks on Microelectrode Arrays”[50], suggests (following the findings of Eytan et al.[57]) that one can modify the neuronal weights in a network using high (1/5 Hz) and low (1/50 Hz) frequency stimulation. They showed that high frequencies seem to reduce the number of evoked APs following a stimulation pulse, while low frequencies would gradually increase the number of evoked APs. Similarly, Dockendorf et al.[55] demonstrated that low frequency stimulation would induce bursts while high frequency would suppress these bursts. Several other studies also confirm these results, showing low-frequency stimuli can evoke bursting[98, 45], while high frequency through causes activity to fade after a few minutes[57]. Morin et al.[106] state that the exact mechanism behind this fade is unclear, but argues that it may be due to exhaustion of the resources of the network or to an unidentified mechanism of adaptation.

Several studies have also showed that stimulation frequency has effects on the synaptic weights within the culture. Jimbo et al.[81] used repetitive tetanic (high frequency) stimulation when training their cultured networks. They found that stimulating just one of the 60 electrodes with the tetanic pulse train, resulted in complex changes in the strength of the underlying connectivity in which both enhancement and depression of synaptic strength were observed.[30] Likewise, Van Steveren et al.[156] concluded that tetanic training experiments did significantly change the network response on some of their electrodes. They found this using 20 Hz tetanic stimulation and two different spike-train protocols: 10 trains of 11 pulses with an interpulse interval (IPI) of 50 ms (20 Hz) and an intertrain interval (ITI) of 5 seconds, and 20 trains of 10 pulses with an IPI of 50 ms and an ITI of 5 s. These findings, demonstrating potentiation with high-frequency training and depression with low, have been noted in several other studies[44, 163, 136] as well, and has also been confirmed by the neuroscientists at INM during Cyborg related discussions. Thus, we conclude that electrical stimulation can be an artificial source of neuronal plasticity.[8]

These effects of frequency based stimulation will be of great importance when training our neuronal culture, which is further discussed in chapter 7.2.

### 6.1.4 Some issues regarding stimulation

One problem with artificial activation of neural tissue is that a single electrode affects a number of cells that are not necessarily close to it.[45] The anatomy and tight circuitry of the network puts neuronal cell bodies and axonal fiber tracts in close proximity.[146] As a result, there is a loss of specification, and the responses obtained within the first ms

after stimulation thus might be attributed to the stimulation itself rather than to synaptic transmission through the network.[106]

Another issue is that recording is usually impossible for the duration of the stimulation, since the amplitude of the stimulus itself is usually at least one order of magnitude bigger than the neuronal signals.[106] This, along with the network wide bursting responses mentioned above, poses challenges for our real-time embodied system.

Potter et al.[128] argue that methods for stimulating a neuronal culture may be better served by communicating through chemical (i.e. neurotransmitters) stimulation rather than electrical. They argue that it would be more natural to communicate with the cells via the very chemicals that neurons use to communicate with each other. Some advances have been made in this direction. One approach is to include a microfluidic system into the MEA for the localized delivery of neurotransmitters or other neuroactive compounds[70]. Another approach is to apply neuroactive compounds via a micromanipulated puffer pipette[93]. Also, the use of optogenetics was discussed in chapter 5.1. For now though, we will make due with our electrical MEA.

## 6.2 Analyzing the culture recordings

Analyzing the neural activity is a very important aspect in The NTNU Cyborg project, as we want to use the output of the neuronal culture to control a robotic platform. One of the difficulties of studying neural computation within these dissociated cortical networks, is the lack of a sound model with which to investigate their computational properties.[49] Better understanding the neural output may additionally lead to huge scientific and medical impacts. In this chapter, we investigate different methods for analyzing the neural activity and how this output may be applied in an embodied environment. Some of these techniques are inspired by other similar studies involving embodiment of neural cultures, while the rest are suggestions of the author.

### 6.2.1 Increasing the MEA resolution using spike-sorting

When recording from neuronal cell cultures, the signals of interest are the electrical spikes recorded from the neurons when they activate. Spike-sorting is useful when using MEAs as it can increase the resolution of the neural culture recordings and thus tell us more about the network activity. Spike-sorting is the grouping of spikes into clusters based on the similarity of their shapes. Given that, in principle, each individual neuron tends to fire spikes of a particular shape, the resulting clusters correspond to the activity of different neurons. The end result of spike-sorting is the determination of which spike corresponds to which of these neurons[138]. This allows for the detection and separation of action potentials from different neurons, or groups of neurons, around an individual electrode. As a result, MEA recordings across the culture permit a picture of the global activity of the entire neuronal network to be formed.[91]. Thus, the activity of multiple neurons can be observed in parallel and network phenomena can be studied. As an example, if we say

that each electrode is sensitive to 5 nearby neurons, then we could theoretically increase the resolution from 60 electrodes to  $60 \times 5 = 300$ .

Basic spike sorting-capability is included in the MC Rack software[150] provided by MultiChannel Systems, described in chapter 5.1. MC Rack can detect spikes either by an individual threshold on each electrode, or by a combination of the slope and amplitude of the signal.

## 6.2.2 Utilizing the networks stimulation response

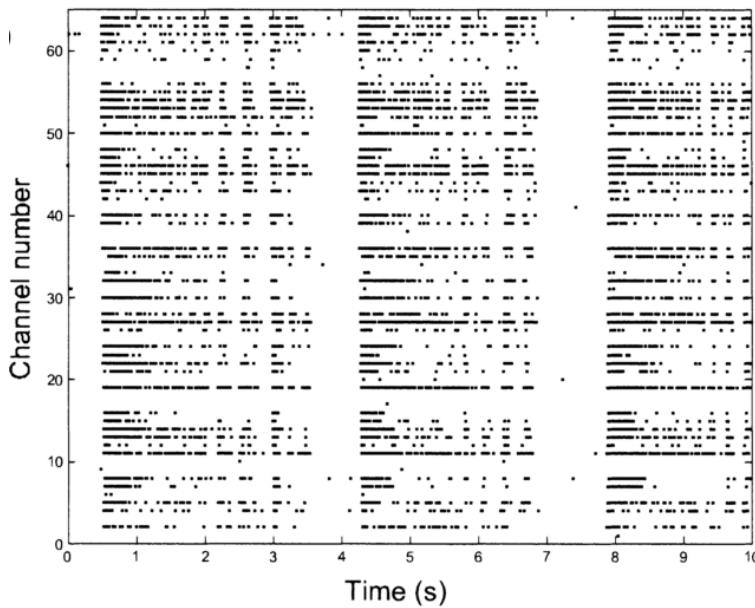
Because of the highly spontaneous and bursting properties of a neuronal culture (chapter 3.7.1 and 6.1.2), assigning robot instructions from the recordings is a challenge and very much up to the imagination of the experimenters. Studies involving embodiment have chosen to tackle this problem in various ways:

Demarse et al.[50] used the networks response to stimulation as an indication of the weights for a proportional controller. They used the average number of APs 150 ms following each stimulation, and calculated the difference between the current amount of APs and the initial amount. This difference became the proportionate weight of their flight controller. With high-frequency stimulation, they decreased the amount of responding APs from a stimuli, which caused a bigger difference between initial and current APs; hence increased proportionate control to the pitch and roll of the aircraft controller. Warwick et al.[162, 163] used the characteristic response curve they witnessed when stimulating two electrodes with varying time delay (termed inter-probe interval (IPI)). These response curves formed the basis for deciding the movement of their robot (forward, backward, left and right). DeMarse et al.[51] clustered the spike patterns observed across all the electrodes of the MEA, and assigned different robotic functions to different clusters. Bakkum et al.[8] used the Center of neural Activity (CA) (analogous to the center of mass), as a form of population coding, which they used as instructions for their Animat.

## 6.2.3 Spatial spike-pattern sorting

In many bio-robotic studies using neuronal cultures, the spatial spike-patterns occurring over the electrodes across the culture are used as motor inputs to a robot (e.g. [51, 8]). A spike-pattern is defined as all the spikes (APs) observed over the network during a short instant of time. As an AP is a very short-lived firing of the neuron, and as the neuron either fires or not, we may represent this pattern as a 1s and 0s matrix (60-bit in the case of our MEA). Figure 6.1 illustrates this type of spike-pattern map. Through pattern sorting, one can dedicate different spike-patterns, or clusters, to represent different motor controls in the robot[50]. We may categorize these spike-patterns by use of pattern recognition algorithms such as clustering or self-organizing maps.

From section 3.5, we learned about the different coding schemes. Pattern-sorting is a good example of using a population coding scheme, where patterns across the network population contain the information.



**Figur 6.1:** Raster plot showing the spike activity for each channel/electrode over time[106]

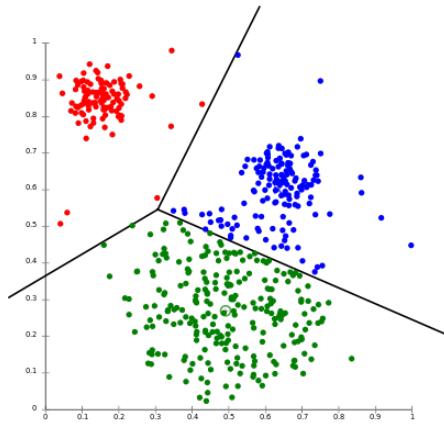
### Clustering

Data clustering is a popular method of extracting motor signals from the neuronal culture[51, 109, 123, 101]. Clustering is the process of classifying data elements by sorting similar elements close to each other (or within the same class) and dissimilar elements far from each other (or in different classes). Different measures of similarity may be used for sorting. Some examples include distance (nearest neighbor method), connectivity, and intensity. There are many types of clustering algorithms, some of the most widely used being k-means, mixture models, fuzzy clustering (where cluster borders are not as hardy defined) and hierarchical clustering. These algorithms are described well in literature[171] and will not be discussed further here.

For our neuronal culture, clustering can be used to group similar spatial spike-patterns. These clusters can then be correlated with different robotic instructions. This way, when the system is monitoring the culture output, it can associate the different firing patterns to different instructions using real-time cluster categorization. In most clustering algorithms, one can choose how many clusters one wishes to create. For a robotic system then, one could for example chose as many clusters as needed motor inputs.

In DeMarse et. al[51], a clustering algorithm was trained to recognize spatio-temporal patterns in the spike train, to use as motor commands for an Animat. They accomplished this using the following procedure:

1. Without stimulation, record from the MEA and register new patterns until no new

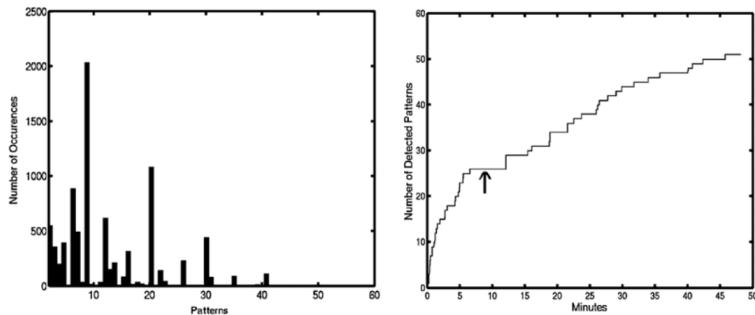


**Figur 6.2:** Example of K-means clustering. By Chire, Wikipedia Commons

patterns emerge

2. With stimulation, register any new occurring patterns until no new patterns emerge
3. Cluster the recorded patterns into N clusters using some clustering algorithm.

Figure 6.3 shows the result of this process.



**Figur 6.3:** Left: The frequency that different patterns would recur. Right: Number of novel patterns that the clustering algorithm detected as the session progressed (total 51). The arrow indicates the plateau reached when no stimulation was provided. When stimulation was provided, the number of patterns increased.[51]

In their study, activity on each channel was integrated and decayed following each spike,  $i$ , by:

$$A_n(t_i) = A_n(t_{i-1})e^{-\beta(t_i-t_{i-1})} + 1 \quad (6.1)$$

where  $n$  is the MEA channel number from 1 to 60,  $t_i$  is the time of the current spike,  $t_{i-1}$  is the time of the previous spike on that channel  $n$ ,  $\beta$  is a decay constant. This produced a vector,  $\mathbf{A}$ , representing the current spatial activity on the MEA. The activity vector was sent through a squashing function,  $P_n(t_i) = \tanh(\delta A_n(t_i))$  which limited the contribution of channels with extremely high spike rates. They clustered this activity by:

$$\begin{aligned} M_k &\leftarrow (\frac{N_k}{N_k+1})M_k + (\frac{P}{N_k+1}) \\ N_k &\leftarrow N_k + 1 \end{aligned} \quad (6.2)$$

where  $M_k$  are the clusters. A pattern was grouped to the nearest cluster using the Euclidean distance that had to be less than a threshold  $\Delta$ , where  $\Delta = 0.9$ .  $N_k$  is the number of occasions this pattern was matched, gradually freezing  $M_k$  as  $N_k$  became large. If  $P$  was not close to any cluster in  $M_k$ , a new cluster was constructed at position  $P$ .

In a talk by Emre Yaksi (Phd), Kavli Institute for Systems Neuroscience, named 'Sensory processing in zebrafish' at NTNU, it was shown through their studies, that neurons in networks like to stay within their clusters (fire with the same neurons) during both spontaneous activity and stimulation. This displays the deterministic behaviour one gets by utilizing clusters as robotic operations.

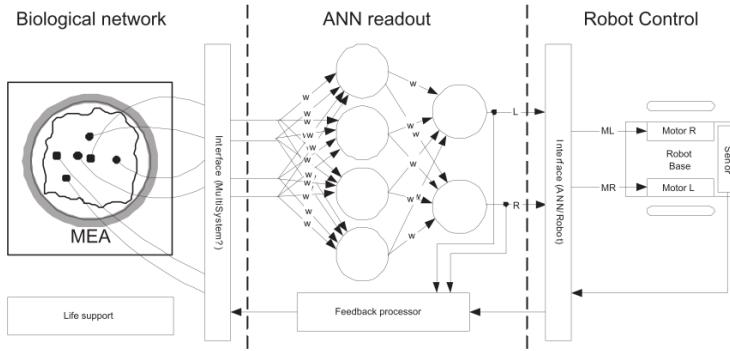
### Self organizing maps

Self-organizing maps (SOMs), a particular group of ANNs utilizing unsupervised learning, were introduced in chapter 4.2.3. The SOM is a sort of clustering algorithm with the advantage of providing continuous real-time clustering; the clusters are constantly adapting to the incoming data, unlike conventional clustering algorithms which have predefined clusters. As with clustering algorithms, SOMs can be configured with a predefined number of clusters.

#### 6.2.4 Liquid state machine

The liquid state machine (LSM) was introduced in chapter 4.2.2. Training a neuronal culture is challenging. One way for us to implement a culture in a robotic system is to not train the CNN directly, but instead utilize its intrinsic non-linear dynamics in a system we can train linearly. This may be done using a LSM (figure 6.4), as done in a similar approach where neurons in the primary visual cortex of cats were utilized[113]. Using a CNN as a liquid in such a system has been verified as possible[55]. DeMarse et al.[49] stated that a LSM is 'capable of producing stable outputs even though the liquid state is a high-dimensional continuously varying pulse train'. A LSM requires only training of the feed-forward weights to an artificial neural output layer.

The feed-forward network may be trained as any ANN; using backpropagation, genetic algorithms etc. The ANN will essentially be trained to classify the response from the biological network to control the robot according to the target task.[112] The output of this



**Figur 6.4:** Example of a liquid state machine in a closed-loop NTNU Cyborg system.[112]

network is forwarded to the motor controllers of the robot. An interesting alternative to the conventional rate-based ANN, is to use a spiking network as the output layer. This would allow for better incorporation of the time aspect; the sequence of spikes. After training the desired behaviour into our LSM, it will be interesting to observe any change in behaviour over time, indicating the adaptation of the CNN.

## 6.2.5 Prediction and anomaly detection using HTM

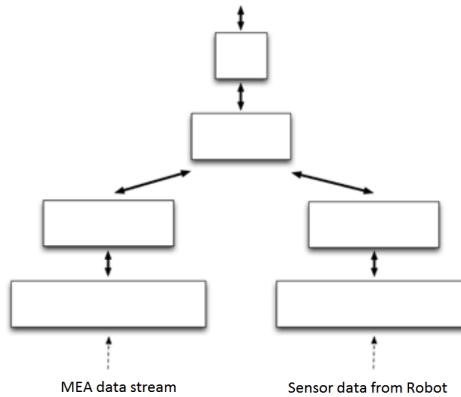
In chapter 4.2.4 we introduced Hierarchical temporal memory (HTM). We found that HTM is efficient at analyzing and decoding unlabeled high velocity time-based data streams, which is exactly what we get from the neural recordings of the CNN. Also, HTM continuously updates to the incoming data stream[69] which, with the constantly adapting neuronal culture, is a great feature. HTM may be useful in the following tasks: Pattern prediction; prediction of next spike pattern based on previous pattern sequences, and anomaly detection; detecting if the current spike-pattern deviates from the expectations.

Prediction may be useful for predicting the single or series of next firing sequences. A simple simulation of HTM's prediction capabilities may be viewed in this video: [58]. Prediction could, for example, enable us to take action (through necessary stimulation) if we see a network wide burst is about to occur. There is also scientific interest in finding out exactly how predictable a CNN really is, telling us something about the degree of spontaneity of the network. If the internal state of the CNN is indeed deterministic, one may ask what this means for networks in our own brain and whether our thoughts may be predicted.

Anomaly detection is sort of the opposite of prediction. It comes into play when the prediction fails to be true. Anomaly detection could alert us when something unexpected occurs in the culture, and will let us further analyze the cause of such an anomaly by going through the log records.

HTM also allows for the merging of senses, meaning that one could use one HTM network

with the spike-patterns recorded from the MEA as input, and another with the sensory data from the robot as input. Then one could merge the two into a single HTM network, which would allow prediction dependent not only on internal state, but also of the external sensory information. This is illustrated in figure 6.5.



**Figur 6.5:** Converging networks from different sensors. Modified from [69]

## 6.2.6 Mining the data

In chapter 5.2, we proposed an infrastructure where all stimulation to, and recordings from, the MEA are logged to a database. With all the data that will accumulate, it is interesting to investigate whether there exist some unseen dependencies within the neural activity. This is where mining comes into the picture.

The overall goal of the data mining process is to extract knowledge and patterns from large amounts of data[67], through methods such as principal component analysis (PCA) and multivariate analysis. Mining involves several classes of tasks:[60]

- **Anomaly detection:** the identification of unusual data records, or sequence of records. Used to anticipate behavior patterns and trends.
- **Association rule learning:** searches for relationships between variables..
- **Clustering:** discovering groups and structures in the data that are in some way or another similar.
- **Classification:** generalizing known structure to apply to new data. E.g. classifying a neuronal spike pattern as a particular movement.
- **Dimensionality reduction:** Representing multivariate data through less fewer dimensions. Such as SOMs attempt to do.
- **Regression:** find a function which models the data with the least error. A property of e.g. ANNs.

- **Summarization:** providing a more compact representation of the data set, including visualization and report generation. This may for example be done by dimensionality reduction.

The reader may notice that some methods described earlier in this chapter also fall into the data mining category. Usually, we talk about mining when we are analyzing logged data, in contrary to the real time data.

The question when conducting any data mining procedure, is if we can actually expect any informative results from mining the MEA recordings. We mentioned the spontaneous activity of the network in chapter 3.7.1. This spontaneous and seemingly random activity may not be much else than just that; spontaneous and random. Or, there could possibly be unseen correlations that we have yet to discover. This is just one of the many things in this project we will have to find out.

We will not go into further detail here about the workings of each mining algorithm, as the topic includes a vast set of methods and algorithms well documented elsewhere[67]. The topic of mining is however, included in the discussion to aware the reader of our intentions with logging data, and what we mean when we say this data is to be mined.

## 6.3 Neural coding

In order to make our Cyborg, we need to better understand how neurons use electrical signals to encode complex information about the world around us. Understanding how neurons encode information is highly important when it comes to analysing and stimulating our neuronal culture. We have to speak the cultures neural language if we hope to communicate with it.

However, this is no trivial task. In the field of neuroscience, unlocking the true mechanisms of this neural brain code remains one of the biggest puzzles yet to solve[99]. Hopefully, the research we are now conducting in the NTNU Cyborg project, may help towards further understanding some of the brains secrets. Developments in this area may contribute to better understanding how brains work, help with rehabilitating brain disorders, help people with spinal injuries, enable us to model the brain, replicate the brain or at least some aspects of functionality. Using neural signals, we may also be able to improve brain-computer interfaces (BCIs).

We discussed neural coding mechanisms in chapter 3.5, such as rate-based, temporal, population and sparse coding. Here, we investigate how we may communicate with our culture is a closed-loop bio-robotic system. Basically, we need to implement two subsystems: (a) culture-to-robot, in which we decode the culture output as robot instructions, and (b) robot-to-culture, in which we feedback sensory information from the robot to the culture.

### 6.3.1 Encoding sensory input to the neural culture

We have talked about the stimulation parameters, but have not yet discussed how we should encode the sensory information about the environment to the neuronal culture. For this, we need to convert sensory information from the sensors on the robot (and maybe even feedback from the robots own movements[51]), into electrical stimulation to the neuronal culture. Most embodied studies accomplish this through the use of dedicated input electrodes together with a rate-based coding scheme. The frequency of the stimulation rate encodes such things as how far a wall is from the robot[163], or how fast the robot is moving[51]. There have been few attempts in using temporal or population based coding as input, in contrary to the opposite mapping; reading from the culture, where population based coding is often applied.

Here are some examples of how others solved this task: DeMarse et al.[51], in their study using an Animat. provided feedback for each movement within the Animats virtual world (four channels; one for each movement) as well as the effects of those movements, such as collisions with walls or barriers (one channel). Feedback into the neuronal network was accomplished by inducing neural activity near one of five possible electrodes that delivered four pulses. Stimulated, or ‘sensory’ channels were chosen to be spatially distributed across the MEA, and capable of eliciting a reproducible response when stimulated. Warwick et al.[163], using a hybot, stimulated the culture through a dedicated electrode every time a laser sensor registered an obstacle 30 cm in front of it. In a follow-up experiment, they used sonar information to directly control (proportionally) the stimulating frequency of two sensory/input electrodes.

Initially, for our own culture, applying the same coding mechanisms as the above studies is certainly a good starting point. We would however, benefit from experimenting with several coding mechanisms, also those that are population based (dense and sparse). E.g, instead of encoding the distance to a wall through frequency, we could use the number of stimulated electrodes as a code for the distance. It would be interesting to see how different coding mechanisms may be used to solve the same problems, and how the culture reacts and adapts to these differences. We may also use a combination of schemes such as population and frequency stimulation. In addition, as we have seen in chapter 3.5, temporal coding may be applied to describe the environment to a much greater degree; assuming that the culture is able to distinguish the temporal pattern from just being a spike-rate.

### 6.3.2 Decoding the output from the neural culture

The RAW MEA data must somehow be converted into robotic instructions. Here too, we must decide upon a coding mechanism. As mentioned above, the popular choice is a population based method, such as clustering, such as in [51]. Other methods were described in chapter 6.2. Essentially, a population based scheme, derives information from the spike-patterns emerging from the culture. After clustering these patterns, one may assign each cluster a robotic function. In another study, Bakkum et al.[8] used a population calculation of the Center of neural Activity. Chao et al.[37] similarly employed population coding for

motor mapping. This popularity of population based coding, may be due to this scheme being known as a robust means to represent movement directions in the primary motor cortex.[63]

There have been a few other methods also though: Cozzi et al.[44] used an average neural activity function between two sets of 8 electrode outputs, while Warwick et al.[163], in their Hybrot study, used one dedicated output electrode to make their robot turn. This electrode was chosen for its ability to become active when the Hybrot sensed a wall (essentially, the input stimulation resulting from the wall being detected, evoked a response on the output electrode).

As with encoding, we gain to experiment with different schemes for decoding as well. While population coding seems efficient, we could also use dedicated output electrodes to control motor behaviour. E.g., we could use the firing rate of two electrodes as the left and right wheel motor velocities. According to Jeff Hawkins (Numenta - developer of HTM) though, this may be a fragile option and the brain does not code this way. With our tool set from chapter 6.2, we do now however have a good starting point to decode the neuronal activity.

### 6.3.3 Why encoding and decoding is difficult

Encoding and decoding were defined in chapter 3.5.1. Both are highly interesting to achieve in our neuronal culture, and also in the brain. In the CNN, encoding would enable us to predict the CNNs behaviour when stimulating the culture, giving us better intuition on how to stimulate efficiently. Decoding would enable us to better understand the CNNs output and use its spiking activity in our robotic system. In the brain, encoding would allow us to stimulate senses directly into the brain, e.g. by connecting a camera directly to the visual cortex, which could enable blind people to see. Decoding, on the other hand could, for example, enable people with paralysis to control bionic prostheses, and through encoding again, perceive touch and feeling. Some attempts have been made at decoding neural networks using Brain-computer interfaces (BCIs):

In 1999, Stanley et al.[145] decoded neuronal firings to reproduce images seen by cats. The team embedded an array of electrodes into the thalamus (which integrates all of the brain's sensory input) of the cats. They targeting 177 brain cells in the thalamus lateral geniculate nucleus area, which decodes signals from the retina. The cats were shown eight short movies, and their neural activity was recorded. Using mathematical filters, the researchers decoded the signals to generate movies of what the cats saw and were able to reconstruct recognizable scenes and moving objects. Similar results in humans have since been achieved by researchers in Japan[102]. In another study, Carmena et al.[33] developed BCIs to decode the brain activity of monkeys. The monkeys learned to control a robotic arm by viewing its movements. In 2011, O'Doherty et al.[118] took this one step further and closed the loop with the BCI sending sensory feedback, through intracortical stimulation (ICMS) in the arm representation area of the sensory cortex, to the monkeys.

These are all impressive feats, however the studies are not necessarily universal in that the mapping functions they developed would be applicable to other cats/monkeys/humans.

They simply made a correlation between the neural signals recorded, and the task at hand, e.g. the images seen by cats. What would be truly interesting would be if we, for example, could tap into the visual cortex of any brain and see exactly what the individual is seeing. The issue here though, is that such a global code may not exist, as no brain is equal. From chapter 3.4.1 we learned that the neocortex shapes itself and learns representations by adaptation. The exact structure and synaptic strengths between neurons is not going to be the exact same across different brains. This definitely causes great challenge for the universal decoding of neural activity. However, encoding may actually be somewhat more approachable. In the study discussed in chapter 3.4.1, we saw how Roe et al.[134] were able to reroute the visual pathway to the auditory pathway of ferrets, demonstrating an inherent property of the brain to process consistent patterns rather than just specific brain region dependent codes.

In the context of neural cultures, we meet some challenges: first of all, the network is highly dynamic, always changing due to synaptic plasticity and neuromodulation (chapter 3.6). This may benefit us when it comes to the input stimulation (as the culture will adapt to the sensory information) but creates a challenge when trying to decode the ever-changing recordings. Even without stimulation, the culture is spontaneously active, which makes it hard to create a map or a transfer function between input stimulation and output response. Lastly, the output of the network is highly dependent on the sequence of states up to the measuring point. This internal state is difficult to replicate in a transfer model.

### 6.3.4 Decoding neural activity across individuals

One might imagine that, one day we will be able to read the thoughts or intentions of another human being, simply by reading their neural activity. In this, we assume that the neural activity is equally coded across individual brains; that the firing patterns and sequences are global. We talk about neural coding as if the brain talks a universal language and that this language is transferable. But is this really the situation?

Looking back at our introduction of the neocortex in chapter 3.4.1, we talked about how the neocortex displays a self-organizing behaviour when decoding sensory input via its several cortical layers. Now let's do a little thought experiment. Imagine that we have two neocortical networks in two different individuals. Through the top layer, comes the sensory input from the outside world, e.g. sound. Now these networks have the same general topology, but looking closer, the weights between neurons don't necessarily have to be identical in both networks. This is due to there being 'more than one way to Rome'; the same functionality may be achieved through different routes. This gives rise to different records of activity in the same regions of different brains but meaning the same things. This would further imply, that the further down we get in the neocortical layers, the more distinguished the patterns become from individual to individual. After all, for what we know, neurons themselves are not aware of other neurons' existence. In the same way, decoding the language of one neuronal culture may not help us decode another.



# Training a cultured neural network

In the previous chapter, we looked at how we may utilize the cultures responses to stimulation as robotic actions. However, this is simply a mapping problem; mapping a neural spike-pattern to a convenient robotic behaviour. In this process, the neuronal culture is not being taught how to behave in its environment, but instead the robot is being programmed to behave according to certain firing patterns. In this chapter we would like to investigate the ability of neuronal cultures to learn.

To begin, we should define what is meant by learning. According to Ruaro et al.[136], 'learning in neurons is associated with changes in synaptic efficacy, leading to a persistent increase in amplitude of the response to the learnt stimulus.' They refer to this as long-term potentiation (LTP). For learning to be distinguished from plasticity, the change in network response must be lasting.[156] In the context of an embodied culture however, we may define learning as: the ability to acquire new behaviour through some form of training procedure. This training procedure also entails unsupervised training, which implies learning by experience.

In this chapter, we will take inspiration from the brains learning mechanisms to inspire learning in our culture. Through experiments with our culture, we may in return inspire new understanding to the learning mechanisms in the brain. We will also investigate how learning algorithms, developed for artificial neural networks (ANNs), may be applied to training the culture.

## 7.1 The ability for neuronal cultures to learn

The ability to train the culture to predefined behaviour would improve performance and possibilities greatly. Training the cultured neural network (CNN) to obtain some predetermined behavior would be highly desirable. However, there is some controversy in the field of neuroscience surrounding whether or not a CNN can learn complex behaviour. Experiments have had limited success in demonstrating a definition of learning that is widely agreed upon. Nevertheless, plasticity in neuronal networks is a phenomenon that is well-established in the neuroscience community, and one that is thought to play a very large role in learning.[160] Several studies have shown adaptive behaviour through plastic modulation using CNNs:

Pizzi et al.[122], who designed their cultures to behave like Kohonen and Hopfield networks, showed the CNN was able to distinguish input bit-map patterns. They showed that the introduction of organized stimuli modified the network structure and increased the information content even after the end of stimulation, which they concluded suggested a form of learning and memorization.

DeMarse et al.[51], in their Animat study, found that over the course of the simulation, there occurred changes in the Animat's behavior due to interaction with its surroundings. They suggested that this change was due to the adapting network due to biological processes such as plasticity. The study did not however attempt to reinforce specific behaviour in their Animat.

Warwick et al.[163] reported that their Hybrot appeared to improve its performance over time in terms of its wall avoidance ability. They hypothesized that neuronal structures/pathways that bring about a satisfactory action tend to strengthen purely though a process being habitually performed; learning due to habit. Such a mechanism has also been observed elsewhere[84].

Ruaro et al.[136], in their study to see if neural culture can perform tasks such as those often performed by ANNs, successfully trained a neuronal culture to discriminate between an 'L' and an inverted 'L' pattern using cells from the hippocampus. They showed LTP through tetanic stimulation using frequencies of 100 Hz and higher. Lower frequencies did not show the same effect.

Jimbo et al.[81] observed potentiation and depression of activity in particular pathways when training their CNNs with repetitive stimulation using tetanic stimulation.

Bakkum et al.[8] created a robotic drawing arm controlled by a neuronal culture, which they termed MEART (MEA + art). For training, plasticity was induced by repetitive stimulation of paired electrodes, termed Patterned Background Stimulation (PBS). A PBS was constructed by pairing the probe electrode with another active electrode (one that evokes network responses) at an inter-pulse-interval of 20 ms, repetitively stimulated for 3 sec. They found that Meart's PBS did induce directional neuronal plasticity, but in an uncontrolled manner. They determined that since neurons at different electrodes can be connected through multiple intermediate neurons and pathways, the effect of a given PBS could not be predicted.

Jackson et al.[80], in a primate motor cortex, repetitively stimulated a neuron 5 ms after the occurrence of an action potential on a different neuron using an electronics implant. After halting the stimulation, subsequent activity of the recorded neuron caused an increase in the firing rates in the vicinity of the stimulated neuron.[8]

Bi and Poo[19] found that for mono-synaptically connected neurons firing within a few 10s of ms of each other, directional spike timing dependent plasticity occurred at the level of the synapse.

Shalaf and Marom[143] defined a criteria for large random in.vitro cortical networks to display general capability of learning: numerous connections, stability of connections, and modifiability by external stimuli. They concluded that these criterias were fulfilled in their study by use of repetitive stimulation. They also showed that the magnitude of such modifications increases with stimulation time.

Other studies have attempted to imprint signal patterns onto the networks via artificial stimulation.[15] This can be done by inducing network bursts[126] or by inputting specific patterns to the neurons, from which the network is expected to derive some meaning (as in experiments with animats, where an arbitrary signal to the network indicates that the simulated animal has run into a wall or is moving in a direction, etc.).[51, 123] The latter technique attempts to take advantage of the inherent ability of neuronal networks to make sense of patterns.[160]

Although the above studies do show results of neural plasticity and a response to training, some studies have had a hard time achieving a lasting effect. Van Staveren et al.[156] trained their cultures with two protocols, i.e. the tetanic stimulation method from the report by Jimbo et al.[81] and a selective adaptation protocol[143]. Tetanic stimulation training changed the network response significantly, but they did not find it to have a lasting effect, neither did the selective adaptation protocol. This, they concluded, could therefore not be called learning. Potter et al.[128] also did not observe any evidence of lasting (above 30 min) changes in the open-loop behavior (driven by spontaneous activity) in their Animat, as a result of closed-loop sessions/training.

In conclusion, these studies demonstrate that there is a possibility for learning in neuronal cultures. To which extent, and the complexity of behaviour one can achieve, is yet to be understood.

### 7.1.1 Discussing the 'learning' seen in similar in-vitro studies

While most of these studies supply good evidence of neural adaptation to input stimulation through synaptic plasticity, they lack to have actually trained their cultures to perform predefined tasks. This is by no means criticizing the studies, they do exactly what they are meant to do: demonstrate the embodiment of a neuronal culture and investigate the adaptation of behaviour to its environment. Through the adaptation of behaviour, these studies demonstrate a form of unsupervised learning. What they do not demonstrate however, is any form of reinforcement learning. They do not train the cultures to distinguish between

good and bad actions, or have them work towards some behavioral goal. Instead the behaviour of the Animat/Hybrot is pretty much programmed: instead of training a behaviour into the culture, they are simply taking the existing behavior of the network and selectively choosing which patterns should be assigned to which motor signal. This is demonstrated in the study by DeMarse et al.[51], where the existing spike patterns are clustered and these clusters are then made to represent actions. In another study by Warwick et al.[162], they used a Klephora robot with laser sensors, with the goal of avoiding walls. Through the use of an electrode that responds to a certain stimuli (when the wall is closer than 30 cm) they assigned this electrode a robotic turn, as to have the robot avoid the wall. Now to be fair, and as mentioned, the fact that the CNN does display a change in behaviour is truly interesting and demonstrates the synaptic plasticity in the network and the adaptive changes that occur due to the form of unsupervised learning. However, for incorporating more goal directed behaviour in our bio-robotic system, there is a need to solve the mystery of reinforcement learning and utilize this mechanism into our culture.

## 7.2 Training methods

We saw in chapter 3.6 how the brain learns through mechanisms such as association, reward and prediction errors. In chapter 4.3 we looked at machine learning mechanisms for training artificial networks. Here we discuss some possible ways to train a neuronal culture inspired by biology and from algorithms developed for artificial neural networks (ANNs).

### 7.2.1 Beginning early

Neurons and neural systems are designed to change in a ‘use-dependent’ fashion: if they aren’t stimulated properly they won’t develop, and if they’re used abusively they will develop abnormally.[162] The brain develops in a sequential fashion and most rapidly early in life[121]. The CNN also shows signs of increased plasticity in the early stages of development (chapter 3.7). For this reason, it is important to embody our neuronal culture as soon as possible, and begin feeding it with sensory input while it is developing. This is important so that the CNN does not develop bad circuitry which continues on in its mature life.[162]

### 7.2.2 Frequency training

We saw in chapter 6.1.3 that tetanic stimulation can induce long-term potentiation (LTP). This property could be used for tasks such as pattern imprinting, associative learning through the feedback sensory information and reinforcement. Further, we may be able to use frequency to train the neuronal culture to achieve some predefined goal behaviour.

### 7.2.3 Learning through embodiment

Re-embodiment of the neuronal culture is not only a cool way to make a Cyborg, but also an important aspect in studying learning. Bakkum et al.[9] suggest that environmental interaction is needed to expose the underlying mechanisms for learning and intelligent behavior. The same notion is implied by Potter et al.[128]. Through embodiment, we are able to observe the behavioral adaptations that we define necessary for it to be called learning. Even without a specific training protocol, simply embodying a neuronal culture and watching how the network adapts to its environment, is essentially a live model of learning by adaptation.

### 7.2.4 The importance of network topology

Looking at the nervous system discussed in chapter 3.4, it is important to note that neural networks function in highly organized and hierarchical structures. In particular, we looked at the neocortex which consists of multiple layers, which are thought to play an important role in decoding incoming sensory information[69]. In fact, it is also thought that the structure of the network is more important than the mere quantity of neurons.[69] E.g., the C. Elegans (roundworm) only has 302 neurons[87], which is far less than the number of neurons often used in in-vitro studies, yet still displays simple behaviour. In ANNs also, network organization is very important for the functionality of the ANN. This begs the question whether our neuronal culture should/must be structured to a much greater degree than simply having it grow into a random network. It would be highly interesting to experiment with different network topologies, and see their effect. Specifically, implementing a neocortical inspired structure would be exciting. By utilizing such a structure, it may also be of less importance how we encode information into the network as long as the inputs are consistent and sufficient. The neocortical structure might be able to, in an unsupervised manner, extract information from any type sort of coding. (chapter 3.4.1).

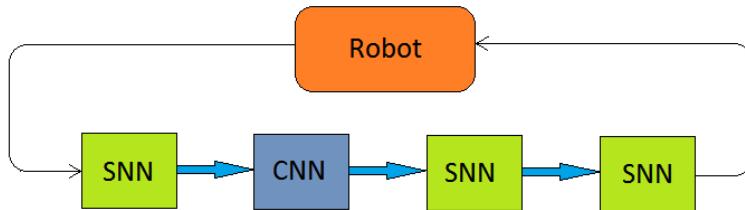
#### The CNN inspired by the neocortex

The neuroscientists at INM have stated that research is currently being conducted to enable the possibility of organizing cultures to a much higher degree than done previously. This will enable the culture to be organized in sections/chambers. By having more control of the network topology, than the randomized networks used today, it would be highly interesting to organize the neuronal culture in a layered, neocortical inspired, fashion. With this emerging technology, this may be possible in the foreseeable future.

#### The CNN organized in an artificial neocortex

Another possibility, closely related to organizing the CNN itself, is to incorporate the CNN, as one layer, in an artificial neocortex. With this solution, there is no need for sectioning the culture itself. The artificial layers can consist of spiking networks, which we

learned in chapter 4.1.3 simulate biological networks quite well. This setup is not dependent in the research mentioned above, and enables better understanding of the importance of network topology. One method would also be to use a HTM network as a structure around the CNN. A simple schematic of such an artificial neocortex network is depicted in figure 7.1.



**Figur 7.1:** Spiking neural networks and cultured network form layers of the neocortex inspired network. Feed forward neurons between the layers forward the signal the the next layer in the hierarchy.

### 7.2.5 The possibility of supervised training

It was argued in chapter 3.6 that supervised learning in the brain, may just be another unsupervised form of learning through association. However, in algorithms developed for ANNs, there is a more clear distinction between those methods that involve supervised training (such as the error back propagation algorithm) and those that involve unsupervised learning (such as incorporation of Hebbian learning). The reason this distinction may be done with ANNs is that, unlike biological systems, artificial systems may implement the notion of a global supervisor. E.g., with the back propagation algorithm, one finds the global error value between the ANN output and the wanted output and changes values of the ANN synapses accordingly. In biological systems, this process must take place locally and be self-organizing.

We are however, not confined to operate exactly as the brain does in our neuronal culture. One advantage we have, is that we are in fact able to make some sort of global supervisor, and could potentially incorporate our own quasi error back propagation algorithm. Supervised Hebbian Learning (SHL) offers a straightforward solution. According to this approach a spike-based Hebbian process is supervised by an additional 'teaching' signal that reinforces the postsynaptic neuron to fire at the target times and to remain silent at other times.[124] This teaching may be transmitted to the neuron through intracellular stimulation with an MEA.

### 7.2.6 Learning by association: Unsupervised training

In chapter 3.6 we discussed how the brain is constantly being moulded by plasticity though the firing and interconnection of neural pathways. As the CNN is a biological network,

we assume the same functionality, which has also been conformed in several studies (see chapter 7.1). So how can we exploit this inherent property of biological networks?

One way of utilizing this property, is to combine several types of sensory input to the neural network, as to allow the CNN to get a 'bigger picture' of the environment in which it is operating. For example, we may encode laser feedback from the robot to the CNN as well a sonar sensor. When the laser input is showing signs that a wall is in close approximation, this will be associated with the sonar input being active. Eventually, it would be interesting to see if a form of prediction occurs in the network; we would like to see if the pathways that are usually activated when the sonar input is active, also fire when the sonar is turned off but the laser sensors are indicating that the wall is close. If so, this could lead to a further understanding (inspired by associative memory) of the predictive nature of the brain, as discussed in chapter 3.6.

Another way to utilize the network plasticity is, as mentioned already, to organize the culture like the neocortex and let the CNN derive meaning from the inputs through its layers.

### 7.2.7 Rewarding the culture: Reinforcement learning

4.3.3 For our Cyborg, reinforcement learning, along with associative memory, is the most attractive learning protocol to employ. In an embodied environment, the ability to enforce good behaviour and steer away from bad, is necessary for the proper functionality of the Cyborg. This type of training also may be the most natural for the biological system compared to the more artificial supervised method. Reinforcement learning gives the Cyborg a goal; a specification to fulfill. In the following, we suggest mechanisms for achieving reinforcement learning in the CNN.

#### Rewarding using tetanic stimulation

We have already talked about how tetanic stimulation strengthens neural pathways. We may use this property when reinforcing certain behaviour patterns into the culture. One way of doing so, would be to track the activity of the CNN leading up to some (good) action to be rewarded (e.g. an Animat obtaining food in a virtual environment). When this event occurs, the neural activity (the sequence of firing pathways) leading up to this occurrence may be replicated with tetanic stimulation of the network: basically stimulating the same pattern sequence, that was recorded, leading to the rewarding event. The hope here is to strengthen the neural pathways leading to the desired behaviour.

#### Utilizing dopamine

When the brain receives an unpredicted reward, or on unpredicted indication of reward (such as in Pavlov conditioning), dopaminergic neurons fire and release dopamine through dopaminergic pathways (chapter 3.6.5). This process is theorized to be highly important in

the animals ability to be trained through reinforcement. Therefor, it would be highly interesting to include dopaminergic neurons into the culture. These neurons can be activated (stimulated) when some behaviour to be rewarded has been performed by the CNN.

The author has not found any embodiment studies utilizing dopaminergic neurons in their studies. Incorporating such neurons into our system, paves the way for a lot of interesting scientific research, and the potential of much more advanced Animat/Hybrot behaviours. As most studies employing embodiment of neuronal cultures have had limited results when it comes to efficient training, this could be theorized to be due to them not utilizing biology's own reinforcement mechanism; dopamine.

### **Actor-critic using a SNN-CNN hybrid system**

Actor-critic models[14] belong to the field of reinforcement learning[148]. More specifically, they are members of the set of temporal-difference (TD) methods[148, 157]. In chapter 3.6 we discussed how it is believed that TD plays an important role in the brain. The actor-critic theory is one of the more popular hypothesised TD mechanisms[125, 157]. In its theory, the critic consists of brain areas contributing to the production and release of dopamine, while the actor consists of brain areas responsible for action selection[157, 115]. The exact mechanisms in which this is employed is still not well understood, but research in this area promises exciting new understanding into how animals learn and adapt their behaviour through reinforcement.

There have been several attempts at modelling (at least certain aspects) the actor-critic though biologically plausible artificial networks[157, 125]. These models are a bit out of the scope of this text, but are mentioned as they may be interesting to investigate further in the NTNU Cyborg project. One possibility would to arrange the CNN to be the actor in such a system, and build an artificial critic around the CNN. In this way, we would be placing the CNN in a configuration more like home; in the structures of the brain. This may yield a better mechanism for teaching the culture new tasks in its embodied environment, and the scientific value is certainly significant.

### **Solving the distal reward problem**

Although this problem is not yet understood in the brain, the problem may be solved in our CNN since we log the firing history and can replicate the firing sequences leading to reward as suggested above.

As a more biologically plausible method, one hypothesis by Izhikevich[78] (as we saw in chapter 4.3.3), is that the STDP process leaves a eligibility trace that dopamine may use to find and strengthen the synapses contributing to a reward. If this hypothesis holds true, utilizing dopamine in our cultures would naturally solve the distal reward problem.

# Modelling a neuronal culture

Here follows a very brief chapter on the topic of modeling a neuronal culture. The modeling of a culture could very well be a thesis in and by its own, and deserves a much larger focus than as a mere sub-chapter here. The topic is however added, for the sake of completion; complimenting our discussion on the necessary aspects of creating a system of an embodied neurons.

Modeling a CNN, is an important task in the NTNU cyborg project, and is highly advantages to achieve. When the culture is up and running it will most likely be heavily used, and not readily available whenever someone wishes to use it. Also, there will be a lot of downtime as growing the network takes time and the culture must spend a certain amount of time in its incubation chamber. A simulation of the culture would be beneficial in that it would be available at all times and allows more people to experiment at once.

Naturally, making a 1-to-1 model replication, incorporating all the dynamics and processes of a biological culture is not something we are likely to have up and running any time soon. For this reason, a model will serve more as a testing ground for investigating aspects such as the bio-robotic infrastructure and the closed-loop system where the simulation can control the robot.

Models also have another advantage; they tend to increase our knowledge about the object we are modeling. The mere fact of being able to model a system requires a thorough understanding of its workings. When creating a model, there is a continuous trial and error process occurring, where one compares how one thinks the object works, with how it actually works. For each iteration, the model becomes closer to the real object. We are essentially learning how our original assumption may or may not have been correct.

In this chapter, we suggest a very simple spiking neural network to test our interface, and discuss some of the challenges that come from modeling the neuronal culture. We also supply the reader with some information regarding existing modeling frameworks. The

reader may also find interest in studying literature on biologically plausible spiking neural network models which there is quite a bit of.

## 8.1 Modeling and simulation frameworks

We are far from the first to explore modeling biological neural networks. There is a great deal of literature covering the topic, some coming from the perspective of computer scientists and others from the field of neuroscience. Luckily for us, a lot of the models and frameworks developed over the years, are openly available for use. The models range from the highly neurologically detailed but somewhat computationally slow (such as NEURON), to the more efficient but somewhat simplistic (such as Brian (python)). Some models are better at describing the behaviour of the network at whole, while others focus on the interacting between pairs of neurons. Here are listed some of the most popular frameworks:

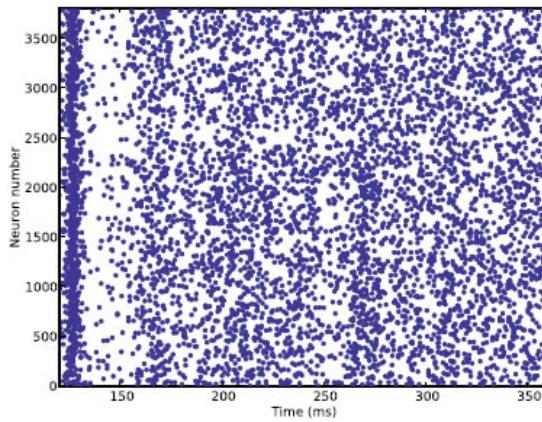
- NEURON
- GENESIS
- HTM (numenta)
- Brian and Brian2
- NEST
- Nengo - Neural Engineering Framework (NEF)
- GeNN
- SpikeFun

These models could potentially serve as a basis for the models of our neuronal cultures. Of these, the Brian and NEURON models were tested. Brian, with its realistic spiking outputs (see figure 8.1) seems to be a good and efficient starting point for testing the Cyborg infrastructure. Brian and Brian2, are written in python, making them easy to use for most programmers and have a short learning curve. Further investigation of the different frameworks must however be conducted.

For a complete overview of modeling and simulation software, the reader is directed to the following sites: 'Comparison of Neural Network Simulators' by the University of Colorado Boulder's Computational Cognitive Neuroscience Lab[43] and Jim Perleowitz's 'Computational Neuroscience' page[120].

## 8.2 Developing a model

An alternative to using a framework is to make the model from scratch. Using a framework enables a quick setup, however we may require a more personalized model to replicate



**Figure 8.1:** Raster plot from the Brian simulator, showing a very similar output as seen in real cultured networks.

our neuronal cultures. Also, we wish to include a model of the MEA interface; we want to stimulate and record from our model through an MEA and not directly at the neuron level as many models incorporate. Here follow a short discussion on the thing we need to consider. As mentioned before, no actual model will be presented.

### 8.2.1 The neuronal culture

For the neuronal culture we can use a networks comprised by spiking neurons. In chapter 4.1 we looked at some example neuron models. We found that the Izhikevich model is able to replicate the mathematical dynamics of real neurons well, with good efficiency (13 FLOPS). We also found the leaky integrate and fire neurons (LIF) to be particularly efficient, although they lacked some correctness. For this reason we propose using Izhikevich neurons in our model.

The number of neurons to be used in the model is yet to be decided, but looking back at chapter 3.7 we can assume 5000-50000 neurons (excitatory and inhibitory) must be modeled. We also need to incorporate plasticity. For this, the STDP model presented in chapter 4.1 may be applied. The effect of STDP may also be varied, as to incorporate the higher plasticity observed in younger cultures; make STDP changes greater during early simulation. In terms of network topology, we can either initiate a random full network or we can attempt to model the growth of the network, as done in the Master thesis of Riikka Havela[68]. We may also experiment with more organized structures (such as neocortical layering) to experiment with the effects of these. We also presented a model for incorporating dopamine into the system in chapter 4.3.3, which may be used to experiment with reward modulated dopamine effects. The Master thesis 'Dopamine modulated STDP and reinforcement learning in an embodied context' by Lars Andersen and Tormund Sandve Haus (IDI, NTNU) implements such a model in which is a good inspiration.

This, off course, is the proposal of a fairly simple model compared to the complexity of a biological network which incorporates effects in which we have yet to truly understand (such as the effects of different stimulation frequencies, LTP, LTD etc.). The model is however, sufficiently accurate for some simpler infrastructure and control-loop related tests, and is a starting point to a more complex model.

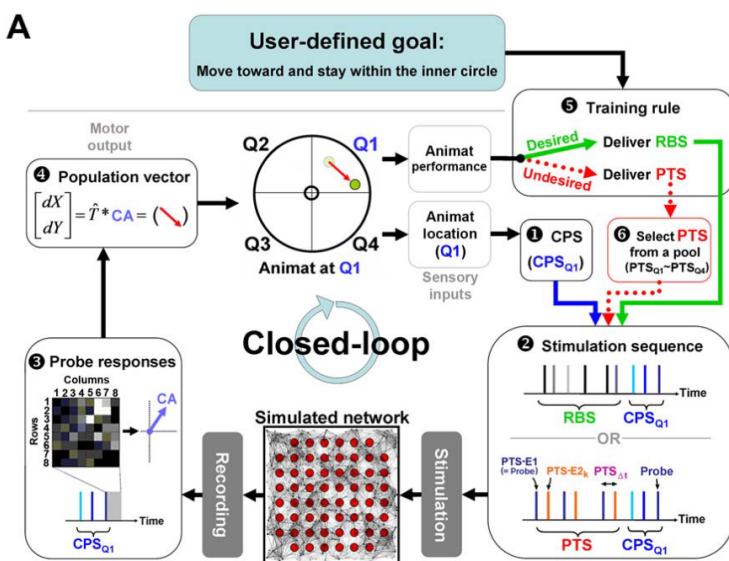
## 8.2.2 The MEA

A model of the microelectrode array (MEA) would also be beneficial. The MEA model should be connected to the above network model. It should incorporate 8x8 (optionally experiment with higher resolution) bidirectional electrodes, and use these electrodes to stimulate and record from the extracellular environment of the neural model.

## 8.2.3 A study using a SNN to simulate an embodied CNN

Chao et al.[37] used a very similar setup to the one suggested above. They had a spiking network simulate a cultured network embodied through an animat (closed-loop setup in figure 8.2). They used the Neural Circuit SIMulator[110] to produce three artificial neural networks[38]. Their models incorporate 1,000 leaky integrate-and-fire (LIF) neurons, with a total of 50,000 synapses. All synapses were frequency-dependent to model synaptic depression. 70% of the neurons were excitatory and utilized STDP. They also modeled the MEA by an 8x8 grid of electrodes, 60 of these were used for recording and stimulation as in a typical real MEA.

In a previous study[38], they showed that a 1000-neuron LIF model and living MEA cultures expressed similar spontaneous and evoked activity patterns, demonstrating the usefulness of the LIF model for representing the activity of biological networks. In another study, they successfully used the simulated network to find a statistic to detect network functional plasticity in living MEA cultures and to demonstrate region-specific properties of stimulus-induced network plasticity[36]. These results clearly demonstrate the potential and advantage of developing and working with a model.



**Figure 8.2:** The closed-loop setup in [37], where a SNN was used to simulate an animat embodied CNN.



# Putting it all together

We have discussed a wide range of topics in this thesis; from the necessary hardware and design aspects for developing the cybernetic system, setting up communication between robotics and neuronal culture, as well as training and modeling the culture. Here, we summarize these chapter by proposing a plan moving forward with The NTNU Cyborg project.

This chapter is a summary and a proposed plan moving with The NTNU Cyborg project.

## 9.1 Cultivation

Cultivation of neurons has already begun at Department of Neuroscience (INM), and this first point is left in their hands. When the MEA is up and running, they will be in charge of culturing and growing the networks and keeping them alive. Once we are up and running, we may play around with different network configurations such as:

- incorporating different types of neurons: cortical, dopaminergic, motor etc.
- experiment with different network topologies (when this is possible)
- experiment with different concentrations of excitatory vs inhibitory neurons
- experiment with different network densities

to name a few. There are many variables to consider when growing these networks, but again, this is mostly in the hands of INM.

## 9.2 Setting up the MEA and infrastructure

### 9.2.1 The MEA

The MEA was shipped by MultiChannel Systems 16.06.2016. Once the MEA2100-60-system has arrived, the system is to be set up in an appropriate lab at St. Olav's University Hospital. The setup will be supported by the manufacturer. The users of the system must become acquainted with the included software, such as the MC Rack program. From here the initial trials recording and stimulating a neuronal culture may begin. We may also now implement and use the built-in spike-sorting functionality of MC Rack to increase the recorded resolution.

### 9.2.2 The distributed infrastructure

Once the MEA2100-60-system is set up and a culture has been cultured on an MEA, it will be possible to use the system locally. Now comes the time to set up the distributed interface across the three institutes (ITK, IDI and INM) and to begin embodying.

Hardware:

- Set up servers/computers at the three institutes
- Set up the logging database at IDI

Software:

- IDI: make the MQTT coordinator handling requests by clients. The MQTT coordinator communicates directly through to the MEA-server at St. Olav
- ITK: make the server to control the Cyborg platform. Since the Cyborg is operating ROS[], a port into ROS must be developed
- Define protocols for data (RAW, robot, etc)

Testing the interface:

- Test each node (alternatively use a SNN for testing)
- Check if real-time closed-loop system works

## 9.3 Closing the loop

encode sensory information into the culture as well as feedback its own robotic instructions.

### 9.3.1 The robot platform

The robotic body to embody the neuronal culture was discussed in chapter 5.3. It is natural to begin our embodied experiments with a software Animat, then scaling up to a real small-scale robot/Hybrot until finally beginning work on the NTNU Cyborg platform. Here follows a plan of what must be developed:

Animat:

- Develop a simple software robot, 'Animat', and a simulated environment for it to operate.
- The Pioneer LX simulator, MobileSim, may be used as an Animat to simulate the NTNU Cyborg navigation platform.
- Develop sensory feedback from the Animat to the MEA. E.g laser/sonar distance telemetry. Also, incorporate feedback of the robots own movements (forward, backwards, turns, etc)
- Control Animat by converting MEA recordings to movements (through methods mentioned in chapter 6.2)
- Use the Animat to test the distributed real-time infrastructure.

Hybrot:

- Purchase a small scale robot for testing purposes, e.g. the Khepera or Koala robot from K-Teams Mobile Robot.
- Transform sensory from the Hybrot to be used as feedback to the MEA. (extension of that made for the Animat)
- Control Hybrot by converting MEA recordings to movements. (extension of that made for the Animat)
- Use the Hybrot to further test the distributed real-time system working with a real robot.

NTNU Cyborg platform:

- Continue development of Cyborg though student projects. [cite my project]
- Decide upon which operation to control on the NTNU Cyborg. (E.g., motor control, troll face, the emotional state of the Cyborg etc.)
- Transform sensory or state of the Cyborg to be used as feedback to the MEA.
- Control Cyborg by converting MEA recordings.

For the application where the CNN is controlling robot movement, we may set some behaviour goals to try to achieve:

- Avoidance of walls and obstacles.
- Avoidance of other bots.

- Following other bots.
- Avoidance of simulated poison and trying to obtain food (rewards).

It is not absolutely necessary to follow this progression, as we may choose to incorporate the culture into the NTNU Cyborg platform right away. The Animat and Hybrot solutions are however, important tools for the more scientific investigations that will be conducted, as apposed to the more showcase functionality of the NTNU Cyborg platform.

### **9.3.2 Single spike stimulation**

From the studies we looked at in chapter 6.1 we found the stimulation protocol for single spikes to typically be performed using bipolar pulses consisting of  $200/400 \mu\text{s}$  width and  $500/600 \text{ mV}$  amplitude. We further found that these pulses propagate through the network and evoke a responsive burst of activity for approximately 100 to 200 ms. As this stimulation protocol is fairly standard in the studies, we suggest adopting the same protocol. We will however, conduct our own tests verifying the effects of these parameters in our cultures.

### **9.3.3 Feeding the neuronal culture with sensory input**

We should attempt to feedback both single- and multi-sensory information to the culture, to see if more senses allow the CNN better 'understanding' of the environment. We may also feedback proprioceptive information such as the movement of the robotic body itself.

We would benefit from trying out different coding schemes (rate, temporal and population) and also combinations of these. We found that the use of dedicated input electrodes using single spike and rate-based encoding was the most widely used method in similar studies.

### **9.3.4 Using the neural recordings as input to the robot**

Following our discussion on in chapter 6.2, we may choose the following methods to map the neuronal outputs to robotic input by using the:

- networks responses to single site stimulation
- populations spike-pattern through methods such as clustering algorithms or SOMs.
- neuronal culture as a liquid in a liquid state machine. This enables us to utilize the non-linearity of the culture by simple training of a feed-forward artificial network.

Here too, we benefit from testing several coding schemes.

### Based on population coding

Based on the spike pattern occurring in the network, we may choose to derive meaning from these patterns through the following methods:

- Clustering:
  - Cluster the occurring patterns as done in [51]: register all observed patterns (with and without stimulation) and then cluster these patterns.
  - Choose as many clusters as needed robot instructions (maybe more if we want some clusters to not lead to instructions).
  - Map spike-patterns in real-time into input instructions for the robot.
- Self-organizing maps (SOM):
  - Make a Kohonen network for clustering the neuronal output.
  - Choose as many clusters/nodes as needed robot instructions (maybe more if we want some clusters to not lead to instructions).
  - Map spike-patterns in real-time into input instructions for the robot.
  - The SOM allows for on-line adaptation to the incoming data.
- Liquid state-machine:
  - Develop a feed forward ANN which may be trained to assign robot instructions to different spike-patterns.
  - Train the ANN using genetic algorithms or error back propagation in order to develop an individual displaying good behaviour according to a set goal.

### Based on rate coding and single-site spike responses

It is also possible to dedicate electrodes as output electrodes and have them control some robot instructions based on the firing frequency of these electrodes. One example, is to have the frequency control the velocity of the robots wheels. Different relative frequencies between the electrodes allows the robot to turn. The output electrodes may be further chosen according to their response to certain stimuli; e.g. if a certain electrode responds to the stimulation meaning a wall is in front of the robot, this electrode can be chosen to correspond to a left/right turn of the robot and the frequency of this electrode the turn rate.

### Combining methods

Rate-based coding may be used in conjunction with population coding. For example, one could integrate the occurring patterns and clusters. For example, instead of a cluster just

indicating a constant rate left turn, we could use the number of times that cluster has fired, within some time frame, to also control the turn rate.

## 9.4 Further understanding the culture output

To further understand our neuronal culture we propose to mine the data logs once experimental data has accumulated. We also wish to investigate the predictability of the culture.

### 9.4.1 Mining

We wish to mine the logged data as to investigate the existence of unseen dependencies and correlations within the culture. Methods for doing so include anomaly detection, association rule learning, clustering, classification, dimensionality reduction, regression and summarization.

### 9.4.2 Prediction and anomaly detection

Hierarchical Temporal Memory (HTM) was a proposed method for predicting the next firing pattern/sequence of the neuronal culture. Through this method we hope to test the predictability of the culture, and whether we can use these predictions to steer away from undesired neural behaviour (such as network wide spike bursts). HTM also allows for anomaly detection, which may give us insight to when the culture is behaving abnormally. This allows us to investigate what factors cause the abnormality.

## 9.5 Training our culture

Here follows a short description of possible future training experiments to be conducted when the MEA is up and running. This section builds upon chapter 7.2.

### 9.5.1 'Proof of concept' training experiments

Besides embodying our neuronal culture, it may be fruitful to conduct some 'out of body' training experiments. The following experiments serve as a 'proof of concept' of neural plasticity, and may also further be used for testing training mechanisms; such as frequency based stimulation.

## Pattern imprinting

This goal of this experiment is to imprint predefined spiking patterns into the culture. From chapter 6.1.3 we saw that the network responds to tetanic stimulation by increasing the strength of the stimulated neural pathway, while decreasing its connections with the rest of the network. The test may be conducted as such:

Training phase:

- Whole pattern spikes: imprint a spike pattern using tetanic stimulation
  - optimal frequencies and durations to be investigated as well as the effect of spike timing
- Single electrode stimulation: imprint a spike pattern using tetanic stimulation of a single electrode, strengthening the evoked neural pathway

Testing phase:

- without stimulation: see if the pattern imprinted in the training phase emerges during spontaneous activity of the network.
- with stimulation: stimulate a single electrode (which is part of the pattern) and see if the rest of the pattern spikes.

If pattern imprinting works out, we may take this a step further by imprinting pattern sequences: We follow one firing pattern with others until we loop around back to the first pattern. We continue these loops for the whole training phase. After the training, it would be interesting to see if this firing sequence has been imprinted into the culture. For example, we could imprint the patterns of the numbers 0 to 9 as one loop.

Further still, we may try to have the culture classify input spiking patterns. By, for example, choosing 5 dedicated output electrodes, we can attempt to get one electrode associated to each of 5 different patterns (or more patterns if we attempt to have the culture cluster the incoming patterns); that is, one electrode responds to one input pattern. This video demonstrates the principle well (the video is using adaptive resonance theory and not using a CNN, but illustrates the idea): [42].

## 9.5.2 Logic gate

Another useful experiment is to achieve the functionality of a logic gate, such as AND, OR or XOR. Here, we wish to use two dedicated input electrodes and one dedicated output. Using tetanic stimulation for strengthening the neural pathways, and low-frequency for decreasing the strengths of others, we hope to manipulate the culture in order to achieve our goal. If this goal is reached, it will show that some form of learning in the culture can be achieved. It will also demonstrate that we are able to manipulate the synaptic connections to produce some pre-determined functionality.

### 9.5.3 Unsupervised training of the embodied culture

Proceeding to our embodied environment, we will primarily look at unsupervised and reinforcement forms of learning in our Animat/Hybrot/NTNU Cyborg.

#### Monkey see monkey do: Apprenticeship learning

Apprenticeship learning[1] is learning by imitation/demonstration. What we would like to achieve here, is to have the culture replicate the behaviour of a pre-programmed bot operating in some environment. While this bot is performing some task in its environment, e.g. staying clear of obstacles, sensory data and motor instructions are fed back to the neuronal culture. The hope is that the neuronal culture, when taking over the motor control of the bot, will conform to the same behaviour as the pre-programmed bot. This is taking advantage of the unsupervised Hebbian nature of the culture. The variables to be experimented with, for this sort of training, is the feedback stimulation frequencies and patterns to be used, as well as the duration of the learning phase.

#### Incorporating structure: Layering like the neocortex

We discussed the importance of network structure in chapter 7.2.4. Following this discussion, we would like to experiment with different network topologies in our culture. Especially, we would like to emulate the structure of the neocortex to the extent possible. This however, will be something we will do down the road, when the research enables it.

Until we can structure the culture by itself, We may investigate using a CNN-SNN hybrid system, where the CNN acts as a layer surrounded by artificial layers in a neocortex layered fashion as suggested in chapter 7.2.4

### 9.5.4 Training the embodied culture though reinforcement

#### Frequency reinforcement

Using inspiration from the back propagation algorithm in chapter 4.3.2, we would like to enforce 'good' actions (actions fulfilling the specification of the agent) and punish the 'bad'. This was discussed in chapter 4.3.3, and may possibly be done by replicating the spike sequence leading to a good action, and using this spike sequence as tetanic stimulation into the culture. The patterns closest to the rewarding behaviour may be repeating more times than those more distant, following a decay evaluation found in temporal-difference algorithms (chapter subsec:annreinforcement).

### Introducing dopaminergic neurons to the culture

Ultimately, when this is possible in our culture, we would like to utilize dopaminergic neurons as discussed in chapter 4.3.3, to train the network.

## 9.6 Modeling

To create a model of the biological culture and MEA, we should develop a mathematically realistic spiking neural network (SNN) that is interfaced via a simulated MEA. Once a basic model has been created, we may further experiment with various network topologies (such a neocortical layering), the introduction of dopamine and other mechanisms we wish to explore.

We may first use this model to test out the infrastructure and closed-loop control of the robot. However, we also wish to make the model biologically accurate as possible for simulated experiments. This model will most likely be in continuous development, incorporating any new properties we, or others, may discover in cultured networks.

Chao et al.[37] provided a great setup for modeling a culture and MEA in an embodied system which we may use for inspiration. Otherwise, we may also want to investigate the use of the established modeling frameworks.



## Discussion

We have, in this thesis, discussed the necessary (and some additional) aspects for developing a bio-robotic system of embodied neural cultures. With a background study into the working of the neuronal cultures home; the brain, and through mechanisms developed for artificial networks, we have tackled the problem from the middle-ground between neuroscience and computer science. We have discussed the MEA hardware needed to interface with our cultures, and also the distributed environment through which it will operate a robot body. We have looked at different bodies; from the virtual Animat to the real Hybrot and NTNU Cyborg platform. We have further discussed how we may communicate with the culture and close-the-loop of our bio-robotic system through the encoding and decoding of information. We also discussed the training of a culture, to achieve more complex and goal-oriented behaviour. As an additional point, we briefly discussed how we may model a neuronal culture, and the benefits of doing so. Finally, we summarized our discussion and proposed a plan moving forward. Looking back at the research questions in the beginning of this thesis, we have attempted to answer these questions to the degree possible without the possibility of conducting our own experiments (yet).

It has been challenges, in writing this thesis, to find all the answers to the questions that have been asked. Hours-long sessions have been spent trying to find relevant literature to subjects, only to find that the research is 'still out'. This especially applies for the mechanisms of learning and training, neural coding, and the effects of neural stimulation. These are among the many unsolved mysteries in the field of neuroscience. An article from Discovery Magazine sums up the questions we have yet to answer, quite well[99]:

- How is information coded in neural activity?
- How are memories stored and retrieved?
- What does the baseline activity in the brain represent?
- How do brains simulate the future?

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- What are emotions?
  - What is intelligence?
  - How is time represented in the brain?
  - Why do brains sleep and dream?
  - How do the specialized systems of the brain integrate with one another?
  - What is consciousness?

It is in these questions that, through the study of neuronal cultures, we ultimately hope the NTNU Cyborg initiative may provide further insight. The applications are vast, ranging from medical contributions to improved artificial intelligent models. In addition, and finally, studying the workings of the brain also gives us little more insight into ourselves.

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# Appendix A: MEA2100-60-System

The MEA2100-60-System specification may be found at:

<http://www.multichannelsystems.com/systems/mea2100-60-system>

The MEA-2100 user manual: [100].

## Data acquisition and analysis software:

- Including a 2 years maintenance package (software updates and support)

## MEA2100-HS60 headstage:

- Pre- and filter amplifier with integrated data acquisition and analog-digital converter
- Gain and bandwidth adjustable via software
- Data resolution: 24 bit
- Direct access to each electrode for stimulation via internal stimulus generator.
- Control of stimulator via the included data acquisition software MC\_Rack
- Integrated blanking circuit for stimulus artifact suppression
- 3 independent stimulus generators
- Current stimulation at max. +/-1 mA
- Voltage stimulation at max. +/-10 V
- Sampling rate max. 50 kHz per channel
- Integrated heating element (20Ω, PT 100 sensor)
- With installed metal plate for fixing perfusion equipment

## MCS-IFB 3.0 multiboot interface board - with signal processor for real-time signal detection and feedback:

- 4x Digital Out: Interface for output bit 0-3 of the 16-bit digital input / output channels (Lemo connector)
- 4x Digital In for synchronization with other instruments (bit 0-3) (Lemo connector)
- Digital IN / OUT: Interface for 16-bit digital input / output channels. Generates or accepts TTL-pulses.

- 
- 1x Stereo Out: Possibility to export 2 channels (e.g on an oscilloscope or headphones making the signals audible)
  - 2x Analog IN: Interface for 2 additional analog inputs (channel 1 and 2) (Lemo connector)
  - Analog IN: 8-channel Analog input (channel 1-8)
  - 1x Ground
  - 1x Universal Serial Bus High Speed for transferring digitally converted data to any data acquisition computer with a sampling rate of up to 50 kHz (USB High Speed cable)
  - 1x USB Serial Bus High Speed for programing the integrated signal processor
  - External power supply: 100-240 V input voltage range