FACULTY OF ENGINEERING

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Neocortex information processing after stroke induction

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1 Abstract

How the brain processes all the incoming information it receives is still an unanswered question, and many different theories about how it is done exist. This thesis expands on one of these theories which views the brain's information processing as a widely distributed network phenomena, which contradicts the currently most popular hierarchical theory consisting of areas dedicated to processing specific types of information in a hierarchical manner. Here we addressed the issue by recording the responses of individual neocortical neurons to a repeatable battery of tactile stimulation patterns in the anesthetized rat *in vivo*. By investigating the potential changes in the responses to direct current (DC) stimulation of cortical areas with a remote location to the recorded neuron, we aimed to get an indication about the distributed nature of processing of tactile information. The effects of the applied DC was also studied in order to see if it could be used as a new and reversible model for stroke induction. The DC stimulation was associated with a statistically significant effect on the responses to the tactile stimulation patterns. The precise nature of those changes remains unanswered, though, and further research needs to be performed in order to find the answer to that question. However, the results support a distributed network processing of the brain's information processing.

2 Preface

The project documented in this thesis report was performed between August 2015 and January 2016 during the fall semester with the group ”Neural Basis of Sensorimotor Control” at the department of Experimental Medical Science belonging to the Faculty of Medicine at Lund University. The degree course was ”Degree Project in Biomedical Engineering” (BMEM01) at the department of Biomedical Engineering at Lund University.

The purpose of this thesis project was to further the understanding of how a brain processes sensory information, and how this is affected by a neurological deficiency. The chosen deficiency was stroke and the project also included an attempt to develop a new reversible stroke model.

I wish to thank my supervisor Henrik Jörntell for his guidance, patience and for introducing me to the project, my co-supervisor Jonas Enander for teaching me the experimental procedures and giving me support during the programming required for the project, Anton Spanne for his help with the statistics used and Kersti Larsson for her assistance during the performed experiments.

The thesis project was preceded by a course in animal handling corresponding to 3 ECTS-credits at Lund University which resulted in a certificate allowing for laboratory animals to be handled by the certificate owner. This was necessary in order for the experiments to be performed. All experimental animal procedures in the project were approved by the Local Animal Ethics Committee of Lund.

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3 Introduction

The tactile sense, which enables us to experience a sensation of touch, is made up of a large number of tactile receptors mainly located in the skin. The signals generated by these receptors are transmitted through the so called primary afferents in the peripheral nervous system to the central nervous system. The signals are partly processed in the brainstem and the thalamus before reaching the primary somatosensory cortex.

Currently there exists several different theories about how the brain processes information. The theory which is currently the most widespread and popular is based on a hierarchical view of sensory processing [1]. In this view information is processed in several different levels of increasing complexity where a neuron in specific processing level receives input from several neurons in the processing level below, creating a pyramid-like model, also known as a bottom-up process. As an example imagine reading a word in a text. Say that a specific neuron reacts only upon viewing a horizontal bar and sends information to a higher level when it detects one. This higher level receives input from several neurons and combines it into the letter A and sends this information to yet another higher level. This level receives input from several below and combines the input to form the word ”Apple”. Other levels even higher up then make you able to understand the meaning of the word and make you think about what an apple is. In the case of tactile sensory information we have the vertical columns in the primary somatosensory cortex which projects onto the secondary somatosensory cortex and onward to the posterior parietal cortex where the perceived touch is given a meaning in the association cortex [1]. With this view one arrives at the detailed homunculus maps that can be found in many of today's neurological textbooks showing which area of the cortex is dedicated to processing a certain specific type of information, such as the one seen in Figure 1.

However, based on some recent studies one can assume that all processing in neocortex is widely distributed [2][3], i.e. that you use nerve cells spread out over large parts of the cortex even in seemingly simple tasks such as the flexing of a single digit or to process sensory information from a small patch of skin.

With this view of how the brain processes information the occurrence of a neurological disease damaging a small portion of the neocortex could have widespread consequences affecting many different brain functions. A stroke

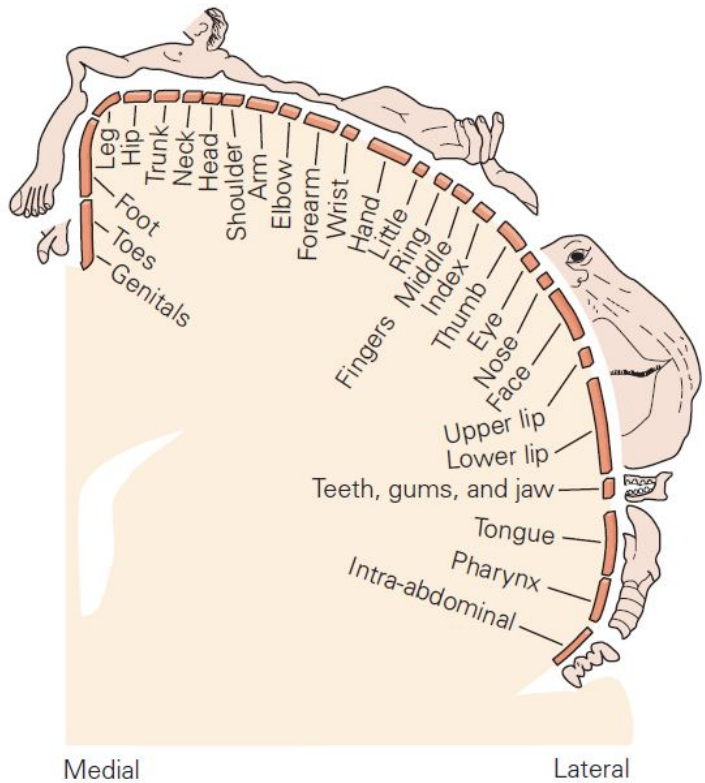

Figure 1: The cortical sensory homunculus showing the sensory somatotopical map. Image from [1] page 364.

occurring in the brain cortex would remove a part of the neuron population in the area of the stroke. Since neurons are interconnected in what can be assumed to be networks of a specific structure such a damaging event would result in a decrease in the functionality of the network.

The term stroke refers to a neurological deficit of cerebrovascular cause, i.e. a problem in the brain's blood supply causes either temporary cell damage or cell death due to the lack of oxygen and nutrients in the area affected by insufficient blood supply. Stroke can be divided into two major classes, ischemic strokes and hemorrhagic strokes[4]. An hemorrhagic stroke occurs when a cerebral blood vessel ruptures and is thus unable to supply blood. An ischemic stroke is caused by a reduction of blood flow due to obstructions in the blood vessel. The blockage of a vessel can be caused by either a thrombus or an embolus[4].

Receiving a stroke is one of the most common causes of death in the industrialised world and the most common neurological cause for hospital admission [4][1]. It is thus very important to study the causes of strokes and the effects it can have on how the brain functions. In order to do this it is imperative to have a good model which can be used to study strokes in experimental animals.

The goal of this thesis is to develop a method to reversibly block out the functionality of a part of the local population of neurons. This can simultaneously work as a stroke model in order to analyse the effects of a stroke on the function of the neural network and at the same time test the hypothesis that the functionality of the brain is distributed over large areas of the brain cortex. The advantage of a reversible stroke model compared to a model where a permanent stroke is induced is that an experiment studying the effects of a stroke can be repeated several times in the same experimental animal. This could possibly enable a considerably larger amount of data to be collected from each experiment thus reducing the number of animals used for each study, saving both animals, money, and time in future research projects.

In order to measure the effect on information processing of a stroke a reasonably complex brain activation had to be produced. The activation additionally had to be reproducible. To achieve this we used spatiotemporal activation of primary afferents in digit 2. The simulation is based on the output of a finger prosthesis used to create spatiotemporal patterns of skin activation, patterns which can be expected to occur naturally. This is important since the structure of the brain's neural network can be expected to be adapted to patterns of sensory activation it has previously been exposed to, occurring naturally when interacting with the physical environment [5][6][7].

After this brief introduction to the purpose, aim and theory behind the project the outline of the remaining report is as follows: first, the procedures of animal preparation and setup is presented, followed by an explanation of the stimulation and recording techniques used combined with lists of the used materials. Then the results from the recordings are presented and explained followed by some meta-analysis of the results. This is followed by the results, the implications of which are examined together with a discussion about restrictions of the project, and ultimately, some recommendations for future research and analysis. Lastly there is a discussion about the largest challenges and what I have gained from the project, followed by a summary of the project.

4 Methods

4.1 Animal preparation

4.1.1 Resources

- Scissors
- Electric razor
- Straight tweezers
- Toothed tweezers
- Hooked tweezers
- Scalpel
- Blunt Scalpel
- Hooks
- Surgical tape
- Catheters
- Syringes
- Surgical needle
- Needle-holder
- Surgical suture thread
- Cotton
- Sodium chloride
- Ketaminol
- Rompun
- Glucose solution
- Ringer's acetate solution
- Euthasol
- Bone scissors
- Dental drill
- Agar
- Paraffin oil
- Beakers
- Magnetic stirrer
- Optical microscope
- Spongostan
- Heated operating tables
- Stereotaxic frame
- Heated water bath

4.1.2 Procedure

For the experiments Sprague Dawley rats (N=32, weight 300-450g) were used. The rats were sedated using isoflurane (3% mixed with air) and given anaesthesia (a mixture of Ketaminol (Ketamine 100 mg/ml), Rompun (Xylazine 20 mg/ml) (proportions Ketamine:Xylazine 50:3) and Sodium Chloride 9 mg/ml) via an intraperitoneal injection and then left to rest for 5 min-

utes to allow the anaesthetic to take effect. That the level of anaesthesia was adequate was made sure of by observing the animal and by an absence of withdrawal reflexes when given a noxious pinch to a hind paw with a pair of toothed tweezers. If the level of anaesthesia was not deep enough an additional intraperitoneal injection was given, otherwise the rat was placed on the operating table and shaved on the top of its head and on the lateral portion of the right hindleg. With the animal lying on its back an incision was made 0.5 cm from the midline along the axis of the femoral bone. By blunt dissection the femoral vein was exposed, and the wound was held open using small hooks. Distal blood flow in the vein was blocked with a ligature. An incision was made into the vein and a catheter was inserted and then secured using surgical sutures. If the procedure was not successful then the whole surgery was repeated on the opposite side of the rat. A suture was placed to close the wound and the rat was moved to a second operating table where additional anaesthesia was given intravenously a mixture of Ketaminol (Ketamine 100 mg/ml), Rompun (Xylazine 20 mg/ml) (proportions Ketamine:Xylazine 20:1), Ringer's acetate solution and Glucose solution (50 mg/ml). A second check for withdrawal reflexes was performed after a few minutes.

The head of the rat was fixated with a stereotaxic frame and a incision was made along the midline of the skull with a scalpel. Blunt dissection was again used to remove the skin and the periosteum in order to expose the right side of the skull. The skull was then carefully thinned down using a dental drill and the last layer of bone was removed using tweezers and different types of bone scissors while making sure that the dura mater was intact and kept moist. This was achieved with intermittent application of saline at body temperature. When the brain was exposed a pool was constructed around the exposed area using wool dipped in warm agar mixed with a sodium chloride solution in order to produce a gel and filled with paraffin oil, chosen for its low reactivity and conductivity. Open brain recordings could then be performed while the brain was constantly kept moist with the oil also acting as a thermally isolating layer in order to keep the brain from cooling down. A majority of the procedure was performed under a optical microscope.

The animal was euthanized 8 hours after the initial sedation by injection of a lethal dose of Euthasol (Pentobarbital 400 mg/ml). Body temperature was kept constant during the whole experiment using heated operating tables.

4.2 Neural recordings

4.2.1 Resources

- Computer
- Spike2 software
- Ball electrode
- Glass micro pipettes
- Micro pipette puller
- Signal amplifiers
- Analog-to-digital converters
- Constant current stimulators
- Needle electrodes
- Hook electrode
- Step motors
- Silver plate electrodes
- Syringes

4.2.2 Recording procedure

Three types of data were collected during the experiments: the activity of individual cells in the form of action potentials and the activity in their local surroundings called field potentials; and the spontaneous activity of the brain. Action potentials are seen as a very rapid increase in a cells membrane potential followed by a rapid decrease. Field potentials are seen as large decreasing shifts in the membrane potential lasting several milliseconds.

Spontaneous brain activity was recorded with the help of Electrocorticography (ECoG) using a ball electrode placed on top of the dura covering the cerebrum. This spontaneous activity was used to study the depth of anaesthesia by looking at the occurrence of sleep spindles as a sign of deep sleep [8][1]. The ECoG was also used to study the occurrence of abnormal brain activity which might have been due to experimental procedures.

The activity of individual neurons and their immediate surroundings was studied using the *in vivo* patch clamp technique in the current clamp mode where the current is kept constant and the change in electrical potential is measured. This technique uses a glass pipette which is created by pulling the pipette apart while heating it using a micro pipette puller in order to produce a very fine tip with an opening of just a few micrometres. The pipette is then filled with a solution that mimics the intracellular fluid of neurons which also conducts electricity. The electrode is then connected to amplifiers and a analog-digital converter. The electrodes were then lowered

down into the brain using a microcontroller connected to the stereotactic frame holding the electrode at very low speeds (between 0.3 μm and 4 μm per second). The recording system was then bridge balanced and capacitance compensated, and the output signal which showed fluctuations in voltage at the electrode tip could then be studied using several displays showing different time spans using Spike2 software (Cambridge Electronics Devices, CED, Cambridge, UK, version 7.03) and a loudspeaker in order to easier find neural activity. A grounding electrode was attached to the neck muscles. The patch clamp recordings were done in two different methods, either the loose patch mode for extracellular recordings or the whole cell mode for intracellular recordings.

In loose patch mode the tip of the pipette is brought into close proximity to a neuron of interest and a low suction is applied in order to form a loose seal between the pipette tip and the cell membrane of the neuron. The advantage of this mode is that the cell membrane is left intact, not damaging the cell or influencing the composition of the cytoplasm by mixing it with the solution in the pipette[9]. This enables several recordings to be made on the same cell over a long time. The loose patch mode is used to record action potentials extracellularly. An additional advantage to the loose patch recording is that it is much easier to achieve a full recording compared to when performing a whole cell mode recording, which is why the majority of the electrophysiological recordings in this thesis were made in loose patch mode. A schematic view of the loose patch mode can be seen in Figure 2.

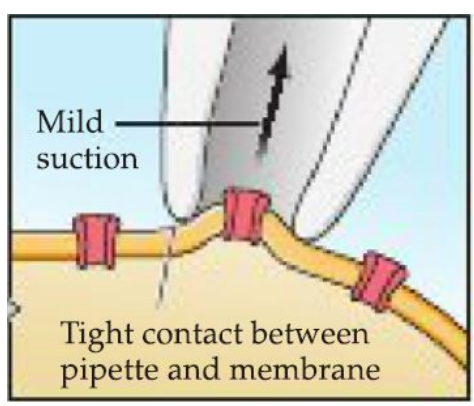

Figure 2: A schematic overview of the loose patch recording mode for a patch clamp recording. Image from [4] page 59

The whole cell patch mode is used when recording intracellular signals. In

this mode the tip of the pipette is again brought into close proximity of a neuron and suction is added so that a seal is formed between the cell membrane and the pipette tip. Even more suction is then briefly applied in order to rupture the cell membrane so that the intracellular fluid is continuous with the solution in the patch pipette [9]. The drawbacks of this technique are that if handled carelessly it can easily damage or kill the cell and over time the solution in the pipette will mix with the intracellular fluid which may affect the behaviour of the cell [4]. This makes it a challenge to perform long intracellular recordings. In order to avoid this the fluid in the pipette is made to be as similar to the intracellular fluid composition as possible. The advantage of this recording mode is that you get a very good electrical connection to the interior of the cell which enables you to study not only the generated action potentials but all the subthreshold potential changes in the cell. In other words you are able to study how the cell membrane potential changes over time due to excitatory and inhibitory input from other neurons connected to the studied neuron, which gives you information on the behaviour of the local neural network. The information content of an intracellular recording is thus much higher than an extracellular recording. A schematic overview of the whole cell patch mode can be seen in Figure 3.

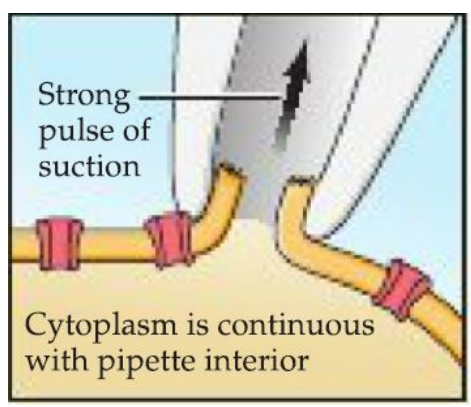

Figure 3: A schematic overview of the whole cell recording mode for a patch clamp recording. Image from [4] page 59.

4.2.3 Stimulation procedure finger electrodes

In order to reproducibly generate different types of tactile stimulations used to create a sensation of touch, eight needle electrodes in four pairs were attached to the index finger of the front paw contralateral of the studied brain

area (in this case the index finger of the left leg) in a specific pattern. During stimulation electrical pulses with an intensity of 0.5 mA and a duration of 0.14 ms was sent between the electrode pairs in twelve specific spatiotemporal patterns, out of which eight are the studied stimulus patterns and four are control patterns. These stimulation patterns generate a tactile sensation similar to that of natural tactile scenes [10], which have been shown to generate different cortical activity than simplified artificial stimulations [5]. A short pause was included after each pattern resulting in each stimulation lasting about 2 seconds. In total 440 stimulations were made for each type of context, so in the case of a electrical stroke experiment (see below) 440 stimulations without stroke stimulus and 440 with stroke stimulus was recorded, for a total of 880 stimulations. After this, recording continued without stimulation for a few hundred seconds in order to record any unusual behaviour resulting in a total recording time of about 30 minutes per recording.

During the experiments including a photothrombotic stroke (see below) 440 stimulations were made before stroke induction in order to observe the behaviour of the neuron without stroke. 880 stimulations were then made during the stroke induction to see how the neuron responded to the stroke and 880 stimulations were made after stoke induction in order to observe behaviour after stroke resulting in a total recording time of about 60 minutes.

4.2.4 Stimulation procedure stroke

Two different types of stroke stimulations were used in the project. The majority of the experiments (N=25) were performed to test the idea of the electrical stroke model using a trans-hemispheric DC stimulation. Two silver electrodes with a diameter of about 2 mm was placed about 1 cm apart along the rostrocaudal axis on the parietal cortex of the brain. A DC was then applied in pulses between them with an intensity varying between 5-70 μA with the majority of experiment being performed at a stimulus current of 10 μA and a duration of 2s. The stimulation pulses were timed so that the stimulation of the finger electrodes occurred at the middle of the stroke pulse duration. There was a short delay of a few milliseconds between each stroke stimulation pulse. The idea was to disrupt the functionality of the neurons located between the electrodes by affecting their membrane potential instead of actually killing them as in an actual stroke. The affected neurons would then hopefully return to their original behaviour once the current was turned off.

Some experiments (N=5) were also performed where the experimental stroke was induced using a well tested stroke model, called a photothrombotic stroke model. In this model a stroke is induced by injecting a photoreactive dye into the blood of the test animal. A laser of a specific wavelength (561 nm) is then directed at the area where the stroke is supposed to be induced. The laser excites the dye molecules causing oxygen radicals to form, which damages the cell membranes of surrounding endothelial cells leading to platelet aggregation causing a thrombus to be formed [11]. This causes an ischemic stroke [12].

4.2.5 Recording positioning

In order to find the approximate position to record from a rat brain atlas was used to find the region of the primary somatosensory cortex of the forepaw. The final recording position was then found by studying the local field potentials which represent the synchronised activity of the surrounding neural tissue [1]. If the occurrence of field potentials continuously matched the occurrence of the search stimulus then one could assume that neurons in the area surrounding the electrode respond to the stimulation. When the occurrence of action potentials matched the occurrence of field potentials and the search stimulus, a recording was started and the stimulating protocol was initiated. An example of a recording can be seen in Figure 4.

The practical part of the thesis also included building, repairing and maintaining all the different types of electrodes used in the project.

4.3 Data analysis

The recorded raw data was converted to .mat-files using the Spike2 software. MATLAB (MATLAB Release 2015a, The MathWorks, Inc., Natick, Massachusetts, United States) was then used to perform the data analysis by first detecting the spiking and stimulation times from the recordings and then creating peristimulus time histograms, continuous spike histograms and calculating moving average values of these. MATLAB was then used in order to compare the response magnitudes for the different stimulation patterns and perform Student's t-test in order to statistically compare the stimulus responses.

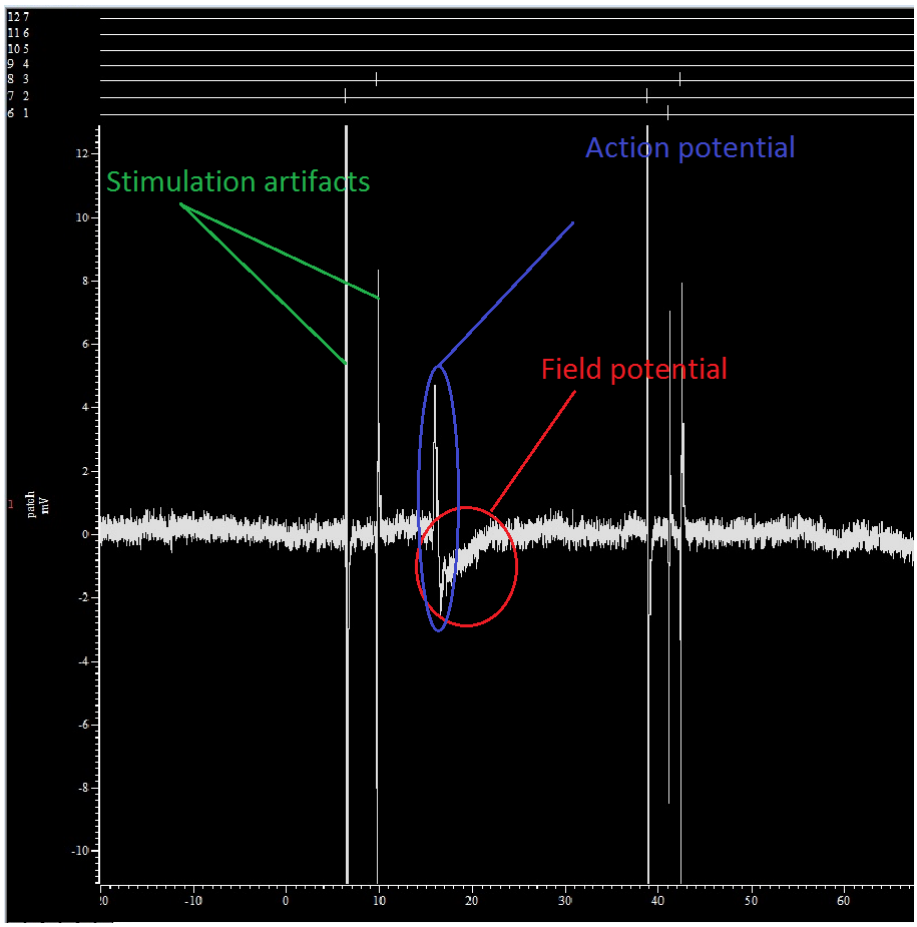

Figure 4: An example of an action potential occurring at the same time as a field potential during one of the experimental recordings.

5 Results

In total 25 experiments were performed in order to test the new stroke model and its effect on neural processing. Out of these 25 experiments only 21 generated any recorded neurons due to problems with either equipment or the used test animal, resulting in 4 experiments without any data being collected. In total 117 recording procedures were performed which in the end resulted in 82 cells surviving the entire recording procedure. Out of these only 57 could be analysed properly due to a low signal to noise ratio, from these 23 cells which had a robust response to the finger stimulation were found.

In order to study the behaviour of a cell and how it responded to the finger and stroke stimulation peristimulus time histograms were created, examples can be seen in Figure 5 and 6. They show the rate and timing of a neuron's action potential firing in relation to stimulus onset. The eight stimulus patterns are named 0.5 fa, 0.5 sa, 1.0 fa, 1.0 sa, 2.0 fa, 2.0 sa, flat fa and flat sa. In Figure 5 and 6 stimulation starts at time t=0s and they both show the stimulus patterns 0.5 fa and 1.0 fa together with their stroke stimulation counterparts for two different cells. In order to determine if a neuron had a robust response to the finger stimulation a visually distinct response profile in relation to spontaneous action potentials which create the background activity is needed. A robust response itself is needed in order to study how a cell's information processing changes when comparing a cell's natural response to finger stimulation to the response it has during the stroke stimulation. In Figure 5 the amplitude of the response patterns increases during the stroke stimulation compared to before stroke stimulation while the opposite can be seen in Figure 6 where it decreases.

Out of the 23 cells which had a robust response to the finger stimulation according to the peristimulus histograms, the change during the electrical stroke stimulation was quite varied. In 12 of the cells the stimulus response seemed to improve. This means that the probability of the cell responding with an action potential to the stimulation increased compared to the spontaneous firing of action potentials. In 4 of the cells the response decreased compared to the spontaneous firing, and in 7 of the chosen cells there were either no distinguishable change or the response seemingly improved for some stimulation patterns while it decreased for others, which makes it hard to decipher how the cell was affected by the stimulation.

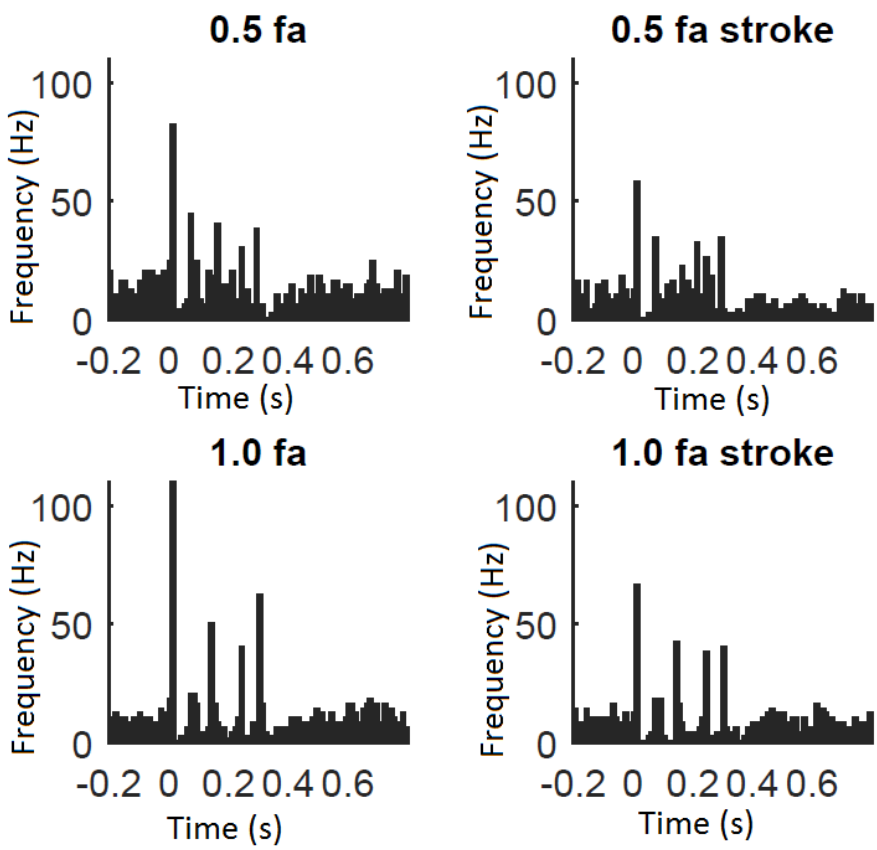

Figure 5: A peristimulus time histogram showing how a single neuron responds to two different finger stimulation patterns before and during electrical stroke stimulation. The cell's response decreases during stroke stimulation, indicated by a decrease in firing frequency compared to spontaneous activity.

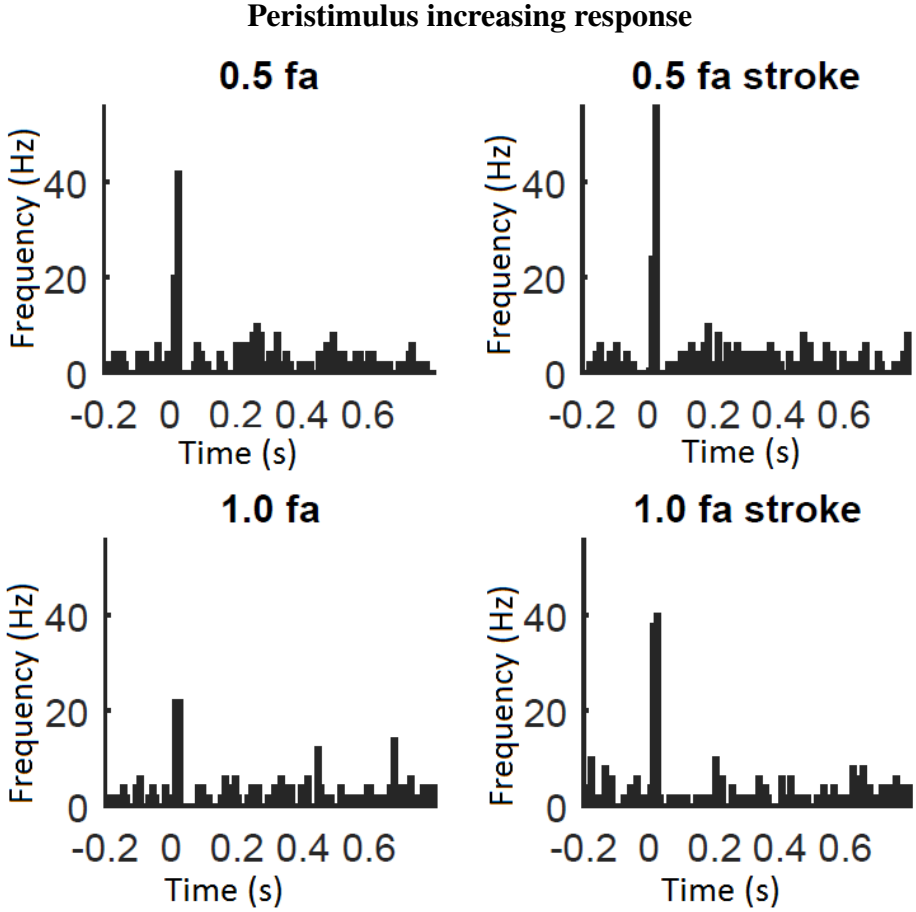

Figure 6: A peristimulus time histogram showing how a single neuron responds to two different finger stimulation patterns before and during electrical stroke stimulation. The cell's response increases during stroke stimulation, indicated by an increase in firing frequency compared to spontaneous activity.

In order to compare the change in activity before and during stroke stimulation more quantitatively the overall activity of the cells was compared before and during stroke. This was done by calculating the difference in mean activity in Hz before and after the onset of the finger stimulation and comparing before and during stroke. A collective mean was calculated for the activity 300 ms before the finger stimulation onset for all stimulation patterns for

each cell before and during stroke according to equation 1.

$$\text{Mean prestimulus activity} = \frac{\sum_{i=pattern1}^{pattern8} \frac{\int_{i=-0.3s}^{0s} \text{Firing rate}}{0.3s}}{8} \tag{1}$$

This in order to get a better measure of the background activity which can be assumed to be the same for all stimulus patterns since they are measured in the same cell. A mean activity was then calculated for the 300 ms after the onset of the finger stimulation for each stimulation pattern for each cell according to equation 2 for activity before stroke and equation 3 during stroke.

$$\text{Poststimulus activity nonstroke} = \frac{\int_{i=0s}^{0.3s} \text{Firing rate in Hz}}{0.3s} \tag{2}$$

$$\text{Poststimulus activity stroke} = \frac{\int_{i=0s}^{0.3s} \text{Firing rate in Hz}}{0.3s} \tag{3}$$

The mean activity after finger stimulation onset was then subtracted by the background activity for each stimulation pattern, before and during stroke, see equation 4 and 5.

$$\begin{aligned} &\text{Prestroke activity} = \\ &\text{Poststimulus activity nonstroke} - \text{Mean prestimulus activity} \end{aligned} \tag{4}$$

$$\text{Stroke activity} = \text{Poststimulus activity stroke} - \text{Mean prestimulus activity} \tag{5}$$

This gave a measure of the response strength of a cell to a specific stimulus pattern in Hz, both before and during stroke. The calculated value for the response strength was then compared before and during stroke by subtracting the activity before stroke stimulation from the activity during stroke stimulation as shown in equation 6.

$$\text{Change in activity} = \text{Stroke activity} - \text{Prestroke activity} \tag{6}$$

If the resulting value was negative then the cell's general response strength was weaker after stroke compared to before for that specific stimulation pattern and the reverse is true if the resulting value was positive.

The resulting change in activity is shown in Figure 7 for all pairs of stimulus patterns of all cells with a robust response sorted by order of occurrence in order to observe the trends in single cells and sorted by magnitude in Figure 8 to observe general trends. When looking at Figure 8 one can observe that the change in activity during the stroke stimulation is rather small for most stimulation pairs and when looking at Figure 7 it is shown that the stimulation pairs where there is a larger effect are originated in a few cells. It thus seems as if the stroke stimulation had a large effect on some cells while it had almost no effect on others.

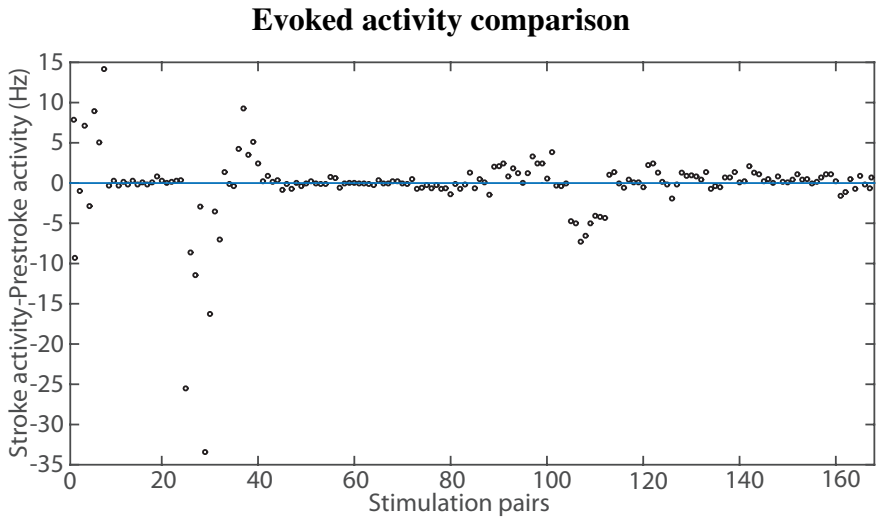

Figure 7: Comparison of activity level evoked by electrical finger stimulation 300 ms after stimulation onset before and during electrical stroke stimulation sorted after occurrence.

In order to study the observed effects in greater detail the poststimulus activity was then studied in time windows of 50 ms ranging from 0 to 400 ms and then the same analysis was performed as above. The results can be seen in Figure 9 and 10. Figure 10 indicates that an increase in activity after stroke was more common in earlier stages of a cells stimulus response than in later stages of the response.
Student's t-test was then performed on the data in order to statistically assess any effects of the electrical stroke stimulation. Here the response distributions of the 50 repetitions of each non stroke finger stimulus pattern was compared to its stroke stimulation counterpart in order to see if there was a significant difference between them, which would indicate that the stroke stimulation really had an effect on the information processing in the individ-

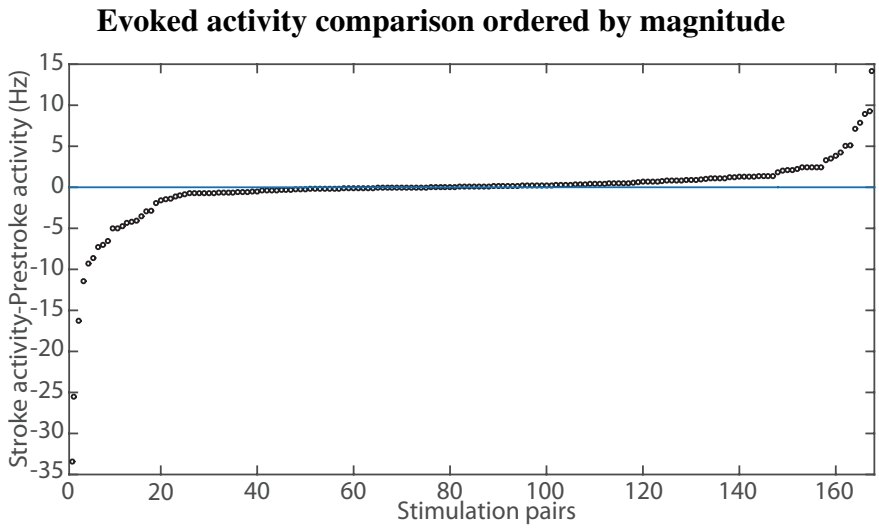

Figure 8: Comparison of activity level evoked by electrical finger stimulation 300 ms after stimulation onset before and during electrical stroke stimulation sorted after magnitude.

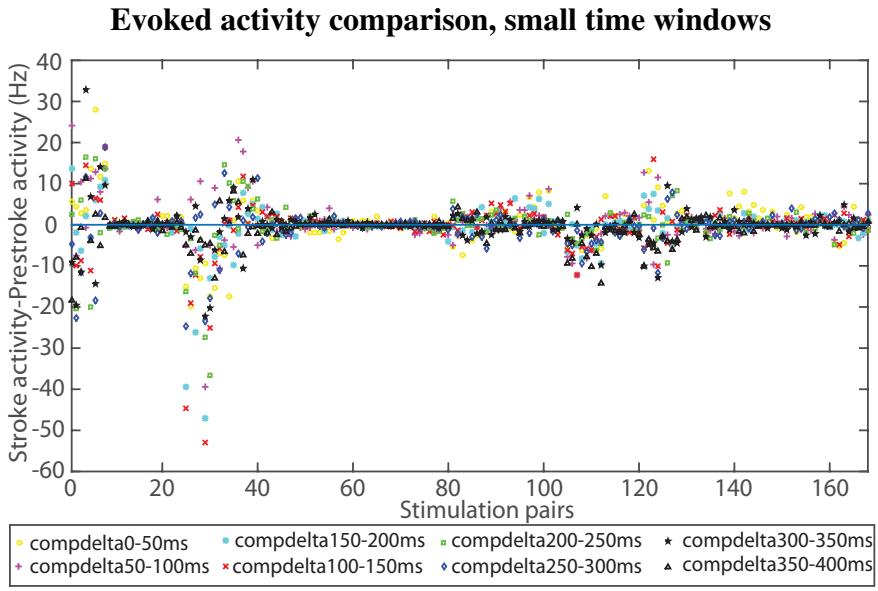

Figure 9: Comparison of activity level evoked by electrical finger stimulation, split into in 50 ms time frames ranging from 0-400 ms after stimulation onset before and during electrical stroke stimulation sorted after occurrence.

ual neuron through cortical network effects. A low p-value means that the

Evoked activity comparison, small time windows ordered by magnitude

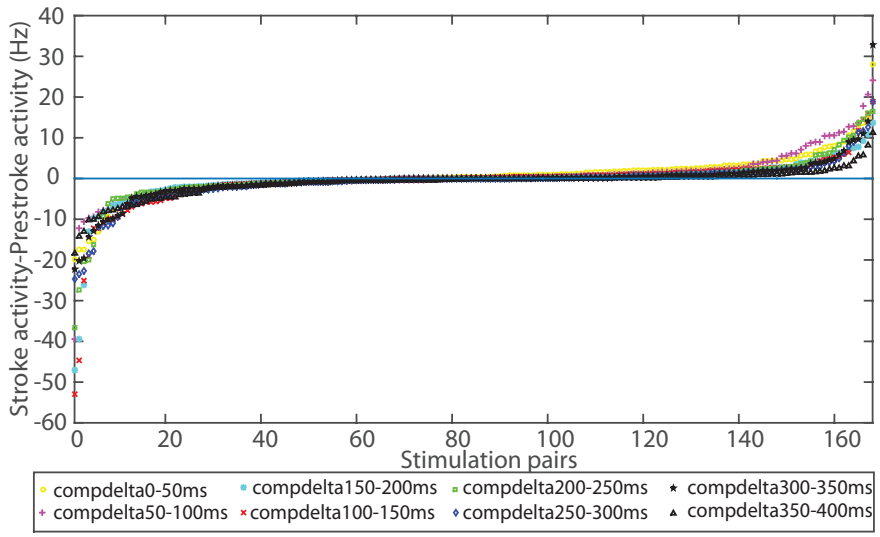

Figure 10: Comparison of activity level evoked by electrical finger stimulation, split into in 50 ms time frames ranging from 0-400 ms after stimulation onset before and during electrical stroke stimulation sorted after magnitude.

probability of the two paired distributions being the same is low. The results are displayed in Figure 11 when comparing the distributions of the first 300 ms after the start of the stimulation pattern and in Figure 12 when comparing the distribution of the eight 50 ms time frames for each pair of stimulation patterns. When looking at Figure 12 one can observe that the effects of the electrical stroke stimulation seems to be larger for the later components of the response as the number of significantly different distributions increases as the studied time frame moves further away from the stimulus onset.

It was also of interest to study the recorded cells firing frequency and how it varied over the duration of the recording. In many of the 57 cases there seemed to be no major change in the firing frequency but in 18 of the recordings the firing frequency increased over the duration of the recording as indicated by the calculated moving average shown in Figure 13. However, since the start of the stroke stimulation at 650 seconds into the recording is mismatched with almost all of the observed increases in firing frequency it is hard to draw any proper conclusions about if the stroke stimulation is the cause of the increase. One effect which was observed during in total 5 recordings is shown in Figure 14. Here the regular firing activity of a cell is interrupted by a sudden and huge increase in the firing frequency followed

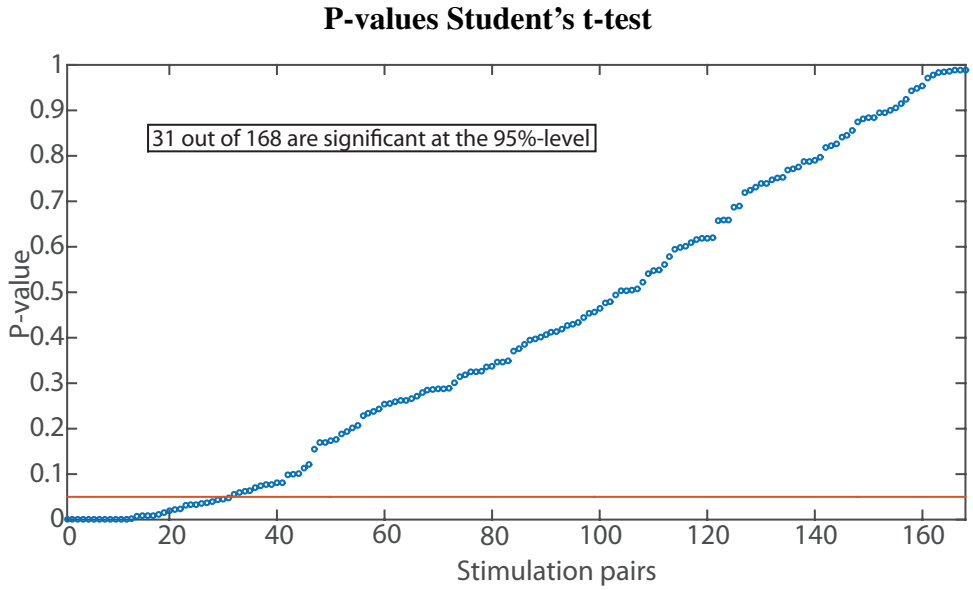

Figure 11: P-values for Student's t-test when comparing the response distributions of a stimulation pattern and its stroke counterpart 0-300 ms after the start of the pattern for all stimulation patterns and all cells with a robust response.

by a period of complete silence. The observed increases and decreases varied in duration and magnitude between the different observations.

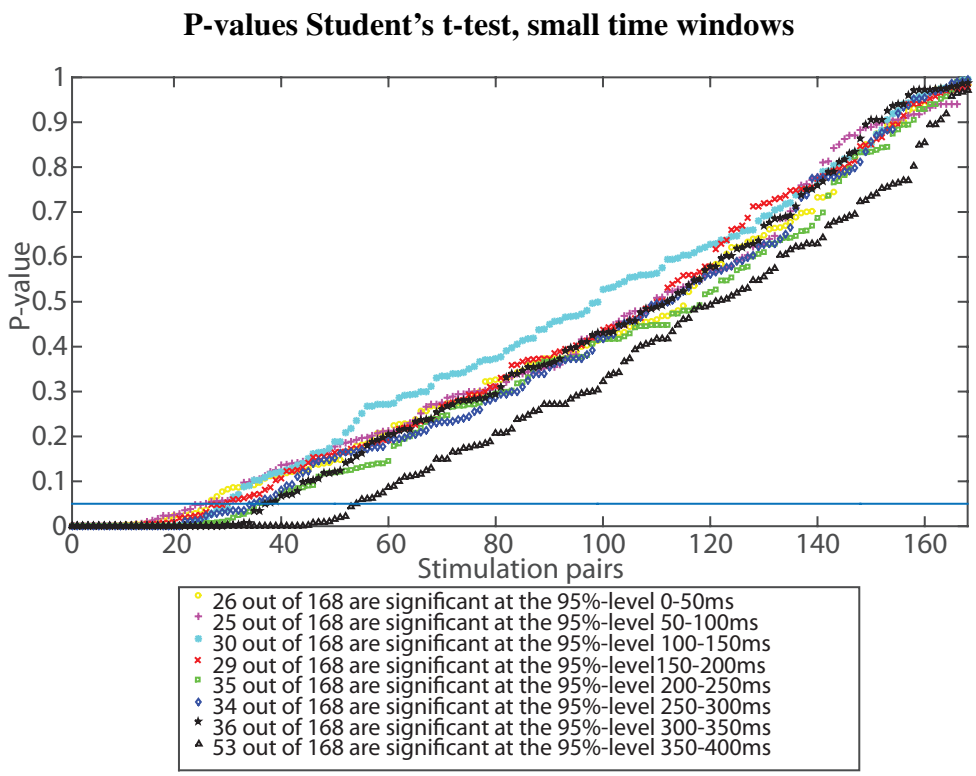

Figure 12: P-values for Student's t-test when comparing the eight response distributions of a stimulation pattern and its stroke counterpart split into 50 ms time frames 0-400 ms after the start of the pattern for all stimulation patterns and all cells with a robust response.

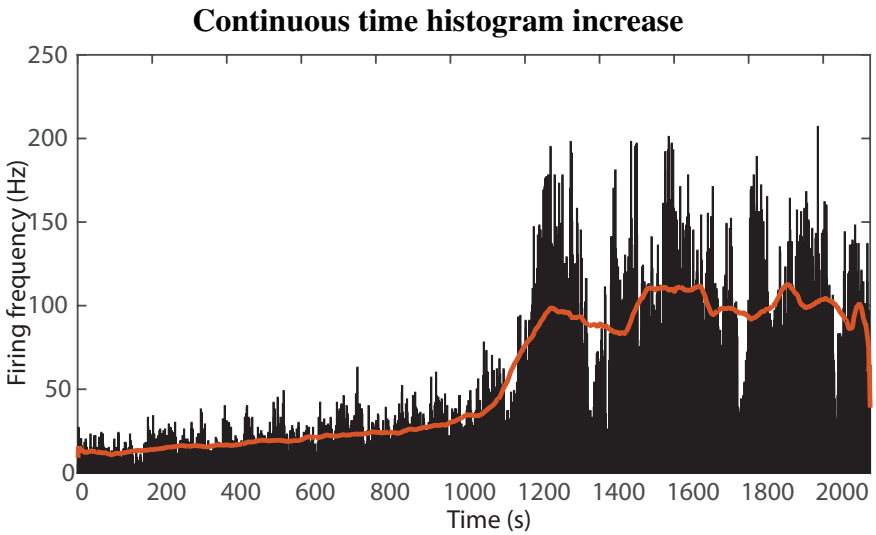

Figure 13: Continuous time histogram showing the firing frequency of a recorded neuron and how it varies over the duration of the recording. The orange line shows the calculated moving average frequency for the recording. In this case the firing frequency of the neuron increases markedly halfway into the electrical stroke stimulation procedure.

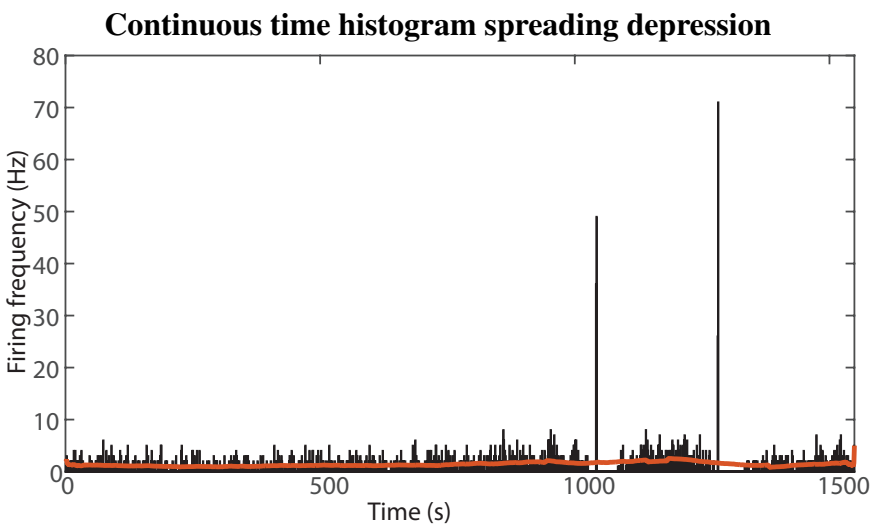

Figure 14: Continuous time histogram showing the firing frequency of a recorded neuron and how it varies over the duration of the recording. The orange line shows the calculated moving average frequency for the recording. A sudden and large increase in firing frequency followed by a period of complete neuronal silence is observed twice in this recording.

6 Discussion

6.1 Conclusions and new hypotheses

When looking at the effect the electrical stroke stimulation had on the information processing capabilities and activity of the recorded neurons the effect was quite varied. In some cases there was an increase in the response, in others a decrease and often there was essentially no change. Two different scenarios can be used in order to explain these observed effects.

In the first scenario the increased response in some of the cells are connected to an increase in their spontaneous activity. It can be explained by a decrease in activity of inhibitory interneurons locally in the area which the current cell is recorded from, or similarly an increased activity in excitatory neurons. In the same scenario the decrease in stimulus response observed in other cells could be explained by an increase in activity in the local inhibitory interneurons or alternatively a decrease in activity in the local excitatory neurons as a response to the electrical stroke model.

One property which speaks against this scenario is that different effects on the cell response can be observed in different phases of the cell responses, as in Figure 12.

In the second scenario, the change in the recorded cells response to the finger stimulation can not be explained by a change in the spontaneous activity in the local area around the recorded site. Instead the change in the response could be explained by a shift in the balance between the activation of excitatory and inhibitory cells in the global network which processes the information generated by the finger stimulation. In this scenario the different effects in the different phases of an evoked cell response as seen in 12 could easily be explained. If the early part of the response is more dominated by direct thalamocortical inputs, as mentioned in the introduction, it would be expected to be less sensitive to changes in the activity balance between excitatory and inhibitory neurons in the global neocortical network. Later phases of the responses, however would be expected to be more sensitive to changes in this balance. A disruption in the global neocortical network would then have a larger effect on the later phases of a cell's evoked response, which is what was observed.

In both scenarios a shift in the excitatory and inhibitory input to the recorded cell would affect its response to incoming excitatory input caused by the

finger stimulation. An excitatory shift would increase the response whereas an inhibitory shift would decrease the response. A conclusion which can be drawn due to the fact that the electrical stroke stimulation had an effect, is that the state of the cortical network at the time of arrival of a particular sensory input has an important effect on the stimulus response in individual neurons. Extrapolating from this one can assume that the state of the brain cortex at the arrival time of sensory input is important for how that input is processed and later perceived, strengthening the findings in [2] and [3]. That is to say, the context in which a stimulus is experienced determines how it is perceived.

The many cases in which the electrical stroke stimulation had no or very little effect might be due to methodological issues since the impedance between the two stroke electrodes might have varied depending on their placement on the brain. Differences in the amplitude of the stroke stimulation artifacts observed in the ECoG indicates that this might have been the case.

When looking at the continuous activity histograms which show a cell's behaviour over the entire recording period, several different patterns were observed. One pattern which is particularly interesting is the one shown in Figure 14 where the firing frequency of the recorded neuron suddenly increases to a very high rate followed by a period of complete silence. This is thought to be due to a phenomenon called cortical spreading depression. Cortical spreading depression is usually caused by traumatic brain injury, migraine or stroke and it's characterised by a wave of hyperactivity spreading out from the point of origin across the cortex [13]. This wave of hyperactivity can last from a few seconds up to several minutes and is followed by a period of lower neuronal activity which have been observed to last for more than an hour in some cases. The presence of the phenomenon at least points towards that the brain experiences something akin to damage caused by stroke.

6.2 Restrictions of the project

Due to time restrictions some restrictions had to be placed on the project. Originally the project included several experiments using the photothrombotic stroke model. The results of these experiments were then supposed to be compared to the electrically induced stroke model in order to test its performance. Five of these experiments were performed, however it was not enough in order to both master the new procedure and obtain useful data.

One of the major drawbacks of this experimental setup is that very little data can be obtained from each experiment since the stroke model in not reversible, hindering a protocol restart after stroke induction. The stroke also takes 20 minutes to induce, thus in order to record before, during and after stroke a cell would be required to have a stable signal for about 60 minutes, which is often very hard to achieve. The stability is challenged further due to the fact that an ischemic stroke may cause changes in local blood pressure causing tissue movement which might shift a cell's position away from the recording electrode.

Also due to time constraints some restrictions had to be placed on the data analysis. The plots showing the moving average of a cell activity pattern are useful to see if there are any characteristic changes to a cell's behaviour during the experiments. However in order to quantify this properly one would also have to perform an analysis of the derivative of the moving average in order to see if a specific change occurs at a specific point of time in the experiments and thus can be linked to the onset electric stroke stimulation or if the appearance and duration of a behavioural change is more variable. Even after this it may be hard to draw any conclusions since the appearance of an effect (if any) may vary due to small changes in recording or stimulation positions and the anatomy of the test animal. The current data analysis focuses on comparing the change in activity of a cell before and during stroke stimulation. But further analysis should be done on exactly how the activation pattern changes for each stimulation type before and during stroke stimulation. This could be done by looking at how a cell's ability to separate different stimulation patterns would be affected by a change in the hypothetical cortical network. This could be done using principal component analysis of the response shapes and using a confusion matrix to see how well a cell can differentiate between stimulation patterns.

6.3 Future research

As stated above the first step in order to further study the new stroke model one would have to perform the mentioned data analysis. After that the next step would be to compare the data from this project with data recorded from cells where no stroke model was used, but the same recording duration was used in order to ascertain that the observed effects are not due to the experimental setup. One could hypothesise that the recording electrode might affect the recorded cell's behaviour over time by pressing against the cell

membrane during extracellular recordings or changing the intracellular fluid composition during intracellular recordings.

Further experiments and analysis should also be done when performing a single or several non stroke stimulation procedures directly after stroke stimulation when recording from the same cell in order to fully access the reversibility of the electrical stroke model.

Another procedure which should be done is to include a colouring agent in the electrode fluid and perform intracellular recordings. This would also colour the recorded neurons so that a histology study can later be performed. This would be useful in order to see if the electrical stroke stimulation would have different effects on different types of neurons such as interneurons or pyramidal neurons, inhibitory or excitatory neurons and also if the effects would differ between different cortical layers.

More experiments in which the phototrombotic stroke model is used should also be performed in order to be able to actually verify how close the effect of the electrical stimulation is to the effects of a stroke, in order to see if the new method can actually be used as a reversible stroke model in the future. These experiments are interesting in their own right since how a stroke would affect how single neurons react to a specific set of stimulations have never been studied before. We know that there are long term effects of stroke such as excitotoxicity, where cells die or are damaged due to excessive neurotransmitter stimulation and that a stroke induces local changes around the stroke area, but it is also very interesting to study how cell behaviour changes over time, during the different phases of a stroke.

6.4 Challenges and what I have gained

The by far largest challenge of this project and what I gained the most from was to learn the experimental procedure. I had never before worked with experimental animals in such an extensive manner, so I had to start from scratch and learn how to even remove them from their cage. It took me a long time to learn how to properly perform all the necessary surgical techniques in order to keep the animal sedated and arrange the recording setup while keeping the preparation as stable as possible. Another part of the challenge was the fact that all animals differ slightly anatomically, which sometimes presented unexpected challenges. The next big challenge was the recording procedure. Not only is it hard to even find a cell, it was also hard to keep

a stable recording. Even things such as the animal taking a deep breath, or tissue movement could cause you to lose or kill a cell you were recording from. In the worst cases rats could die seemingly spontaneously, often causing many hours of work to go to waste. The project was also a great training method for patience since it seems that during every experiment a new unexpected error could occur. A slip of the hand, electrodes breaking and thus needing immediate repair, the electronic equipment not working properly, or as mentioned previously the animal unexpectedly dying are just some of the problems which could arise. Another challenge with the experiments was to learn to identify neurons which responded to the stimulation, that is to say that they were not only spontaneously active. It took me quite a few full day experiments and subsequent data analysis in order to be able to separate usable data from unusable.

I enjoyed challenging my programming skills when I was required to for the first time write my code in a way that did not only solve a problem once or a couple of times, but code that could adapt to different data sets with slightly different structure without any manual changes in the code in order to adapt existing code received from the lab to my specific project.

Some time was also spent on teaching. When I had finally started to master the experimental procedures I got the opportunity to teach them to other students in the lab. This both presented me an opportunity to review all the steps myself and really think about what I did, and receive questions from others which enabled me to improve my own performance as well. I also enjoy teaching and it gave me an opportunity to hone my skills in that field as well.

Lastly I really enjoyed the opportunity to challenge most of what I had been taught about how the brain processes information. The opportunity to be part of a team that seeks to revolutionise the field of neurophysiology was a truly thrilling experience.

7 Summary

This thesis sought to answer two different questions: how does a brain process sensory tactile information, and could a DC applied directly to the brain cortex work as a reversible stroke model in animals? This was done by studying individual neurons in anaesthetised rats and how they responded to specific types of tactile information from one of the rat's digits. An electrical current was then applied to the brain cortex in areas different from the recorded area, and the same set of tactile stimulation was presented. The stroke stimulation had a visible effect on the neurons response to the tactile stimulation, however the effects were not well defined. Sometimes the stroke stimulation would increase a neuron's response strength to a specific tactile stimulation, other times it would decrease the response while leaving other neurons completely unaffected. The effects are hypothesised to be caused by the stroke stimulation causing a shift in the inhibitory or excitatory input evoked by the tactile stimulation to the recorded cells by affecting the network of neurons connected to the recorded cell. This points towards that the information processing of a brain is widely distributed in a large network of neurons covering large parts of the brain cortex. Further research is needed however to answer whether the applied DC could work as a stroke model, although there are some similarities to a real stroke. Further research would also be necessary in order to answer whether the new stroke model is reversible or not.

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