AALBORG UNIVERSITY

Faculty of Engineering, Science, and Medicine
Department of Health Science and Technology
Fredrik Bajers Vej 7C
Phone +45 96 35 87 00
Fax +45 98 15 17 39
http://www.hst.aau.dk

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Changes in neuroplasticity and type of recovery in motor cortex, according to onset of rehabilitation following ischemic stroke.

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Group members:
Lars Fjeldborg
Michael Nielsen
Kasper Ottesen

Supervisor:
Winnie Jensen

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Synopsis:

Stroke is a leading cause of disability and mortality worldwide. Neuroplasticity is believed to play a key role in functional recovery. Our objective was to characterize the long-term effect of ischemic stroke, in reference to the timing of the "rehabilitation process" and type of recovery according to the terms "true recovery" and "behavioral compensation". Intracortical signals were obtained from the motor cortex (i.e. the prenumbra zone). 11 Sprague-Dawley rats were instrumented with a 16-ch electrode array. The animals were trained to reach and retrieve food pellets. The 11 rats were split up into Group A (n=6) and Group B (n=5). Intracortical signals were obtained as baseline recordings for both groups during behavioral training sessions, before a photothrombotic stroke was applied. For Group A recordings were made on Day 1, 2, 4, 7 and 10 following the stroke. For Group B Day 4, 5, 7, 10 and 13. Analysis of the rats pellet rate, an ANOVA test and a detection in feature changes on the PSTH plots were made on basis of the data.
Based on the ANOVA test, differences between the neuroactivity could be detected between the two groups. For Group A computed PSTH responses revealed similar trends between the animals. It was found that at day 7 and 10 the responses appeared similar to the baseline responses. Based on pellet rate and feature detection it was found that many rats in this group behaved in a similar pattern.
For Group B, no similar patterns could be detected from either of the analysis.
It could be concluded that the onset of the rehabilitation affects the brain plasticity. An early onset of training was found to result in a more organized and uniform rehabilitation period, and it was judged that a higher degree of "true recovery" was possibly associated with this.
Variability across animals should be expected and must be investigated further. A better understanding of the effect of stroke on motor patterns, may assist to a better understanding of the plasticity mechanisms involved in functional recovery and to a more evidence based design of stroke rehabilitation.

Preface

This report is composed by the project group 10gr1085c, in the project period of 3rd and 4th semester, at the master education in medical systems at the "Faculty of Engineering, Science and Medicine" at Aalborg University. The report has partly been prepared in corporation with the Biomedical Department of "University of Illinois at Chicago", UIC, on which location most of the introductory work has been conducted.

A series of appendices that in details describe the procedures and techniques used as part of the experimental work is attached to this report. This is considered recommended reading for any reader who might be interested in replicate the experiment or wants to evaluate the preconditions the project builds upon. The data recorded through the experiment is included as enclosures at the end of the report.

Reference method is based on the Harvard Style, where writers surname and date of publication are used in the text to represent a citation. If the reference is placed after a full stop, the source is used in the entire paragraph. If it is placed before, it is only used for the actual sentence.

We would like to thank our primary supervisor Winnie Jensen for exceptional good co-operation and feedback. We would also like to thank Dr. Patrick Rousche and his colleagues from the Department of Bioengineering for valuable supervision during the time the project group spent at UIC. At last, we would like to thank the staff at Pathological Institute, "Aalborg Sygehus Nord" for their great assistance during the animal experiments conducted as part of this work.

Kasper Ottesen

Michael V. Nielsen

Lars Fjeldborg

Contents

Chapter 1

Introduction

Stroke is the second leading cause of death worldwide and the third most common cause of death in the western world [Selzer et al., 2006], [Lopez et al., 2006]. It is assumed that 1/6 of the entire human world population will suffer from a stroke once or several times during their life [Durukan and Tatlisumak, 2007].

Stroke has a mortality rate between 15% and 35% [Dobkin, 2003]. In 2001, 5.4 million individuals died of stroke worldwide. Around 1.0 million of those lived in high income countries. [Lopez et al., 2006]

Since a major part of the stroke survivors to some extent suffer from some disabilities, stroke is a major problem - not only for the patients and their relatives, but also for the society. In the United States, the estimated cost of stroke in 2005 was $ 56.8 billion. Of those the $ 21.8 billion corresponds to losses in productivity. [Selzer et al., 2006] In Denmark the total estimated cost of stroke in 2001 was found to be DKK 7 billion. [Hjernesagen, 2005]

In general, stroke can broadly be defined as either ischemic stroke or hemorrhagic stroke. Ischemic stroke is caused by a clot in a blood vessel in the brain, either as a result of a thrombosis or an embolism [Becker et al., 2009]. Hemorrhagic stroke, which is less common compared to ischemic stroke, is characterized by an accumulation of blood, and happens when a blood vessel ruptures and bleeds into the brain or the surrounding tissue in the skull [Zieve, 2009].

Even though a person survives a stroke, the person may still suffer from a series of disabilities. The nature of those depends on in which part of the brain the stroke occurs. In many cases, the stroke victim will suffer from problems related to motor control. Hemiplegia is a common effect of a stroke, and it is found in 70 - 86 % of all patients who has experienced their first stroke [Dobkin, 2004]. If the damage occurs in one of the hemispheres, there is a great risk that the movement of the opposite part of the body is affected. [National-Stroke-Organisation, 2009a] Right-hemisphere stroke patients may, in addition to this

also suffer from problems with their motor coordination, since their ability to judge distances, speed, position and similar sensory inputs are processed in this part of the brain. [National-Stroke-Organisation, 2009a]

The effect of a stroke is related to the amount of brain tissue that has been damaged during and after the incidence. It is important to distinguish between the ischemic core and the ischemic penumbra of the stroke. The ischemic core is defined as the area where the blood flow is significantly decreased, and the brain tissue as a result dies only a few minutes after the stroke event. Structural damage in this area has been identified as early as 2 minutes after the stroke [Murphy and Corbett, 2009]. The ischemic penumbra is defined as the area around the core, where the blood flow is decreased enough to cause damage, but not irreversible damage as in the core. Depending on the perfusion in this area, it might take hours or days before the cells in this area suffers from necrosis. Around the penumbra is a third area where the blood supply is decreased, but not enough to cause dysfunction of the neurons. The connection between the three areas can be seen on Figure 1.1. [Davis et al., 2006]

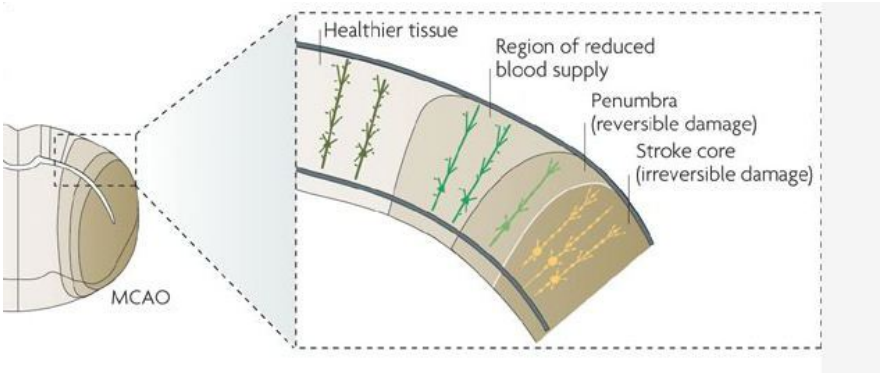

Figure 1.1: A cross selection of a brain cortex showing the core of the stroke, the penumbra and the area with reduced bloodflow in relation to each other. [Murphy and Corbett, 2009]

The main purpose of rehabilitation is to recreate as much of the lost function in the brain as possible. If the condition of the patient allows it, current guidelines recommends that this rehabilitation process should be initiated around 48 hours after the onset of the stroke. It is highly relevant to identify novel techniques and methods that can ensure that victims surviving a stroke ends up with the lowest possible loss of functionality. This may be related to the conservation of the ischemic penumbra, and thereby making sure that as little brain tissue as possible becomes permanent damaged [Jauch and Kissela, 2009] [Dobkin, 2004].

In order to make evidence based improvement of the post-stroke rehabilitation it is of high relevance to understand the process that takes place inside the brain during and following the stroke. Since ischemic stroke is by far the most common

type of stroke, it is especially important to understand the process behind this disorder.

It has therefore been selected that the overall focus of the present work is:

Describe the neurological effects of ischemic stroke and how the treatment of this disease affect the neurological outcome.

The project focused only on strokes that affect the motor cortex area of the brain and the disabilities related to motor control caused by such a stroke.

To measure and evaluate the brain processes, it was selected that the overall model would build on various behavioral analysis in combination with **intracortical signals (IC signals**). By analyzing IC signals, it is possible to pick up data from individual neurons and distinguish each action potential. The disadvantage associated with this technique is, as the name implies, that it would be required to place electrodes within the brain tissue. This naturally introduces various risks for the subject. These risks, however, was considered worth taking considering the much larger resolution obtainable compared to non-invasive methods of neuro-analysis.

In order to understand the focus area, the following topics will first be described:

1. The neurological mechanism that are involved with cell damage during and following the stroke. This is referred to as *the ischemic cascade*
2. Neurological mechanisms related to stroke recovery.
3. Approaches for treatment of stroke: the acute treatment and the rehabilitation process (chronic phase).
4. Assessment of neurological function after stroke, with focus on describing the principles of IC measurements

The analysis of those questions will form the base for outlining a specific research question later.

1.1 Terminology

In this report, the word **acute** is used to define effects or actions with a duration of less then two days. For instance, the acute experiments, conducted as a part of this project and described later on, defines an experiment with a duration of a few hours, where no long term effects are registered. In opposite, the word **chronic** describes experiments and effects that are maintained over several days. **Acute**

treatment of stroke describes the direct treatment of the source of the stroke. The main purpose the acute treatment phase is to make sure that the patient survives with as few neurological injuries as possible. The **Chronic phase/Rehabilitation period**, in opposite, describes the longer lasting period following the acute phase, where the main objective is to reconstruct function loss caused by the stroke and prevent new strokes from occurring. The word **rehabilitation** describes actions taken in the post-stroke phase, in order to improve the patients neurological outcome. **Recovery** is defined as improvement in performance following a stroke no matter if this improvement is caused by rehabilitation or by other factors.

It is also of importance to define the terms ”experimental” and ”clinical”. The word **experimental** is used to define treatments that are still not fully investigated and/or treatments that are only used on few locations. **Clinical** treatment defines treatments that are considered normal clinical practice.

Chapter 2

Pathology of ischemic stroke

On a cellular level, the ischemic processes in the brain, following a stroke, is referred to as the "ischemic cascade". This process is initiated only seconds after a stroke.

Five different events have been identified as being related to neurological cell death. These are either the direct result of the lack of oxygen and glucose in the ischemic area, or triggered as a result of those first events. However, the process of revealing the entire cascade is still under development, and those five mechanisms possibly does not give the complete picture of what actually happens. [Doyle et al., 2008]

Those mechanisms are:

1. excitotoxicity, acidiotoxicity and ionic imbalance
2. oxidativ/nitrative stress
3. inflammation
4. apoptosis
5. peri-infarct depolarization

[Gonzalez et al., 2005] [Doyle et al., 2008]

In the following those mechanisms will be described separately, however it is important to notice that there is a lot of interactions between them. This also complicates the process of identifying the factors that actual causes a specific effect in the post-ischemic brain.

2.1 Excitotoxicity, acidiotoxicity and ionic imbalance

This event basically covers the primary effects caused by the lack of energy in the ischemic area of the brain. This process is therefore initiated when the cerebral blood flow decreases from its normal level, which is approximately between 10 and 50 ml/100 g/min [Davis et al., 2006].

In order for the neurons to propagate action potentials, the ionic pumps on the cell membrane must maintain the intercellular concentrations of Na+ and K+. The level of K+ must be high and the level of Na+ must be low in the intracellular space, compared to the level in the extracellular space. In order to maintain this disequilibrium it is important that ATP is available. ATP generation requires a large amount of oxygen. Since oxygen is not present in an ischemic brain, the neurons will start to depolarize. [Gonzalez et al., 2005], [Doyle et al., 2008]

The lack of energy also inhibits the cells mechanisms that regulate the intercellular calcium level. The amount of calcium in the affected cells will therefore start to increase. A lot of this effect is caused by glutamate dependent processes.

The depolarization leads to an increase in the release of a series of neurotransmitters. One of those is glutamate. An increased concentration of glutamate in the synapses causes calcium permeable ion channels to open, which increases the calcium level in the cells further. Acidosis caused by anaerobic metabolism in the stroke area initializes a glutamate-independent process that results in a further calcium overload. [Doyle et al., 2008]

This increase of calcium is thought to initialize a series of cytoplasmic and nucleus events [Dirnagl et al., 1999]. This activates a series of enzymes, which eventually leads to necrosis in essential cellular structures [Doyle et al., 2008].

2.2 Oxidativ/nitrative stress

Oxidative stress can be defined as an imbalance between the production of free radicals in particular, reactive oxygen species, and the organisms ability to defend itself against them [Alexandrova and Bochev, 2005]. In the ischemic brain, the level of Ca2+, Na+ and ADP will be increased. ADP is increased due to the fact that it cannot be transformed into ATP because of insufficient energy in the ischemic area. It is the level of those substances that causes the oxidative stress. The high quantity of those substances causes the cell mitochondrias to produce reactive oxygen species, like superoxide and hydroxyl [Gonzalez et al., 2005]. Brain tissue is vulnerable to those kinds of oxygen since it does not contain the level of endogenous antioxidant enzymes that would be required to neutralize this.

The reactive oxygen species will therefore damage the lipids, proteins, nucleic acids and carbohydrates in the brain tissue. This process is linked to ischemic cell death. [Gonzalez et al., 2005]

Another effect of ischemia is the generation of nitric oxide, a free radical. Combined with superoxide, a potent oxidant called peroxynitrite will be produced in order to modulate the effect of the free radical. The increased production of nitric oxide has been linked to DNA damage. [Gonzalez et al., 2005]

2.3 Inflammation

Inflammation typically happens on the onset of stroke, and are associated with stroke-related tissue damage. If the patient suffers from stroke as an result of arterial thrombosis, this process will initiate within the arterial wall as a result of the thrombosis. [Gonzalez et al., 2005]

However, inflammation is also triggered by ischemic stroke itself. An inflammatory cascade is initiated when a ischemic stroke occurs. Different inflammatory mediators will be released in waves by the immune system and the neurons and astrocytes in the brain. The first wave is initialized only minutes after the stroke occurs. During this the gene encoding transcription factors c-Fos and c-Jun are released. Those two factors belong to the immediate early gene family. Their purpose is to send generic information from the cell's DNA to the mRNA, and thereby provide a rapid response to stimuli. A second wave of the so-called heat shock genes are released 1-2 hours after the incidence. The concentration of these decreases again after a period of 1-2 days. A third wave of inflammatory mediators is released 12 - 24 hours after the incident. [Gonzalez et al., 2005] Even though the function of those processes are to protect the cells, there is evidence indicating that post-ischemic inflammation contributes to ischemic brain injury. However the actual reason for this has not been totally identified. [Dirnagl et al., 1999]

2.4 Apoptosis

Apoptosis is programmed-cell death. Many of the processes causing this depends on the enzyme caspases. Caspases are protein-cleaving enzymes that have the ability to dismantle proteins and enzymes with responsibility for cellular repair. Especially neurons are affected by this factor [Pendlebury et al., 2009]. [Gonzalez et al., 2005]

This effect must not be mistaken with necrosis, since necrosis is premature cell death while apoptosis is a natural occurrence. Even though this process is initiated by the organism, it is not necessarily beneficial. It has been showed by

several experiments, that a inhibition of the events associated with apoptosis will reduce ischemic injury. [Doyle et al., 2008]

A cells resistance to apoptosis contra necrosis depends on its type, age and location in the brain. In general, mild ischemic injuries causes more of its damage through apoptosis rather than necrosis, compared to major incidence. [Gonzalez et al., 2005] The caspases-dependent mechanisms behind apoptosis requires the presence of ATP. Therefore apoptosis mostly cause damage in the ischemic penumbra. [Doyle et al., 2008]

2.5 Peri-infarct depolarization

Peri-infarct depolarization can be defined as spontaneous self-propagating wave of electrochemical activity that propagates through the ischemic penumbra. It is assumed that this process is caused by the release of potassium and excitatory amino acids from the ischemic core. Similar spontaneous waves occurs in the healthy brain as well, and are associated with the regulation of a series of processes in the brain [Gonzalez et al., 2005]. However, since they occur with a much higher frequency in the ischemic brain, this might result in a accumulation of calcium in the neurons. [Doyle et al., 2008]

It is assumed that this mechanism causes a lot of the reversible damage in the ischemic penumbra to become irreversible. This however has only been shown in rat models, and the actual effect of this mechanism in humans still remains unclear. [Gonzalez et al., 2005]

Chapter 3

Neurological mechanisms involved in stroke recovery

This chapter will describe the neurological basis for stroke recovery.

When describing the overall elements in stroke recovery, two definitions are commonly used.

The **first definition** is associated with the terms two terms:

- True recovery
- Behavioral compensation

This is used to define the types of changes during the stroke recovery. True recovery of the motor function is defined as changes that cause the body to perform the same way as before the stroke. Behavioral compensation covers situations where a function is regained, but through a different motor program where other muscle groups, body angles and rotations are used to execute the movement, compared to before the stroke. Both elements increase the so-called "functional recovery" following a stroke [Murphy and Corbett, 2009]. It is, unlikely to obtain a 100 % true recovery after stroke, since neurons with highly specific functions will typically be lost. However, if the stroke is minor, there is a possibility to obtain relatively true recovery, since only a minimum of crucial brain tissue is destroyed during the incidence. Even though, recovery is typically made mainly with behavioral compensation. This can be done by using the plastic properties of the brain. [Murphy and Corbett, 2009]

The **second definition** is applicable when analyzing a recovery based on internal or external factors. The overall recovery is divided into two mechanisms:

- Natural spontaneous neurologic recovery

- Plasticity

The major difference between these mechanisms is that the neurological mechanisms involved in natural spontaneous neurologic recovery are unaffiliated of external influences, while plastic changes are caused by a external stimuli. Natural spontaneous recovery, initiate just after a stroke, will continue to be in effect for many months afterwards, although most of the effect from this mechanism is seen within the first six months. The mechanism include the processes of recovering partially damaged ischemic neurons, repairing of local edema, resorption of local toxins and enhancing local circulation in the stroke-affected area. [Bruno-Petrina, 2010] Due to the definition of the mechanisms, rehabilitation, which can be seen as an external influence, can only effect the recovery process through plastic changes. For that reason, plasticity is the most interesting element in relation to the focus of this project. This factor will therefore be thoroughly described during the rest of this chapter:

3.1 Plasticity

Plasticity, is the utilization of the brains synaptic plasticity. This means the brains ability to change structure and function. The plasticity mechanism has a much broader timeline than the spontaneous recovery, and can enhance the patient's function the rest of his/her life. This adaption is in general caused by maturation, learning, challenges from the environment or due to neural diseases [Filippo et al., 2008]. Since the neurons in the ischemic core have been permanently damaged, it is required that other brain areas captures the effects of the remaining inputs and thereby make a readaptation of the lost functions. [Selzer et al., 2006]

3.1.1 Types of plasticity

In general, there has been identified two ways where plasticity can influence the stroke recovery:

1. Neighboring area remapping
2. Connectivity changes

[Murphy and Corbett, 2009]

The first of those describes the local changes that takes place in the brain area close to the stroke. It has been shown that tissue neighboring the damage tissue

has been shown to undergo structural and functional remodeling after a stroke. They make foundation for the probability that this tissue partly takes over the function of the damaged tissue.

The second term describes multi-region changes. This builds on the fact that the neurons contributing to a skilled movement are typically spread over different parts of the brain. Those parts are connected in a network. In many cases it will therefore not be all the areas associated with a movement that are lost during the stroke. This makes it possible for the parts of the network not affected by the stroke to readapt. Redundancy in the neuronal processing is a factor likely to improve this readaptation. An example of this mechanism can be seen on Figure 3.1. This figure shows the activity associated with hand movements in the ventral premotor cortex in a squirrel monkey. The two pictures show the activity before and 12 weeks after a stroke is given in the hand representation area of the primary motor cortex. The visual representation is constructed by a computer algorithm, using signals from IC micro-stimulations made in the area, based on where this stimulation resulted in a movement. As it can be seen, a large number of differences in the representation areas exist on the two images. This indicates that even though the ventral premotor cortex physically is placed away from M1, it is still affected by damage caused in this area. [Frost et al., 2003]

An interesting element associated with connectivity changes is, that bilateral activation have been observed following a stroke. This implies that the opposite hemisphere has taken over some of the function from the contralateral site. Such findings are, however typically associated with large strokes, where the patients typically has little chance of complete recovery. [Dobkin, 2004], [Murphy and Corbett, 2009]

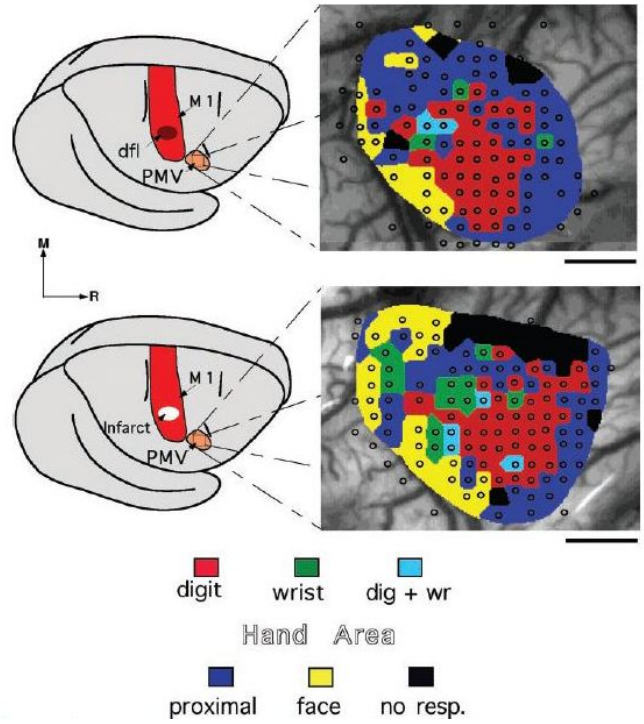

Figure 3.1: Activity in premotor cortex associated with hand movements, before and 12 weeks after a ischemic stroke is made in the hand representation area of M1. [Frost et al., 2003]

3.1.2 Time window of plasticity

Recent research has found that the plastic mechanisms after a stroke have parallels to the same plastic behavior that is present during the maturation of the nervous system. [Murphy and Corbett, 2009]

During this early brain development it has been found that many genes and proteins that influences neuronal growth are at a high level, compared to later in the persons life. It has been found that a similar increase of those substances are seen following a stroke. Those changes, however can only be observed in a limited time. The theory therefore is that during this period, defined as the ”critical period”, the brain is more adaptable to plastic changes. This element might therefore be linked with the initiation time of a rehabilitation process, as this theory suggest that relatively early rehabilitation in general might be more efficient. [Murphy and Corbett, 2009]

Figure 3.2 shows, what is found as the critical period of rehabilitation in a rodent study. In this period, a sustained upregulation of growth-promoting genes predominates can be seen. At the same time most of the growth-inhibitory genes are at a low level early after stroke, and increases gradually. The dashed lines corresponds to some genes that has been found to be regulated transiently during the early and "middle" part of the recovery period. [Murphy and Corbett, 2009]

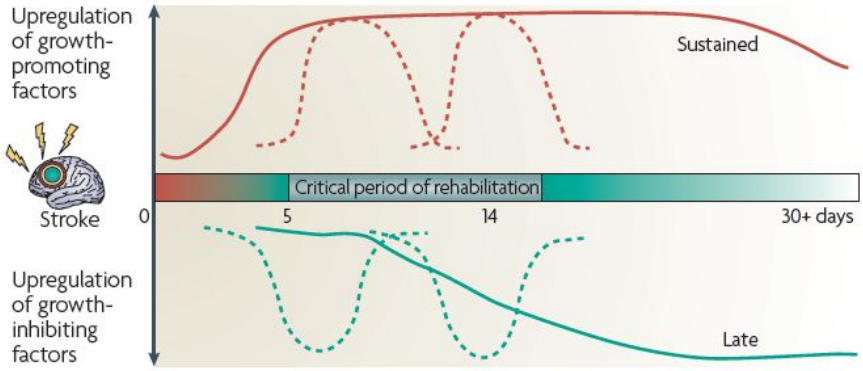

Figure 3.2: Regulations of growth promoting factors and growth inhibiting factors from a rodent study. The dashed lines represent some of the factors the tends to be transiently up- and down regulated. A critical phase of recovery has been identified based on the level of the factors. [Murphy and Corbett, 2009]

3.1.3 Mechanisms of plasticity

Today it is believed that the ways neurological changes in practice occurs in the brain, can be divided into two types: Homeostatic plasticity and Hebbian plasticity. [Murphy and Corbett, 2009]

Homeostatic plasticity

Homeostatic plasticity is a negative feedback mechanism, whose purpose is to keep the activity level of a neuron as close to a certain level or set point as possible. This set point is dependent on the activity of the entire neurological network. This prevent the circuit from becoming hyper- or hypoactive. [Turrigiano and B.Nelson, 2004]

After a stroke, interruptions can be seen in the synaptic activity, probably due to the loss of inputs from the affected area of the brain. Findings indicates that the homeostatic plasticity might be able to compensate for this, either by resetting the activity level of the working neurons or by triggering the formation of new synapses. Findings also suggest that this mechanism might be more activated

than normal at a certain time window, right after the stroke. However, results from some clinical studies indicated that a very early start of physical recovery therapy after the stroke might be harmful to the recovery. This might indicate that it is important that the brain uses this time window to reestablish itself, without too much intervention from the surroundings. This is, however, not fully investigated yet. [Murphy and Corbett, 2009]

Hebbian plasticity

In opposition to homeostatic plasticity, Hebbian plasticity is a positive feedback mechanism. This mechanism can be used to strengthen the connection between presynaptic neurons and postsynaptic neurons if this connection is commonly used. This mechanism is therefore a fundamental part of learning, since it can determine which neurological connections should be strengthened.

One of the most investigated processes related to this mechanism is the use of long-term potentiation (LTP). LTP is involved in when readapting is made during rehabilitation and when new movement patterns are learned and is by that reason of particular interest in relation to ischemic neural-damage. [Selzer et al., 2006] The strength between synaptic connection of two neurons, and thereby the ability to transmit signal between those, is basically affected by the LPT/LTD (long-term depression) relationship. LTP can occur when the involved neurons is stimulated synchronously. [Draganski and May, 2008], [Calabresi et al., 2003]. This process is therefore most likely used to encode new representations of movement skills. The basis of this kind of development lays in repeated associated inputs onto the neurons in motor cortex. When a skilled movement, like playing a tune on a piano, is performed, the neurons connected to the single movements become associated. If a specific movement pattern is repeated, the neurons involved and the neurons that are linked to them will represent this specific movement. [Dobkin, 2004]

Figure 3.3 shows an example of a training induced neurological reorganization. This figure shows the hand representation area of the motor cortex in a squirrel monkey before and after its digits has been trained. As it can be seen, both an increment in size of the areas that maintain the digits, and a general reorganization, in some ways like the one that could be observed on Figure 3.1, is present.

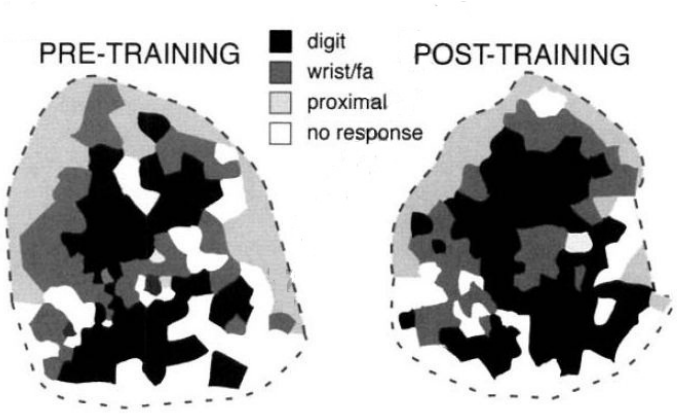

Figure 3.3: Activity in motor cortex associated with hand movements, before and after training of the digits. [Levin and Grafman, 2000]

A neuron can form a part of in several different movements. It is therefore the pattern of activation and not the activation of the single neurons themselves that can be used to characterize a movement. [Dobkin, 2004]. It has actually been shown that both the direction, acceleration and force used during a certain skilled movement might be determined for the firing rate of the neurons used during the movement. In order to make a strong movement pattern not only must the same skilled movement be repeated, but is must also be repeated with the same direction, acceleration and force. Sensory feedback has also shown to have an important effect on reshaping these motor abilities. [Dobkin, 2004]

The speed of this learning process can be modulated by the presents of various neurotransmitters. The sensitivity to those is affected by LTD. It has also been shown the more skilled the movement is, the stronger is the synaptic connection between the neurons responsible for performing the action. [Dobkin, 2004]

Besides from the two types of plasticity, an optimal rehabilitation must also consider the reward system of the brain. This system lies within the basal ganglia and is activated by motivations and feedback. It is connected to memory regions in the frontal lobe, so if a positive ”experience” is associated with a movement, this can contribute to the rehabilitation process. [Dobkin, 2004]

3.1.4 Plasticity in relation to the ischemic cascade

Although the neuronal plasticity related to stroke is typically described in the chronic context, another theory links mechanisms in the acute ischemic cascade closely with the brains ability to utilize its plastic properties. It builds on the theory that many of the processes in the cascade not only leads to cell death, but also induces changes in physiological plasticity meant to recover the brain function. [Calabresi et al., 2003] This theory challenges the traditional theory that sees the ischemic cascade as a mechanism where the brain damages itself due to a unforeseen situation. According to the new theory, a part of the purpose with the ischemic cascade is to abandon the damaged part of the brain, but at the same time lay the foundation for the most suitable environment for plasticity to occur, so a minimum function is lost during this.

Findings showing that the excitability around cortical ischemic lesions in the brain is increased supports this theory, since this can increase the brains ability to adapt to changes. [Calabresi et al., 2003] There has also been identified similarities between molecular mechanisms involved in LTP and mechanisms triggered by ischemic damage. For instance, in investigations of regions in the hippocampus it was found that LTP requires activation of calcium ions. As written in Section 2.1 on page 9, one of the main event related to the ”Excitotoxicity, acidiotoxicity and ionic imbalance”-mechanism in the ischemic cascades is a major increment in calcium ions. The nitric oxide balance has also been shown to be of importance, which is affected by the ”nitrative stress” mechanism during the ischemic cascade. This may indicate that the underlying purpose of plasticity and the ischemic cascade has at least some similarities. [Filippo et al., 2008], [Calabresi et al., 2003] If this theory is correct, it is possible to state that the process of synaptic plasticity adaption in the brain already starts on the onset of the stroke.

3.2 Summary

All this research only reveals parts of the overall mechanisms behind synaptic plasticity. However, if one assumed those findings is correct, it is possible to get a basic idea of the mechanism and how to take advantages of it. Based on the theory behind Homeostatic plasticity and Hebbian plasticity, both the brain itself and rehabilitation process plays a role in stroke recovery, and the time for initiation of training after stroke is probably of importance.

Based on its relation to the ischemic cascade it is logical to assume that the process is a natural response to ischemia and an integrated part of the brains regulation process. It can also be stated that such changes affect the whole brain and not only the local area, and that they can be achieved by skill-oriented repetitions of movement patterns. Positive response increases the learning rate

and can therefore be dependent for which movement patterns one develops after a stroke.

Chapter 4

Treatment of stroke

It is possible to divide the treatment of a stroke into the acute treatment of a stroke and the following chronic phase. The acute treatment phase is the period where the patient is hospitalized, diagnosed and given medicine to prevent further damage to the neural network. The chronic phase includes the rehabilitation process to obtain the best possible quality of life after the stroke.

Those two topics will be addressed separately in the following sections.

4.1 Acute treatment

The main purpose of the acute stroke treatment is to limit the neurological damage of the stroke and make sure that the patient survives. The strategy for fulfilling this differs from treatment site to treatment site. However some general guidelines for stroke treatment have been composed. In Denmark the "Danish National Board of Health" has made a report containing clinical guidelines and recommendations for treatment of stroke patients [Sundhedsstyrelsen, 2006]. This material is regularly updated. In the United States a panel appointed by the "American Stroke Association", a subdivision of the "American Heart Association", published a guideline for the management of the acute stroke patient in 1994 [Adams et al., 2003], which was revised in 2003. This has been followed by a series of short texts, containing research findings and recommendations related to the stroke treatment.

This section will therefore be based on these recommendations for an ideal practice in the United States and Denmark, instead of describing the actual clinical practice on a specific location.

The key issue to a successful acute stroke treatment lies in a rapid diagnosis. This is therefore an important issue in both the Danish and the American recommendations [National-Stroke-Organisation, 2009b], [Sundhedsstyrelsen, 2006]. The

following four steps must be addressed correctly in order to provide an optimal stroke treatment:

1. A fast identification of the conditions based on the warning signs
2. Rapid contact to emergency services
3. Fast transport to the hospital
4. Rapid diagnosis and intervention at the hospital

[Davis et al., 2006]

As it can be seen from this list, already in the pre-hospital phase the outcome of the stroke is affected by how fast the patient and his/hers surroundings recognize the early stroke symptoms and react on them. Such symptoms could be weakness or numbness associated with one side of the patients body, difficulty in speaking, balance problems or severe headache. As it can be seen by comparing with 1, the type of symptoms correspond to the type of possible long-term effects of the stroke. [Gan and Ramani, 2008]

After the warning signs have been recognized, it is important that the patient is rapidly transported to a hospital where the person can be properly diagnosed and treated. [Davis et al., 2006]

The infrastructure and process of receiving the patient on the hospital is also a vital part of this process. A streamlining of those elements is shown to reduce mortality by more than 20% and improve the functional outcome of the patient [Davis et al., 2006]. It is important that the patient, if necessary, is stabilized and that the stroke is diagnosed as fast as possible.

If a person is diagnosed within 3 hours of the incidence, it is possible to use the drug "intravenous recombinant tissue-type plasminogen activator" (rt-PA) in the treatment. This substance can cause the conversion of plasminogen into another enzyme, which dissolves an eventual blood clot [National-Stroke-Organisation, 2009b]. This has been shown to increase the blood flow in the ischemic penumbra and thereby improve the patient outcome if used in the proper time window. The use of this drug is therefore highly recommended worldwide and is recommended in both Denmark and the United States [Sundhedsstyrelsen, 2006]. In the Danish guidelines, however, it is clearly stated that such a treatment must not be initialized before intracranial bleedings has been ruled out by a CT or a MR scanning of the patient. The rate of symptomatic intracerebral hemorrhage is three-fold for patients receiving this treatment, but the therapeutic advantages outweighs this risk. The level of salvaged tissue is greatly correlated with the onset of treatment time, thus the benefits from the treatment diminishes the later the treatment. Although benefits from the treatment has been reported for up to

6.5 hours after stroke onset, the clinical procedure is not to administer this treatment later than 3 hours after stroke symptoms initiated [Butcher et al., 2003]. This is one reason why it is important to initialize the stroke treatment as rapid as possible. [Davis et al., 2006]

When the condition is diagnosed and the rt-PA treatment, if possible, has been initialized, the next step is to identify where the stroke is located in the brain and what kind of stroke it is. The first can be obtained by analyzing what kind of symptoms the patient has, and compare those to the properties of this specific brain area. Scoring systems will typically be used as a part of this diagnosis. In Denmark the use of a scoring system is considered important, especially the "Scandinavian Stroke Scale", SSS, is recommended [Sundhedsstyrelsen, 2006]. This scoring system gives a standardized measurement for the patients state of mind, including factors like consciousness, the patients ability to move parts of the body and speech problems.In the United States, the "National Institutes of Health Stroke Scale", NIHSS, is widely used as a standardized unit of measurement, included in a lot of the stroke related research and trials [Adams et al., 2003]. This scales score is similar to the SSS.

Hemorrhagic strokes have been shown to be difficult to distinct from ischemic strokes based on scoring systems. It is therefore important also to perform a CT or an MRI scans on the patient. If possible, this should be done within 24 hours of admission. This is used as standard both the United States and Denmark [Adams et al., 2003], [Sundhedsstyrelsen, 2006]. Many times patient data, scoring systems and scans can give an idea of the actual cause of the stroke, but this is not always the case. [Gan and Ramani, 2008] A cerebral angiography, where the blood vessels are visualized, can in some cases be used together with the imaging techniques in order to identify intracranial or extra cranial arterial occlusions that might have caused the stroke. This technique can be used to identify if an acute treatment will be necessary, in order to decrease the risk for more bleedings. [Adams et al., 2003], [Sundhedsstyrelsen, 2006]

A system, called the "Penumbra System", has been available for use in the United States since 2008 and in Europe since 2007. The system contains a series of mechanical tools that can be used to re-open blocked blood vessels, and thereby restore the bloodflow to the brain area. This system can be used within an eight-hour window since the stroke occurred and can allow revascularization of occluded vessels and helps restore the blood flow in the brain. Because of the enlarged time-window this method has some benefits, compared to rt-PA treatment. This system is not mentioned in the Danish guidelines or stroke literature. [Sundhedsstyrelsen, 2006]. [National-Stroke-Organisation, 2009b]

4.1.1 Stroke risk factors

The risk for a person to develop a stroke increases with the age. Males also have a larger risk of getting the condition than females. Other risk factors include increased blood pressure, smoking, alcohol abuse, obesity and the use or misuse of a series of drugs. If a person already has suffered from a stroke, the risk for another stroke is increased. [Hjernesagen, 2005]. Irregular cardiac rhythm, arterial fibrillation, diastolic blood pressure and abnormal visual field and/or eye movement are among the symptoms of the person suffering from a stroke. It has been discovered that 20-30 % of patients diagnosed with stroke actually suffered from another disease with similarities in their symptoms. This can be anything from brain tumor to migraine. [Gan and Ramani, 2008]

4.2 Rehabilitation of motor function after ischemic stroke (chronic phase)

After the acute treatment of the stroke, strategies must be initiated to prevent reoccurring stroke. This will most often include that the patient lifestyle changes. This is done according to the stroke risk factors. [Dobkin, 2003] [Hjernesagen, 2005]

In this section, some basis approaches and modern techniques for rehabilitation in general will be described briefly. This form the base for a more detailed description of the mechanisms that makes neurological changes possible.

The kind of rehabilitation applied depends on where in the brain the neurological damage has been caused. Since this report deals with stroke in motor-cortex, only rehabilitation of the musculoskeletal system will be addressed in this section. It is therefore mostly rehabilitation of hemiparesis that will be focused on. Hemiparesis patients can, beside weakness in their arm and leg, also suffer from complications like pain, spasticity and joint contracture [Dobkin, 2008].

Several professionals are involved in the rehabilitation process, including doctors with a specialization in the field, physical therapists, that can help the patient regain movements. [Dobkin, 2003]

Based on information about brain organization and synaptic plasticity, it is possible to divide the factors that rehabilitation training must affect into a series of functional categories:

- Skill training: When a new skill is learned, this will result in motor map reorganization.

- Strength training: An increase of strength is caused by an altered excitability of spinal motor neurons. Affects the rate of torque development and the discharge rate of motor units but not the organization of the motor map.
- Endurance training: Improves the person's fitness by affecting cardiovascular parameters. This does not change the organization of the motor map. This might in some cases however cause changes in spinal reflexes, depending on the particular task performed.
- Resistance training: This training increases the volume of muscle fibers as a responds to stress.

[Dobkin, 2008]

In order to understand the entire rehabilitation process, it is essential to distinguish between different training strategies, even though, in practice, it will probably not be possible to create any exercise that only affects one of those parameters. However, it is clear that all of those elements must be affected during of a rehabilitation program, for en enhanced results to show. For instance it is not effective help to train a person's strength in some part of the body, if the program for executing the desired motion has not been established.

Professor Bruce H. Dobkin, Director of the Neurological Rehabilitation and Research Program on UCLA and Editor-in-Chief of the journal "Neurorehabilitation and Neural Repair", has stated the following general guidelines for development of a post-stroke training program.

- Decide which impairments, disabilities and daily activities are reasonable goals for training
- Practice components of impaired movements of the affected limbs to achieve task-related actions
- Practice should be progressive in intensity and at levels of difficulty near maximal performance
- Improve strength and endurance

[Dobkin, 2008]

4.2.1 Approaches to rehabilitation of the extremities

It has been shown that task specific training benefits stroke patient more than healthy subjects. It has been proven difficult to present a quantitative effect of such training, but this might be a tool to improve daily function. [Dobkin, 2008]

In the upper extremities an experimental task-specific training method called constraint induced therapy (CIT) has been shown to give good results of improvements. The concept behind that method is to create as much ”true recovery” as possible, and minimize the ”behavioral compensation”. This is done by restraining the other side of the body so the affected side will be used as much as possible. The patient unaffected arm will therefore be placed in a sling and the patient must then perform intense training with the other side of the body. This is based on the theory that when the patients develop new movement patterns after a stroke, they will tend to exclude the affected side in those because of initial pain, slow and high-effort attempts related to the used of this hand [Dobkin, 2004]. [American-Stroke-Association, 2006] A number of robotic devices are also being developed for assisting in this type of rehabilitation.

Beside the technique mentioned above, other methods like virtual reality systems and the integration of functional MRI to provide feedback of the rehabilitation are also research areas in progress. This is only on the experimental level [Dobkin, 2004]

Chapter 5

Assessment of neurological function after stroke

When investigating the neural activity during and after a stroke, one of more standardized methods are usually used. These methods vary greatly in complexity, precision, availability and other factors such as risks involved. This chapter will introduce the principles behind *functional assessment* and *IC signals*. As mentioned in Chapter 1 on page 4, those are the methods selected for this project.

5.1 Functional assessment

In humans, scoring of mental and motor functionality is important for judging the amount of damage caused by a stroke. As mentioned in Chapter 4 on page 21 such scoring systems are commonly used in both Denmark and the United States. Later on, such methods also becomes a tool in judging the rehabilitation process. An example of those methods are the "Stroke Impact Scale". This scale uses 8 different scoring criteria, including mobility, memory and emotion, in order to make a complete evaluation of the stroke victims recovery [KU-Medical-Center,]. Other tests, like the "Fugl-Meyer" looks specific on motor function following a stroke. Along with scorecards, video recordings used for automatic analysis can be included in a behavioral analysis in order to obtain data related to motor-control [Gladstone et al., 2002].

The advantage of functional assessment is that often no specific equipment is necessary in order to make the analysis. This makes it possible to use this kind of method in most circumstances. The disadvantage is, that although the resulting movement is seen, the neurological firing remains a black box. This means that any neurological damage in the stroke area remains unclear - rendering this method insufficient in studies focusing on particular neuron behavior. This type

of analysis can therefore be excellent as supplementary analysis, but are not always fulfilling.

Not all techniques used for evaluation humans can a be applied on animals as well. Since no direct communication is possible with the animal, more behavioristic methods must be used in order to make a functional assessments. For an analysis of motor function, this could include a recording of precision and/or velocity of limb movement. Some assessment methods for rodents are described in Appendix A.

As an example of a behavioristic animal study of ischemic stroke, [Biernaskie et al., 2004] is worth to mention. This study aim to reveal the plastic changes due to rehabilitation using mainly functional assessment. This is focused solely on the chronic phase of the ischemic stroke. This study followed three groups of rats after they were given an ischemic stroke. Their rehabilitation process were then initiated at three different timings, (5, 14 and 30 days after a stroke), and the rats were then assessed during 5 weeks of rehabilitation. The rats performed a skilled movement during this process and their velocity and precision were recorded. This study indicated that an early initiation gave a faster velocity when performing the skilled movement test but also the least precise one. [Biernaskie et al., 2004]

5.2 IC Signals

IC signals or extracellular spike signals are signals recorded in the extracellular fluid. This method uses the electrical properties of the neurons. As the signal pathway progress through the neurons, each and every neuron cell will experience a depolarization generally from its dendrites onward through the axon of the cell.

To sustain a fast and reliable electrical conduction, some neuron axons are covered by myelin sheets. These myelin sheets, that are created by specialized neuroglia cells, wrap around the axons, increases the electrical resistance across the cell membrane and therefore function as electrical chord insulation. Between each sheet of myelin, there is a small uninsulated node called Node of Ranvier. This Node of Ranvier is the key to fast conduction in the nervous system. In non-myelinated axons, the conductance move in a wave-form through the axon as expected. But in myelinated axons, the conduction ”leaps” or ”jumps” from one node to the next. This way of conduction is also called saltatory conduction. As the cytoplasm of the axon is electrical conductive, the depolarization of one Node of Ranvier is sufficient to cause a depolarization at the next, hence the ”jump conduction”. This enables a much faster conduction. [Huxley and Stompfli, 1949]

As depolarization occur along the Node of Ranviers of the axons, it is possible to capture these using intracortical electrodes. The closer to the Node of Ranvier and the larger the neuron, the bigger will the recorded action potential be. As

axons from one neuron are placed in the vicinity of multiple others, the recording from a single probe is capable of capturing depolarizations from multiple axons. This method gives a uniquely high spatial and temporal resolution and is capable of giving neurological data on cell level in vivo. An example of a single channel extracellular recording setup can be seen in Figure 5.1.

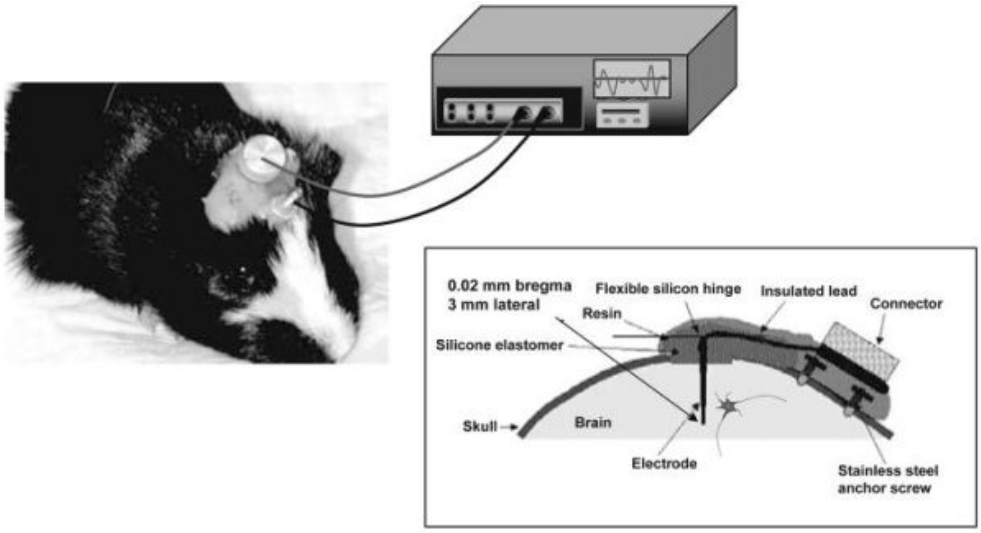

Figure 5.1: Illustration of an extracellular recording in a chronically implanted guinea pig using a Michigan electrode. This particular setup records signals from 3 mm lateral into the brain of the guinea pig. Notice the electrode probe is near a single neuron. In reality, each channel in the probe might pick up signals from multiple neurons which are distinguishable from each other depending on signal to noise ratio and signal processing algorithms. [Kim et al., 2007]

IC signals has previously been used in acute studies of ischemic stroke, such as [Jensen et al., 2006]. This study aims to reveal the acute mechanisms in the ischemic cascade during the first hours after ischemic stroke onset by investigating the spike activity in a rat using IC signals close to the infarct site. As described earlier in this chapter, the IC signals gives a very high temporal and spatial resolution within the brain tissue, giving a unique method to establish information about what happens on cellular level in vivo. [Jensen et al., 2006]

Chapter 6

Project hypothesis

Stroke is the third most common cause of death in the western world, and therefore naturally receives a lot of attention in the department of health sciences around the world [Selzer et al., 2006]. Within the context of ischemic stroke, we defined two categories:

The acute phase, which covers the self-repairing process that starts immediately after stroke onset with the apparent purpose to limit the damages caused by the stroke.

The chronic phase covers the following processes that utilize the plastic properties of the brain to help intact brain matter take over the functions that were lost during the stroke and acute phase.

Both phases are of cause interacting with each other, and a new theory described in 3.1.4 on page 19 and presented by [Calabresi et al., 2003], introduces the concept that the damaging mechanisms in the acute phase might actually have beneficial properties, and helps/boosts the plastic properties of the surrounding neurons in the chronic phase.

A key part to rehabilitation, in the chronic phase, is believed to be related to the plasticity of the brain, and it seems that there is a lot of knowledge hidden in the firing patterns during post-stroke rehabilitation. Earlier studies indicated a link between the onset of the rehabilitation process and the outcome. [Murphy and Corbett, 2009] [Biernaskie et al., 2004]

When human patients have suffered from stroke, the rehabilitation training process is initiated as soon as the patient is stable, usually after 48 hours. Some studies indicate that the natural spontaneous neurologic recovery, initiated just after a stroke, needs rest to function optimally. An earlier rodent study divided rats into three groups each with different rehabilitation onset (5, 14 and 30 days after a stroke). The results indicated that, although early initiation gave the best results in terms of how many times a movement was initiated by the rat,

later initiation gave slower but more precise movements. [Murphy and Corbett, 2009] [Biernaskie et al., 2004]

If these findings are valid, they could affect the way we look at rehabilitation. It might indicate that if the rehabilitation is initiated early, the individual will use other muscles or use the same muscles differently in a greater extent than otherwise. This is referred to as ”behavioral compensation”. On the other hand, later initiation may support ”true recovery”, where neurons are reprogrammed to take over the function of dead neurons.

Based on the previous chapters, it can be seen that a lot of research within the area of cerebral stroke, and rehabilitation has been carried out. Those studies are, however, mostly made with either a observational approach, where the subjects overall motor capability are analyzed, or by a biochemical approach, where findings builds on the ions, drugs or substances that can be registered in the brain at given times. Furthermore, it was evident that the studies that did use IC signals were solely focused on the acute phase. There seems to be a gap of information due to the lack of studies using methods with high spatial and temporal resolutions in chronic phase studies of ischemic stroke. This gab is especially significant according to the terms ”true recovery” and ”behavioral compensation”, as it has been found extremely difficult to describe the outcome of those mechanisms, based on behavioral observations. [Murphy and Corbett, 2009]

We speculate that a timing window within days after stroke influence the ability to receive ”true recovery” compared to ”behavioral compensation”, and a chronic rehabilitation process including IC signals might reveal more within the nature of the plasticity of the post-stroke brain.

The hypothesis of the present work is therefore:

The timing of the onset of the rehabilitation process affects the brain plasticity, hereby also the type of recovery in the chronic phase following stroke.

Chapter 7

Experimental design

The primary focus of the experiment was to obtain data of the recovery process of the two groups of rats. However, in order to evaluate a recovery process following a stroke, the effectiveness of the stroke model had to be verified. This was the secondary objective.

Before the experiment began, a series of pilot studies were performed in order to improve the practical skills of the project group, and to fine-tune various details associated with the conduction of the experiment. The pilot studies will not be described in this report, however, it will be mentioned, when results from these studies determined the experimental setup.

In the following section a description of the methodological choices for the experiment will be made. This is followed by a section with a strategy for obtaining the data. Finally the considerations behind the selection of the data-analysis will be described.

7.1 Methodological choices

In order to address the hypothesis, a wide range of data must be obtained. Based on the hypothesis and limitations stated, the following five elements are considered as cornerstones in the experimental design:

Animals: Since IC signals have been selected as the method to assess plasticity in this project, experiments must be conducted on animals. It was chosen to use rats, because they, compared to other animals, are relatively cheep, easy to work with and have been used in other similar intracortical experiments [Jensen and Rousche, 2006]. A total 24 rats were enrolled, with the purpose that only the 12 best were selected for electrode implantation.

IC signals: The electrode was implanted in the primary motor cortex of the animals in order to obtain IC signals. It was chosen to use a 16-channel tungsten micro-wire array electrode. This type of electrode was chosen because it can provide IC signals from a large area of motor cortex and because 16 channels is the maximum capacity of the equipment available for project group. This type of electrode has also previous shown good results in chronic rat trials, where IC signals have been synchronized with an event [Jensen and Rousche, 2006]. IC signals were recorded for each rat, before the stroke was applied (baseline recording), this ruled out a separate control-group, because each rat was their own control.

Two-group comparison: The rats in the experiment was divided into two groups, where the time of rehabilitation onset after a stroke is different. This makes it possible to associate the rehabilitation initiation time with differences between the two groups, observed during the experiment.

Reaching test: In order to detect changes associated with the rats motor function, it was chosen to correlate the IC signals with an "event" caused by a specific movement made by the rats. It was selected to let the rats perform a so-called "reaching test". During this test the rat reach out multiple times through a small opening in the cage in order to obtain a food pellet. An advantage of this method, is that the test requires no specific equipment, the movement is relatively identical from time to time and the rat can learn the procedure in a few weeks. This reaching test is basic setup similar to the one mentioned by [Biernaskie et al., 2004].

Stroke model: Based on experiences from the pilot study, photothrombosis (see Appendix E on page 99) with Rose Bengal injected through the tail vein was chosen as the most optimal stroke model. The electrodes were designed with a small tube, to induce the stroke through.

7.2 Strategy for obtaining the data

Figure 7.1 shows the analysis chosen to be performed, the purpose of the tests, along with the type of data collected.

	Focus area:	Data type:	Analysis:	Purpose:
Primary focus	**Evaluation of training onset**	IC signals + behavioral	PSTH-plots	Show trends in the data qualitatively.
			ANOVA	Statistically prove differences in the data
			PSTH Quantification	Quantify significant changes observed in the PSTH-plots.
		Behavioral	Pellet rate plot	Detect the rats improvements in the reaching test over time
Secondary focus	**Evaluation of stroke model**	Anatomical	Histology	Detect the size and location of the stroke
		Physiological	Micro-stimulation	Identify plastic changes caused by the stroke
		Behavioral	Cylinder test	Identify behavioral changes caused by the stroke

Figure 7.1: A description of the tests performed during the experiment. For the primary focus, the purpose is to observed if changes between the groups can be attributed to differences in true-recovery/behavioral-compensation between the groups. For the secondary focus, the purpose is to evaluate the effectiveness of the stroke model.

7.2.1 Flow of the data collection process

Figure 7.2 shows the final design of the overall experiment. As it can be seen each group is trained over 5 session, where IC signals are measured, and the last training session takes place 10 days after the training initialization. Since measurements were made in both groups at day 4,7 and 10 after the stroke it was possible to compare the groups according to both time and training. One of the 12 rats, selected for electrode implantation, died after surgery of unknown reasons. Therefore only 5 rats were included in ”group B”. The rats were randomly allocated to the two groups using (”www.random.org”).

It was chosen to perform the cylinder test as a baseline measurement before the stroke, post-stroke and after the last recording of the IC signals. The IC micro-stimulation test was only conducted as baseline measurement and after the last

recording, since micro-stimulation may effect the recovery process. Of practical reasons, only 7 rats were used for the micro-stimulation analysis (4 rats from group A and 3 rats from group B).

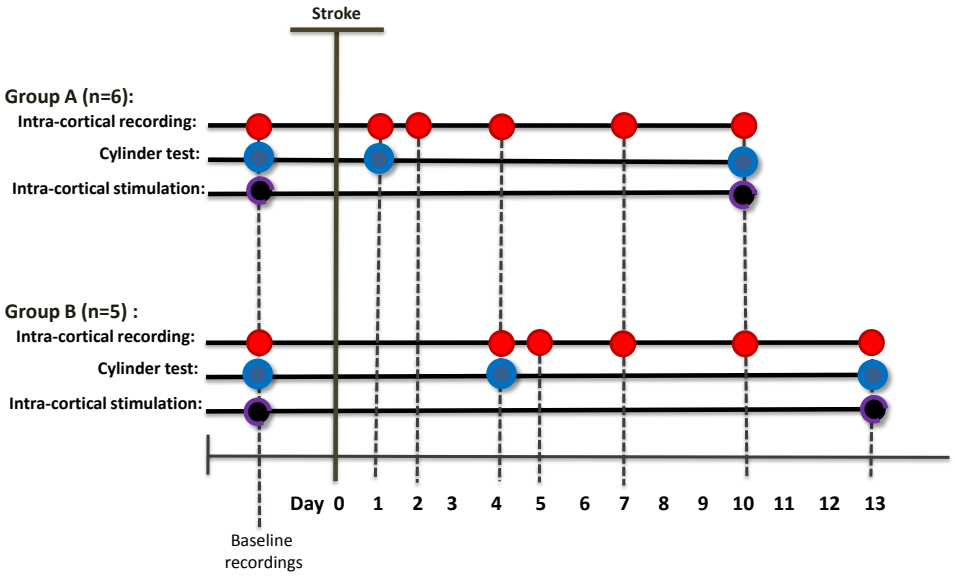

Figure 7.2: The overall flow in the experimental phase. Stroke was applied on ”day 0”, and on ”day 10” and ”day 14” post-stroke the experiment was terminated for the two groups, respectively.

One of the main challenges in the design of the experiment was to decide when the individual tests and analysis should be performed. Some of those practical challenges lies in the fact that the recording sessions themselves in the cage would train the rats, thus it is not possible to acquire IC signals from the rats in our experiment without training them.

The design was based on our pilot studies. During those studies it was discovered that the rats seemed unaffected by the stroke a few days after the stroke was induced, when looking at the behavioral data. Therefore it was assumed that the rehabilitation time following the stroke was relatively short. Based on this, it was decided to terminate the experiment within two weeks after the stroke. Five measurements was made with a maximum of three days between measurements and more frequent measurements in the first days after the stroke, since it was assumed that the largest changes would take place in this phase. The initiation time for the training after stroke, was selected to be one and four days for the two groups respectively. It was desired that one of the groups started on the rehabilitation as early as possible, since this strategy can be compared to the strategy of optimal 48 hours before rehabilitation used for humans. Based on observations during out pilot studies it was found that the rats must have at least one day to recover from the photothrombosis procedure. The initiation

time of four days after the stroke for "group B" was also chosen based on our pilot studies. Due to the quick recovery observed in the rats, it was considered likely that three days in difference between the two groups was enough to show significant changes in the neurological-plasticity, if such changes existed.

7.3 Selection of data-analysis

The following will give a brief motivation of the data-analysis stated in Figure 7.1. A more detailed description of the analysis will be given in the next chapter.

7.3.1 Data-analysis to evaluate the effect of the training onset

PSTH: PSTH, Peri Stimulus Time Histogram, was selected because it is a simple and useful way to obtain an overview of the IC signals. This method is used as indication for neuronal activity and timing syncronised with an event activity such as a skilled movement.

ANOVA: ANOVA was chosen because it is a commonly used method that can show differences between a series of factors. A main purpose of the ANOVA test was to identify if differences could be observed between the two experimental groups. It was also used to test a series of other relationships in the data.

PSTH Quantification: While the other analysis were standard methods commonly used in the literature, the PSTH quantification analysis was a method developed by the project group. The method was a mixture of subjective evaluations and quantifications, and was build on the PSTH-plots. When the PSTH-plot was described qualitatively, factors that represents the changes, were identified. It was then, based on subjective analysis, counted when those changes could be observed for the two groups. This method is of cause a major simplification of the neurological following a stroke. However, it was assumed that it could be used to identify some general tendencies and help define what can actually be seen on the plots.

Pellet rate: The purpose of the last method is to analyze the behavioral data, without correlation with the IC signals. This is important because this data, has only been used as a trigger point in the first three methods. The purpose of this analysis is basically to plot the behavioral results, and detect if any differences over time or according to rat can be found.

After each method was conducted, the results from the individual analysis was compared. This comparison was used to interpret the data in order to identify differences between the groups, that could be attributed to differences in the true-recovery/behavioral-compensation.

7.3.2 Data-analysis to evaluate effectiveness of the stroke model

The cylinder test: This test show if any changes in their behavior occurs over time as a result of the stroke. As a secondary purpose the test can also be used to indicate if the two groups of rats behaves differently from each other.

IC micro-stimulations: This was chosen to do cortical mapping and to identify plastic changes in the rats brain during the experiment.

Histological analysis: This analysis reveals the anatomical abnormalities after stroke, that helps evaluate how vast the stroke induction was.

It was assumed that those tests, when held together, can be used to describe the effectiveness of the stroke model.

Chapter 8

Materials and Methods

This chapter provides an overview of the choices regarding materials and methods used in this project. In some of the sections there is a reference to appendix for a more detailed description. The chapter is divided into the following sections:

1. **Data-collection:**
    - Animal preparation
    - Behavioral training
    - IC signals
    - Pellet rate
    - Ischemic infarct
    - Cylinder test
    - IC micro-stimulation
    - Histology
2. **Data-analysis:**
    - Evaluation of training onset
    - Evaluation of stroke model
3. **Documentation and management of the research**

8.1 Data-collection

8.1.1 Animal preparation

Approval for all experimental procedures was obtained from the Danish Committee for the ethical use of animals. 24 male Sprague-Dawley rats (weight of

350-400g at the time of implant) were included in the study. Advantages of using this race includes the fact that they are relatively calm and easy to handle.

The rats were 3-4 weeks old when enrolled and had a weight at around 100-120 g.

Inclusion criteria for electrode implant were:

- The rats had to be able to obtain at least 80 pellets with one paw during four consecutive training sessions (duration of 15-20 min each). This is to insure sufficient data to be collected during the rehabilitation process.
- The weigh of the rats must be above 350 g as pilot studies showed that the skulls of the rats were not developed enough to perform the craniectomy and implant the electrode.

It took approximately 45 days (16 training sessions) to reach the inclusion criteria and the 12 most suitable rats that had reached the criteria were enrolled for the next part of the experiment. To instrument the animals with IC electrodes, the animals were anaesthetized with a 0.5 ml subcutaneous injections of Ketamine (100 mg/kg), Xylazine (5 mg/kg) and Acepromazine (2.5 mg/kg). A dose of approximately 0.1 ml / 100 g body weight were given every hour to maintain the anaesthesia. A craniectomy was performed over the primary motor cortex (M1) related to forelimb movement (2-4 mm postal, 2-4 mm lateral relative to Bregma [Kolb and Tees, 1990]. A 16-channel tungsten micro-wire array (wire diameter = 100 μm, spacing 500 μm, depth 1.7 mm) was implanted after retraction of the dura, see Figure 8.1. Two stainless steel bone screws were placed outside the cortical implant area to both serve as ground reference points and mechanical stabilization points.

See Appendix for a more detailed description of the animal preparation (B on page 86), electrode manufacturing (C on page 90) and electrode implantation (D on page 94).

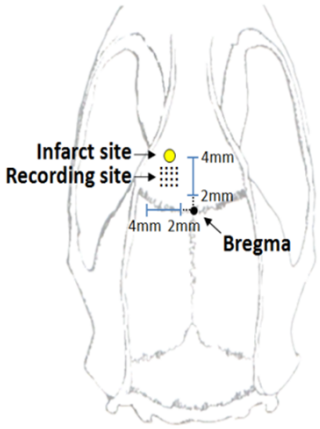

Figure 8.1: A 16-channel micro-wire array was placed in the M1 area in the hemisphere contra lateral to the rat's preferred limb. A 1.5 mm diameter, ischemic infarct was created anterior to the electrode.

8.1.2 Behavioral training

Prior to electrode implantation, the rats were trained to perform a reaching task. The movement the rat performs during this test can be seen on Figure 8.2. We used standard psychophysical techniques to train the rats to perform the reaching task in return for a food reward. Rats were kept food restricted and maintained on 90% of their normal body weight during the training and experiment sessions. Water was always provided ad lib and additional food was provided to maintain the target weight (above 350g). The training cage was a simple, square box where a 1.2 cm opening was place on one side. The pellets were placed at a distance of 2 cm from the opening. When the pellet was successfully retrieved by the rat, a new pellet was manually placed in the same position by the conductor of the experiment.

To provide synchronization between the reaching task and the IC signals, a photosensitive resistor was placed below the pellet, that provided a digital high signal when regular light hit the sensor, i.e. at the point when the rat was removing the pellet.

A manual trigger-button was also implemented, that a test conductor pressed when the rat caught a pellet. This input was used to eliminate false-positive inputs from the photosensitive resistor. This was to exclude events where the rat caught the pellet with its corresponding paw.

See Appendix B on page 86 for a more detailed description of the behavioral training.

Figure 8.2: The rat training to reach out for a pellet.

8.1.3 IC signals

The IC signals were filtered (800 Hz - 8 kHz) before sampled at 24 kHz (Tucker Davis Technologies). On-line spike detection was performed using a lower threshold at approximately 1.5 times the SNR of the raw data. The threshold was determined based on spontaneous motor cortex firing activity before the behavioral sessions began. All spike and synchronization timestamp data were saved for off-line analysis.

8.1.4 Ischemic infarct

A localized, ischemic infarct was created by activation of a photosensitive dye in the animal's blood stream. Rose Bengal dye solution (Aldrich Chemicals, 10 mg/ml saline solution, (0.3 ml/100 g body weight) was administered intravenously through a tail vein catheter while the animal was placed under general anaesthesia (same KXA dosage as described for electrode implant procedure). A fiber optic light source of white light (1.5 mm diameter) was lowered through a guide-tube to be positioned directly over the brain surface 2 mm anterior to the micro-wire array. The guide-tube was an integrated part of the electrode. The target location for the ischemic infarct was chosen to be the primary motor cortex (M1). The setup can be seen on Figure 8.3.

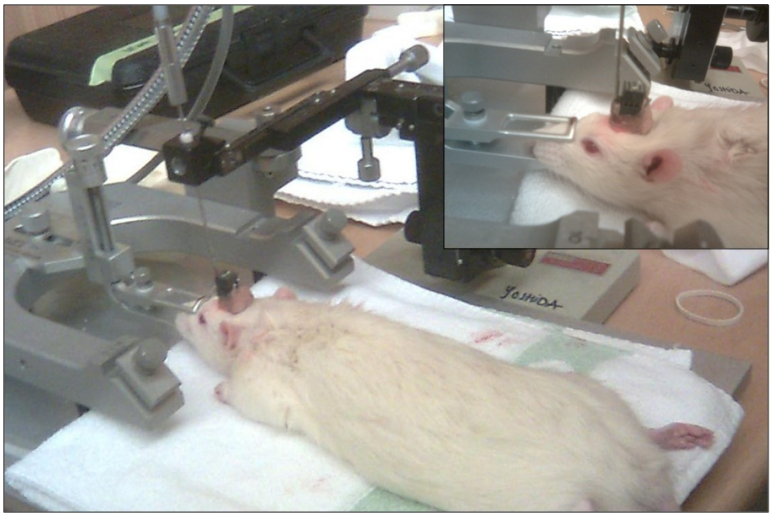

Figure 8.3: The setup during the photothrombosis.

See Appendix E on page 99 for a more detailed description of applying the stroke.

8.1.5 Cylinder test

In order to conduct this test, the rats were placed in a cylinder with the diameter of 20 cm, made of transparent plastic. A video camera was placed above the cylinder. Figure 8.4 shows a rat in the cylinder, placing the paws on the surface. The duration of the test was set to 5 minutes. During this time the rats behavior in the cylinder was recorded. Afterwards it was manually counted how many times the rat touched the surface of the cylinder with respectively its right and its left paw.

Figure 8.4: The rat reaching out in the cylinder.

8.1.6 IC micro-stimulation

When stimulating the various channels in the electrode and evaluating the resulting behavioral function, it is possible to get an idea of the cortical mapping throughout the electrode area. By performing cortical mapping analysis before and after stroke rehabilitation, it is possible to get an idea and indication of the plasticity changes occurred during the rehabilitation. The analysis was performed by connecting the ground wire of the rat and one of the channels in the electrode to a current stimulator. The rat was positioned in such a way that there were no support on the forelimbs. A 100 Hz stimulus train was then delivered. The pulse train had a duration of 200 μs. It was then detected if the rats made any involuntary movements. The amplitude of the pulses were regulated independently according to the specific channel and rat. Typically pulses with the amplitude of 150-200 μA was administered. If no response were seen, the amplitude were increased to a maximum of 350 μA. This procedure was repeated for all 16 electrode channels for each rat. If a specific movement could be seen when a channel was stimulated, this movement was qualitatively described as detailed as possible.

8.1.7 Histology

The rat brain was taken out, as a final task in this animal test. The brain surface was marked with dye approximately where the infarct was induced, this was done to make it more easy to identify the infarct after it have been formalin-fixed. The formalin-fixed brain were cut into a section of 4-5 mm, including the infarct site. This section was further paraffin-embedded to make very thin slices directly through the infarct site. A total of three cuts where made in the coronal plane. The first slice was made in the middle of the section. The second and third slice were made 1 mm anterior and posterior respectively to the first slice. The cut in the coronal plane made it possible to have the contralateral side as a control. The

slices were stained using H&E (hematoxylin and eosin), to give better contrast.

8.2 Data-analysis

8.2.1 Evaluation of training onset

Due to the nature of the synchronization techniques used during experiments, the data from TDT's recording software was imported into Matlab and was processed by an algorithm to ensure that all synchronization points were true positives.

Peristimulus Time Histogram (PSTH)

The PSTH is basically a summation of Perievent Raster plots, see Figure 8.5. This makes it much easier to evaluate how much more the neuron(s) is firing in different periods of the time compared to the event trigger. The PSTH is created by dividing the x-axis into small intervals called bins. All events throughout all repetitions within a single bin are summed which provides the total number of action potential firing within this bin-size. An example, based on the same data as the Perievent Rasterplot in Figure 8.5, with bin size ten milliseconds is shown in Figure 8.6.

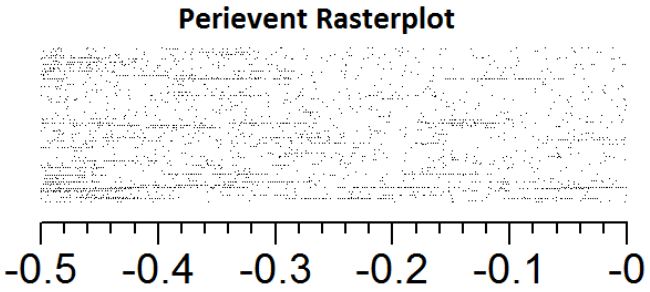

Figure 8.5: Perievent Rasterplot. Each row represents timing of neuron firing for this channel. Each row on the vertical axis represents one repetition of the experiment and the x-axis is the timing in seconds compared to the event trigger. In this example it seems like there is a bit more firing going on until around 0.25 seconds prior event trigger.

By looking at the PSTH plot in Figure 8.6 it is quite easy to see in which timing compared to the event trigger the activity peaks. This makes the PSTH a very powerful tool to analyze Spike Trains when dealing with some sort of event. The selection of bin size is very critical. If one selects the bin size too large, the time-dependent spike rate information is lost. If, on the other hand, one selects the bin size too small, the resulting PSTH fluctuates greatly making it

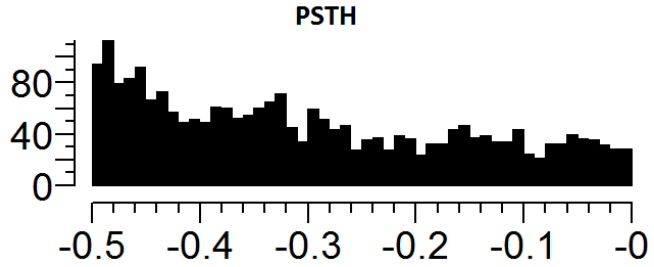

Figure 8.6: The PSTH shows the neuron firing tendency throughout the entire experiment length. The y-axis is the total number of spikes per second. The x-axis shows the timing compared to the triggered event (reaching for food). This example shows the neuron firing in the interval between the event trigger and 0.5 seconds prior, and has a bin size of ten milliseconds. It is based on the same data as the Perievent Rasterplot in Figure 8.5.

impossible to read the underlying spike rate pattern. There have been attempts at creating procedures for objectively choosing the optimum bin size, but it is still more common to subjectively choose the bin size that one see fits best for data-presentation. The procedure of choosing the optimum bin size objectively is further hindered as different experiment sessions and even the different channels in each session have different data and therefore different optimal bin sizes.

The procedure for constructing PSTH plots is:

1. Align all n spike sequences of period T with the event triggers.
2. Split the spike sequence period T into N bins of length λ.
3. Count all spikes from all spike sequences that fall into the bin i.
4. Plot a bar-graph histogram, where each bar is the bin size length, and the y-axis is number of spikes per second (in this example).

The bin size for all measurements was 5 ms, and the PSTH range was -0.5 seconds to 0.05 seconds, where 0 ms is the event where the rat grabs the food pellet.

Statistical characteristics and normalization

To normalize the PSTH data, the PSTH mean was calculated for each channel in each recording and subtracted from the PSTHs. This normalization reveals the inhibitory and exhibitory behavior of the spike trains, despite the threshold setting and signal-to-noise ratio varies for each recording session. This allows us to do inter recording and inter rat comparisons.

PSTH ANOVA

A 4-way analysis of variance (ANOVA) with two interactions was used to test the two group populations towards each other, as well as test how much other factors affect the data. The normalized PSTH from all channels were divided into five sub-periods: -500 ms to -401 ms, -400 ms to -301 ms, -300 ms to -201 ms, -200 ms to -101 ms and -100 ms to 0 ms, see illustration in Figure 8.7. The mean for all subcategories were then calculated and were then organized into a data-matrix with the following factors:

- Group - Which group the data belong to (A or B)
- Distance - Distance from stroke (2.0 mm, 2.5 mm, 3.0 mm or 3.5 mm)
- Interval - Which subperiod in the PSTH the data belongs to (-500 ms to -401 ms, -400 ms to -301 ms, -300 ms to -201 ms, -200 ms to -101 ms or -100 ms to 0 ms)
- Day - which number recording (0, 1, 2, 3, 4 or 5).

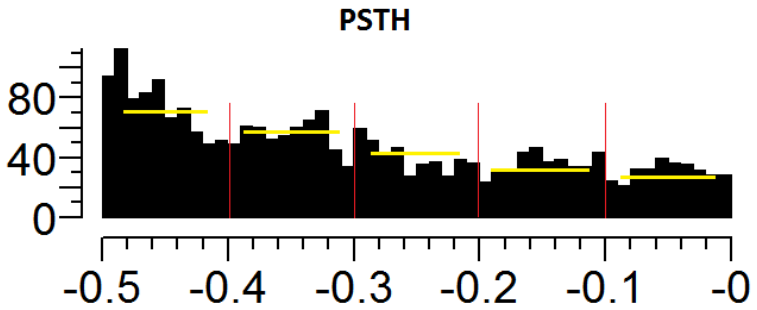

Figure 8.7: The PSTH shows the neuron firing tendency throughout the entire experiment length. The y-axis is the normalized number of spikes per second. The x-axis shows the timing compared to the triggered event (reaching for a pellet).

PSTH quantification

Based on the findings from the PSTH analysis, it was decided to use shift of peak and positive/negative changes in number of peak as factors for this analysis. The PSTH plots was averaged according to distance, and it was detected for all the data if one of those changes could be registered. A shift in peak was defined as a shift larger then 50 milliseconds.

8.2.2 Evaluation of stroke model

Data-analysis of the cylinder test

Quantitative analysis was used to evaluate the entire population of rats. The following two cases were used:

1. All the rats were seen as one population. This was used to indicate if any significant differences in the data can be observed before/after the stroke and before/after the rehabilitation.

2. The rats were seen as two populations, according to their experimental group. This can be used to show if any significant changes can be seen between the groups at the different stages in the experiment. It is most interesting to discover if the two groups behaves differently after the rehabilitation.

Two factors were analyzed: the amount of paw placements registered during the 5 minutes in the cylinder, and the ratio between the use of paws (primary paw/corresponding paw).

In the first case, where all the rats are seen as one population, no normal distribution can be assumed through the entire experiment. For the two first sessions, it might be justified to look at the rats as a normal distributed group. However, if the initiation time of the rehabilitation, as claimed, affect the functional outcome, then the rats will behave as two groups after the rehabilitation phase. Because of that, it has been chosen to use a Wilcoxon signed rank test on the data. This non-parametric test can be used on repeated measurement data that can not be assumed as normal distributed, and is therefore ideal for this situation. The method can pair data from two populations and test if the median differs significantly from 0, and thereby if the populations are different from each other.

When the rats are seen as two populations, a normal distribution can be assumed. A two-sample t-test were therefore used. This test also works on the hypothesis that the mean of the two groups are identical. In all tests, a 95 % confidence interval are used to show statistical significance.

Data-analysis of the IC micro-stimulation

The data from the IC micro-stimulation was analyzed qualitatively. A color-mapping was to used to represent the different movement registered on each single channel. This tool can be used to identify general trends concerning plasticity in the rats brains. Based on this analysis single rats and/or channels were withdrawn and their IC signals were analyzed individually, and tendencies in those recordings were compared with general tendencies.

Histology

The histology slices were analyzed qualitatively. Abnormalities near the stroke cite were identified, then categorized to be either caused by stroke or something else.

8.3 Documentation and management of the research

In order to document the experimental process and make sure that all relevant information concerning the rats were saved, a series of tools and routines were developed. Those are the following:

A data logging software for the rat training Data logging software was developed. This software was used to write down information concerning the state of specific rats on specific dates. After each session of rat training, comments concerning each rats performance were logged, as well as the weight of the rat. This can be used to:

- Evaluate how well the rat performs the ”reaching test”
- Ensure that the rat does not starve
- Document that the rats weight enough for surgery

An excel sheet containing basis information concerning each rat This sheet contained information regarding what paw the rat preferred to use, their status in the experiment, including which experimental group the rat was enrolled in, and general comments regarding their behavior. This document was used to provide the project group with an overall picture concerning the current status and whereabouts of each rat.

Routines for data management Those routines were developed in order to make sure that all the data from the experiment was saved in a consistent way. This included that all the experimental data was saved in specific folders regarding the rat, date, rat number and measurement.

A list describing the quality of the inter-cortical signal This sheet contained a qualitative measurement of the IC signals from each of the rat. Before each of the recordings during the experiment was made, each of the 16 channels in the electrode of each rat was examined qualitatively. The individual channels were, based on its signal quality and the amount of spikes, scored as ether: ”Dead”, ”containing few and/or small spikes”, ”acceptable”, ”good” or ”excellent”. This sheet was used to get an overall impression of the signal

quality, which was considered important in connection to the analysis and evaluation of the intracortical data.

Chapter 9

Results

9.1 Evaluation of training onset

9.1.1 PSTH Plots

Six PSTH plots and a description of the features registered on those is included in this chapter (from the rats A2, A3, A6, B1, B5 and B5). All plots and descriptions can be seen in Enclosure D on page 114. It was chosen to include those specific plots, because they contains features representative for the general observations. This section describes the PSTH obtained during the skilled movement. The PSTH plots are presented with each row representing one day of recording, and each column is the plot at a particular distance from the stroke site (2.0 mm to 3.5 mm). This means that each subplot contains four PSTH plots due to the fact that a 4x4 channel electrode was used for recording.

Rat A2

The PSTH for this rat can be seen on Figure 9.1. Two waves of higher activity is visible a Day 0; one at -450ms and one at -215 ms. The first day post stroke (Day 1), the two waves seems to have merged together but is still visible - an effect that is most noticeable in the channels furthest away from the stroke area. At Day 2 the overall activity is diminished, but the two waves are still visible. More hyperactivity is visible around 0 ms, which is probably due to withdrawal in the paw movement after the rat grabs the pellet. There is a lot of modulation from Day 2 to Day 4, where only one wave is visible (around -370 ms). This could indicate a behavioral rehabilitation process. Day 7 and Day 10 both show indications of true rehabilitation as the two waves also seen in Day 0 becomes more and more apparent again.

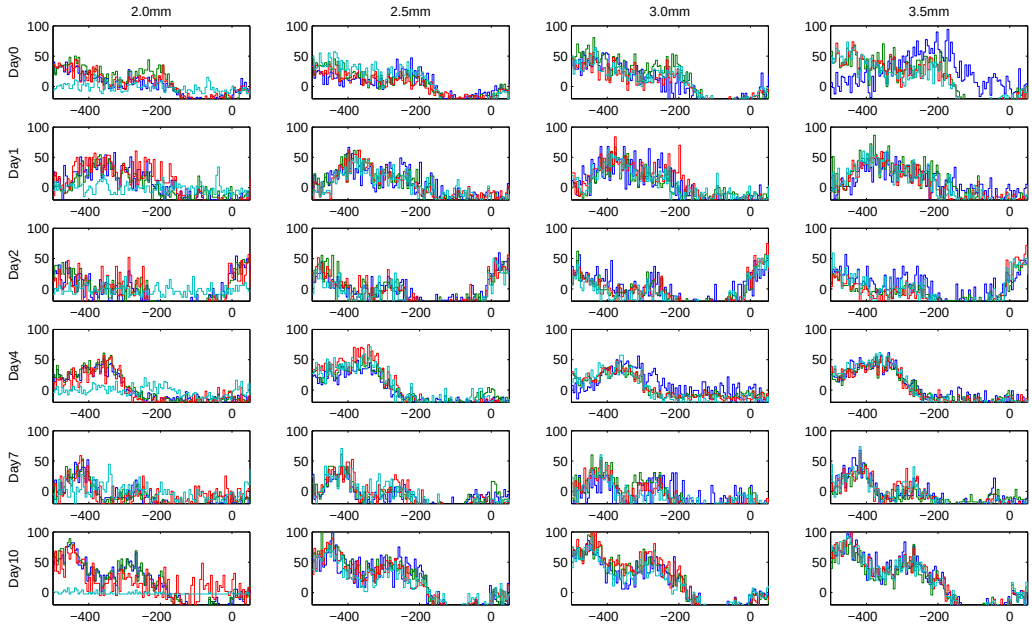

Figure 9.1: **Rat A2:** PSTH recorded during skilled movement pre- and post-stroke. The data is categorized into distance from stroke (2.0 mm - 3.5 mm) and time of recording session (Day 0 - Day 10).

Rat A3

The PSTH for this rat can be seen on Figure 9.2. In most of the channels at Day 0, there are two waves showing higher firing at -500 ms and around -200 ms. The channels listed under 3.5 mm from stroke are exceptions from this, since only one peak can be observed. At Day 1 after stroke higher frequency can still be observed, and it seems to be shifted a bit towards the trigger event and only one wave can be registered. This could indicate that the rat is using a different motor program during the skilled movement. The same patterns can be seen for almost all channels at Day 2. However, the single wave of excitatory spike activity is now smaller. At Day 4 there is a large wave of activity centered around -290 ms and the plot shows traces of hyperactivity around 500 ms prior the event trigger. At day 7 only one wave, centered around -500 ms, is seen. This wave remains until 'Day 10' where another wave around -200 ms occurs. This pattern is similar to the pattern observed on the baseline measurement at 'Day 0'. This indicates that the neural network associated with this exact movement is returning to the normal situation we see at the baseline measurement.

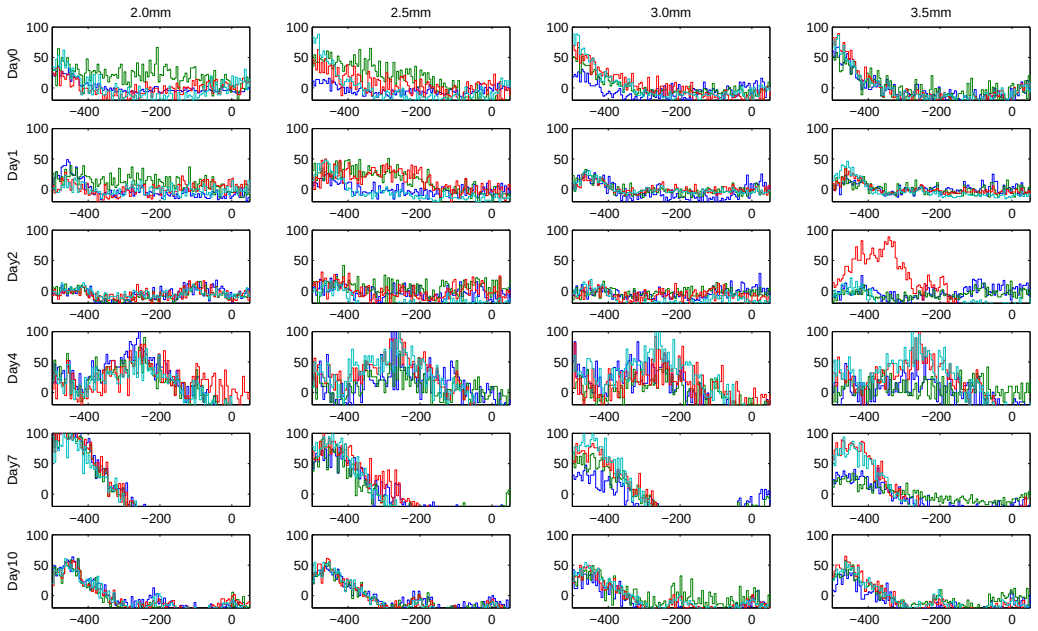

Figure 9.2: **Rat A3:** PSTH recorded during skilled movement pre- and post-stroke. The data is categorized into distance from stroke (2.0 mm - 3.5 mm) and time of recording session (Day 0 - Day 10).

Rat A6

The PSTH for this rat can be seen on Figure 9.3. The baseline measurements at 'Day 0' show higher activity i the interval -500 ms to around -200 ms. After stroke (Day 1), the activity close to the stroke area diminished to almost nothing. The rest of the channels still shows some higher activity just around -300 ms. At Day 2 this modulates as an enlarged activity from -500 to -200 ms has reemerged on all the channels. Interestingly, channel 13 (blue at 3.5 mm) where a characteristic activity could be observed at 'Day 0' is gone at Day 2 where it follows the general trend. At 'Day 4', a wave is visible again at around -300 ms. This modulates until Day 7, where the activity from -500 to around -200 ms is visible again and remains until Day 10.

Rat B1

The PSTH for this rat can be seen on Figure 9.4. 1-2 waves can be seen on the recordings from day 0. For all channels a wave placed around -270 ms is seen. In about two third of the channels, a second wave is placed around -400 ms. At Day 4, a large decrease in activity can be registered, and only a slight elevation is visible around -400 ms. Day 5 shows a larger activity around the -400 ms mark. At Day 7, still only 1 wave can be seen, but it now covers the period -500 ms to

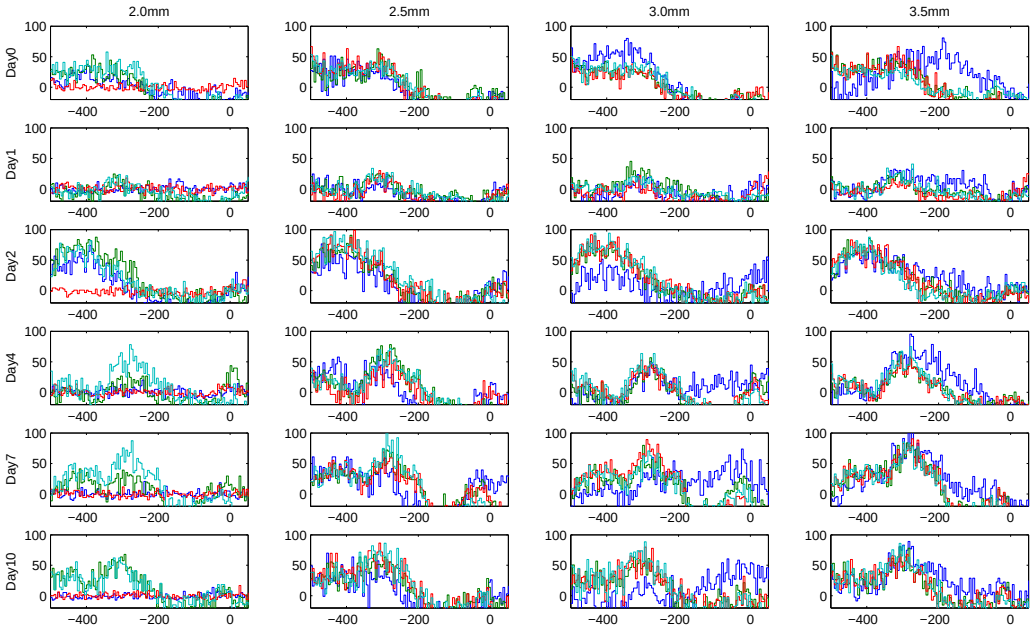

Figure 9.3: **Rat A6:** PSTH recorded during skilled movement pre- and post-stroke. The data is categorized into distance from stroke (2.0 mm - 3.5 mm) and time of recording session (Day 0 - Day 10).

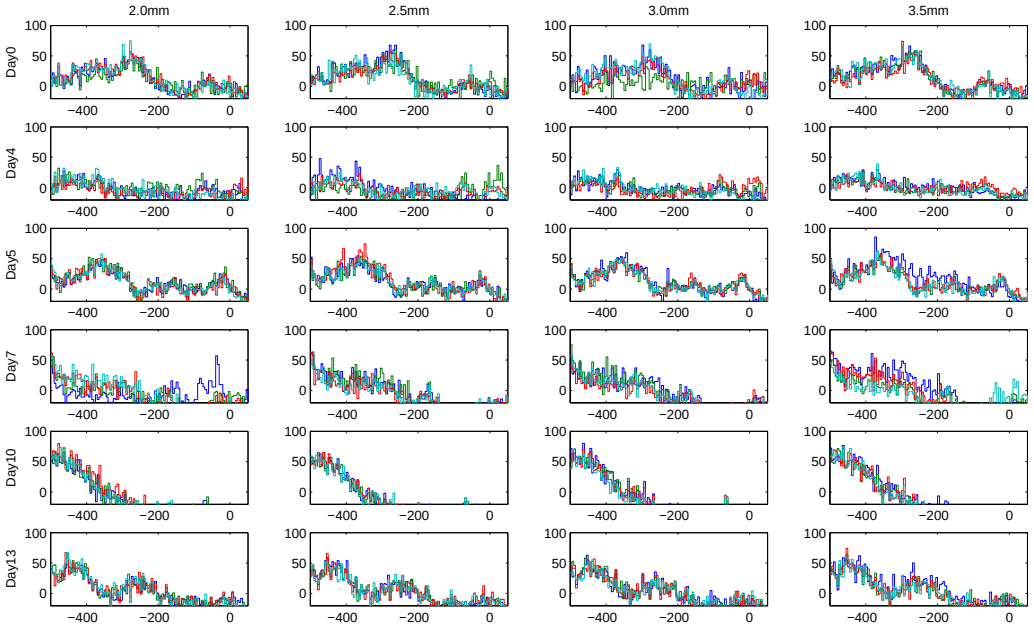

Figure 9.4: **Rat B1:** PSTH recorded during skilled movement pre- and post-stroke. The data is categorized into distance from stroke (2.0 mm - 3.5 mm) and time of recording session (Day 0 - Day 10).

-210 ms. At Day 10, this modulation changes. A shortening of the wavelength can be seen and a small peak around -270 ms is revealed. This pattern is most visible in the channels 2.5 mm to 3.5 mm from stroke. When analyzing the data from day 13, it indicates that this pattern might be the beginning of a returning to the patterns seen at Day 0, since two waves are now fully visible.

Rat B4

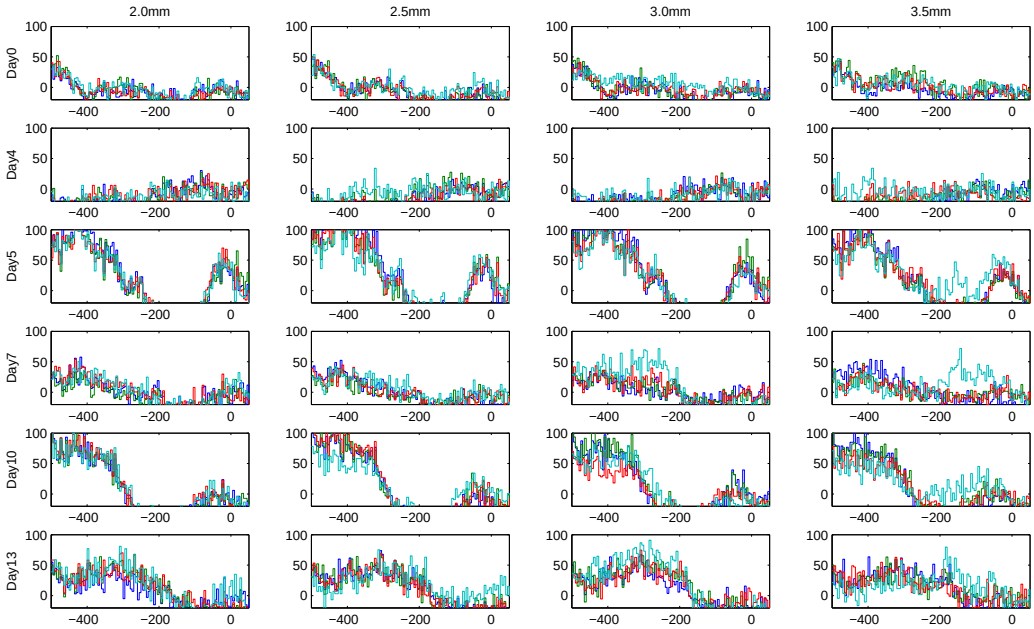

Figure 9.5: **Rat B4:** PSTH recorded during skilled movement pre- and post-stroke. The data is categorized into distance from stroke (2.0 mm - 3.5 mm) and time of recording session (Day 0 - Day 10).

The PSTH for this rat can be seen on Figure 9.5. At Day 0 two waves is seen; one at -500 ms and one at -300 ms. At Day 4 the wave at -500 ms is gone and a new is found at around -100 ms. It is further modulated at Day 5, where a large amount of activity is seen from -500 ms to -220 ms. At the same time another wave is seen around 0 ms. The wave around the trigger event is probably a result of the paw withdrawal movement. The same is seen at Day 7 and 10, but in Day 13 the pattern is slightly modulated back towards what we see in the baseline measurements.

Rat B5

The PSTH for this rat can be seen on Figure 9.6. At Day 0 there is increased activity between -500 and -200 ms, with a peak around -290 ms. One exception

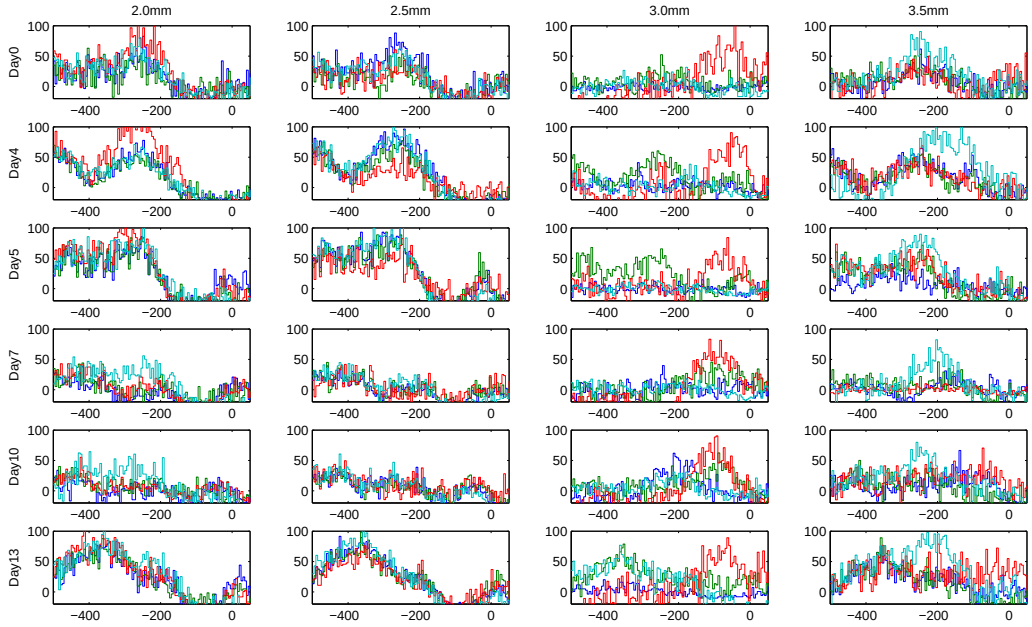

Figure 9.6: **Rat B5:** PSTH recorded during skilled movement pre- and post-stroke. The data is categorized into distance from stroke (2.0 mm - 3.5 mm) and time of recording session (Day 0 - Day 10).

is channel 11 (red graph at 3.0 mm) where a peak around -100 ms is seen. This particular neuron(s) does not change firing pattern throughout the experiment, and it due to its timing believed to be encoding for the withdrawal movement. At Day 4 two waves can be seen; one around -500 ms and one around -290 ms. The same pattern is seen on Day 5, even though it is not as distinct as the previous day. At Day 7 and Day 10, activity in same area can still be observed even though it is less evident. At Day 13 a wave of activity is seen from -500 ms to around -200 ms, like registered on the baseline recording. The peak of this wave is shifted compared to Day 0 and is now around -380 ms. This might indicate that the rat is performing the movement slightly differently then before the stroke.

Summary

All the rats has been analyzed the same was as the PSTH data described above. During those analysis, a major focus has been to describe the changes between the baseline recording at Day 0, and the recording from the last day. The outcome of this has been described as the ”rehabilitation modulation”. Changes that takes place during the single recordings have have been defined as ”process modulation”. Along with a description of the individual rats, a short description of the rehabilitation and process modulation is included in Enclosure D on page 114.

Besides a series of similarities, two overall differences were found between the group and their PSTH tendencies. For group A, a pattern similar to the baseline firing can be observed on the first measurement after stroke in three rats (A2, A3 and A4), and partly also on the following recording. The same behavior could only be observed for on rat in Group B (B5). Another apparent difference observed, is that features in firing pattern at the last day of rehabilitation is often similar to the baseline firing pattern in Group A. This is not the case for Group B.

9.1.2 PSTH quantification

The data from this analysis can be seen in Table 9.1 and 9.2 for respectively group A and group B. The complete sheet of data can be seen in Enclosure E. As it can be observed from the summations, it is difficult to detect any changes according to electrode for each of the groups and/or analysis. On the other hand, some interesting elements can be observed when one compares the changes in event over time.

Group A					
Distance to stroke:	**2**	**2.5**	**3**	**3.5**	**Total (day)**
Period	***Number of peak shifts***				
Baseline - Day 1	1	3	1	4	9
Day 1- Day 2	2	2	2	2	8
Day 2 - Day 4	3	1	2	2	8
Day 4 - Day 7	3	2	3	3	11
Day 7- Day 10	1	1	2	1	5
Total (distance)	10	9	10	12	41
Period	**Positive changes in peak**				
Baseline - Day 1	0	0	0	0	0
Day 1- Day 2	3	4	4	4	15
Day 2 - Day 4	1	1	2	1	5
Day 4 - Day 7	4	4	3	3	14
Day 7- Day 10	1	1	0	0	2
Total (distance)	9	10	7	8	36
Period	**Negative changes in peak**				
Baseline - Day 1	3	3	4	5	15
Day 1- Day 2	0	0	0	0	0
Day 2 - Day 4	4	4	4	4	16
Day 4 - Day 7	1	1	1	1	4
Day 7- Day 10	0	0	0	0	0
Total (distance)	8	8	9	10	35

Table 9.1: The three tables shows respectively shift in peak and positive and negative changes in peak for group A (Based on PSTH's averaged according to distance)

Group B					
Distance to stroke:	**2**	**2.5**	**3**	**3.5**	**Total (day)**
Period	***Number of peak shifts***				
Baseline - Day 4	3	3	3	2	11
Day 4 - Day 5	2	2	2	2	8
Day 5 - Day 7	2	2	2	2	8
Day 7- Day 10	1	1	1	1	4
Day 10 - Day 13	5	5	5	5	20
Total (distance)	13	13	13	12	51
Period	**Positive changes in peak**				
Baseline - Day 4	2	1	3	2	8
Day 4 - Day 5	2	3	2	2	11
Day 5 - Day 7	2	2	2	1	9
Day 7- Day 10	0	0	0	1	1
Day 10 - Day 13	1	1	1	2	5
Total (distance)	9	7	8	8	32
Period	**Negative changes in peak**				
Baseline - Day 4	1	2	3	3	9
Day 4 - Day 5	1	1	1	1	4
Day 5 - Day 7	1	1	2	2	6
Day 7- Day 10	2	2	2	2	8
Day 10 - Day 13	0	0	0	0	0
Total (distance)	5	6	8	8	27

Table 9.2: The three tables shows respectively shift in peak and positive and negative changes in peak for group B (Based on PSTH's averaged according to distance)

Figure 9.7 shows the sum of each of the elements over time for both groups. This is shown accordingly to number of recording session and time after the stroke.

It is possible to observe some general differences between the groups. For one thing, the shifts between increased and decreased number of peaks changes rapidly over time for group A. The same significant changes can not be observed for group B. When looking at the activity according to shifts, it is possible to make two conclusions, depending on if you observe the data according to number of recording session or to time. On the graph representing number of recording sessions, a significant peak can be observed for group B, a behavior not seen in group A. When looking at the graphs according to time, it can be seen that those differences might be due to the fact that no data recorded later then 10 days after the stroke exist for group A.

Even though those finding is based on a subjective quantification, it is likely

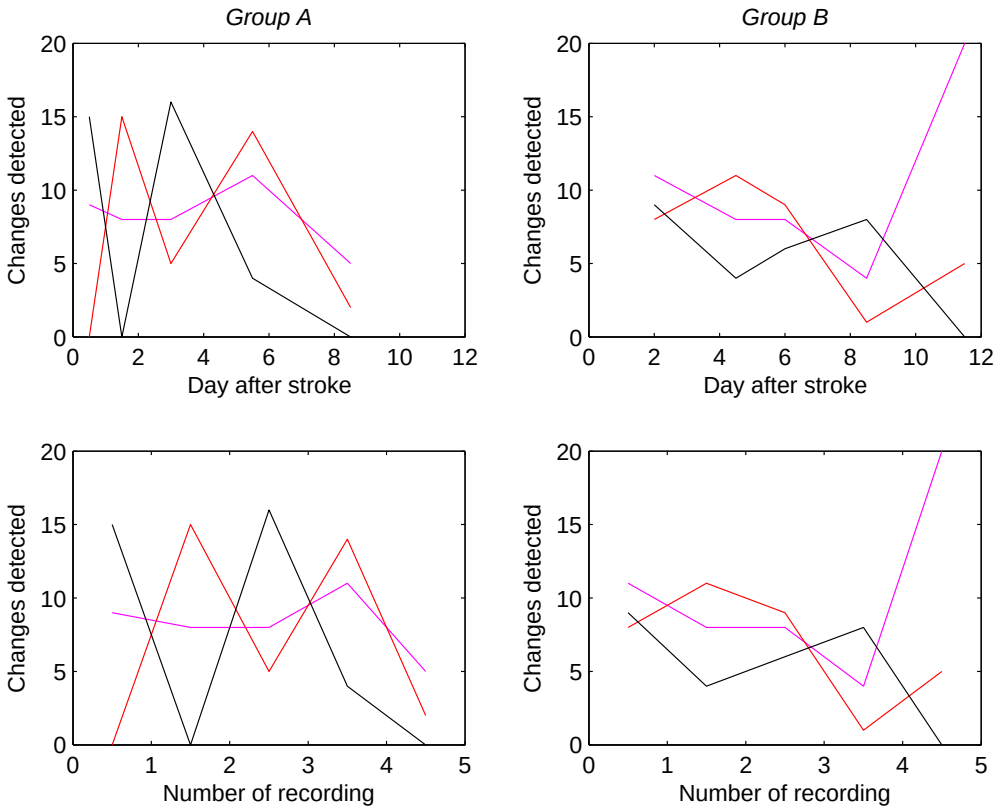

Figure 9.7: Graphs showing number of changes between recordings identified on PSTH plots, according to time after stroke and amount of training. Results from group A is shown to the right and group B is shown to the left. The read and black lines represents respectively positive and negative changes, and the purple line represents shifts in the peak

that those differences in some way can be linked to true-recovery and behavioral-compensation.

9.1.3 PSTH Mean ANOVA test

The Analysis of Variance results is seen in Table 9.3.

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob>F
Group	25026.2	1	25026.2	74.328	0.00000
Distance	5993.2	3	1997.7	5.9	0.00049
Interval	966132.3	4	241533.1	717.4	0.00000
Day	75331.9	5	15066.4	44.7	0.00000
Group*Distance	1929.1	3	643.0	1.9	0.12569
Group*Interval	2403.7	4	600.9	1.8	0.12890
Group*Day	10949.9	5	2190.0	6.5	0.00000
Distance*Interval	20214.3	12	1684.5	5.0	0.00000
Distance*Day	2204.4	15	147.0	0.4	0.96893
Interval*Day	103835.4	20	5191.7715	15.4	0.00000
Error	1753193.2	5207	336.6993		
Total	2976598.2	5279			

Table 9.3: ANOVA with constrained (Type III) sums of squares.

9.1.4 Pellet rate

Figure 9.8 shows the average number and 95 % confidence interval of pellets obtained each minute for each group of rats. The pellet rate is plotted against the training session. These results can also be seen in Table 9.4.

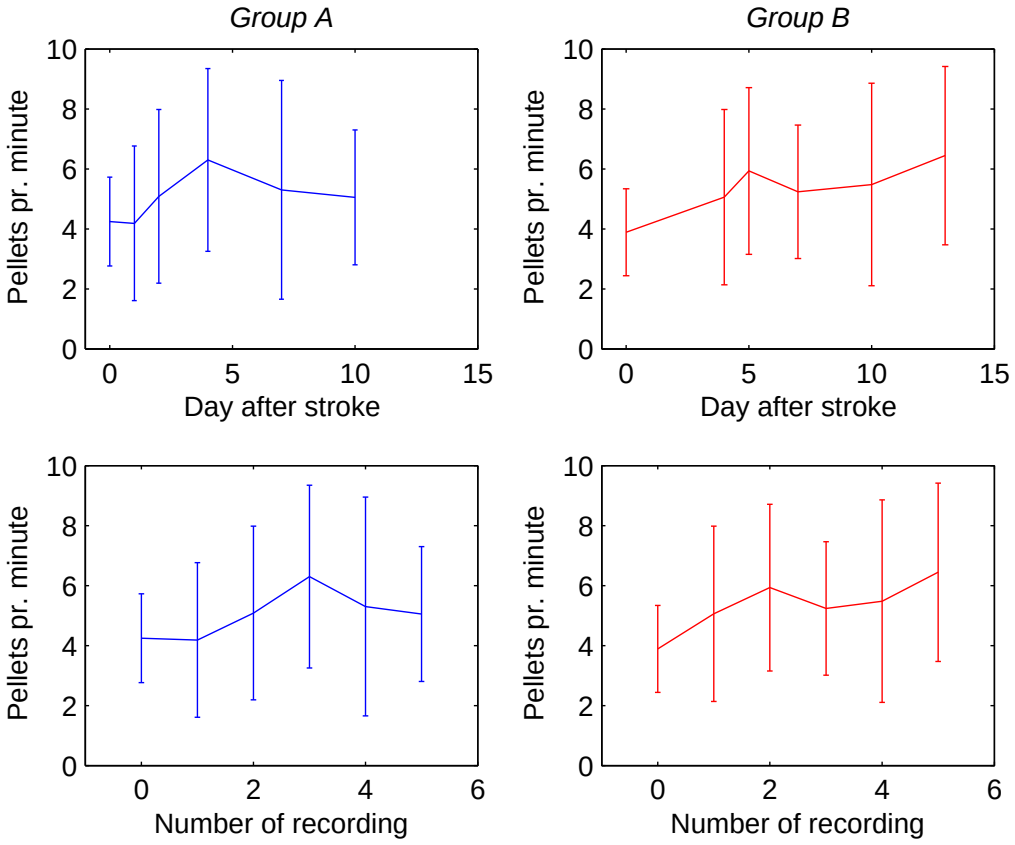

Figure 9.8: Graphs showing average pellet events pr. minute in the two groups. Plotted against date of recording and number of rehabilitation sessions.

Recording/Group	Group A	Group B
Baseline	4.25 ± 1.48	3.89 ± 1.45
1	4.19 ± 2.58	5.06 ± 2.92
2	5.09 ± 2.89	5.93 ± 2.78
3	6.30 ± 3.04	5.24 ± 2.22
4	5.30 ± 3.65	5.48 ± 3.38
5	5.05 ± 2.25	6.44 ± 2.97

Table 9.4: Mean-value and 95% conference intervals for pellets pr. minute.

As it can be seen from the graphs and the table, it is not possible to detect any

significant differences between the two groups or between the individual training sessions or day after stroke. It is, however, important to notice that the confidence interval in the baseline measurement is considerable smaller than the confidence intervals in the period after stroke. This may be an important factor, when interpreting the results. In Figure 9.9 pellet rates according to day can be seen for the individual rats. It can be registered that some of the rats in group A (A1, A2, A4 and to some extent A5) seems to follow the same pattern. A similar behavior can not be observed for group B, even though rat B1 and B2 have some similarities.

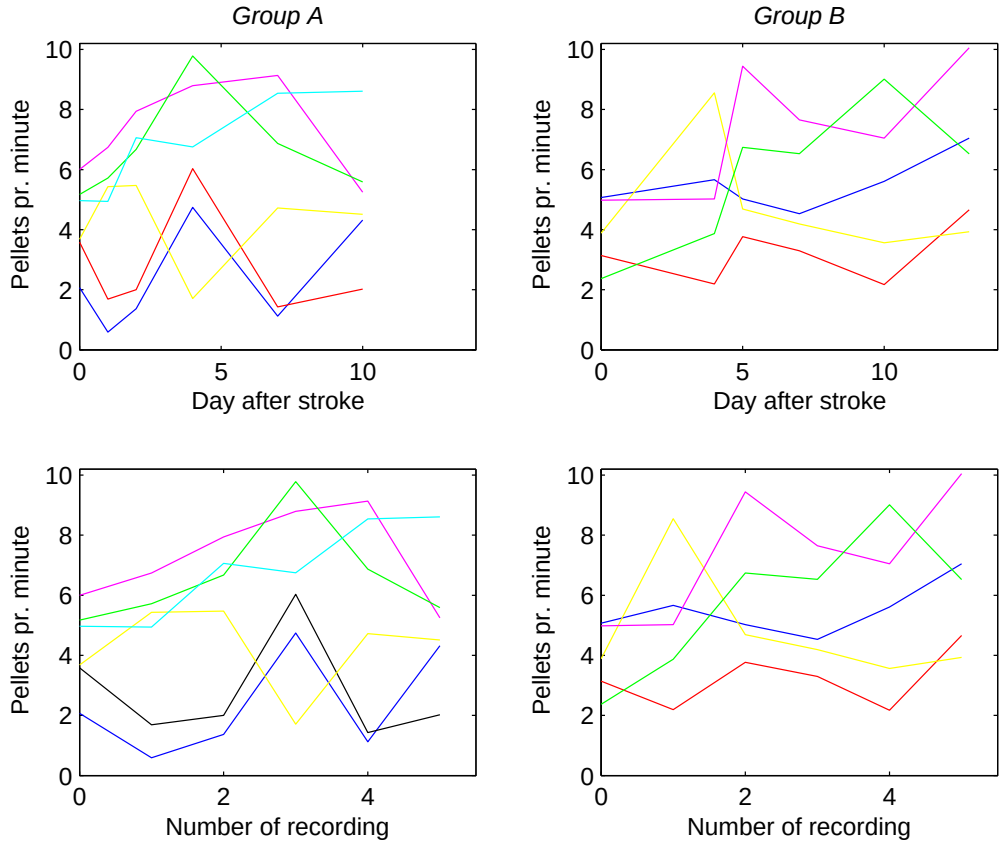

Figure 9.9: Graphs showing average pellet events pr. minute for the single rats in the two groups. Blue line = Rat A/B 1, red line = Rat A/B 2, yellow line = Rat A/B 3, purple line = Rat A/B 4, green line = Rat A/B 5, light-blue = Rat A6.

9.2 Evaluation of stroke model

9.2.1 Cylinder test

Qualitative analysis

Most of the rats used their paws almost equally during the test. A few of the rats showed major changes in their paw preference during the experiment. One of the rats (right pawed), for instance, has a 60.8% right-paw preference before the stroke. After the stroke this decreased to 11.1% and increased to 28.5% again after the rehabilitation period. It is important to notice that the rats, in some situations, only touches the cylinder a very few times during a training session. In one case, the rats did not touch the cylinder during the entire session.

Quantitative analysis

Changes during experiment. This analysis is made using the "signrank" implementation of the Wilcoxon signed rank test in Matlab. The results can be seen on Figure 9.5.

Data paired:	**Number of touches:**	**Use of paw ratio:**
Baseline and first day of rehabilitation	p = 0.0088	p = 0.012
Baseline and last day of rehabilitation	p = 0.20	p = 0.065

Table 9.5: P-values for Wilcoxon signed rank test on paired data. Number of touches in total during the cylinder test and the ratio between the use of (primary paw/corresponding paw). NB: Only data from 10 of the 11 rats were used i the paw ratio test, since one ratio from the last rat could not be calculated (division with zero)

With a 95 % confidence interval ($\alpha = 0.05$) it can be stated that there was a change in number of touches before and after the stroke, but that no significant changes can be seen during the rehabilitation period. In the same way is is shown that the ratio changes significantly during the stroke, but not during rehabilitation, even though the p-value is only a little higher than 0.05. It is, however, important to notice that only a few touches where registered during some of the sessions, which may have made the ratio more extreme.

Table 9.6 and 9.7 shows statistical data from the t-test. During those analysis it is tested if a significant difference between the two groups of rats in any phase of the experiment can be seen as two individual populations. As it can be seen on the p-values, this is not the case in any of the situations. However, the p-value for differences in total touches after the rehabilitation are relatively small. It is plausible that a significant difference would have been seen, if a larger number of rats has been used in the experiment.

Experimental phase	**P-values**	**Confidence interval**
Baseline	0.95	[-30.68:32.42]
Post-stroke (First day of rehabilitation)	0.34	[-19.20:7.40]
Post-experiment (After last day of rehabilitation)	0.073	[-16.27:0.87]

Table 9.6: P-value and 95% conference intervals from the two-sample t-test. It is tested if the two groups of rats in all phases of the experiment can be seen as individual populations based on number of total touches during cylinder test.

Experimental phase	**P-values**	**Confidence interval**
Before stroke	0.25	[-0.24:0.79]
Before rehabilitation	0.59	[-0.70:0.43]
After rehabilitation	0.45	[-1.03:2.10]

Table 9.7: P-value and 95% conference intervals from the two-sample t-test. It is tested if the two groups of rats in all phases of the experiment can be seen as individual populations based on the (primary paw/corresponding paw)-ratio during cylinder test.

9.2.2 IC micro-stimulations

In general, a few trends can be observed, when comparing the baseline mapping with the end-experiment mapping. One significant trend is that a lot of changes takes place, between the two mappings for each rat. Many of the same movements can be registered in the same areas on the recordings, but if one compare the active channels before and after, they are in most cases not identical. This effect can be caused by a movement in the position of the electrode, but compared with the increased counter-corresponding activity it is logic to assume that some significant plastic changes has taken placed in the area of measurement. The most conspicuous is the fact that the counter-corresponding body side in many situations react on the stimuli. Before the stroke, only one channel in one rat

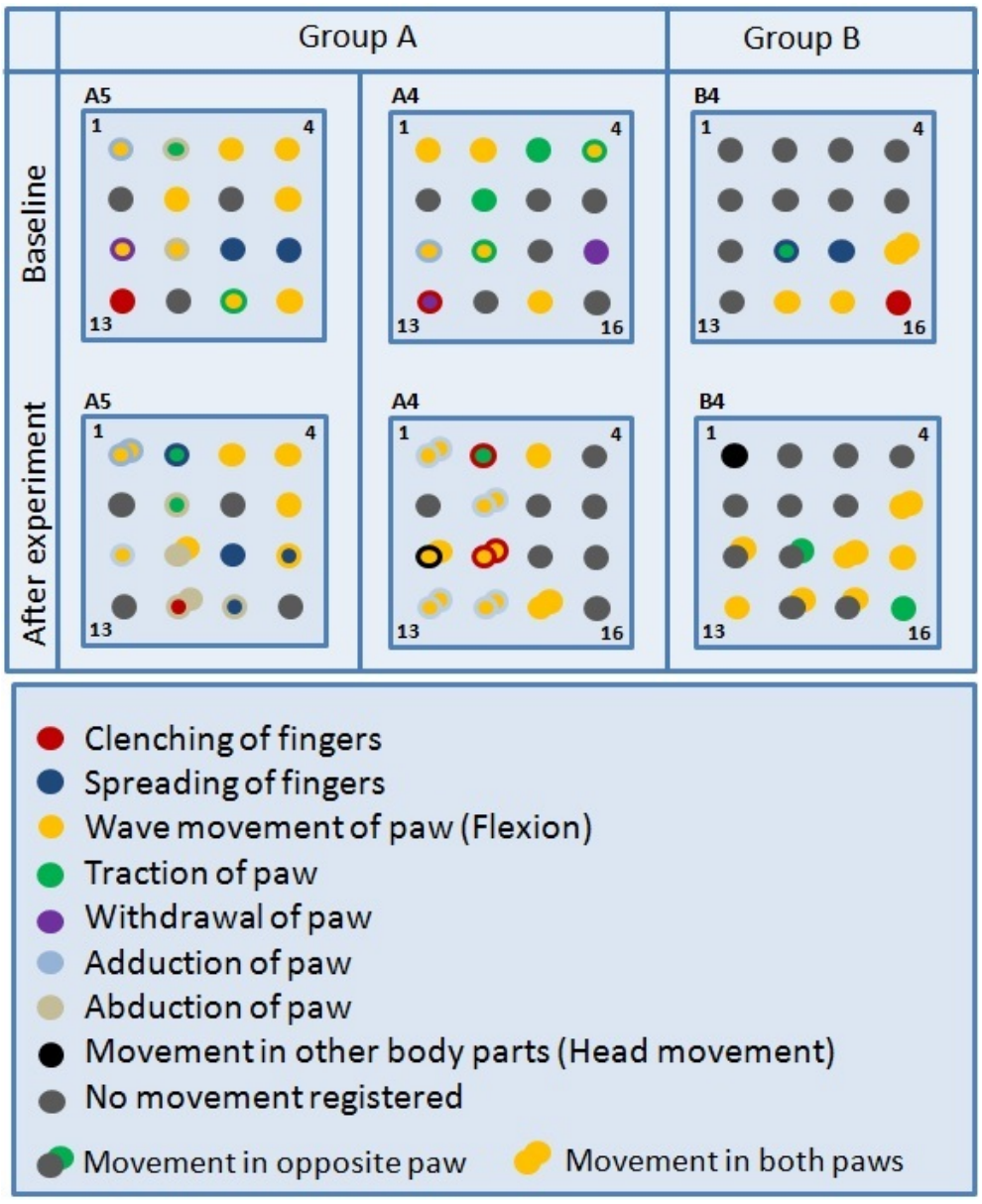

Figure 9.10: Results for selected rats from the electro stimulation. A5 represents a rat with only small plastic changes, A4 is judged as a rat with large plastic changes, and B4 shows a lot of counter-corresponding activity (movements in opposite paw)

could be associated with such a pattern. At the last recording this number has increased to a total of 25 channels, and was observed in all the rats.

Figure 9.10 shows the IC micro-stimulation mapping for three of the rats. Two of those was from group A, and the last one from group B. Those rats are shown because they each contains come distinct characteristics that makes it interesting

to analyze them further. The first of those rats, ”A5” is chosen because only a low degree of changes can be seen when comparing its two mappings. It is therefore interesting to discover if the IC signals from this rat shows a smaller change over time then the entire population. ”A4”, on the opposite possess a very large degree of modifications. Almost no common features can be seen when observing the two mappings. It will therefore be interesting to compare this rat with the entire population and with the ”A5”-rat, where only a small degree of modifications could be observed. The last rat, ”B5” were chosen because a lot of its activity were changed to counter-corresponding activity, during the experiment. It will be interesting to see how this is represented on the recordings. The data from the remaining four rats can be seen in Enclosure C on page 112.

9.2.3 Histology

As seen in Enclosure F on page 129 some of the brain slices are in bad shape. But it is possible to see abnormalities, in some of the other brain slices, that might be caused by stroke, for example as seen on Figure 9.11. However, it is difficult to determine if the abnormality is due to the stroke, or if it is where the electrode has been placed.

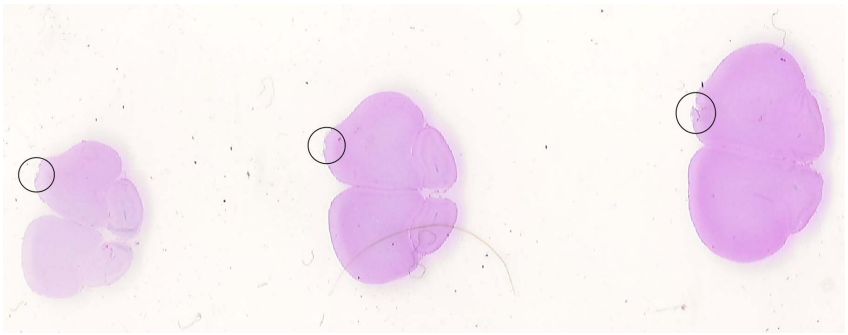

Figure 9.11: Three slices of brain from rat A5, right where the stroke site should be. The abnormality in each slice is marked with a black circle. ⟵ anterior

Chapter 10

Discussion

10.1 Methodological considerations

The rat training element of the experiment was in general executed as expected. Due to settings associated with the experimental setup, it was possible for the rats to vary the movements performed during the pellet reaching. This can be seen as both a weakness and a strength in the experiment. The negative aspects is that it makes it more difficult to make conclusions based on changes over time in the PSTH plots since each upper limb movement cannot be assumed identical. On the other hand, this setup gives a better simulation of the rats behavior, and changes in the rats motor function caused by the stroke as it allows for different postures during the movement, and behavioral compensation movements is allowed. In order to take fully advantages of this setup, it will, however, be required to make a comparison between the PSTH and the rats behavior. The videos made during each recording session could be a useful tool for such an analysis. This data have not been used in this experiment, due to the resources required to perform such analysis.

Although all the rats did have a primary paw, they could at times either use their corresponding paw or both paws to retrieve the pellets. To insure a consistent data-collection, we manually pressed a button each time the rat successfully retrieved the pellets, so that no false-positives event triggers were obtained. It would however be more optimal if the cage was designed in a way that prevented the use of the corresponding paw. This would also improve the data provided for pellet rate analysis.

Based in the consistency in the results, the overall method used in the data-collection phase has proven itself suitable. However it is important to keep in mind, that the channels in the electrode might change a little in position between the measurements. This might cause that the nero-signals recorded does not

always come from the same neurons. Another issue is the fact that the stroke is given in the border area of the primary motor cortex (M1). Since it is not possible to instrument the electrode at exactly the same position each time, it can not be precluded that the stroke, in some of the rats, affect mainly M1. This will of cause affect the rats behavior following the stroke. However, this source of error is difficult to eliminate, since the physical size of the M1 area is small compared to the surface area of the electrode and stroke tube diameter. The data obtained was timestamps of neuron action potentials which makes data-analysis more simple than using real continuous signals. The disadvantage is, however, that a threshold must be set at each recording session and this threshold is different from session to session due to the nature of neuron movement and time-dependant noise issues. If continuous signals were used, post recording-session data-processing might make it possible to extract more information from the signals.

A disadvantages in the experimental setup is the length of the experiment. Before the experimental phase was initiated, it was, based on the pilot studies, judged that the rats would have almost recovered within two weeks following the stoke. If one looks at the rats behavior only, this assumption seems correct. However based on the PSTH analysis, changes can be detected until the end of the experiment. It would therefore have been interesting to observe how the rats would behave later on after the stroke. Another element associated with the choice of measurement point are associated to the baseline measurement for all the analysis. It would have been relevant, if more then one baseline recording was made on each animal. In that way it would be possible to observe how large a variance that was present before the stroke was given. This could be used to evaluate if the detected changes actually are the result of plastic changes caused by the stroke, and how large a factor the variance is within the experimental setup itself.

10.2 Results

10.2.1 Evaluation of training onset

Qualitative PSTH analysis

The tendency for both groups is, as expected, that the neural firing timing in primary motor cortex, seems to slowly return to normal (baseline tendencies) during the rehabilitation process. Interestingly, it appears that the rats in group A generally return to what looks like baseline measurements around Day 7 and Day 10 and does it more than rats in Group B. In group B, two of five rats (B3 and B4, see Figure D on page 122 and D on page 123) seems to have similar PSTH's at Day 13 compared to Day 0. This is compared to Group A, where five out of six (A1, A2, A3, A5 and A6, see Figure D on page 114, D on page 115, D

on page 116, D on page 118 and D on page 119) does seem to return to pre-stroke patterns.

It was also found, for the data from Group A, that the baseline recording in many ways was similar to the recording from day 1. This might indicate that the early mechanisms from the stroke around the electrode array is very active the first couple days after which the firing pattern changes dramatically. No such tendency was found in Group B, which makes sense as the first recording post-stroke is made no earlier than 4 days after stroke.

PSTH Quantification

Based on the PSTH quantification, it was possible to quantify some of the tendencies registered on the PSTH plot. Based on a subjective evaluation, it was found that those changes follows a different pattern for the two groups of rats. Even though this analysis is associated with a large amount of uncertainty it is still likely that this observation can bring one closer to a understanding of the processes that takes place following a stroke, and to the changes that causes true recovery and behavioral compensation. It is however important to keep in mind that some of the observed changes in the IC signals can be a result of a minor dislocation of the electrode channels, rather then plastic changes. However since most of the channels in the electrode typically shows the same overall tendencies this in not likely. When analyzing the changes in shifts, one can make one of two conclusions, depending on if this factor is training depended or not:

1. The factor depends on training: A change in shift of peak values exist between the two groups. This occur late in the training process.

2. The factor does not depend on training: No significant changes can be observed, and it is expected that the value of shift for Group A would increase if the experiment had contenued.

PSTH ANOVA test

The ANOVA test reveals that there is a significant difference in Group, Distance, Interval, Day, Group*Day and Interval*Day, and no significance were found in Group*Distance, Group*Interval and Distance*Day. Keep in mind that the Day factor is 0-5 and is actually number of recording and not the day post-stroke. A different approach would be to check only the dates after stroke where both groups share the day (Day 4, Day 7 and Day 10).

That the group is a significant factor indicates that the qualitative and quantitative analysis of the PSTH plots were correct, as different trends were found

in the different groups. The distance to stroke is, not surprisingly, also a significant factor. The interaction analysis suggest that Day of measurement is the most important factor when comparing the two Groups, which corresponds to the different onsets of the rehabilitation process.

Pellet rate

It was found that the pellet rate alone could not be used as a factor to detect significant differences between the two groups or between the individual training sessions or day after stroke. The confidence intervals might help to interpret this result. For both groups, small confidence interval are seen for the baseline measurement, compared to the confidence interval in the later recordings. This might indicate that the rats react very differently to the stroke. The same tendency can be observed when one looks at the complete data-set for the pellet reaching, presented in Enclosure A. Some of the rats appears to be very affected by the stroke, while other rats actually improves their pellet rate after the stroke. The largest confidence interval is associated with measurement number 4, which might indicate that at this point are some of the rats are relatively recovered, while others are still very affected by the stroke. In general, this variety implies that a much larger amount of data would be required in order to make a conclusive evaluation based only on the rats pellet rate.

It is surprising that some of the rats actually increase their pellet rate after the stroke. A theory, that might explain this, is related to the increase in the growth-promoting genes following a stroke, described in Chapter 3.1.4. If the stroke is so small that the rats are almost unaffected by it, but an increased amount of growth-promoting genes is still released, then this might actually be a beneficial situation for the animal. In itself, this theory might be quite interesting, and worth to investigate further.

10.2.2 Evaluation of stroke model

Cylinder test

The analysis of the cylinder tests showed with statistical significance that the rats behavior in the cylinder changed during the stroke. Both the rate where the rats touched the cylinder and the (primary paw/corresponding paw)-ratio were seen changed. Even though it could not be stated with the desired 95 % statistical significance, the same studies indicates that the rats behavior in the cylinder might also change during the rehabilitation period. This can, along with the other supplementary analysis be used to validate the experiment. It can be claimed that the size of the stroke are big enough to affect the rats motor-control, and that the training possibly has an effect.

Based on the measurements, it is not possible to claim that the difference between the two groups rehabilitation process affects their behavior in the cylinder. However, the p-values of total touches during cylinder test where relatively low, and it is therefore likely that a larger population of rats would have given a different result.

In some sessions the rats were relatively unwilling to touch the cylinder. This might partly be associated with the fact that Sprague-Dawley rats in the literature have been described as generally unwilling to perform the cylinder test [Whishaw and Kolb, 2004]. However since most of the rats in general were willing to touch the cylinder several times (in average ≈ 35 times) this unwillingness is possibly caused by the stroke. No matter the reason, this decreases the credibility of the analysis made on basis of the (primary paw/corresponding paw)-ratio.

The first session for the rats in the cylinder, might have confused the rats and caused them to touch the cylinder more then they would have if they were more familiar with the procedure. Based on subjective observations during the experiment this factor is considered to most likely be of only minor influence.

IC micro-stimulation

Based on the results from the IC micro-stimulation analysis, it can be claimed that plastic changes takes place in the cortex mapping of the brain during and/or after the stroke. Since the results from the cylinder test indicates the same, it is evident to conclude that the used stroke model works. It is, however important to keep in mind that no micro-stimulations were made just after the stroke. The changes registered when comparing before and after measurements, corresponds to changes caused by both the stroke and the following rehabilitation. No general variations could, based on the qualitative analysis, be observed between the two groups. This test can therefore only be used to investigate the stroke model in general and not conclude anything concerning the onset for stroke rehabilitation.

Histology

From the histological analysis, it was found that some abnormalities could be registered on the brain slices. However, it is difficult to determine if those changes are a result of the stroke or other factors. This might partly be due to the method used to extract the brains from the rat. A lot of force was required for this process, and a few of the brains were partly damaged. Other methods should therefore be reconsidered if such an analysis was to be repeated. Another element is related to the fact that the members of the experimental group are not experienced in analyzing histological data. It was therefore difficult to determine if a change was caused by the stroke, or by the placement of the electrode. It would therefore be

recommended that more experienced specialists analyzed this data. This has not been done so far, because this area of the experiment are considered to have low priority.

Chapter 11

Conclusion and future work

Conclusion

During this project, an experiment has been conducted in order to identify changes in brain plasticity and type of recovery, according to onset of rehabilitation following a stroke. The motivation for this was bound to a theory that an early rehabilitation might be related to a large level of "behavioral compensation" and would thereby not necessarily be beneficial in the long term. If this theory is correct, it would contradict current clinical practice.

An rat-model based experiment where IC signals were correlated with behavioral data was conducted. PSTH plots from this correlation were analyzed both qualitatively and quantitatively, and was used as input for an ANOVA test. The pellet rate itself was also analyzed. In order to validate the used stroke model, cylinder tests, IC micro-stimulations and histological analysis were made.

The project hypothesis can overall be divided into two parts. In order to answer this hypothesis, the two parts will be answered individually:

11.0.3 The timing of the onset of the rehabilitation process affects the brain plasticity

Based on the ANOVA analysis conducted, it is possible to state that there is a significant difference between the two groups of rats enrolled in the experiment. Since no other factor than the onset of rehabilitation differs between the groups, it is likely to assume that this difference is the result of plastic changes. Findings from the pellet rate analysis, PSTH qualitative analysis and the PSTH quantification further indicates that different patterns between the two groups can be observed in the period after the stroke. Since this can be observed in both

behavioral data and in neurological activity for the rats, it is likely that those differences originates in plasticity changes between the groups.

A positive answer can therefore be given to the first part of the hypothesis.

11.0.4 Hereby also the type of recovery in the chronic phase following stroke

As mentioned above, different patterns between the two groups of rats can be associated with both behavioral data and neurological activity. In general, the rats in Group A responds in the most homogeneous way after the stroke, while no general patterns can be observed when comparing the single rats in Group B. This might indicate differences in ”true recovery” and ”behavioral compensation” between the two groups. When investigating the PSTH for the two groups, it can be seen for five out six rats in group A, that the PSTH plots seems to have turned back to a similar appearance when comparing the baseline recording with day 7 and day 10. For Group B this behavior can only be observed for two out of the five rats, and is not seen before day 13. Based on that, it is possible to claim that a higher degree of ”true recovery” has taken place in group A compared to group B. This claim is based on the assumption that identical patterns in neurological activity for the individual rats corresponds to a similar movement pattern.

In general the main conclusion to the second part of the hypothesis is that an earlier onset of rehabilitation is associated with a larger degree of ”true recovery”. This might be reflected in a more controlled and homogeneous rehabilitation period according to both physical behavior and neurological activity. Those findings support the current clinical practice and is in contrary to the theory that a staggered onset of rehabilitation promotes ”true recovery”.

Future work

This section will brief summarize our recommendation for future work in a similar research setup as ours.

Future work could possible include biomechanical sensors on the animal body or the use video-analysis more widely, this would enable angles, velocities and accelerations to be measured and evaluated. These measures could give a more precise view of the behavioral recovery.

More baseline recordings would give a clearer view of the variance of the signals without the influence of stroke/recovery. This can be used to evaluate if changes between the recordings represents actual changes or uncertainties associated with the experimental design.

It could be interesting to correlate our results with the level of growth-promoting and growth-inhibitory genes. It was found that those factors might be associated with changes in time on the PSTH plots, and thereby with plastic changes. Any correlation would also help to link ”true recovery” and ”behavioral compensation” closer to a time window for rehabilitation.

Our results plus future reach would give a better understanding of the effect of stroke on motor patterns. This may assist to a better understanding of the plasticity mechanisms involved in the time onset of functional recovery. If the results of a more extensive study are consistent with the findings in this report, this might lay the foundation of a more evidence-based approach, according to rehabilitation onset following stroke.

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Appendix

Appendix A

The rat as a model of a human

This appendix describes the primary motor control differences between rats and humans. Differences in skilled movements, limbstructure (forelimb) and motor and sensory brain areas are topics that will be addressed.

Compared to primate studies, rat studies are in general cheaper. The cortical structure of rats appears to be simpler than that of primates, so one may argue that if signal patterns cannot be found in rats, it would be very difficult, if not impossible in primates [Kolb and Tees, 1990]. This makes rats good candidates for early research in many areas, including ischemic stroke.

A.1 Definition and description of skilled movement

Skilled movements are defined as movements used to manipulate objects and movements performed in order to eg. walk, climb, swim or similar actions. In rats, the term skilled movement only defines such movements, where the mouth or upper limbs are used. [Suckow et al., 2006]

One of the main differences between rats and humans, when it comes to skilled movements, is the sensory control of those. Humans mostly direct skilled movements based on visual inputs, where rats primarily uses their olfaction, audition and vibrissae palpation [Whishaw, 2003].

The skilled movement performed by the rat, is much like humans. E.g. in reaching where aiming, transport and withdrawal is performed mainly by upper arm movement. Rats shape their digits in anticipation of the size of the object they are about to grasp, as do humans [Whishaw, 2003].

In order to evaluate skilled movement abilities in rats, trials like beam walking and forelimb evaluation are commonly used methods. A beam walking trial, tests

the rats ability to walk on a narrow beam. A variation of this is the rotorod test, where the rat tries to maintain balance on a rotating rod. An evaluation of forelimb function can be constructed by setting up an experiment where the rat recieves a reward, like a pellet of food, by performing a specific skilled forelimb movement. [Suckow et al., 2006]

A.2 Comparison of limb structure in relations to control of movement

The rat has approximately the same structure, anatomy and functionality of the forelimbs as humans. The forelimb of the rat is comprised of similar bones and muscles and it can make a similar range of movements as humans. However, their are some differences in the way the movements are generated.

Relative to the humerus, their scapula can make about 10 degrees of ventral and dorsal movement and 45 degrees of anterior and posterior movement. Scapula can also move together with the humerus and clavicle, which together form the shoulder joint as in humans. The muscles are the main attachment of the shoulder and is also the main restriction of movement [Whishaw, 2003].

The humerus can rotate about 10-20 degrees in its socket with the scapula, move from 20 degrees flexion to 108 degrees in the anterior-posterior plane, and move over 145 degrees in the medial-lateral plane. The ulna and radius can make almost no rotation in their elbow joint with the humerus. At this joint they can move about 10 degrees in the medial to lateral direction in the horizontal plane. They can flex to an angle of 10 degrees and extend to an angle of 160 degrees in the anterior-posterior plane [Whishaw, 2003]. The rat's paw can rotate about 20 degrees, relative to the radius and ulna, flex and extend by approximately 90 degrees in each direction, move about 45 degrees in each lateral direction. Rats have more parasagittal movement and less horizontal movement at the scapula, less rotation at the shoulder, more limited backward movement at the elbow, but otherwise similar movement compared to humans. Rats cannot rotate their paw by rotating the radius over ulna, because these are fused, unlike humans. However, the rat's wrist is much more flexible, this enables the rat to make similar rotation in their wrist, as humans [Whishaw, 2003]. In general it can be said that rats have the same degrees of freedom to move their arm as humans and that the movement in rats are almost the same as in humans. However there is an anatomical difference between how this freedom is achieved. Humans, for instance has ball-and-socket joints in their shoulder and is able to rotate radius and ulna. Rats scapula is tethered with muscles and their hands can be rotated around their wrists. Those mechanisms provides the rats with ability for similar hand-movements as humans. [Whishaw and Kolb, 2004]

The digits of rat can flex and extend almost in the same way as humans. The rat can only flex the thumb, and it is smaller relative to the other digits, and the thumb has a nail, whereas the other digits have claws. The rats small finger (digit 5) is more versatile than the humans, because it is able to adduct to some extent [Whishaw, 2003].

A.3 Comparison of neurophysiology in relation to control of movement

In general, rats have the same major structures in the motor system, as humans. Rats have a motor cortex, caudate nucleus, red nucleus, and descending pathways to the spinal cord, including corticospinal tracts and a rubrospinal tracts. The corticospinal tracts of rats have their origins in three fields of the pyramidal neurons in the rat, as in humans. The corticospinal tract is the main descending pathway from the cerebral cortex to the spinal cord. It shares a lot of characteristics in all mammalian species [Nudo, 2007] [Whishaw, 2003].

There are, however, some differences in the organization of the motor system between rats and humans. The motor cortex and the primary somatosensory cortex of rats are overlapped, while they are separated in humans [Giovanni and Lamarche, 1985].

For rats and humans, at least two somatosensory representations, S1 and S2, have been identified in the parietal cortex, see Figure A.1. In humans, S1 can be divided into additional areas. In rats, two motor areas have been identified, the CFA (caudal forelimb area) and the RFA (rostral forelimb area).

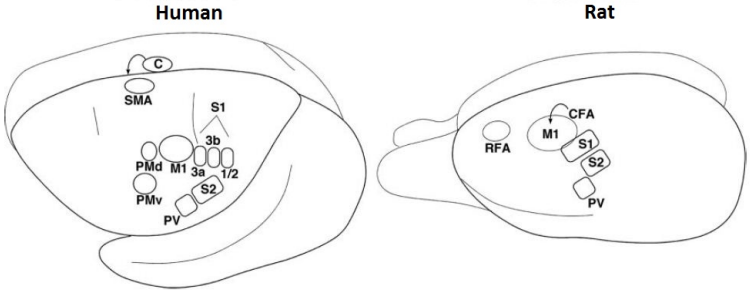


Figure A.1: This figure shows different areas in the primate (e.g. human) and the rat brain. ⟵ anterior. [Nudo, 2007]

In humans, the frontal cortex has been divided into at least five regions including M1, supplementary motor area (SMA), PMv, dorsal premotor cortex (PMd), and cingulate motor areas (CMA) [Stein et al., 2009]. Each of these areas has

been further divided into fields. There has been considerable discussion regarding the similarity of the rat RFA and various human motor areas. RFA shares some anatomical features with SMA and some with premotor cortex, but the functionality cannot be compared directly with the human motor areas [Nudo, 2007].

The caudate and the putamen are not separate in the rat, as they are in humans. When entering the spinal cord, there are two main branches of the rat corticospinal tracts, a dorsal crossed tract and a ventromedial uncrossed tract. These pathways are likely homologous to the lateral and ventral corticospinal tracts of humans. The corticospinal tracts in humans have direct connections with motor neurons of lamina IX, it is unclear if those exist in rats [Whishaw, 2003].

Appendix B

Experimental procedures: Behavioral training

B.1 Cage design

The cage in which the training took place were a 35 cm x 35 cm cage. A grate covered the bottom of the cage. One end of the cage had an opening with the width of 1.2 cm. The size of this opening were a tradeoff. Since some of the rat tended to use both paws simultaneously to obtain the pellet, it was important that the size of the opening was so small that only one paw can be reached through the opening. On the other hand, the larger the opening was, the easier it was for the rat to discover the food pellet and reach out for it. A picture of the cage can be seen on Figure B.1

Outside the opening, a wooden block was placed. An opening was made in the block, and a smooth cavity was made with the use of dental acrylic (Heraeus Kulzer Paladur). A thin bridge made of plastic runs from the block through the cage opening. This was crated in order to minimize the risk of the rat dropping the pellet to the bottom of the cage. The wooden block was designed to contain a single pellet in a hole in the middle and in a way that the rat could not reach the pellet with its mouth.

When a rat was in the cage, a black piece of cloth was folded over the cage, such that only the end containing the opening was uncovered [Suckow et al., 2006]. This helped the rats to focus its attention to the open part of the cage.

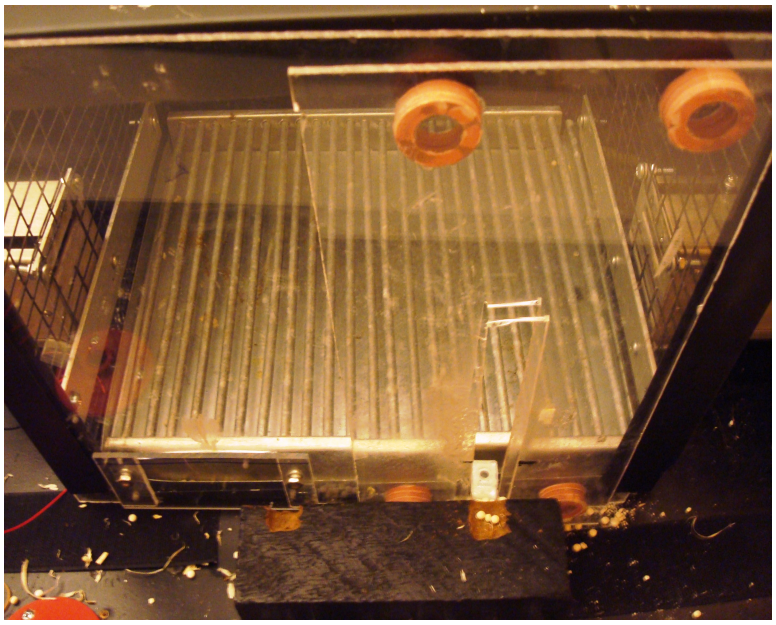


Figure B.1: The cage used for the experiment.

B.2 Behavioral training

The rats were taken from three litters - 8 rats in each. Each rat was labeled on the tail. The three litters were labeled with a separate color, and each rat within the litter was given a specific symbol corresponding to a number. The rats could therefore be identified on basis of their number and their color. When the experiment was initiated, a new identification system based only of the rats experimental group was introduced. At the beginning of the training, the rats were physically located in cages with four rats in each.

The overall training process was divided into the following phases:

1. Hand training
2. Cage training
3. Pellet reaching training

The hand training phase occupied the first 2-3 weeks of training. The purpose of this phase was to let the rats get used to human contact, and to make them feel safe when handled. The rats were trained 2-3 times each week, during 15 minutes long training sessions. In each training session each rat was handled by a member of the project grouped and was petted during the entire session. After the end of the session, the rat was weighed and the weight and individual comments were noted in the log.

When the rats were used to human contact, the next step was to make them familiar with the cage in which the later experiment was conducted. During this phase the 2-3 weekly training sessions was of 15 minutes, but only 2-3 minutes were used for hand training and the rest took place in the cage. After the hand training, the rat was placed in the cage.

The purpose of this phase was:

1. To make the rat comfortable with the cage itself
2. To make sure that the rat was aware of the ”opening area” in the cage, and associate this area with food.

In the first few training sessions, it was accepted that the rat ate the pellets directly by the mouth, without using the paw. Later on, the pellets were placed on a rectangular log - the same log that was used in the further experiment. The pellets were placed so far away from the cage opening, that the rat must use its paws in order to reach them. This can be seen on Figure B.2. It was preferable that the rat used only one paw to reach out for the pellet, and that the use of this hand was consistent, so the electrode could be implanted in the corresponding lobe of the brain. However many of the rats tend to use both paws, like it can be seen on Figure B.2, or shift between the paws. It was not possible to eliminate such behavior totally. However, a preferred paw was identified for all the rats.

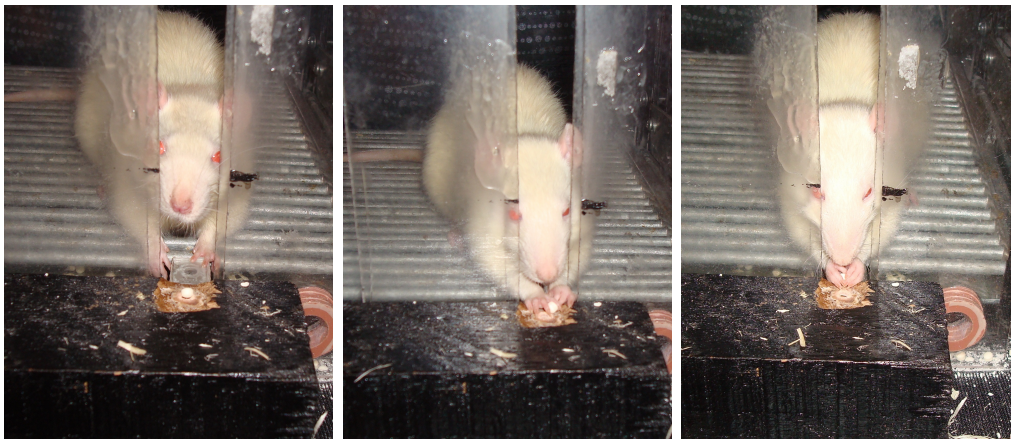


Figure B.2: The rat reaching out for a pellet.

At the initiation of the ”Pellet reaching” phase, the rats were placed on a diet. This was important for the rats motivation to learn the task. If the rats were not hungry, they were not really interested in making an effort to reach out of the cage, only for obtaining a small amount of food. Their basic amount of food was set to 15 g pr. day. This amount would, however, be increased on days where no training of the rat tool place. In order to make sure that all the rats obtained this

amount of food, and no rat starved, the rats were divided from their multi-rat cages and placed in separate cages. The training sessions in this phase were 15-20 minutes long, and besides from 1 minute of hand training, this phase was spend entirely in the cage.

After a completed day of training, the cage was cleaned, by removing pellets and feces from the bottom of the cage, and disinfect the cage with alcohol. During the entire training, the rats reaching performance was tracked. An important indicator for the rats abilities regarding the experiment, was how naturally it was for the rats to use their paw to reach out for the pellets. Based on this evaluation, rats were excluded if they did not perform well. In the end, only the best 12 rats were chosen to continue the experiment and receive the cortical implant.

Appendix C

Experimental procedures: Electrode design and manufacturing

This appendix contains a guide, describing the procedure developed for creating the electrodes used in this project. The electrode was a 4x4 electrode array manufactured for implantation in the motor cortex of a rat.

The following materials were used for creating such an electrode:

- Two board-to-board sockets with the dimensions: 2x4 rows, row pitch of 2 mm (Harwin M22-7140442)
- Ground wire
- Teflon Insulated tungsten wire (100 μm in coated diameter, 50 μm in bare diameter), length of 80 mm, 16 wires for each electrode.
- Shrinking-tube with the diameter of 2 mm, length of 16 mm.
- Components for making cold-curing resins prosthetics (Heraeus Kulzer Paladur)
- Solder

The following equipment was required to create the electrode:

- Microscope
- Lighter
- Wire wrapping tool
- Soldering iron

- Superglue
- 16 crocodile clamps
- Graph paper
- Aluminum foil
- Acupuncture needle (0,30 mm x 30 mm)
- Micro-scissor

C.1 Procedure

- Use the lighter to burn the insulation of one of the ends of the wires. The insulation must be removed for approximately one centimeter of the wires.
- Wrap the uninsulated part of the wires onto the two sockets (8 wires on each).
- Solder the wrapped wires onto the sockets, like shown on Figure C.1 (Left).
- Glue the graph paper to the aluminum foil. This was used as a mould for constructing the rest of the electrode.
- Perforate the graph paper using the needle. A 4x4 pattern with a half millimeter between the dots was made. A larger hole for the shrinking tube was made so the center of this is 2 mm away from the closest wires. This can be seen on Figure C.1 (Right).

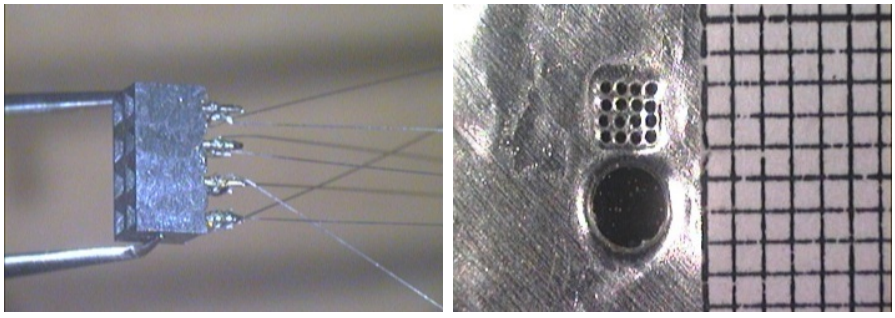


Figure C.1: Left: A socket with 8 tungsten wires was soldered on. Right: The mould used for shaping the electrode.

Figure C.2: The wires are pulled through the mould.

- Each of the wires were pulled through the hole in the paper corresponding to its position (see Figure C.2). The orientation of the wires and the guide holes were matched. When a wire was pulled through the hole, a crocodile clamp was attached to the end of the wire to keep it in place.

- The two 4 x 2 sockets were attached by using the super glue, so they together form a 4 x 4 socket like seen on Figure C.3.

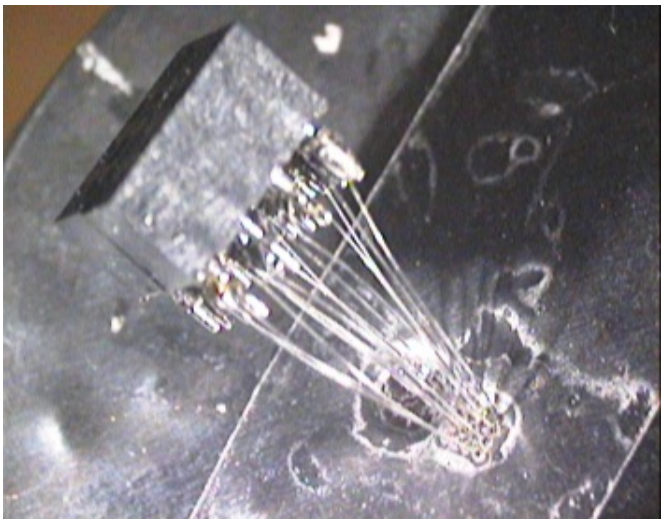


Figure C.3: The complete 4 x 4 wire-array

- Attach the shrinking-tube to the socket like it is shown on Figure C.4. A needle was used to fixate the tube.

- The prosthetics material was made by mixing the Paladur powder component with the Paladur liquid component in the proper ratio. This material was applied around the soldering, and the wires at a distance of approximately one centimeter. The electrode was then molded in two steps: a)

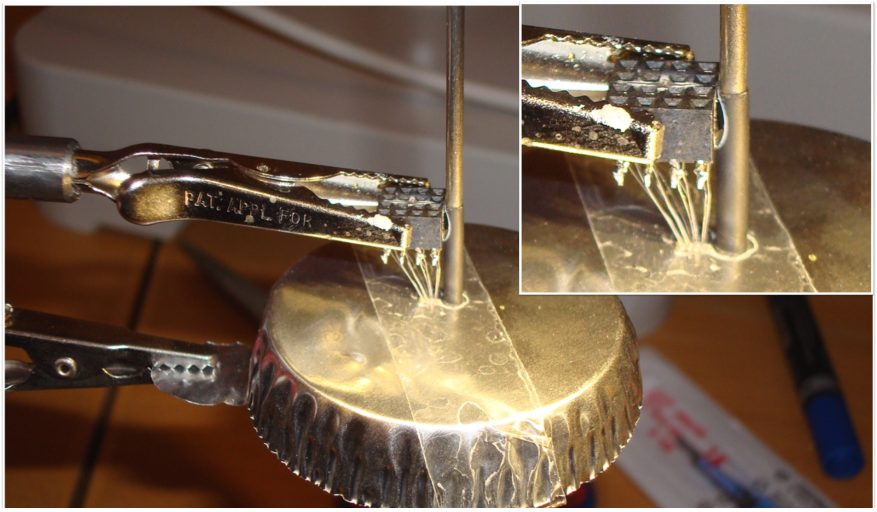


Figure C.4: The tube is glued to the electrode socket

The foil was placed as close to the sockets as possible and mold the area between the foil and the socket b) then the foil was lowered a bit and mold the wires together, starting from the earlier molded part, going to the foil. The wires in this area should be vertical. The result of those steps can be seen in Figure C.5.

- Let the material solidify for at least one hour, then carefully cut it down, using a micro-scissor. The electrode now appear like the electrode shown at Figure C.5. A ”dummy-connector”, was created by gluing two sockets together. This dummy must be connected to the electrode when not in use, in order to prevent dust from getting down in the electrode, and thereby blocking the connection.

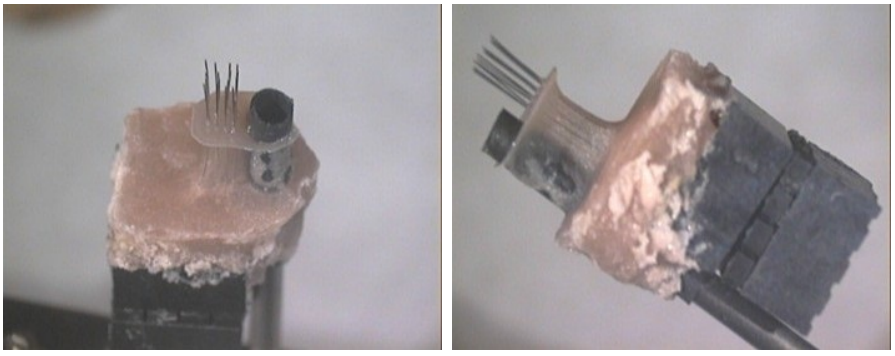


Figure C.5: The complete electrode with a ”dummy connector” attached.

Appendix D

Experimental procedures: Electrode implantation

The durtio of the operation was on average 1.5 - 2 hours. Two persons were required to perform the surgery: a sterile surgeon and a non-sterile assistant. The environment for the operation as well as the surgical tools used during the procedure had to be sterile. The complete setup for the surgery also included a microscope containing a camera, connected to a television. This made it possible for both the surgeon and the assistant go maintain a proper overview during the different phases of the procedure. A picture of the complete setup can be seen on Figure D.1.

To instrument the animals with intra-cortical electrodes, the animals were anaesthetized with a 0.5 ml subcutaneous injections of Ketamine (100 mg/kg), Xylazine (5 mg/kg) and Acepromazine (2.5 mg/kg). A dose of approximately 0.1 ml / 100 g body weight were given every hour to maintain the anaesthesia. When anaesthetized, the rats should have a very slight corneal reflex and a very slight or non-existent paw pinch reflex. During the surgery, the assistant checked with regular intervals if the rat was still properly anaesthetized. The muscle tone of the rat was used as the primary indicator for this judgment.

When the rats were properly anesthetized, their heads were shaved and prepared for sterile surgery using a 1% iodine solution. When this was performed, their heads was fixated using a stereotactic frame. Sterile cloth was used to cover the remaining body of the rat. A fixated rat, prepared for surgery can be seen on Figure D.2.

The main part of the surgery was then initiated. A craniotomy was performed over the primary motor cortex area in the hemisphere corresponding to the rats' primary hand (the opposite side of the body). The motor cortex area was identified by stereotactic coordinates and/or blood vessel landmarks. The primary

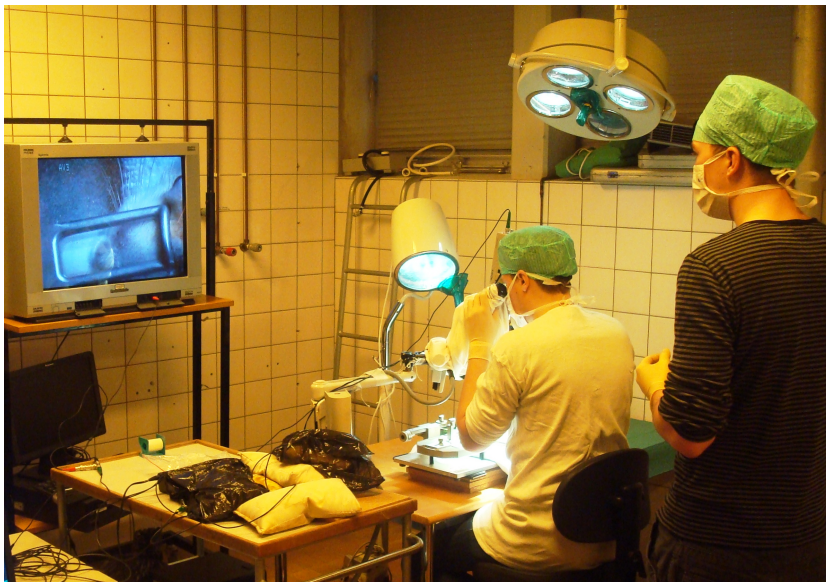


Figure D.1: The setup used during the operations. This includes a sterile area for the surgery and a microscope connected to a television.

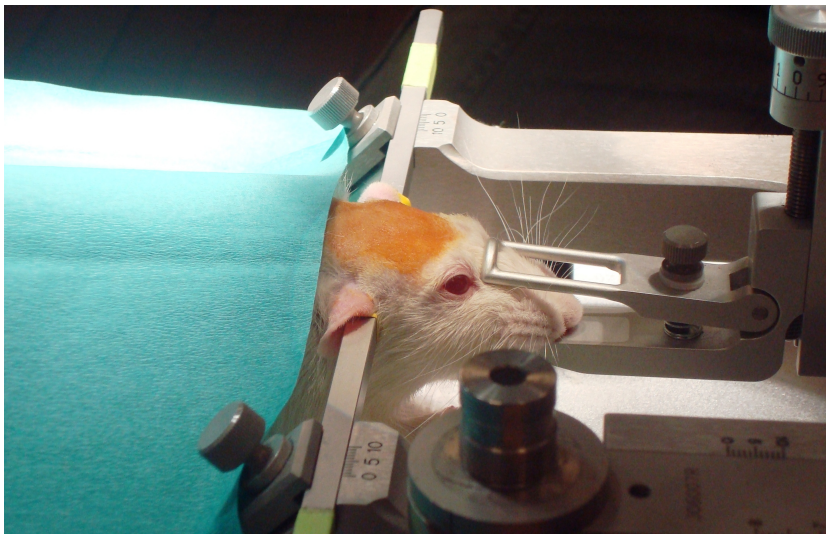


Figure D.2: A rat ready for surgery. Its head is shaven, sterilized and fixated.

motor cortex area related to limb and trunk movement is located +2 mm to +4 mm anterior-posterior and +2 mm to +4 mm medial-lateral relative to Bregma. The size of the craniotomy was matched to the size of the implant (approximately 5 mm x 2 mm). The size of the electrode channel array was 1.5 mm x 1.5 mm plus a tube for the photothrombosis equipment.

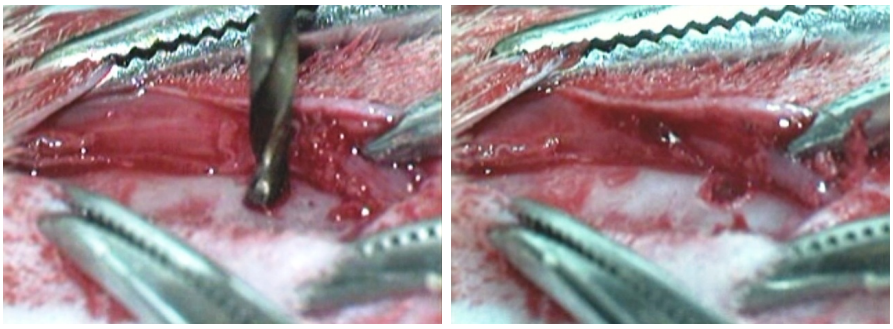


Figure D.3: Left: A hole is being drilled. Right: The finished hole.

The craniectomy was initiated by drilling three holes in the skull of the rat, using a 2 mm hand drill. This can be seen on Figure D.3. One hole was drilled in the area close to the desired location of the electrode. It was important that this hole was not made too anterior to the motor cortex area, since the bone here is relatively thick in that part of the skull, and that it was not made through midline or Bregma, since that will increase the risk of bleeding. The other two holes were used bonescrews. Those holes were made posterior to the first hole.

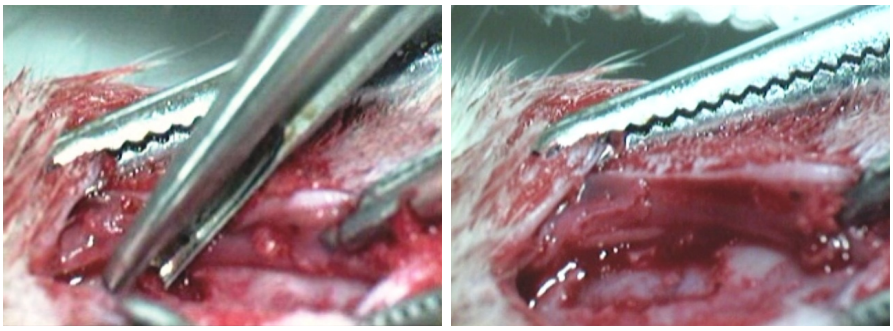


Figure D.4: A hole for the electrode were made: The screw hole was expanded by cutting small pieces of skull of the edges (Left). The size of this hole was increased, until it had the a proper size for the electrode (Right)

A rongeur was used to expand the first screw hole into a size that fitted the dimensions of the electrode. This process can be seen on Figure D.4. A large part of the brain would then be revealed. Before the electrode was inserted, the dura that protected the brain had to be removed over the implant site. This was done using a small needle (30G) to catch the dura, and a small surgical scissors to cut it up. This procedure can be seen on Figure D.5.

The two bonescrews were then screwed down into the two remaining holes. They stabilized the electrode, when implanted. Wires used for grounding were coiled around the screws. Two grounding wires were considered most optimal, since the risk of one of the wires getting bend or damaged was relatively high.

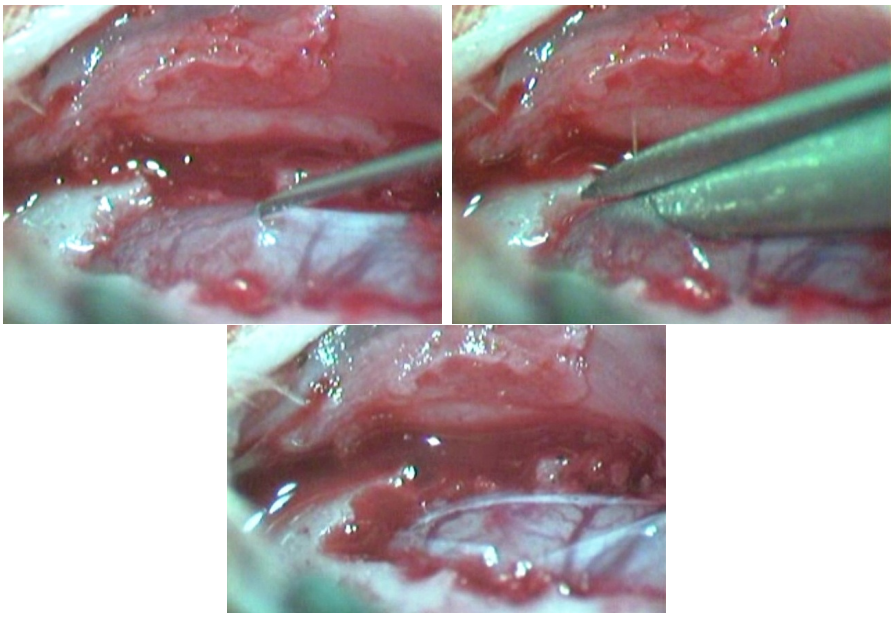


Figure D.5: Making a hole in dura. Right: Make a opening with a 30G needle. Left: Expanding the hole using a micro-scissor. Bottom: A difference in color between the brain area with and without the layer of dura can clearly be observed.

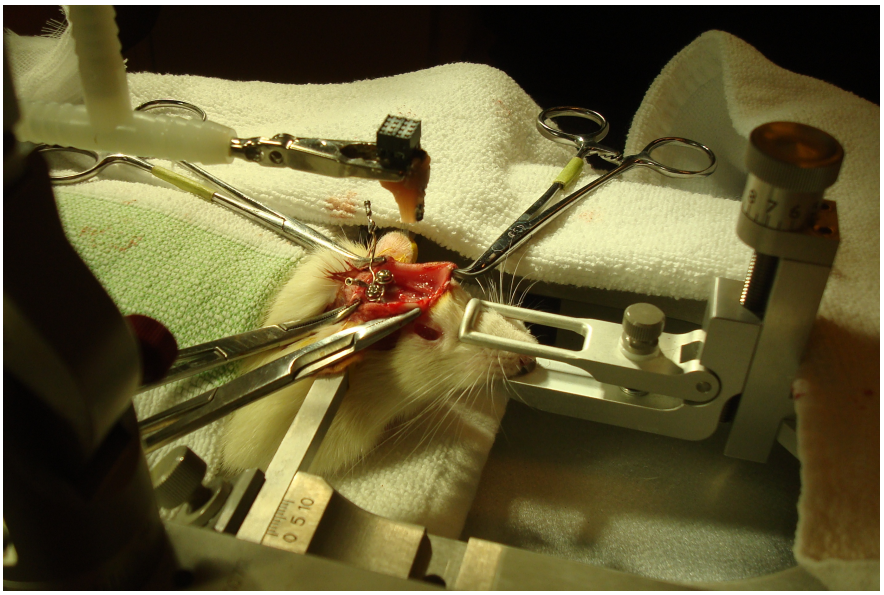


Figure D.6: Setup for lowing the electrode into the brain.

The next step was to insert the electrode into the primary motor cortex of the rat. This was done through the pia by a manually operated micromanipulator. Figure D.6 shows the rat and the micromanipulator. The electrode was positioned over the created hole and lowered to a depth of 1.7 mm into the brain. On Figure D.7,

closeups of a electrode being lowered into the brain can be seen. The electrode did not penetrate pia at this point, due to the small spacing between the wires and the surface tension of the pia. In practice this was therefore done by lowering the electrode to a depth of 2.0 mm in order for the electrode to penetrate the tissue. When this had taken place, the electrode was elevated 0.3 mm so the correct height at 1.7 mm below the pia surface was reached.

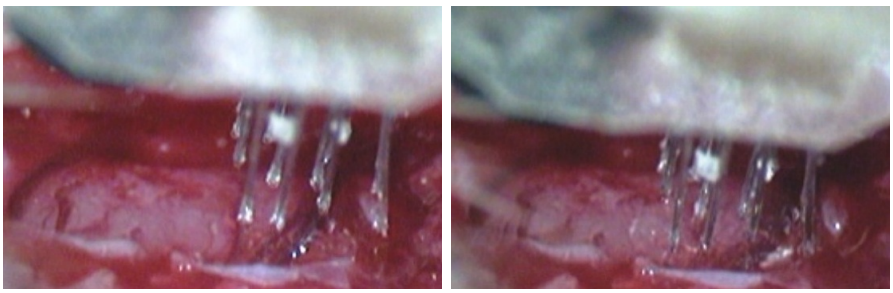


Figure D.7: Lowering the electrode through the tissue.

A protective layer of collagen-based Gelfoam was placed to recover any exposed pia. Dental acrylic (Heraeus Kulzer Paladur) was then be used to seal and protect the craniectomy and to hold a percutaneous electrode connector in place against the implanted bonescrews. This can be seen on Figure D.8. It was important that the stroke tube and all the wires were covered with the dental acrylic, such that no sharp edges caused irritation.

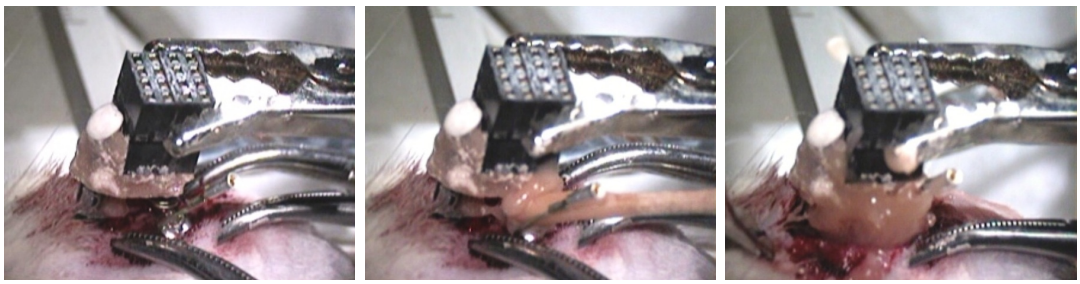


Figure D.8: Moulding the electrode on to the bonescrews.

The skin was closed with 2-0 suture (SN-758 from Monosof) around the now protruding mound. Typically two stitches were made posterior to the electrode and one stitch made anterior. This, however, depend on the ratio between length of the original cut and the size of the electrode.

Within 0.25 - 1 hours, depending on the time the last dose of anesthesia was given, the rat should start to wake up. The rat received pain-reliving injections four days following the surgery.

Appendix E

Experimental procedures: Applying the stroke using Photothrombosis

The main aim of this procedure was to infuse a photosensitive dye called "Rose Bengal" into the rat. One of the properties of this dye is, that when exposed to light with a wavelength of 561 nm, it creates singlet oxygen species leading to blood clotting and microvascular occlusion. A white light probe was inserted through the stroke tube in the implant, and a clot was thereby formed in this area. The advantage of this model, is the possibility to control the stroke size by varying dye plasma concentration and light beam intensity. [Macrae, 1992] [Murphy and Corbett, 2009]

Before the procedure was initialized, the rat was anesthetized. The procedure for this is similar for the procedure also used when implanting the electrode and its head was fixated (see Appendix D. When the rat was anesthetized, a rubber band was placed around the root of the rat's tail. This helped to widen the blood vessels. A 24GA catheter (venflon) (dimensions: 0.7mm * 19mm) was then used to puncture the tail and placed in the tail vein of the rat. Since the tail of the rat is made from fibrous cartilage, this required a certain amount of force. Blood entering the catheter indicated that the catheter was positioned in the vein. A mixture of Rose Bengal and saline, in the proportions 10 mg Rose Bengal pr. 1 ml saline, was then injected. For each 100 g of the rats body weight 0.3 ml of this solution was injected. The Rose Bengal/saline solution was injected slowly through the vein over a 2 minute period of time, in order to minimize the risk of heart failure. It was important that the Rose Bengal was exposed to as little light as possible before injected, due to its photosensitive properties.

A light probe was then inserted into the stroke tube. This probe was fixated in a proper angle and lowered down through the 1.6 cm long tube, until it nearly touches the surface of the brain. It was remained at this position for 30 minutes.

When this was done, a clot has been formed in the brain preventing perfusion, and the procedure was thereby completed.

Appendix F

Experimental procedures: Intracortical recordings

The recording was made on specialized hardware from Tucker-Davis Technologies (TDT) collecting spike train data from 16 channels.

An overview flow diagram of the experiment setup is seen on Figure F.1.

F.0.1 Hardware

RA16CH 16 Channel Chronic headstage

The 16 channel 1x gain headstage was manufactured by TDT and weighed only 1.2 g which made it useful in applications such as small rodent recordings. The job for the headstage was to perform a low profile stable connection between the implant and the preamplifier.

RA16PA 16-channel preamplifier

The preamplifier from TDT sampled and filtered the signal from the headstage and transmits the digital signal to the RX5 via an optical connection. The preamplifier bandwidth was 2.2 Hz - 7.5 kHz. The sampling frequency was 25 kHz. To reduce noise, the preamplifier was battery driven.

RX5 Pentusa Base Station

The RX5 Pentusa Base Station (RX5) filters and analyzes the time continuous signals from the preamplifier and reduces the signals into binary spike train data based on a user defined threshold detection. As can be seen on Figure F.1, the

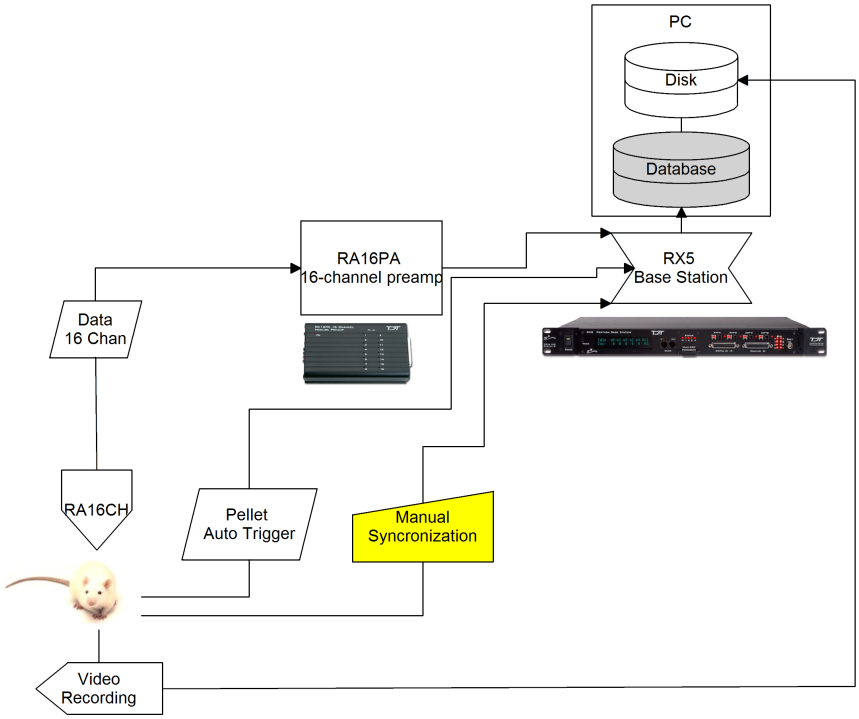


Figure F.1: Flow diagram of the recording setup.

RX5 also got input from two sensors correlated with pellet dispensing. One of the sensors was automatic, and triggered whenever the food pellet was taken from the food box. This sensor was, at times, hypersensitive - which was why there was a manual trigger override that was manually triggered by an observant whenever the pellet was taken from the food box. Any automatic triggers in a period of three (-.05 to +1) seconds with no manual triggering, was ignored in the later data analysis. The sensors concerning synchronization with the pellet action was connected to the digital ports, and it required the voltage input to be between 0 and 5 V. To distinguish between logical high and low, the voltage change must cross 2.5 V.

Hardware features of the TDT system

The hardware features of the TDT system is as follows:

The data channel from the preamplifier was connected to the base station through one of the fiber optic ports and was sampled at 25 kHz. This data was filtered digitally within the RX5 with a bandpass filter from 800 Hz to 8 kHz.

DSP (Digital Signal Processors)	Five 100 MHz Sharc ADSP
Memory	128 MB SDRAM
Max sampling rate	50 kHz
Frequency response	DC - Nyquist
Sample delay	4 samples
Digital I/O	40 bits programmable
Fiber optic ports	Four 16-channel inputs

Pellet action synchronization sensors

The circuit layout for both the automatic and manual trigger is seen on Figure F.2.They were both powered by a 9 V battery. The pellet synchronization triggers were very simple circuits. The manual trigger was a simple pushbutton with an attached resistor across the pushbutton pins creating a 4.5 V difference over the pushbutton when not pushed, and 0 V difference when it was pushed. The manual trigger was directly fed into a digital port in the RX5.

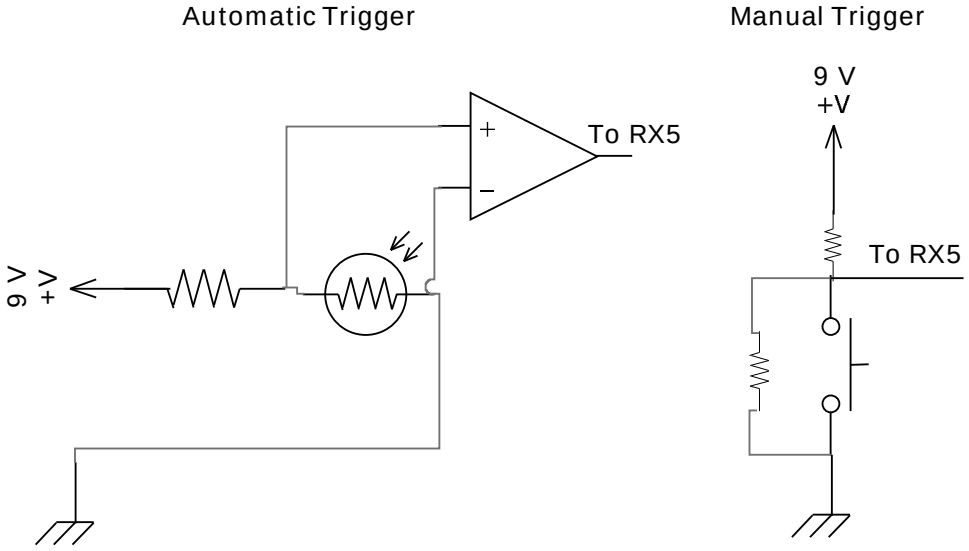


Figure F.2: Electric circuit of the pellet synchronization sensors.

The automatic sensor was a light sensitive resistor (LSR). The resistance ranges from around 0 Ω to 0.5 $M\Omega$ with the lowest resistance occurring when light is induced on the LSR. The LSR was placed inside the pellet block just beneath the spot where the food pellets are positioned. An indirect light was placed above the food pellets, so that whenever a food pellet was taken from the pellet block, the light increased on the LSR inducing a resistance drop.

F.0.2 Software

During recording sessions, two different software packages were used. For the intracortical signals and the event triggers, a custom made TDT System 3 program was used. For video capturing, Windows Movie Maker was used.

TDT OpenEx Suite

The TDT OpenEx Suite includes software for programming the RX5 hardware, visualizing the raw signal input and the spike train raster plot output, exporting data from the native TDT TTank database format into .nex format readable by software packages such as NeuroExplorer and Matlab.

Before any experiments could be done, the programming of RX5 hardware was necessary. The programming was done in TDT's own software package, and featured a graphical flow-based programming style. In this project, the data flow for the RX5 can be seen in Figure F.3. Two digital inputs in the RX5 listens for a voltage rise and voltage fall and saves the timestamps for these under the parameters "'SynUp"' and "'SynM"' which was the corresponding Synchronization Up, and Synchronization Manual. This was where the pellet sensor and the manual sensor was connected. Each time an event was detected, a timestamp was recorded.

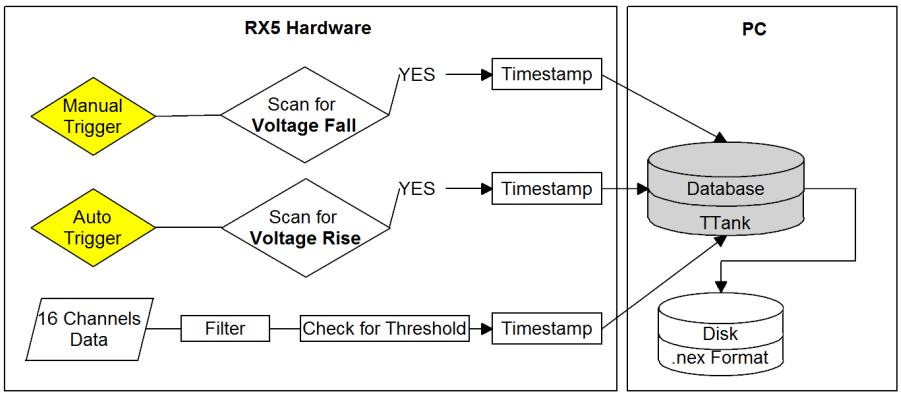


Figure F.3: Detailed flow diagram of the dataflow in the RX5 and the exportation to the PC.

The digital 16 channels from the preamplifier were processed according to threshold setting, and timestamps for all events were also recorded. The TDT software writes the data continuously into TDT's own database format, TTanks. After the recording session, the data was exported to .nex format so other analyzing software can read it.

F.0.3 Recording procedure

Prior recording

The system was setup by creating a new datatank with the filename corresponding the ratID, date and recording session in the filename, for instance "A1_05-15_Day4". The hardware setup program was then loaded into the RX5 base station through the TDT software and the system was now ready to record data. Windows Movie Maker was then booted, and setup to capture the video footage. The video file name was in the same format as the datatank created. The system was now ready for the recording session.

The rat was carefully held in place, while the headstage was connected to the implant. The preamplifier was now turned on, and the 16 channels were carefully examined and the quality of these channels were written down in a log. A threshold was set for each channel after studying the signal to noise ratio by inspecting the filtered signal plot in the TDT software and by listening to the signal through speakers. After all channels had been set, the rat was put into the cage and after a final noise-inspection on all channels, the system was ready for recording.

During recording

The recording was started in TDT and the video capture was enabled. To synchronize the data from the IC signals with the video feed, the manual trigger was pushed in front of the camera. Two persons were handling each recording session. One fed the pellet box after the rat had finished eating the previous one (to prevent chewing noise artifacts in the recordings), while the other manually triggered the manual sensor after each successful pellet reach. The goal was to get between 80 and 110 reach repetitions during each recording, and the recording time therefore varied. No recording session would last longer than 30 minutes. When the rat had reached the needed repetitions, the spike recording and video capture were stopped and the rat was taken out of the cage, headstage disconnected.

After recording

The data was archived into the TTank, and ready for exportation. This was done using TDT's browser. The data that solely consisting of timestamps for the 16 data channels and the two synchronization channels were exported into .nex files for further analyzing.

Enclosures

Enclosure A

Events from Group A and B

Rat	Meassurment	Events	Total time (sec.)	Events/min
A1	Baseline	84	2429	2.07
	Day 1	12	1216	0.59
	Day 2	33	1443	1.37
	Day 4	98	1240	4.74
	Day 7	32	1718	1.12
	Day 10	93	1293	4.32
A2	Baseline	95	1594	3.58
	Day 1	46	1630	1.69
	Day 2	40	1201	2.00
	Day 4	98	975	6.03
	Day 7	47	1977	1.43
	Day 10	59	1749	2.02
A3	Baseline	94	1532	3.68
	Day 1	99	1093	5.43
	Day 2	98	1075	5.47
	Day 4	37	1296	1.71
	Day 7	84	1067	4.72
	Day 10	82	1092	4.51
A4	Baseline	98	980	6.00
	Day 1	98	872	6.74
	Day 2	99	748	7.94
	Day 4	105	717	8.79
	Day 7	103	677	9.13
	Day 10	98	1121	5.25
A5	Baseline	103	1195	5.17
	Day 1	91	955	5.72
	Day 2	100	900	6.67
	Day 4	109	669	9.78
	Day 7	102	891	6.87
	Day 10	91	976	5.59
A6	Baseline	116	1401	4.97
	Day 1	89	1081	4.94
	Day 2	104	884	7.06
	Day 4	105	933	6.75
	Day 7	102	717	8.54
	Day 10	95	662	8.61

Rat	Meassurment	Events	Total time (sec.)	Events/min
B1	Baseline	101	1196	5.07
	Day 4	96	1018	5.66
	Day 5	99	1184	5.02
	Day 7	96	1272	4.53
	Day 10	98	1048	5.61
	Day 13	116	987	7.05
B2	Baseline	76	1453	3.14
	Day 4	74	2024	2.19
	Day 5	98	1558	3.77
	Day 7	75	1363	3.30
	Day 10	59	1.631	2.17
	Day 13	94	1210	4.66
B3	Baseline	83	1284	3.88
	Day 4	103	723	8.55
	Day 5	90	1151	4.69
	Day 7	85	1217	4.19
	Day 10	92	1549	3.56
	Day 13	72	1100	3.93
B4	Baseline	101	1216	4.98
	Day 4	97	1159	5.02
	Day 5	95	604	9.44
	Day 7	108	847	7.65
	Day 10	100	851	7.05
	Day 13	100	597	10.05
B5	Baseline	78	1974	2.37
	Day 4	93	1442	3.87
	Day 5	88	783	6.74
	Day 7	107	983	6.53
	Day 10	103	686	9.01
	Day 13	91	837	6.52

Enclosure B

Cylinder test

Rat	Measurement	Number of paw placements			% distribution		Ratio
		Left	Right	Total	Left	Right	
A1	Baseline	6	13	19	31.58	68.42	2.17
	After stroke	0	0	0	-	-	-
	End experiment	9	9	18	50.00	50.00	1.00
A2	Baseline	24	24	48	50.00	50.00	1.00
	After stroke	4	1	5	80.00	20.00	0.25
	End experiment	14	12	26	53.85	46.15	0.86
A3	Baseline	9	14	23	39.13	60.87	1.56
	After stroke	8	1	9	88.89	11.11	0.13
	End experiment	15	6	21	71.43	28.57	0.40
A4	Baseline	17	23	40	42.50	57.50	1.35
	After stroke	11	13	24	45.83	54.17	1.18
	End experiment	12	11	23	52.17	47.83	0.92
A5	Baseline	44	43	87	50.57	49.43	0.98
	After stroke	8	5	13	61.54	38.46	0.63
	End experiment	7	5	12	58.33	41.67	0.71
A6	Baseline	5	4	9	55.56	44.44	0.80
	After stroke	16	8	24	66.67	33.33	0.50
	End experiment	1	4	5	20.00	80.00	4.00
B1	Baseline	9	7	16	56.25	43.75	0.78
	After stroke	6	6	12	50.00	50.00	1.00
	End experiment	16	14	30	53.33	46.67	0.88
B2	Baseline	9	39	48	18.75	81.25	0.23
(Left)	After stroke	3	14	17	17.65	82.35	0.21
	End experiment	5	20	25	20.00	80.00	0.25
B3	Baseline	24	23	47	51.06	48.94	0.96
	After stroke	18	17	35	51.43	48.57	0.94
	End experiment	12	14	26	46.15	53.85	1.17
B4	Baseline	24	22	46	52.17	47.83	1.09
(Left)	After stroke	4	11	15	26.67	73.33	0.36
	End experiment	12	13	25	48.00	52.00	0.92
B5	Baseline	12	15	27	44.44	55.56	1.25
	After stroke	7	6	13	53.85	46.15	0.86
	End experiment	10	10	20	50.00	50.00	1.00

Enclosure C

Results from electrical stimulations

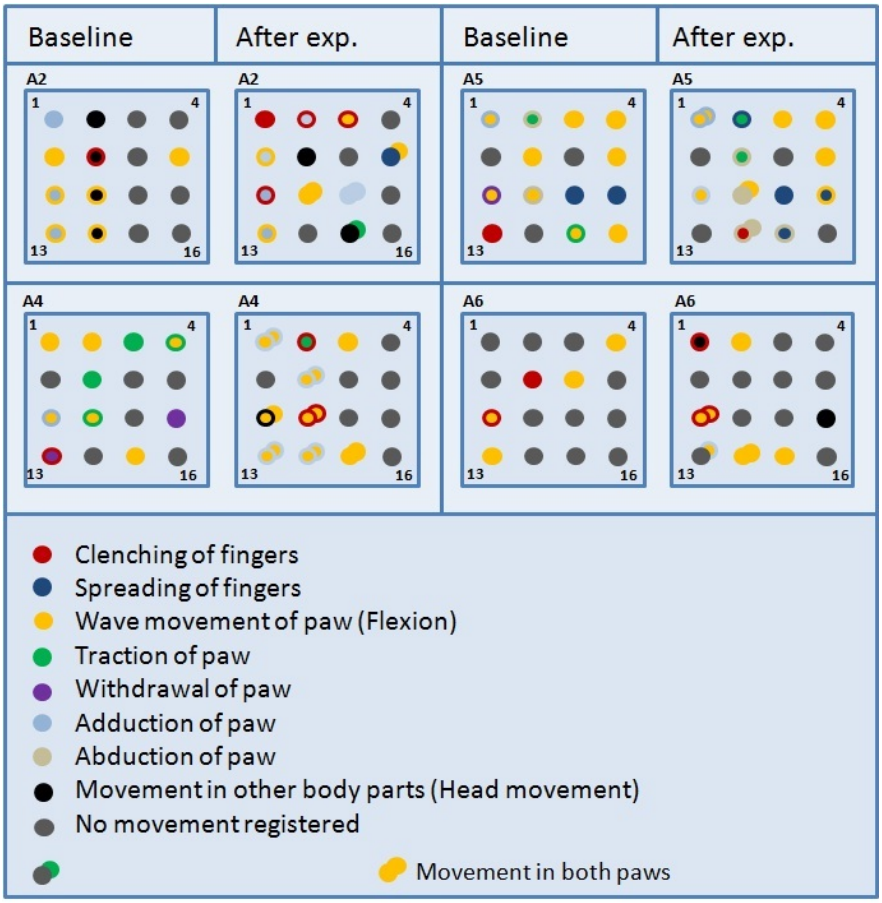


Figure C.1: Results from the electro stimulation - group A.

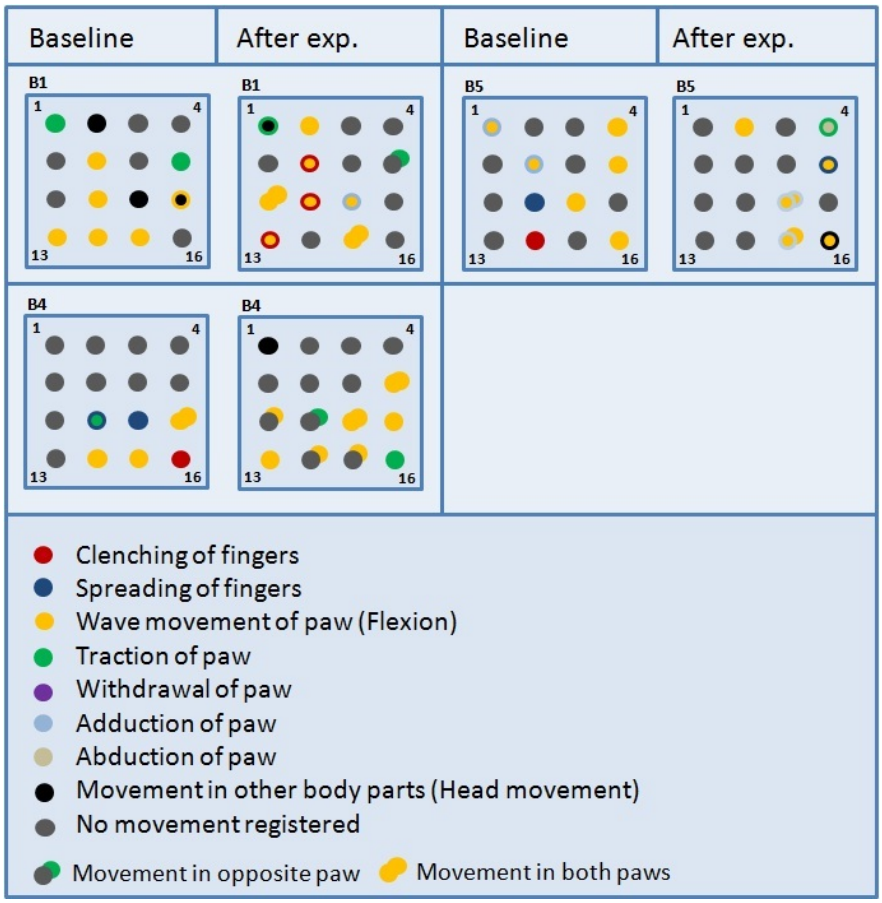


Figure C.2: Results from the electro stimulation - group B.

Enclosure D

PSTH results for all rats

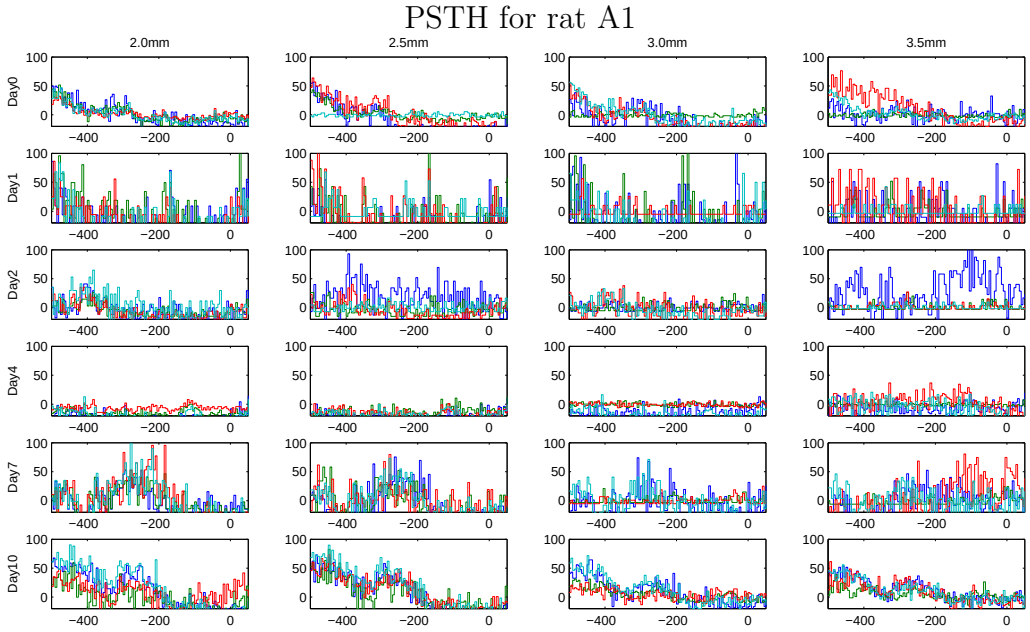


Figure D.1: A high firing pattern is seen at 'Day 0' around -470 ms and around -300 ms before event trigger. As the rat made too few repetition at 'Day 1' recording session, it is not possible to see any firing pattern. At Day 2, there are only one wave at around 390 ms but it is rather small. Channel 13 (blue at 3.5 mm) does not follow the general pattern, though. Day 4 shows almost an iso-electric line, giving no clues of any firing pattern. The two waves from the baseline seems to return at Day 7 and even more at day 10 indicating that the neural network associated with this exact movement is returning to the normal situation we see at the baseline measurement.

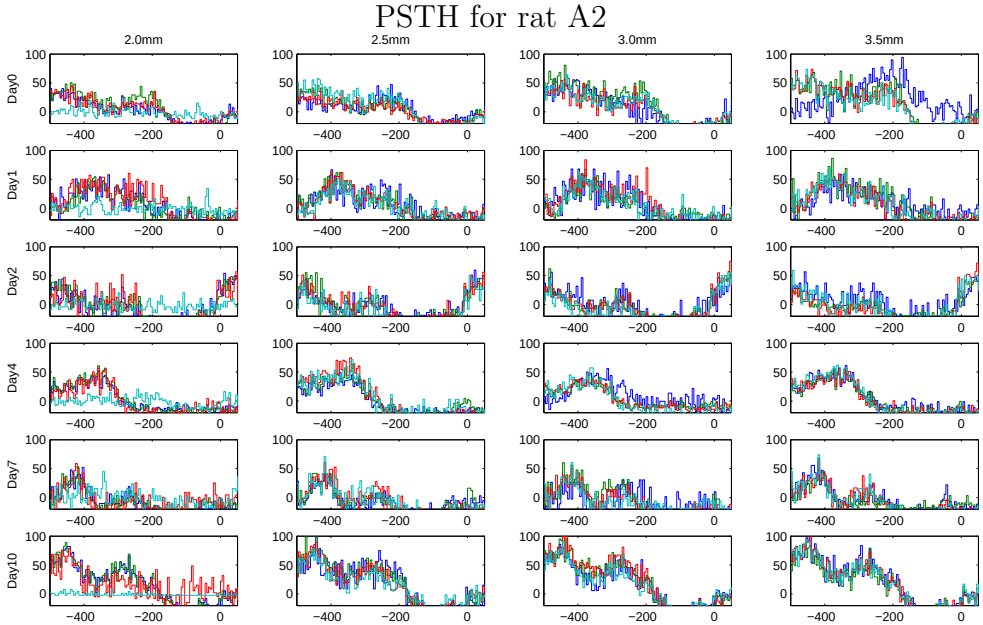


Figure D.2: Two waves of higher activity is visible a 'Day 0', one at -450ms and one at -215 ms. The first day post-stroke (Day 1), the two waves seems to have merged together but is still visible - an effect that is most noticeable in the channels farthest away from the stroke area. At 'Day 2' the overall activity is diminished some, but the two waves are still visible. More hyperactivity is visible around 0 ms, which is probably due to the withdrawal paw movement after the rat grabs the pellet. There is a lot of modulation from Day 2 to Day 4, where only one wave is visible (around -370 ms) - this could indicate a behavioral rehabilitation process. Day 7 and Day 10 both show indications of true rehabilitation as the two waves also seen in Day 0 becomes more and more apparent again.

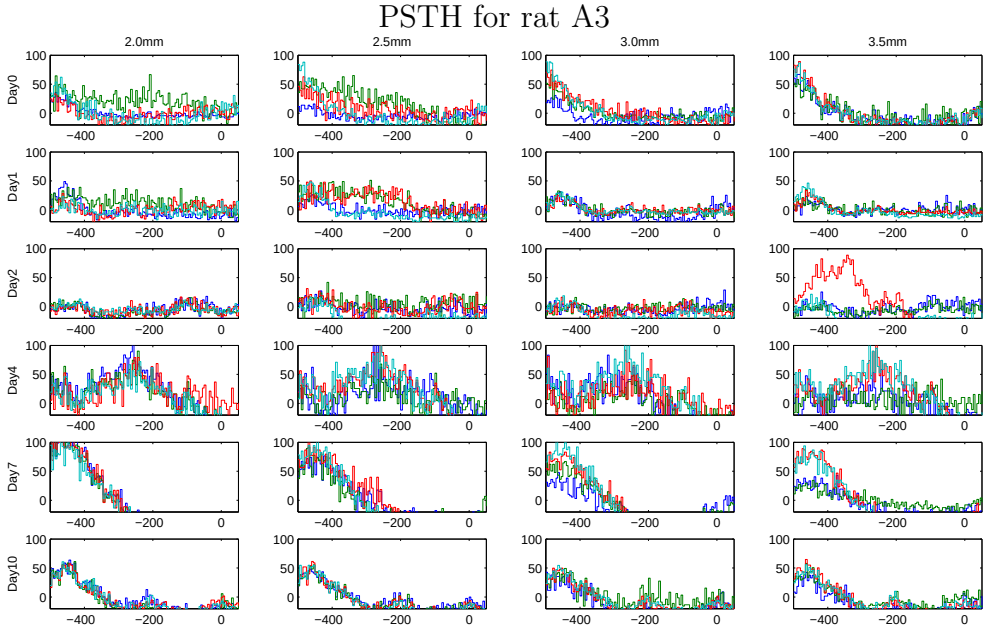


Figure D.3: In most of the ”Day 0” channels, there are two waves showing higher firing at -500 ms and around 200 ms - except for the channels listed under 3.5 mm from stroke where only one ’peak’ is seen. ’Day 1’ after stroke still indicate a higher frequency, but this time it seems to be shifted a bit towards the trigger event and there and there are only one ”wave”. This could indicate that the rat is using a different motor program during the skilled movement. The same thing applies to almost all channels at ’Day 2’ except that the single wave of excitatory spike activity is now even smaller. At ’Day 4’ there is a large wave of activity centered around -290 ms as well as the plot shows traces of hyperactivity around 500 ms prior the event trigger. At ’day 7’ only one wave is seen, centered around -500 ms. This wave remains until ’Day 10’ where another wave around -200 ms is seen - which is similar to the baseline measurement at ’Day 0’. This indicates that the neural network associated with this exact movement is returning to the normal situation we see at the baseline measurement.

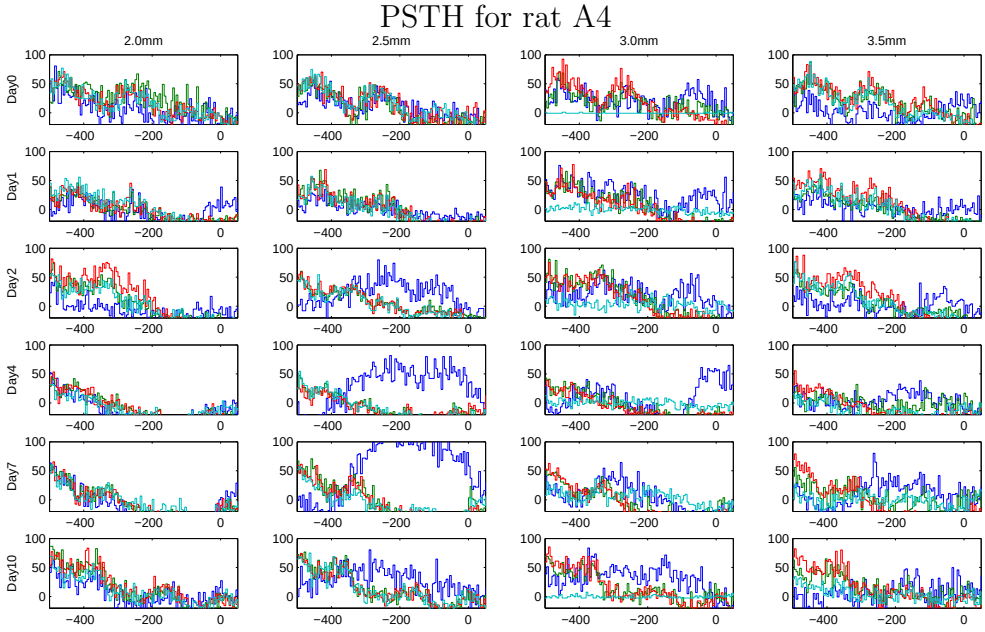


Figure D.4: The recordings from 'Day 0' shows two very distinctive waves are around -260 ms and -440 ms. This two hyperactive timings are also apparent in 'Day 1' and 'Day 2' . Blue channel at 2.5 mm (Channel 5) shows a very distinctive activity in 'Day 2', 'Day4' and 'Day 7'. As the activity across -100 ms and to 0 ms, it might be because this neuron(s) has taken over some of the function in the paw withdrawal movement. At 'Day 7' and especially 'Day 10' the patterns are very irregular, but at least in the channels in 2.0 mm and 2.5 mm two waves is visible just like in the baseline recording. One of them seems to have shifted around 50 ms though which indicates that the rat is performing the skilled movement differently 10 days post stroke than before stroke.

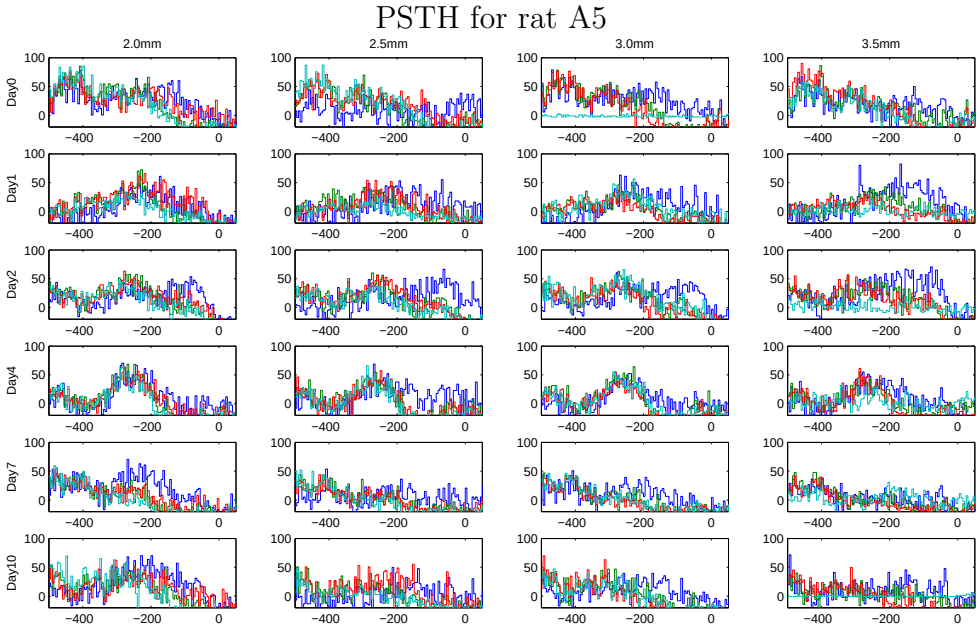


Figure D.5: At 'Day 0' the plots show a high activity at -450 ms and -200 ms. At 'Day 1' the first wave seems to have vanished and only the wave at -200 ms is still there. Channel 13 (blue graph in the plot at 3.5 mm) have higher activity between -200 and -100 ms at Day 1 and 'Day 2'. At 'Day 2' the first wave is visible again in almost all channels, and the modulation observed in the later channels is very little except in the channels that lie furthest away from the stroke site, where the activity at Day 10 have decreased noticeable compared to the rest of the measurements.

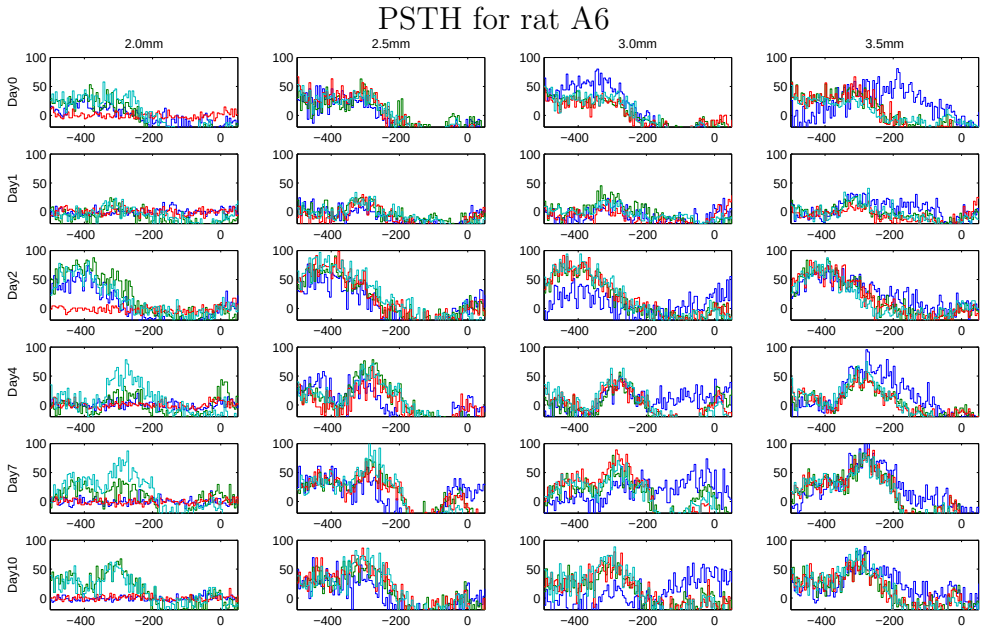


Figure D.6: The baseline measurements at 'Day 0' show higher activity from -500 ms to around -200 ms. After stroke induction (Day 1), the activity close to the stroke area diminished to almost nothing. The rest of the channels still shows some higher activity just around 300 ms. At 'Day 2' this modulates as the higher activity from -500 to -200 ms is visible again in all the channels. Interestingly, the very characteristic activity from channel 13 (blue at 3.5 mm) at 'Day 0' is gone at Day 2 where it follows the general trend. At 'Day 4', a wave is visible again at around -300 ms - this modulates until 'Day 7' and 'Day 10 where the activity from -500 to around -200 ms is visible again.

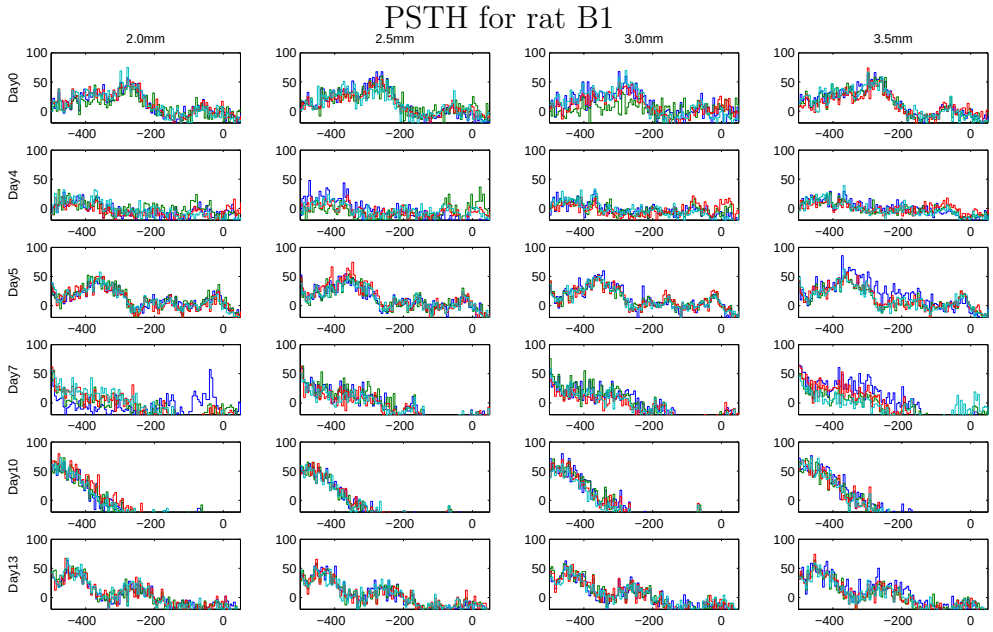


Figure D.7: The Day 0 recording reveals 1-2 waves. One, which is in all channels, is placed around -270 ms and the other which is visible in about two third of the channels, is placed around -400 ms. 4 Days post stroke shows a large decrease in activity, and only a slight elevation is visible around -400 ms. Day 5 shows a larger activity around the -400 ms mark, but still no elevation at -270 ms. Day 7 also show only 1 wave now, but its width is increased and covers the period -500 to -210 ms. At Day 10, this modulation changes and shortening the wavelength and reveals a slight 'bump' at around -270 ms. This 'bump' is most visible in the channels at 2.5 mm to 3.5 mm from stroke. When seeing the data from day 13, this bump could be the beginning of the neurons returning to the two waves that were visible at Day 0 - they are fully visible at Day 13.

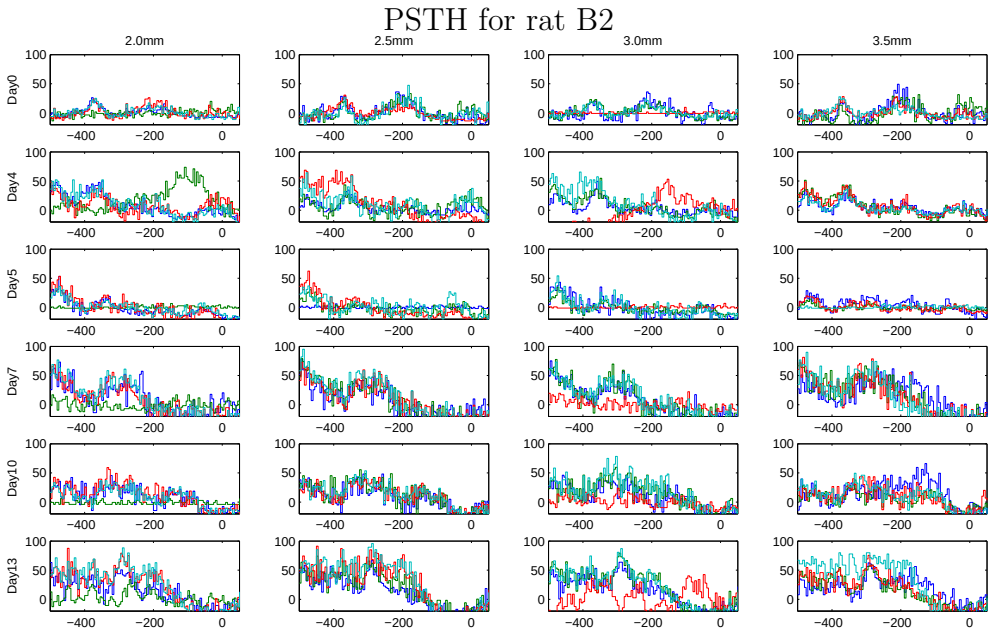


Figure D.8: The baseline measurements at Day 0 show two waves at -380 ms and -200 ms. There are still the same tendencies at 'Day 4' but the green graph at 2.0 mm (Channel 2) shows a lot of activity at -100 ms - the same is seen in channel 11 (red graph at 3.0 mm). This activity is seized at Day 5, and only a little activity is seen at the -380ms mark, but more activity is seen around -500 ms. The same is seen at Day 7 but it is more obvious now. After Day 7, there is no apparent modulation, and although this is 13 days after stroke, the activity is still very different from the baseline measurement.

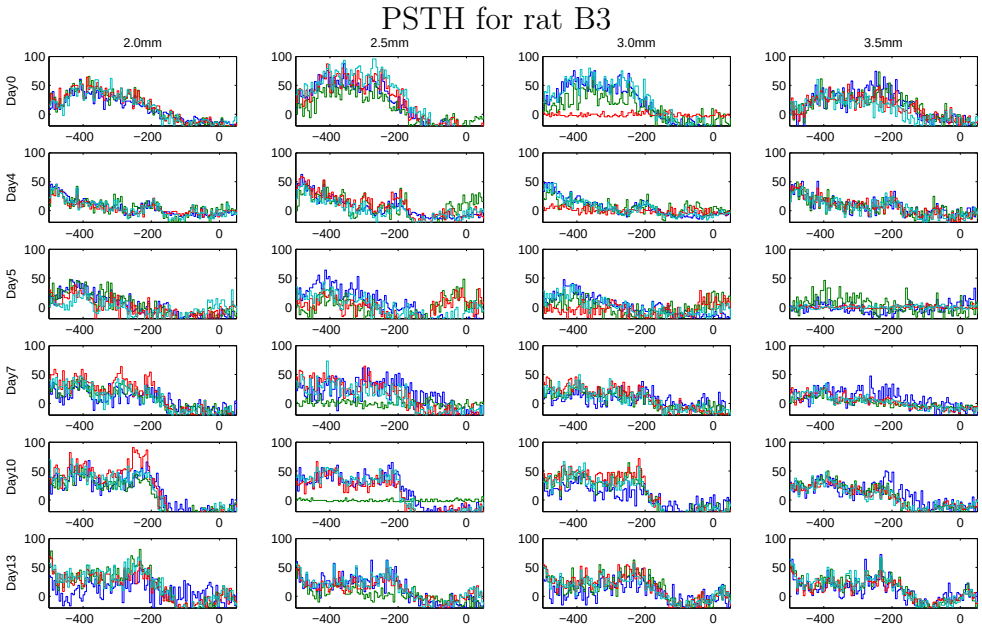


Figure D.9: A long wave from -480 ms to -200 ms is seen in the baseline measurement at Day 0. At Day 4, the wave disappears and modulates into two waves, one around -500 ms and one around -200 ms. The PSTH modulates the following recording sessions and seems to return to pre-stroke status at Day 10. See Figure D for full page illustration.

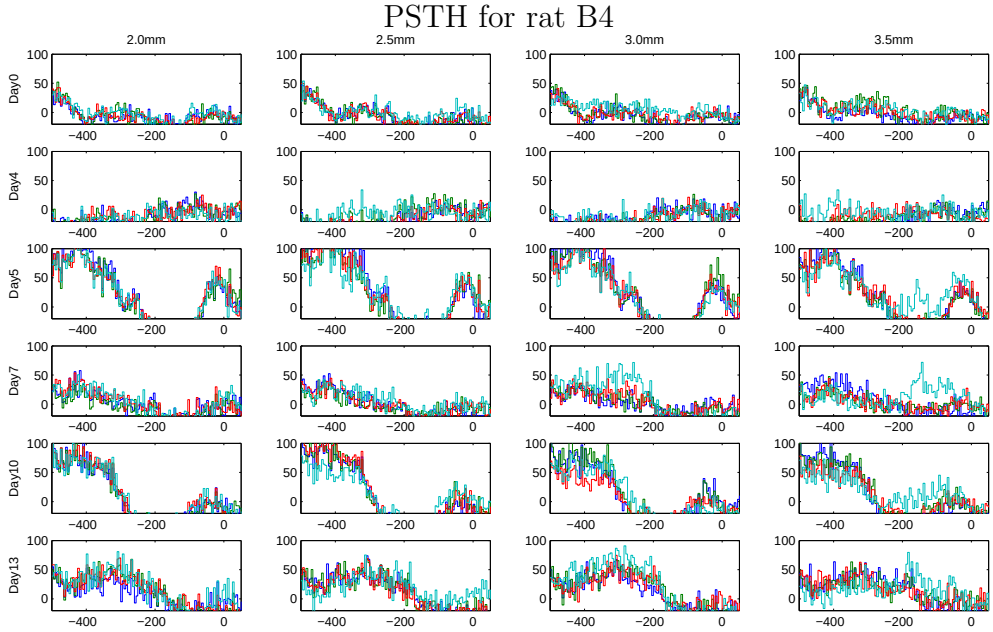


Figure D.10: At Day 0 two waves is seen, one at -500 ms and one at -300 ms. At Day 4 the wave at -500 ms is gone and a new one is found at around -100 ms. It is further modulated into Day 5, where a large amount of activity is seen from -500 to -220 ms and another wave is seen around 0 ms. The wave around the trigger event is probably the paw withdrawal movement. The same is true at Day 7 and Day 10, but in Day 13 the pattern is slightly modulated back towards what we see in the baseline measurements.

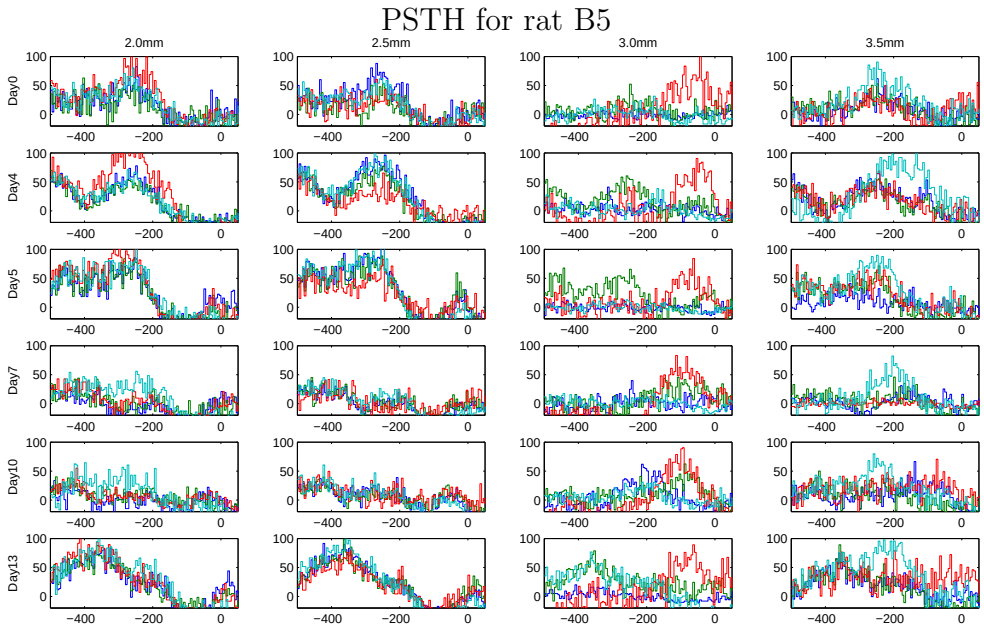


Figure D.11: At Day 0 there is high activity from -500 to -200 ms with a peak around -290 ms. One exception is Red graph at 3.0 mm (Channel 11) which have a peak around -100 ms. This particular neuron(s) does not change firing pattern throughout the experiment, and it seems to be encoding for the withdrawal movement (due to its timing). At Day 4, the activity is more divided into two waves, one around -500 and one around -290ms. This is also true at Day 5, although the division is less apparent. At Day 7 and Day 10, the activity is still around the same area, but is less evident. This modulated into Day 13 where the firing pattern again is high from -500 ms to around -200 ms. The high peak is shifted compared to Day 0 - it is now around -380 ms. It does seem like the rat is performing the movement slightly differently when looking only at the PSTH data.

Table D.1: **Table of modulations - Group A**

Rat	Process mod.	Rehabilitation mod.	Notes
A1	Modulates through Day 1 to Day 7 showing no particular pattern	Returns to pre-stroke patterns after rehabilitation ending	Day 1 recording had too few repetitions to make any useful evaluation
A2	The baseline firing pattern is visible at Day 1. Modulates from Day 1 to Day 4 and returns towards pre-stroke at Day 7. Baseline firing pattern tendency is seen through Day 1 and Day 2 but seem to modulate a lot from Day 2 to Day 4	Returns to pre-stroke patterns after rehabilitation ending	Channel 13 (Blue at 3.5 mm) fires at a unique pattern at Baseline measure - this tendency was not observed in the rest of the plots
A3	The baseline firing pattern is visible at Day 1, but modulates through Day 4 to Day 7	Returns to pre-stroke patterns after rehabilitation ending	Very little firing is seen at Day 2 recordings which might indicate much noise, although Channel 14 (Red at 3,5 mm) show a very characteristic firing pattern which might indicate some other cause
A4	Baseline firing pattern is distantly visible in Day 1 recording, and modulates until Day 7	The Day 10 measurement is different from the baseline measurement	Channel 5 (Blue in 2.5 mm) have very high activity from -400 ms to 0 ms at Day 1 to Day 7 recordings.
A5	Day 1 to Day 4 is very similar, before the firing pattern modulates to Day 7, which is more like the baseline measurement.	The Day 10 is slightly different from the baseline measurement, but still shows the same tendencies	
A6	Modulates from almost no characteristic at Day 1 through one wide wave at Day 2 and a narrow wave at Day 4. At Day 7, the pattern is again similar to baseline firing pattern.	Returns to pre-stroke patterns after rehabilitation ending	

Table D.2: **Table of modulations - Group B**

Rat	Process mod.	Rehabilitation mod.	Notes
B1	The firing pattern modulates with no apparent underlying pattern, and baseline pattern is not visible in Day 4 recording	The Day 13 measurement is different from the baseline measurement	The firing pattern modulation continuous throughout the experiment, and does not seem to approach an equilibrium through the 13 days
B2	Baseline firing pattern is not visible in Day 4 recording, but Day 7 and Day 10 is very similar	The Day 13 measurement is different from the baseline measurement	Day 7, Day 10 and Day 13 is very similar indicating some equilibrium, although it is different from the baseline measurement
B3	Baseline firing pattern is not visible in Day 4 recording. The pattern modulates further until Day 7 and Day 10	Firing pattern is different between Baseline and Day 13, but some correlation is seen between the two	
B4	Baseline firing pattern is not visible in Day 4 recording. A large modulation takes place from Day 4 to Day 5 after the same tendencies is seen until Day 10.	Returns to pre-stroke patterns after rehabilitation ending	Firing around 0 ms is introduced in measurements after Day 4
B5	Baseline firing pattern is still visible in Day 4 and Day 5 recordings, after which it modulates slightly.	Returns to pre-stroke patterns after rehabilitation ending	Channel 11 (Red at 3.0 mm) shows a very high activity around -100 ms throughout all recordings

Enclosure E

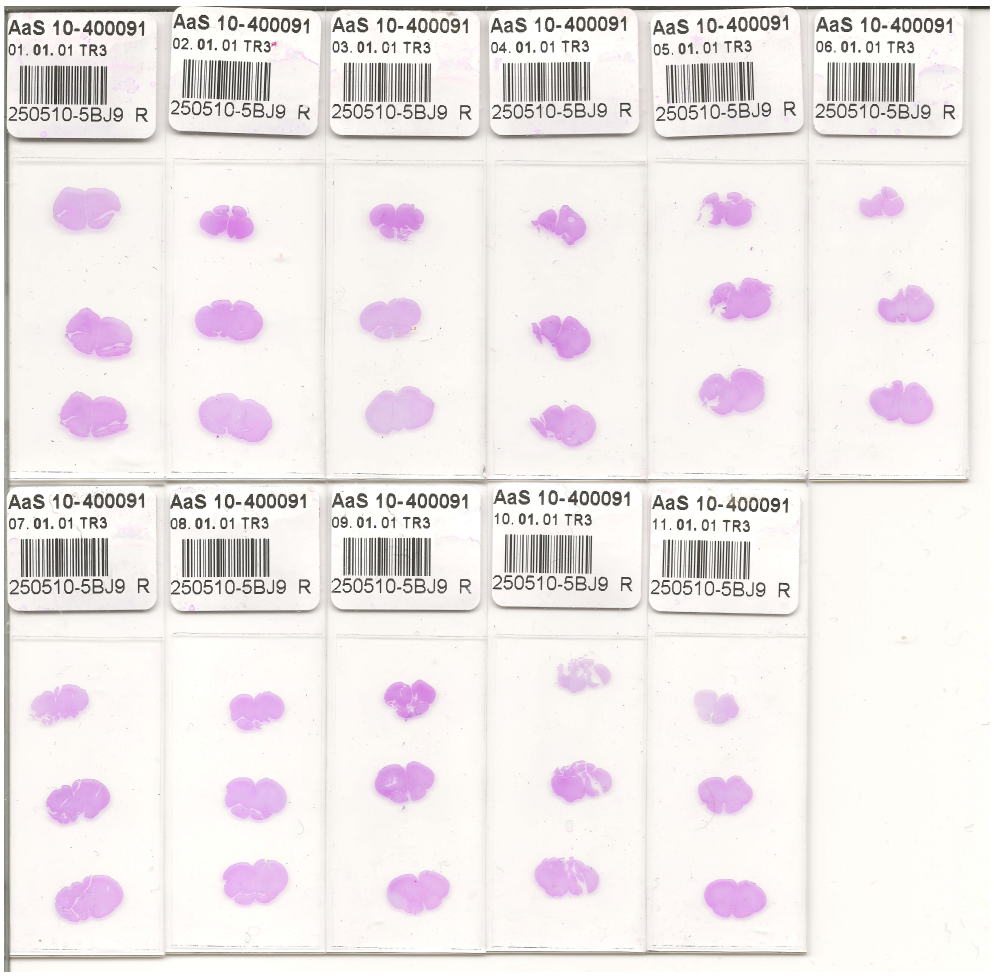
PSTH analysis

Rat	Period	Shift of peak				Number of peaks			
		2	2.5	3	3.5	2	2.5	3	3.5
A1	Baseline - Day 1					-	-	-	-
	Day 1- Day 2					+	+	+	+
	Day 2 - Day 4					-	-	-	-
	Day 4 - Day 7					+	+	+	+
	Day 7- Day 10								
A2	Baseline - Day 1				-				-
	Day 1- Day 2					+	+	+	+
	Day 2 - Day 4	-	-	-	-	-	-	-	-
	Day 4 - Day 7	-	-	-	-	+	+	+	+
	Day 7- Day 10			-					
A3	Baseline - Day 1								
	Day 1- Day 2								
	Day 2 - Day 4					+	+	+	+
	Day 4 - Day 7					-	-	-	-
	Day 7- Day 10								
A4	Baseline - Day 1		-		-			-	-
	Day 1- Day 2	-	-	-	-		+	+	+
	Day 2 - Day 4	-		-	-	-	-	-	-
	Day 4 - Day 7	-		-	-	+	+	+	+
	Day 7- Day 10								
A5	Baseline - Day 1		-		-	-	-	-	-
	Day 1- Day 2					+	+	+	+
	Day 2 - Day 4					-	-	-	-
	Day 4 - Day 7	-	-	-	-				
	Day 7- Day 10	-	-	-	-	+	+		
A6	Baseline - Day 1	-	-	-	-	-	-	-	-
	Day 1- Day 2	-	-	-	-				
	Day 2 - Day 4	-						+	
	Day 4 - Day 7					+	+		
	Day 7- Day 10								

Rat	Meassurment	Shift of peak				Number of peaks			
		2	**2.5**	**3**	**3.5**	**2**	**2.5**	**3**	**3.5**
B1	Baseline - Day 1	-	-	-	-	-	-	-	-
	Day 1- Day 2					+	+	+	+
	Day 2 - Day 4	-	-	-	-	-	-	-	-
	Day 4 - Day 7	-	-	-	-				
	Day 7- Day 10	-	-	-	-	+	+	+	+
B2	Baseline - Day 1					+	+	+	+
	Day 1- Day 2					-	-	-	-
	Day 2 - Day 4					+	+	+	+
	Day 4 - Day 7					-	-	-	-
	Day 7- Day 10	-	-	-	-				
B3	Baseline - Day 1	-	-	-	-	+		+	+
	Day 1- Day 2	-	-	-	-				
	Day 2 - Day 4					+	+	+	
	Day 4 - Day 7					-	-	-	-
	Day 7- Day 10	-	-	-	-				
B4	Baseline - Day 1	-	-	-		-	-	-	-
	Day 1- Day 2	-	-	-	-	+	+	+	+
	Day 2 - Day 4								
	Day 4 - Day 7								
	Day 7- Day 10	-	-	-	-				
B5	Baseline - Day 1						-	+	
	Day 1- Day 2						+		
	Day 2 - Day 4	-	-	-	-			-	-
	Day 4 - Day 7								+
	Day 7- Day 10	-	-	-	-				+

Enclosure F

Histology



Histology number:	1	2	3	4	5	6	7	8	9	10	11
Rat ID:	A3	B2	B3	A1	B1	A2	B4	A6	B5	A4	A5