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# ELECTROPHYSIOLOGICAL ASSESS-MENT OF NEUROPROTECTION AND REHABILITATION INTERVENTIONS IN ISCHEMIC STROKE

**BY**
**RASMUS KRAGH NIELSEN**

DISSERTATION SUBMITTED 2016

AALBORG UNIVERSITY
DENMARK

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Dissertation submitted 2016

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R. K. Nielsen, K. Yoshida, and W. Jensen, "Global and focal features extracted from intracortical multi-unit signals for investigation of electrophysiological changes in a rat model of ischemic stroke", (IN PREPARATION), 2016

R. K. Nielsen and W. Jensen, "Low-frequency Intracortical Electrical Stimulation Decreases Sensorimotor Cortex Hyperexcitability in the Acute Phase of Ischemic Stroke", *IEEE Trans. Neural Syst. Rehabil. Eng.,* (ACCEPTED), 2016

R. K. Nielsen and W. Jensen, "Low-frequency Intracortical Electrical Stimulation Impedes Spatial and Temporal Spreading Hyperexcitability in the Acute Phase of Ischemic Stroke", *IEEE Trans. Neural Syst. Rehabil. Eng.,* (UNDER REVIEW), 2016

R. K. Nielsen, K. L. Samson, D. Simonsen, and W. Jensen, "Effect of Early and Late Rehabilitation Onset in a Chronic Rat Model of Ischemic Stroke — Assessment of Motor Cortex Signaling and Gait Functionality Over Time", *IEEE Trans. Neural Syst. Rehabil. Eng.,* vol. 21, no. 6, pp. 1006-1015, 2013

**Conference papers**

R. K. Nielsen and W. Jensen, "Features for tracking spatial intra-cortical, electrophysiological changes in a rat model of ischemic stroke", *International Society of Electrophysiology and Kinesiology*, Chicago 2016

R. K. Nielsen and W. Jensen, "Application of low-frequency intracortical electrical stimulation to minimize neuronal hyperexcitability in a rat model of ischemic stroke: preliminary findings", *Annual Meeting of the Society for Neuroscience 2014*, Washington D.C. 2014

R. K. Nielsen and W. Jensen, "Novel approach for investigation of neuronal alterations following ischemic stroke in a rat model", *ICNR 2014*, Aalborg 2014

R. K. Nielsen, D. Simonsen, K. L. Sørensen, and W. Jensen, "Modulation of intracortical motor cortex responses during walking in rats", *IFESS 2012*, Alberta 2012

D. Simonsen, K. L. Sørensen, R. K. Nielsen, and W. Jensen, "Assessment of the effects of ischemic stroke on intracortical motor cortex responses during walking in rats", *IFESS 2012*, Alberta 2012

J. B. Manresa, R. W. Horup, R. K. Nielsen, D. Simonsen, K. L. Sørensen, D. He, and O. K. Andersen, "Human model of central sensitization using high-frequency conditioning electrical stimulation: psychophysical and electrophysiological assessment using reflex receptive fields", *Annual Meeting of the Society for Neuroscience 2011*, Washington D.C. 2011

**Book contributions**

M. Molinari, A. Esquenazi, A. A. Anastasi, R. K. Nielsen, O. Stoller, A. D'Andrea, and M. B. Calatayud, "Rehabilitation Technologies Application in Stroke and Traumatic Brain Injury Patients", In: J. L. Pons, R. Raya, and J. González ed. *Emerging Therapies in Neurorehabilitation II*, Biosystems & Biorobotics: Springer International Publishing, pp. 29-64.

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# PREFACE

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*Aalborg, July 2016*

# ENGLISH SUMMARY

In Denmark, approximately 12,000 people each year suffer from the occlusion of one or several blood vessels within the brain, and approximately 30,000 - 40,000 people are currently living with impacts related to stroke incidents. The pathophysiological processes that occur after an ischemic stroke evolve over time and space and are dependent on the severity and location of the ischemia. As a consequence, it is difficult to monitor and predict how ischemia will develop, and it is highly complicated to design effective neuroprotection and rehabilitation interventions. As such, there is a need for alternative and innovative techniques that can monitor ischemic stroke and that can assess the potential benefits of novel approaches for neuroprotection and rehabilitation interventions.

This thesis hypothesizes that utilizing microelectrode arrays to monitor intracortical electrophysiological signals will provide novel insight into ischemic stroke and can be a useful tool when testing novel approaches for neuroprotection and rehabilitation interventions.

To address this hypothesis, four studies were designed based on an ischemic stroke model in rats. The objective in **Study I** was to develop and test a novel microelectrode array and data analysis techniques for use in the ischemic stroke model. The results showed that the techniques allowed for a detailed spatial and temporal characterization of the acute electrophysiological changes. **Studies II & III** investigated the neuroprotective effect of low-frequency electrical stimulation (lf-ES) applied to the ischemic brain in the acute phase following ischemic stroke. **Study II** demonstrated that ischemic stroke resulted in acute hyperexcitability of the neural tissue located within the penumbra and/or lesser ischemia-affected regions. Lf-ES applied to the ischemic brain reduced the overall amount of hyperexcitability, which suggests that the ongoing expansion of the ischemic core can possibly be delayed and reduced in volume. **Study III** showed how hyperexcitability spread along a complex path influenced by time, space and neural connectivity and that the application of lf-ES minimized this spatial and temporal spreading within the affected tissue. **Study IV** explored how the timing of the rehabilitation onset affects the recovery within the subacute and chronic phases of ischemic stroke. The study indicated that rehabilitation initiated at seven days post ischemic stroke resulted in improved motor function and neural activity patterns that were more similar to those of the healthy state compared to initiating the physical training on day one post ischemic stroke. The results support the existence of a critical period where rehabilitation may be more efficient in promoting recovery and indicate that initiating rehabilitation prior to this period may exacerbate the ischemic injury.

The thesis showed that intracortical electrophysiological recordings are a useful tool to provide new insights into ischemic stroke and demonstrated the beneficial effects of novel approaches for neuroprotection and rehabilitation interventions. This work may be a basis for further studies on ischemic stroke and may assist in the design of more effective interventions for stroke patients in the future.

# DANISH SUMMARY

I Danmark rammes ca. 12.000 mennesker årligt af en blodprop (dvs. okklusion af én eller flere blodårer) i hjernen og omkring 30.000 - 40.000 mennesker lever aktuelt med følgervirkninger heraf. De patofysiologiske processer, som forekommer efter en blodprop, udvikler sig over tid og sted, og afhænger af okklusionens omfang og placering i hjernen. Således kan det være svært at forudsige, hvordan iskæmien udvikler sig og dermed yderst kompliceret at designe effektive vævsbevarings- og rehabiliteringsinterventioner. Af denne grund er der brug for nye metoder til at monitorere iskæmisk apopleksi samt undersøge effekten af innovative vævsbevarings- og rehabiliteringsinterventioner.

Hypotesen i denne afhandling er, at brugen af mikroelektrode arrays til at monitorere hjernens intrakortikale elektrofysiologiske kommunikation kan give ny viden om iskæmisk apopleksi samt være et nyttigt redskab ved test af innovative vævsbevarings- og rehabiliteringsinterventioner.

Fire studier blev opstillet for at undersøge hypotesen. Alle studierne gjorde brug af en rottemodel af iskæmisk apopleksi. Målet med **Studie I** var at udvikle og teste en ny mikroelektrode array og nye metoder til dataanalyse. Resultaterne viste, at de nye tiltag gjorde det muligt at karakterisere de akutte ændringer i hjernens kommunikation med en høj temporal og spatial nøjagtighed. **Studie II & III** undersøgte den vævsbevarende effekt af lavfrekvent elektrisk stimulation (lf-ES), udført direkte på den påvirkede del af hjernen, under den akutte fase efter en blodprop. **Studie II** viste, at der opstod akut hyperexcitabilitet indenfor de lettere påvirkede områder af hjernen. Lf-ES reducerede den totale mængde af hyperexcitabilitet, hvilket potentielt kan indikere, at interventionen kunne forsinke og reducere vævsskade. **Studie III** viste, at hyperexcitabilitet spredtes over tid og sted igennem de neurale forbindelser i hjernen. En spredning som lf-ES viste sig at være i stand til at reducere. **Studie IV** udforskede, om tidspunktet for opstart på rehabilitering påvirker, hvor meget hjernen kommer sig efter en blodprop. Studiet indikerede, at rehabilitering, som blev opstartet syv dage efter blodproppen, resulterede i forbedret motorisk funktion samt hjerneaktivitetsmønstre, som var mere ens med raske væv. Opstart på rehabilitation en dag efter blodproppen resulterede derimod i dårligere motorisk funktion samt mere forskellige hjerneaktivitetsmønstre. Samlet set underbygger resultaterne eksistensen af en kritisk periode efter blodpropper, hvor opstart på rehabilitering er mere givtig og indikerer samtidig, at opstart på rehabilitering tidligere end den kritiske periode potentielt kan forværre skaden.

Overordnet demonsterer afhandlingen, at intrakortikale elektrofysiologiske målinger kan være et nyttigt redskab til at opnå ny viden om iskæmisk apopleksi og dokumenterede positive effekter af innovative vævsbevarings- og rehabiliteringsinterventioner. På denne måde kan afhandlingens resultater være basis

for fremtidige studier indenfor iskæmisk apopleksi og være brugbare i forbindelse med udvikling af mere effektive interventioner til behandling i fremtiden.

# CHAPTER 1. INTRODUCTION

Ischemic stroke is a consequence of a permanent or transient decrease in blood flow within the region of the brain supplied by the affected vessel(s). The typical causes of ischemic stroke are embolic or thrombotic events causing the occlusion of the affected blood vessel(s) and resulting in dysfunction and death of neurons within the brain (Kunz, Dirnagl, & Mergenthaler, 2010). In Denmark, approximately 12,000 people each year suffer from the occlusion of one or several blood vessels within the brain, and 30,000 - 40,000 people are currently suffering from impacts related to ischemic stroke (Rostrup Kruuse, 2014).

The acute pathophysiological processes that occur following the ischemic insult evolve over time and space and are dependent on the severity and location of the ischemia. To further increase the complexity, several of the processes have a *'Janus-faced'* nature, with both beneficial and damaging effects (Endres et al., 2008; Wieloch & Nikolich, 2006). The direct consequence of the ischemic insult (i.e., cell death and injury) is observed within the most severely affected ischemic territory, whereas changes in non-injured neural tissue may occur as the connections between neural networks are destabilized (Carrera & Tononi, 2014). Thus, the behavioral deficits observed within the acute phase are reflected not only in the amount of dead or injured tissue but also in the degree of malfunction in non-injured neural networks (Witte, Bidmon, Schiene, Redecker, & Hagemann, 2000). Following the acute phase, recovery may occur via repair of the injured neural networks and neural plasticity within the networks surrounding the lost tissue (Wieloch & Nikolich, 2006).

The goal of neuroprotection interventions is to limit the infarct size by targeting the acute cellular and molecular processes of the progressing ischemia (i.e., processes occurring within minutes to hours after the insult (Fagan, Hess, Hohnadel, Pollock, & Ergul, 2004)). Rehabilitation interventions instead aim to enhance recovery by restoring neural functionality during the subacute (i.e., hours to days after the insult (Fagan et al., 2004)) and chronic phases (i.e., days to months after the insult (Fagan et al., 2004)) (Gladstone, Black, & Hakim, 2002; Lee, Zipfel, & Choi, 1999; Moretti, Ferrari, & Villa, 2015). Optimal recovery, though, is difficult to achieve due to the complexity and heterogeneity of ischemic stroke and the lack of monitoring methods that are able to properly determine the degree of recovery (Krakauer, 2006).

Microelectrode arrays (MEAs) inserted in the brain enable direct analysis of the electrophysiological characteristics of the neural tissue and may therefore provide novel insight into ischemic stroke (Jensen, Rousche, & Chiganos, 2006). This can be done by monitoring both the progression and extent of tissue loss and the functional state of the non-injured brain following the ischemic stroke and during recovery.

MEAs may also assist in elucidating the underlying mechanisms of neuroprotection and rehabilitation interventions.

The focus of this thesis was therefore to study how MEAs may be utilized to investigate the ischemic brain, while testing novel approaches for neuroprotection and rehabilitation interventions.

# CHAPTER 2. BACKGROUND

This chapter first introduces the pathophysiological processes of ischemic stroke and how these are expressed throughout the ischemic territory (see Sec. 2.1). Second, the brain's innate neuroprotective and recovery mechanisms (see Sec. 2.2) and the approaches and targets of ischemic stroke interventions are reviewed (see Sec. 2.3). Finally, because the use of MEAs is a new approach to investigate ischemic stroke, it must be tested in animal models. Thus, the chapter is concluded by describing various aspects of experimental animal models (see Sec. 2.4).

## 2.1. ISCHEMIC STROKE

Ischemic stroke leads to a decline in blood flow, causing metabolic and biological functions to become suppressed within the neural tissue supplied by the affected blood vessel(s). The severity of the ischemic impact and the cascade of pathological processes (denoted as the *ischemic cascade*) vary throughout the affected tissue.

### 2.1.1. THE ISCHEMIC TERRITORY

The concept of *viability thresholds* utilizes critical blood flow levels to differentiate and demarcate the ischemic territory into regions with different levels of suppressed metabolic and biological function (Hossmann, 2012). The *upper viability threshold* is defined by the blood flow level at which the neural tissue experiences electrical failure, whereas the *lower viability threshold* is defined by the level at which cell membrane failure occurs (Hossmann, 1994).

The most heavily impacted region is the *ischemic core*, where cells are mortally injured within minutes due to blood flow levels below the *lower viability threshold* (see Fig. 2-1). The least affected region is the *benign oligemia*, where no functional or metabolic disturbances are apparent, because the blood flow levels range between normal unobstructed blood flow and the *upper viability threshold* (T. H. Murphy & Corbett, 2009). Intermediately, the *penumbra* is located. This region receives sufficient blood to initially uphold metabolic activity to preserve the morphological properties of the tissue (Astrup, Siesjo, & Symon, 1981; Astrup, Symon, Branston, & Lassen, 1977; Lo, 2008). The blood supply shortage within the *penumbra* is highest closest to the *ischemic core* and decreases towards the healthy neural tissue because this region may be supplied by other artery systems that may not be affected by the stroke (T. H. Murphy & Corbett, 2009).

Initially, the *lower viability threshold* is approximately 15% of the normal unobstructed blood flow level, but it will increase over time, particularly within the *penumbra*, due to a progressively increasing mismatch between the blood flow and metabolism caused by the ongoing *ischemic cascade* (Hossmann, 2012). As a result,

the *ischemic core* will over time expand into the *penumbra* (Dirnagl, Simon, & Hallenbeck, 2003; Kunz et al., 2010).

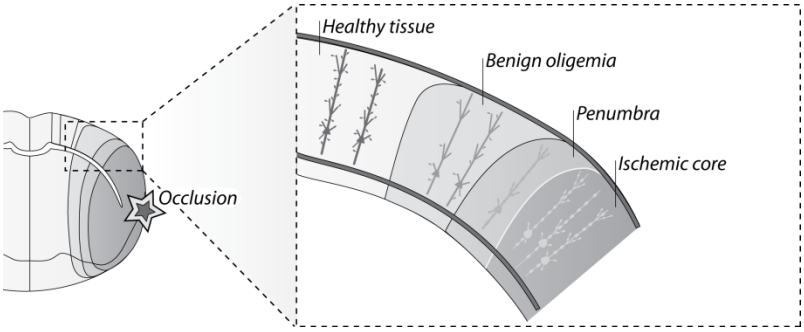


*Figure 2-1. The ischemic territory as demarcated by the viability thresholds. Adapted from* (T. H. Murphy & Corbett, 2009).

## 2.1.2. EVENTS OF THE ISCHEMIC CASCADE

The *ischemic cascade* involves a complex series of pathological processes that evolve over time and space following ischemic stroke and that are dependent on the severity and location of the ischemia as well as the cellular context of the affected tissue (Wieloch & Nikolich, 2006).

### Excitotoxicity

*Excitotoxicity* refers to a process where excessive amounts of excitatory neurotransmitter (predominately glutamate) accumulate in the extracellular space (Choi, 1988). This accumulation is a consequence of the excessive neurotransmitter release of cells that become necrotic and impeded re-uptake due to energy failure (Mehta, Manhas, & Raghubir, 2007). *Excitotoxicity* results in an excessive influx of $Ca^{2+}$ (denoted *$Ca^{2+}$ overload*), which triggers nuclear and cytoplasmatic processes that cause membrane degradation, inflammatory responses and *apoptosis* (Sattler & Tymianski, 2001; Woodruff et al., 2011). At the same time, swelling of the cell occurs due to $Na^{+}$ and $Cl^{-}$ influx with relatively little $K^{+}$ efflux, which leads to the passive transport of water into the cell (Endres et al., 2008).

### Peri-infarct depolarization

*Peri-infarct depolarizations* (*PIDs*) are waves of depolarizations that spread from the *ischemic core* and outwards throughout the *penumbra* (Lauritzen, 1994; Risher, Ard, Yuan, & Kirov, 2010). The neural networks of the *penumbra* may experience multiple depolarizations and repolarizations due to the excessive amounts of neurotransmitter within the extracellular space (Dirnagl, Iadecola, & Moskowitz, 1999). This leads to elevated energy consumption within the tissue, and the *ischemic core* has been documented to expand following each *PID* (Doyle, Simon, & Stenzel-poore, 2008; Strong et al., 2007). For example, Hartings et al. (Hartings, Rolli, Lu,

& Tortella, 2003) showed that *PIDs* predominantly occur within the first 24 hours following ischemic stroke in a rat model. The highest *PID* incidence rates were concentrated around two phases, i.e., the initial ischemic insult (0 – 2 hours after ischemic stroke) and the period of infarct maturation (8 – 18 hours after ischemic stroke).

### Inflammation

Pro-inflammatory mediators are secreted by various cell populations (e.g., endothelial cells, astrocytes, microglia, and neurons) within the ischemia-affected neural tissue following the insult. The mediators include both destructive (e.g., toxic metabolites and enzymes) and protective factors (e.g., erythropoietin). As a consequence, the overall role of the inflammatory factors may differ over time and space (Woodruff et al., 2011). Thus, protective and regenerative activities occur days to weeks after the ischemic insult, whereas the *blood-brain barrier* may rapidly become dysfunctional, resulting in poor perfusion within the *penumbra* (Endres et al., 2008).

### Necrosis

*Necrosis* is an uncontrolled form of cell death where the cellular content is extruded as the cell disintegrates, thereby potentially inflicting damage to surrounding cells due to *excitotoxicity* and the triggering of inflammation. *Necrosis* is a result of energy failure due to the blocked blood supply, and necrotic cells first undergo swelling followed by the disruption of the nuclear, organelle, and plasma membranes and structure, and then the cells shrink and disintegrate (Neumar, 2000).

### Apoptosis

*Apoptosis* is a programmed form of cell death where the cells are systematically disassembled rather than disintegrated. *Apoptosis* results in less inflammation and less release of genetic material compared to *necrosis* (Lee et al., 1999). As result, the effect on the surrounding tissue is minimal (Neumar, 2000). The severity and duration of the ischemia determines whether the cells undergo rapid *necrosis* or slower-progressing *apoptosis* (Mehta et al., 2007). Accordingly, *apoptosis* is the predominant form of cell death within the *penumbra* (Broughton, Reutens, & Sobey, 2009). *Apoptosis* is triggered by factors such as free radicals, DNA damage, and mitochondrial injury (Dirnagl et al., 1999) and can be induced by *excitotoxicity*, $Ca^{2+}$ *overload* and *oxidative stress* (Mehta et al., 2007). One of the most distinctive markers of *apoptosis* is the activation of caspases (a group of protease enzymes), which play an essential role in the later stages of programmed cell death (Lee et al., 1999).

## 2.1.3. JANUS-FACED NATURE OF THE ISCHEMIC CASCADE

To further complicate the ischemic stroke pathology, several of the processes that occur following the ischemic insult mediate cell death during the acute phase but may promote recovery within the *penumbra* at a later point in time (Lo, 2008). For

example, excessive amounts of glutamate are detrimental in the acute phase by mediating *excitotoxicity*, but are suggested to promote the endogenous *neurogenesis* that occurs later on (Arvidsson, Kokaia, & Lindvall, 2001; Bernabeu & Sharp, 2000). Similarly, *apoptosis* induces cell death, but at the same time inhibits inflammation (Zipfel, Babcock, Lee, & Choi, 2000). Thus, the same element of ischemic stroke (e.g., molecules, signaling pathways or cells) will engage in destruction or repair, depending on factors such as the severity and location of the ischemia as well as the cellular context of the affected tissue and the elapsed time from the ischemic insult (Endres et al., 2008; Wieloch & Nikolich, 2006).

### 2.1.4. SPATIAL AND TEMPORAL PROGRESSION OF THE ISCHEMIC CASCADE

The stereotyped progression of the *ischemic cascade* initial involves energy failure due to the blocked blood supply. This results in *necrosis* that leads to *excitotoxicity* within the neighboring tissue and subsequently *PIDs*. Secondary damage occurs as inflammation develops within the affected tissue, and at the same time cell death occurs within cells that have been triggered to undergo *apoptosis* (see Fig. 2-2) (Endres et al., 2008). The *ischemic cascade* progresses rapidly following the ischemic insult, and the neuroprotective potential is the largest within the first hour following the stroke, where the *penumbra* constitutes up to 50% of the tissue directly influenced by the ischemia (Saver et al., 2010). From here on, the amount of salvageable tissue rapidly declines as the *ischemic core* progressively expands to include the *penumbra* until approximately 3 – 6 hours after the insult, when the *ischemic core* is close to its full extent (Hossmann, 2012). *Apoptosis* has, however, been shown to result in significant cell death in regions where reperfusion occurs. Thus, following reperfusion, the cells of the *penumbra* may recover the ability to generate action potentials for 24 – 72 hours, after which this ability is lost again and the cells undergo *apoptosis* (Lee et al., 1999; Witte et al., 2000). This could likely be one of the key pathological mechanisms of cell death within the exterior part of the *penumbra*, where the cells are initially rescued by spontaneous recanalization or improvement of collateral blood flow (Witte et al., 2000).

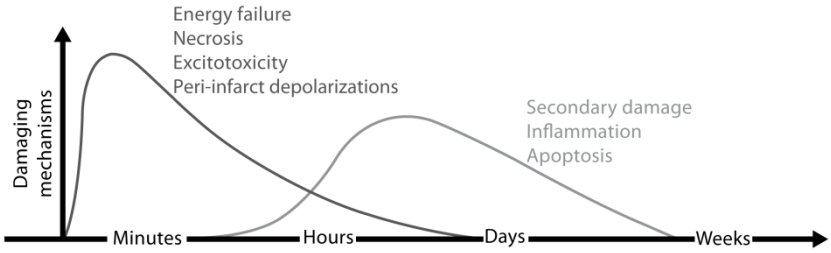


*Figure 2-2. Conceptual illustration of the ischemic cascade. Adapted from* (Endres et al., 2008).

### 2.1.5. INDIRECT EFFECTS OF THE ISCHEMIC CASCADE – DIASCHISIS

The brain constitutes a complex network of multiple and intricate neural connections, and ischemic stroke exerts wide effects on the neural networks and their communication. The direct consequences of the ischemic insult are observed within the *ischemic core* and *penumbra*, whereas indirect effects occur within the surrounding tissue (Witte et al., 2000). *Diaschisis* describes neurophysiological changes in remote brain areas that are not directly affected by the ischemia (Witte et al., 2000). Instead the changes within the remote areas are a direct inhibitory or excitatory effect of the injuries within the ischemic tissue. *Diaschisis* occurs early after stroke (within minutes), and the etiologies can broadly be split into the following categories: **1)** brain edema, **2)** inflammation, **3)** changes that are consequences of the spreading depression, and **4)** changes in projection areas (Carrera & Tononi, 2014; Witte et al., 2000). For example, many studies on humans and animals have demonstrated increased responses in the contralateral homotopic region to the ischemia-affected neural networks (Carrera & Tononi, 2014). Mohajerani et al. (Mohajerani, Aminoltejari, & Murphy, 2011) attributed this increased excitability to the loss of inhibition exerted by the ischemic hemisphere via the transcallosal pathways.

## 2.2. THE BRAIN'S OWN DEFENSE AGAINST ISCHEMIC STROKE

### 2.2.1. ENDOGENOUS NEUROPROTECTION

Endogenous neuroprotective mechanisms occur during the acute phase to prevent cell death as a result of the progressing *ischemic cascade* (see Fig. 2-3) (Auriel & Bornstein, 2010). For example, anti-excitotoxic, anti-inflammatory, and anti-apoptotic mechanisms have been found that can protect the neural tissue from detrimental damage while the *ischemic cascade* progresses (Dirnagl et al., 2003). The anti-excitotoxic mechanism was found to upregulate inhibitory neurotransmitters and alter the composition of the neurotransmitter receptors in response to NMDA-receptor activation (Heurteaux, Lauritzen, Widmann, & Lazdunski, 1995).

### 2.2.2. RECOVERY MECHANISMS

Recovery of behavioral function (e.g., motor function) occurs after ischemic stroke (Edward Taub, Uswatte, & Elbert, 2002) and is most marked during the initial 30 days, but it progresses for at least six months, although with less pronounced gains during this period (see Fig. 2-3) (Duncan, Min Lai, & Keighley, 2000). The initial loss of behavioral function is a result of both the direct and indirect effects of the ischemic stroke, i.e., **1)** cell death in the *ischemic core*, **2)** cell malfunction and aberrant neurotransmission due to altered spine morphology in the *penumbra*, and **3)** *diaschisis* in remote functionally connected regions (Witte et al., 2000).

## Neural repair and reorganization

Within the surviving penumbral tissue and the *benign oligemia*, the resolution of *diaschisis* and mechanisms of cell repair occur first, followed by *functional cell plasticity* and, at a later point in time, *neuroanatomical plasticity*, resulting in the formation of new neural connections (Wieloch & Nikolich, 2006). The neural repair and reorganization of cells is facilitated by the stimulation of anabolic processes via growth-promoting factors (e.g., survival, repair, and plasticity genes) that are upregulated twice within the first 24 hours after ischemic stroke (directly after ischemic stroke and 9 - 24 hours after ischemic stroke) and succeeded by a longer period of expression within the first week after the ischemic insult (Küry, Schroeter, & Jander, 2004; Rickhag et al., 2006). Subsequently, growth-inhibitory genes are upregulated, thereby potentially regulating the efficiency of the neural plasticity by restricting outgrowth and repelling sprouting axons (T. H. Murphy & Corbett, 2009; Wieloch & Nikolich, 2006). During this period, the repaired *penumbra* and non-ischemic neural networks surrounding the lesion as well as the homotopic contralateral neural tissue initially become hyperexcitable, most likely due to deafferentation (Buchkremer-Ratzmann & Witte, 1997; Wieloch & Nikolich, 2006). In these hyperexcitable tissues, *long-term potentiation* (*LTP*) is enhanced, the number of $GABA_A$ *receptors* is lowered, and the number of *NMDA receptors* is increased, causing axonal sprouting to occur (Hagemann, Redecker, Neumann-Haefelin, Freund, & Witte, 1998; Jones & Schallert, 1992; Qu et al., 1998; Witte et al., 2000). As such, recovery is mediated via reorganization of the repaired *penumbra* and the functionally connected non-ischemic tissue, whereby these neural networks take over the function of the ischemic tissue (R J Nudo, Plautz, & Frost, 2001). The most rapid behavioral improvements (within the first few days after ischemic stroke) have been suggested to be due to the activation/strengthening of the redundant silent neural connections (denoted as *silent pathways*) (Dancause & Nudo, 2011; Metz, Antonow-Schlorke, & Witte, 2005).

## Neurogenesis

The adult brain is continuously generating new neurons (Ming & Song, 2011). *Neurogenesis* occur in the subventricular zone and the subgranular zone of the dentate gyrus and has been shown to increase following ischemic stroke in rats (Jin et al., 2001). For example, neural stem cells have been shown to migrate into the ischemic striatum following *middle cerebral artery occlusion* (*MCAO*), where they express markers of developing and mature cells similar to those that were lost due to the ischemic stroke (Arvidsson, Collin, Kirik, Kokaia, & Lindvall, 2002). The fraction of dead cells that are replaced is, however, minimal (Arvidsson et al. (Arvidsson et al., 2002) estimated that 0.2% of the dead cells were replaced at 6 weeks), and stem cells that are recruited to damaged regions either undergo *apoptosis* or form mature neurons that survive for some months (Thored et al., 2006). This failure in long-term survival is presumably due to the lack of trophic support and functional neural connections (Arvidsson et al., 2002). Therefore, it is not yet firmly established whether *neurogenesis* can contribute to recovery.

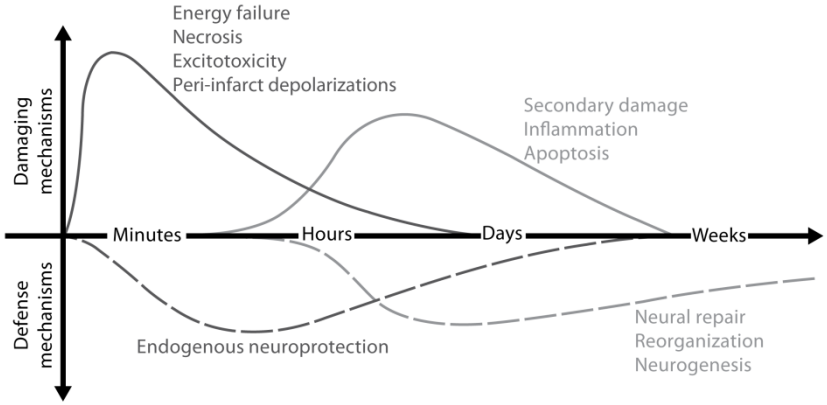


*Figure 2-3. Conceptual illustration of the ischemic cascade, endogenous neuroprotection, and recovery mechanisms (e.g., neural repair, reorganization, and neurogenesis). Adapted from* (Endres et al., 2008).

## 2.3. INTERVENTIONS TO COUNTERACT ISCHEMIC STROKE

As discussed in Section 2.2, the recovery from ischemic stroke is dependent on the extent of cell preservation in the acute phase and the repair of damaged neural tissue and engagement of neural networks that can help compensate for the lost brain functions during the subacute and chronic phases (Wieloch & Nikolich, 2006). The ultimate goal of ischemic stroke interventions is to restore the behavioral function and neural morphology to what was in place prior to the ischemic insult to achieve *true recovery*. In reality, it is not possible today to obtain 100% *true recovery*, and instead recovery occurs through altered behavior and new neural response strategies provided by the reorganization of the preserved neural tissues, i.e., *behavioral compensation* (M. F. Levin, Michaelsen, Cirstea, & Roby-Brami, 2002; M. Levin, Kleim, & Wolf, 2009; Metz et al., 2005; Whishaw, 2000).

### 2.3.1. NEUROPROTECTION INTERVENTIONS

Neuroprotection interventions target the *ischemic cascade* to block the pathological processes and prevent cell death (Auriel & Bornstein, 2010). Pharmacological interventions have targeted various mechanisms including *excitotoxicity*, calcium influx, nitric oxide production, inflammatory reactions and *apoptosis* to achieve neuroprotection (Moretti et al., 2015). Many of these pharmacological interventions have been applied with success in animal models, but later results from clinical trials have been discouraging (Dirnagl et al., 1999; Ginsberg, 2008; Kahle & Bix, 2012; Lo, 2008). It has been suggested that the negative results may be explained by the interventions only targeting one of the multiple pathophysiological pathways, thereby rendering them very sensitive to proper treatment time (Fisher et al., 2009;

Gladstone et al., 2002; STAIR, 1999). *Hypothermia* is one of the only interventions that has shown promising results, as it targets a wide range of mechanisms including *excitotoxicity*, *apoptosis* and inflammation (van der Worp, Sena, Donnan, Howells, & Macleod, 2007; Yenari & Han, 2012). Recently, low-frequency electrical stimulation (lf-ES) has been tested as an alternative neuroprotection intervention and was found to trigger several neuroprotective mechanisms that may diminish behavioral deficits, reduce apoptotic and necrotic cell death, block microglial activation, and promote *angiogenesis* (Baba et al., 2009; Plow & Machado, 2013).

### 2.3.2. REHABILITATION INTERVENTIONS

Interfering with neural plasticity can either block or enhance reorganizational processes, and increased functional reorganization following ischemic stroke has been associated with behavioral recovery (Randolph J Nudo, Wise, Sifuentes, & Milliken, 1996). Behavioral experience has been shown to be a potent modulator of functional reorganization and repetition and temporal coincidence, as apparent when repetitively performing skilled movements like this induces plastic changes (denoted *hebbian plasticity*) (Hummel & Cohen, 2005; Randolph J. Nudo, 2013). An important concept of reorganization (e.g., observed in the motor cortex) relates to specific functions having multiple and overlapping representations that are distributed within the neural networks and are highly interconnected (Bütefisch, 2004). Altering the strength of these connections via intrinsic and extrinsic stimuli facilitates the reorganization and acquisition of novel behavioral patterns (Randolph J. Nudo, 2013). As such, functions that are lost following ischemic stroke are believed to be recovered through reorganization within closely related networks, which may also be located within the homotopic contralateral cortex (Dancause & Nudo, 2011; Kopp et al., 1999). The most successful recovery is, however, thought to occur in individuals in whom reorganization has mostly taken place in the ischemic hemisphere, whereas bilateral cortical reorganization, typically after larger strokes, results in less complete recovery (Cramer, 2008; Ward, Brown, Thompson, & Frackowiak, 2003).

The rehabilitation of motor function has been investigated while using various forms of functional retraining to recover the otherwise lost function (Dancause & Nudo, 2011). In both animals and humans, repetitive-training protocols and *constraint-induced movement therapy* (*CIMT*) have been used and shown to improve the motor function and result in cortical reorganization (Liepert et al., 1998; Randolph J Nudo et al., 1996; E Taub et al., 1993). This use-dependent plasticity has been shown to be facilitated or inhibited by excitability-enhancing (Butefisch et al., 2002; Feeney, Gonzalez, & Law, 1982) or excitability-reducing pharmacological agents (Blin et al., 2001). Direct or indirect electrical and transcranial magnetic stimulation has also been documented to improve motor function when combined with functional retraining during the chronic phase of ischemic stroke (Hummel & Cohen, 2005).

### 2.3.3. TIMING OF REHABILITATION INTERVENTIONS

In relation to the onset time of rehabilitation, a *critical period* where the rehabilitation may be especially efficient due to a favorable interplay between growth-promoting factors (i.e., proteins and genes) and growth-inhibitory factors has been postulated (T. H. Murphy & Corbett, 2009). In rat models of ischemic stroke, this *critical period* is located approximately 5 to 14 days after ischemic stroke (Biernaskie, Chernenko, & Corbett, 2004). Intensive rehabilitation or forced use initiated before the *critical period* has been shown to result in less recovery of behavioral function and enlargement of the ischemic territory, potentially due to excessive energy demands (Bland et al., 2000; Humm, Kozlowski, James, Gotts, & Schallert, 1998; Kozlowski, James, & Schallert, 1996). Human studies have provided similar results, supporting the existence of a *critical period* following ischemic stroke (Horn et al., 2005; Salter et al., 2006) and indicating that very early and intensive rehabilitation can lead to worse outcomes (Dromerick et al., 2009, 2015).

Interference with *homeostatic plasticity* mechanisms is presumed to be accountable for some of the detrimental effects of very early and intensive rehabilitation (T. H. Murphy & Corbett, 2009). Conceptually, *homeostatic plasticity* defines mechanisms that ensure that neurons receive an adequate amount of neural input. As such, homeostatic mechanisms seek to restore a certain level of synaptic activity within the neural networks by acting as a negative feedback loop (Turrigiano & Nelson, 2004). During recovery, homeostatic mechanisms presumably stabilize the interrupted synaptic activity originating from the infarcted tissue by **1)** resetting the activity levels of specific neurons, **2)** forming new connections, and/or **3)** changing the activity at the already existing synapses, thereby possibly unmasking *silent pathways* (T. H. Murphy & Corbett, 2009). In rodent models of ischemic stroke, *homeostatic plasticity* has been attributed to the expanded and less-specific receptive fields as well as the increased spontaneous activity occurring within the first week following the ischemic insult (Schiene et al., 1996; Winship & Murphy, 2008).

## 2.4. EXPERIMENTAL ANIMAL MODELS OF ISCHEMIC STROKE

The complexity of ischemic stroke and subsequent recovery calls for the use of large patient groups when performing clinical research to avoid the confounding effects of disease diversity (Bacigaluppi, Comi, & Hermann, 2010a). Because of these challenges, experimental animal models that enable larger degrees of control and monitoring of physiological variables are extensively used within ischemic stroke research.

Different stroke models have been developed (i.e., focal/global ischemia and transient/permanent occlusion) and applied in different animal species (including higher-order primates (Randolph J Nudo et al., 1996; Plautz et al., 2003), rodents (Biernaskie & Corbett, 2001; Reglodi, Tamas, & Lengvari, 2003), and pigs (Sakoh,

Rohl, et al., 2000; Sakoh, Østergaard, et al., 2000)). The majority of the ischemic stroke models are designed for use in rodents, and rats have especially been extensively utilized (Durukan & Tatlisumak, 2007). Rats have a physical size that allows for reasonably easy monitoring of their physiological parameters, and several methods of stroke induction may be applied due to the vascular structures of the rat (Mhairi Macrae, 1992). Finally, the low cost and greater acceptability from ethical perspectives facilitate the use of rodents in early investigations that seek to establish the effects of novel ischemic stroke interventions or investigate physiological mechanisms (Durukan & Tatlisumak, 2007). The physiological characteristics of primates and pigs, however, have a larger similarity to humans (e.g., a gyrencephalic brain), and as such they would be highly suited for use at later phases (Traystman, 2003).

### 2.4.1. ISCHEMIC STROKE INDUCTION METHODS

Several stroke induction methods exist, and the main objective for all is to generate a reproducible infarct with a minimum of surgical intervention (Carmichael, 2005). The following provides an overview of the focal ischemic stroke models, as these may be used to induce an ischemic stroke that is close to the size of a survivable human stroke (i.e., strokes that affect 5 – 15% of the hemisphere) (T. H. Murphy & Corbett, 2009). These methods primarily target the *middle cerebral artery* (*MCA*) and lead to deficits in motor and sensory functions (Moretti et al., 2015; Traystman, 2003). The most commonly used methods are described below (Bacigaluppi, Comi, & Hermann, 2010b; Carmichael, 2005; Durukan & Tatlisumak, 2007; Mhairi Macrae, 1992; Sicard & Fisher, 2009; Traystman, 2003).

**Transection method**

| | |
|---|---|
| **Craniectomy** | Craniectomy may be necessary, but the orbital route to *MCAO* is preferred because this is a less traumatic procedure |
| **Procedure** | Electrocauterization of vessel(s) |
| **Transient occlusion** | No |
| **Permanent occlusion** | Yes |
| **Tissue damage** | Lesion location, size and severity are dependent on the site of ligation |
| **Advantages** | High correlation between the area of reduced blood flow and the area of neuropathological injury |
| **Disadvantages** | Highly invasive<br>Requires extensive surgical skills |

**Ligation method**

| | |
|---|---|
| **Craniectomy** | Craniectomy may be necessary, but the orbital route to *MCAO* is preferred because this is a less traumatic procedure |
| **Procedure** | Ligation of vessel(s) by use of microclips or ligature snares |
| **Transient occlusion** | Yes |
| **Permanent occlusion** | Yes |
| **Tissue damage** | Lesion location, size and severity are dependent on the site of ligation |
| **Advantage** | High correlation between the area of reduced blood flow and the area of neuropathological injury |
| **Disadvantage** | Highly invasive |

| | |
|---|---|
| | Requires extensive surgical skills |

## Intraluminal occlusion method

| | |
|---|---|
| **Craniectomy** | Not required |
| **Procedure** | A monofilament is maneuvered into the targeted vessel(s) to perform the occlusion |
| **Transient occlusion** | Yes |
| **Permanent occlusion** | Yes |
| **Tissue damage** | Lesion size 5 - 50% of hemisphere |
| **Advantages** | Reproducible *MCA* territory infarcts<br>Large *penumbra* shortly after *MCA* occlusion<br>Minimal invasiveness |
| **Disadvantages** | Lesion size is often larger than in clinical studies<br>Vessel rupture during insertion of filament |

## Thromboembolic method

| | |
|---|---|
| **Craniectomy** | Not required |
| **Procedure** | Injection of non-biological microspheres or artificially clotted blood into circulation |
| **Transient occlusion** | The thromboembolisms that occur after the injection of artificially clotted blood may undergo spontaneous reperfusion |
| **Permanent occlusion** | Yes, when using non-biological microspheres |
| **Tissue damage** | Widespread and multifocal cerebral ischemia |
| **Advantages** | Minimal invasiveness and close resemblance to human stroke |
| **Disadvantages** | Minimal control of the ischemia<br>Spontaneous reperfusion may occur |

## Photothrombosis method

| | |
|---|---|
| **Craniectomy** | Not required but may ease the illumination procedure |
| **Procedure** | Illumination of a photosensitive dye that has been injected into the circulation. Thromboembolic ischemia occurs within the tissue supplied by the illuminated blood vessel(s) |
| **Transient occlusion** | No |
| **Permanent occlusion** | Yes |
| **Tissue damage** | Localized and fairly demarcated thromboembolic ischemia |
| **Advantages** | Lesion size, location and severity are highly controllable and result in reproducible infarcts |
| **Disadvantages** | Small penumbral region<br>May result in vasogenic edema and *blood-brain barrier* breakdown |

## Endothelin-1 method

| | |
|---|---|
| **Craniectomy** | Required |
| **Procedure** | Injection of endothelin-1 results in the vasoconstriction of cerebral vessels in the region of injection |
| **Transient occlusion** | Yes |
| **Permanent occlusion** | No |
| **Tissue damage** | Similar to ligation methods |
| **Advantages** | Gradual reperfusion<br>Control over duration and location of ischemia |
| **Disadvantages** | The dose of endothelin-1 needs to be carefully standardized to assure proper control over the duration of the decreased blood flow |

## 2.4.2. MONITORING METHODS

Different monitoring methods have been used in experimental studies to evaluate the progression of ischemic stroke and the effects of ischemic stroke interventions. The methods can roughly be split into three groups with their individual focuses: **1)** pathological consequences of ischemic stroke, **2)** neurophysiological consequences of ischemic stroke, and **3)** behavioral and functional consequences of ischemic stroke (Durukan & Tatlisumak, 2007; Sicard & Fisher, 2009). The methods for the assessment of the pathological and behavioral and functional consequences of ischemic stroke are by far the most commonly utilized (Kahle & Bix, 2012).

### Assessment of the pathological consequences of ischemic stroke

Methods for assessing the pathological consequences of ischemic stroke, such as histological analysis and magnetic resonance imaging (MRI), seek to directly quantify the pathological impact of the ischemic insult on the brain. Histological analysis may be used to quantify the infarct size or investigate more subtle morphological and chemical changes (Sicard & Fisher, 2009). MRI may be used to detect the ischemic lesion (denoted as diffusion-weighted imaging (DWI)) and the hypoperfused region (denoted as perfusion-weighted imaging (PWI)) (Neumann-Haefelin et al., 1999). Reliable quantification of the histological infarct volume is difficult to achieve until 24 hours after the ischemic insult (Liu, Zhen, Meloni, Campbell, & Winn, 2009), whereas MRI can be used to investigate the lesion-induced changes within minutes of injury (Durukan & Tatlisumak, 2007). One drawback is that the pathological assessment has been shown to not always correlate well with the behavioral improvements and should therefore be combined with behavioral assessment (Cheng, Al-Khoury, & Zivin, 2004; Hunter, Mackay, & Rogers, 1998).

### Assessment of the neurophysiological consequences of ischemic stroke

Methods as electroencephalography (EEG), voltage-sensitive dye imaging (VSDI), electrophysiological recording and microstimulation mapping by use of MEAs allow for direct evaluation of the neurophysiological aspects of the ischemia-affected brain (Durukan & Tatlisumak, 2007). These methods provide information about the electrophysiological state of the brain following ischemic stroke, and the plasticity changes that occur following ischemic stroke have been shown to be related to the behavioral function of the subject (Dancause & Nudo, 2011). MEAs can be used to record the electrophysiological properties of the neural networks with a high spatial and temporal resolution (M. A. L. Nicolelis, 2009) and can be used to record over long periods of time (i.e., up to several months) (Vetter, Williams, Hetke, Nunamaker, & Kipke, 2004). As such, MEAs have previously been used in a rat model of acute ischemic stroke to perform preliminary investigations on the ischemia-affected motor cortex (Jensen et al., 2006).

## Assessment of the behavioral and functional consequences of ischemic stroke

The most frequently used methods for functional and behavioral assessment are sensorimotor function tests, as the experimentally induced ischemia results in these deficits (Kahle & Bix, 2012; Schaar, Brenneman, & Savitz, 2010). Cognitive tests may also be used, as learning and memory impairments are common after experimentally induced ischemic stroke (Schaar et al., 2010). Sensorimotor function tests generally focus on detecting and quantifying asymmetries that occur after unilateral ischemic stroke (Hua et al., 2002). Tests of sensorimotor function in rodents assess the deficits by, for example, quantifying **1)** the ratio of the uses of the affected limb and the non-affected limb (e.g., *Cylinder test* and *Reaching/Grasping tests*), **2)** the number of faults made during the uses of the affected limb and the non-affected limb (e.g., *Rung walking*, *Grid walking*, *Beam walking*, and *Reaching/Grasping tests*), and **3)** the rate and total number of successful executions of a task (e.g., *Reaching/Grasping tests*) (Schaar et al., 2010). Composite tests (e.g., the *Modified Neurological Severity Scores* (*mNSS*)) rate the neurological function of the animal based on a series of tests (i.e., motor, sensory, reflex and balance tests) and therefore have the ability to assess multiple deficits (Schaar et al., 2010). Sensorimotor function tests that assess similar deficits exist in non-human primate models of ischemic stroke (Cook & Tymianski, 2012). A drawback of the sensorimotor function tests is that they do not exhibit similar rates of recovery following ischemic stroke (Hunter et al., 2000), as they are sensitive to measuring deficits dependent on the particular areas of damage (Schaar et al., 2010). Additionally, the majority of the methods only provide limited information on the movement pattern, and it is therefore unknown whether the animal uses an alternative movement strategy to accomplish the task goal (Krakauer, 2006). Whishaw (Whishaw, 2000) has, however, used video records to investigate movement patterns and found that the animals recovered to within 10% of their healthy state success level on a skilled movement task. Nevertheless, from the video data it could be observed that the recovered performance on the task was mediated via altered movement patterns.

# CHAPTER 3. HYPOTHESIS

The large population of people suffering from impacts related to ischemic stroke calls for further research on understanding the pathophysiological mechanisms and neuroprotection and rehabilitation interventions. MEAs may be used to further investigate various aspects of ischemic stroke because the technique **1)** may allow for the monitoring of the electrophysiological signals with high spatial and temporal resolution, **2)** may be used to monitor the development of the ischemic stroke as well as the recovery processes from the acute to the chronic phase, and **3)** may potentially monitor both the progression and extent of tissue loss and the functional state of the non-injured brain following the ischemic stroke. The hypothesis of this Ph.D. thesis was therefore

***Utilizing microelectrode arrays to monitor intracortical electrophysiological signals will provide novel insight into ischemic stroke and can be a useful tool when testing novel approaches for neuroprotection and rehabilitation interventions.***

## 3.1. EXPERIMENTAL STUDIES

Four experimental studies were designed to address the hypothesis, and each of them has resulted in a journal paper. The motivation for the individual studies and the experimental methods as well as the publication status of each paper is described in the following. The published paper(s) can be obtained from the respective journals.

**Study I:** R. K. Nielsen, K. Yoshida, and W. Jensen, "Global and focal features extracted from intracortical multi-unit signals for investigation of electrophysiological changes in a rat model of ischemic stroke", (IN PREPARATION), 2016

MEAs can be designed to meet the custom needs of each specific experiment. Furthermore, the high spatial and temporal resolution of the MEA recordings results in large amounts of complex data and allows many forms of data analysis. However, to the best of the author's knowledge, no standardized MEA design or data analysis methods exist for use in ischemic stroke research. The objective of **Study I** was therefore to develop and test a novel MEA and data analysis techniques for use in the ischemic stroke model.

**Study II:** R. K. Nielsen and W. Jensen, "Low-frequency Intracortical Electrical Stimulation Decreases Sensorimotor Cortex Hyperexcitability in the Acute Phase of Ischemic Stroke", *IEEE Trans. Neural Syst. Rehabil. Eng.*, (ACCEPTED), 2016

**Study III:** R. K. Nielsen and W. Jensen, "Low-frequency Intracortical Electrical Stimulation Impedes Spatial and Temporal Spreading Hyperexcitability in the Acute

Phase of Ischemic Stroke", *IEEE Trans. Neural Syst. Rehabil. Eng.,* (UNDER REVIEW), 2016

Neuroprotection interventions that target a wide range of cell death mechanisms may have a high potential for successful translation into the clinical setting (Gladstone et al., 2002). Lf-ES of the ischemic brain may be one these interventions, but it has been used only sporadically in experiments (Baba et al., 2009; Plow & Machado, 2013). The objective of **Studies II-III** was to investigate the neuroprotective effect of lf-ES applied to the ischemic brain in the acute phase following ischemic stroke. **Study II** utilized a set of global features to characterize the global state of the cortical tissue following the ischemic insult, whereas **Study III** utilized a set of focal features to further investigate the spatio-temporal changes to the cortical tissue.

**Study IV:** R. K. Nielsen, K. L. Samson, D. Simonsen, and W. Jensen, "Effect of Early and Late Rehabilitation Onset in a Chronic Rat Model of Ischemic Stroke — Assessment of Motor Cortex Signaling and Gait Functionality Over Time", *IEEE Trans. Neural Syst. Rehabil. Eng.,* vol. 21, no. 6, pp. 1006-1015, 2013

MEAs allow for the monitoring of the ischemic brain directly after the insult and into the chronic phase. Therefore, the MEA may be used to test the postulated existence of a *critical period* during which rehabilitation is especially efficient and determine if rehabilitation prior to this period may exacerbate the injury (T. H. Murphy & Corbett, 2009). Thus, the objective of **Study IV** was to explore how the timing of rehabilitation onset affects the recovery of ischemia-affected neural networks and behavioral function within the subacute-chronic phase.

# CHAPTER 4. EXPERIMENTAL METHODOLOGIES

The ischemic stroke model was based on male Sprague-Dawley rats and the photothrombosis method because these animals are low-cost and the photothrombosis method is easy to control and results in reproducible ischemic strokes (see Sec. 2.4.1). Additionally, preliminary data using this model existed (Jensen et al., 2006).

To record the electrophysiological characteristics of the ischemic brain, the activity-related neural responses of the sensorimotor cortex were investigated. This neural activity was correlated to a given event in all studies, i.e., either to sensory inputs artificially induced by sciatic nerve stimulation (**Studies I-III**) or to motor outputs to control the gait pattern (**Study IV**).

A 28-channel intracortical MEA (IC MEA) with which an ischemic stroke could be induced within the center of the recording area was designed and utilized in the acute experiments (**Studies I-III**, see Fig. 4-3). This type of MEA design was patented by Rousche et al. (Rousche, Chiganos, & Jensen, 2009) but has to the best of the author's knowledge not previously been used in an experimental setting. A 16-channel IC MEA design was instead used in the chronic experiment (**Study IV**, see Fig. 4-4).

Novel analysis methods to quantify the neural activity were designed and used in the acute experiments (**Studies I-III**) with the aim of obtaining measures that reflect the spatial and temporal progression of ischemia. In the chronic experiment (**Study IV**), more conventional analysis methods, focusing on the neural activity obtained from individual electrodes, were used.

Table 4-1 provides an overview of the experimental methodologies that were used in each study of the thesis.

*Table 4-1 Overview of experimental methodologies*

| **Experimental protocol (Sec. 4.1)** | | | | |
|---|---|---|---|---|
| | Study I | Study II | Study III | Study IV |
| **Animals** | *Control* group (six rats) | *Intervention* group (nine rats)<br>*Control* group (eight rats) | | *Early-onset* group (five rats)<br>*Late-onset* group (four rats) |
| **Focus** | Methodology | Neuroprotection | | Rehabilitation |
| **Phase of stroke** | Acute | Acute | | Subacute-chronic |
| **Exp. timeline** | See Fig. 4-1 | | | See Fig. 4-2 |

| **Animal preparation (Sec. 4.2)** | | | | |
|---|---|---|---|---|
| | Study I | Study II | Study III | Study IV |
| **IC MEA** | See Fig. 4-3 | | | See Fig. 4-4 |
| **Other devices** | Cuff electrode on right sciatic nerve | | | Kinematic markers on right hindlimb |

| **Photothrombosis intervention (Sec. 4.3)** | | | | |
|---|---|---|---|---|
| | Study I | Study II | Study III | Study IV |
| **Fiber optical probe** | φ = 1 mm, white light | | | φ = 3 mm, white light |
| **Rose Bengal sodium salt** | Concentration: 20 mg dye/ml saline<br>Injection dose: 0.3 ml/100 g body weight<br>Injection time: 2 min | | | |
| **Illumination time** | 30 min | | | |
| **Stroke induction site** | Center of IC MEA (see Fig. 4-3) | | | Rostrally to IC MEA (see Fig. 4-4) |

| **Interventions to counteract ischemic stroke (Sec. 4.4)** | | | | |
|---|---|---|---|---|
| | Study I | Study II | Study III | Study IV |
| **Neuroprotection** | NA | Low-frequency intracortical electrical stimulation (lf-ICES) | | NA |
| **Rehabilitation** | NA | | | Beam traversals<br>Treadmill walking |

| **Data acquisition (Sec. 4.5)** | | | | |
|---|---|---|---|---|
| | Study I | Study II | Study III | Study IV |
| **IC recordings** | Sensorimotor cortex responses to sciatic nerve stimulation | | | Sensorimotor cortex activity during treadmill walking |
| **IC microstimulation mapping** | NA | | | Visual assessment of motor response |
| **Behavioral function** | NA | | | Video recording of beam traversals and treadmill walking |

| **Data analysis (Sec. 4.6)** | | | | |
|---|---|---|---|---|
| | Study I | Study II | Study III | Study IV |
| **IC recordings** | Global and focal neural response patterns | Global neural response patterns | Focal neural response patterns | Neural response patterns for subgroups of electrodes with similar hindlimb association |
| **IC microstimulation mapping** | NA | | | Grouping of motor responses |
| **Behavioral function** | NA | | | Foot drops per beam traversal<br>COG of hindlimb during treadmill walking |

## 4.1. EXPERIMENTAL PROTOCOL

### 4.1.1. STUDIES I-III

In total, 17 male Sprague-Dawley rats were included in studies I-III. The rats were assigned to either the *Intervention* group (i.e., nine rats receiving low-frequency intracortical electrical stimulation (lf-ICES) via the IC MEA) or the *Control* group (i.e., eight rats where the stroke was allowed to progress without any neuroprotection intervention).

Two baseline recording sessions were performed prior to the photothrombosis intervention (referred to as B1 and B2), and 15 recording sessions (referred to as P0 - P14) were conducted over a seven-hour period after the photothrombosis intervention (see Fig. 4-1). For the *Intervention* group, the neuroprotection intervention was initiated directly after P0 and maintained for four hours, only being interrupted in a seven-minute period during the recording sessions.

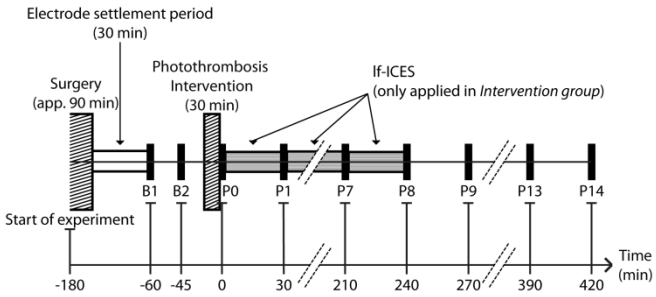


*Figure 4-1. Timeline of the experiments conducted in Studies I-III.*

### 4.1.2. STUDY IV

In total, nine male Sprague-Dawley rats were included in study IV. The rats were assigned to either the *Early-onset* group (five rats that initiated their rehabilitation training on day one after ischemic stroke) or the *Late-onset* group (four rats that initiated their rehabilitation training on day seven after ischemic stroke).

Two baseline recording sessions were performed prior to the photothrombosis intervention (referred to as B1 and B2), and seven recording sessions (referred to as D1 - D7) were performed during four weeks of rehabilitation (see Fig. 4-2). The rehabilitation training consisted of 3 x 1 min treadmill walking and 25 beam traversals five days per week and was similar for both groups. IC microstimulation mapping was performed just before recording session B1 and after recording session D7.

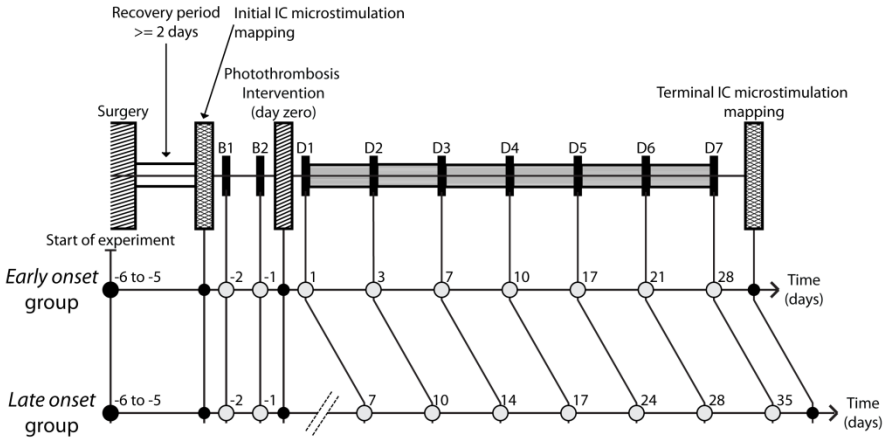


*Figure 4-2. Timeline of the experiments conducted in Study IV.*

## 4.2. ANIMAL PREPARATION

### 4.2.1. STUDIES I-III

Studies I-III were carried out as acute (i.e., one-day) experiments, and the rats were anaesthetized throughout the experiment. A cuff electrode was implanted around the sciatic nerve of the right hindlimb and used to generate a sensory input that could be recorded by the 28-channel IC MEA that was inserted in the hindlimb input layer of the left sensorimotor cortex (see Fig. 4-3).

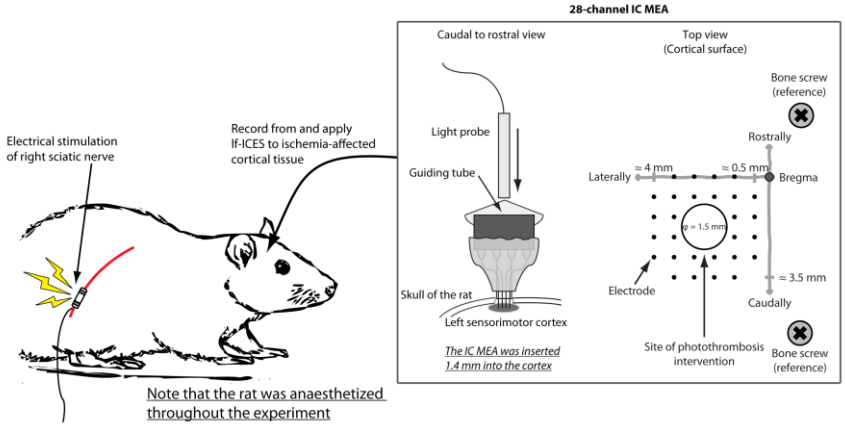


*Figure 4-3. Animal preparation for Studies I-III.*

### 4.2.2. STUDY IV

The animal experiments in Study IV were carried out as chronic (i.e., up to six weeks from the animal preparation to the end of the rehabilitation training) experiments. A 16-channel IC MEA was chronically implanted in the sensorimotor cortex related to hindlimb function to record the neural activity. During the recordings, four markers were temporarily placed on the rats' right hindlimbs to obtain the kinematics of the hindlimb while the rats were walking on a motorized treadmill (see Fig. 4-4).

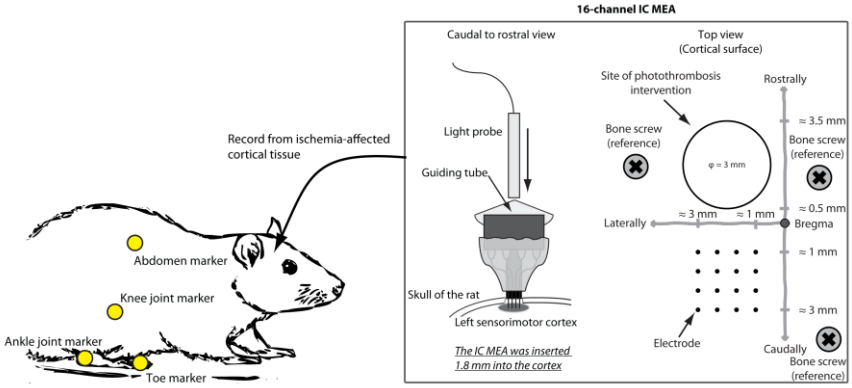


*Figure 4-4. Animal preparation for Study IV.*

## 4.3. PHOTOTHROMBOSIS INTERVENTION

### 4.3.1. STUDIES I-IV

The photothrombosis method, where illumination causes a reaction between the circulating blood and a photosensitive dye, was used to induce an ischemic stroke (Watson, Dietrich, Busto, Wachtel, & Ginsberg, 1985). A hollow tube (referred to as the *guiding tube*) was integrated into the IC MEA and provided access to the cortical surface for an optical probe (see Fig. 4-3 and Fig. 4-4) to illuminate the cerebral blood vessels.

## 4.4. INTERVENTIONS TO COUNTERACT ISCHEMIC STROKE

### 4.4.1. STUDY I

No intervention to counteract ischemic stroke was applied in study I.

### 4.4.2. STUDIES II-III – NEUROPROTECTION INTERVENTION

The neuroprotection intervention consisted of 23 min. sessions of lf-ICES applied simultaneously on all electrodes of the IC MEA. The stimulation was designed as cathodic-first, charge-balanced biphasic constant-current pulses (frequency = 2 Hz, pulse width = 0.2 ms, amplitude = 50 μA). The paradigm was based on observations from studies by Hess & Donoghue (Hess & Donoghue, 1996), Froc et al. (Froc, Chapman, Trepel, & Racine, 2000), and Baba et al. (Baba et al., 2009), where the first two induced *long-term depression* (*LTD*) using similar stimulation parameters and the last showed a neuroprotective effect of the stimulation.

### 4.4.3. STUDY IV – REHABILITATION INTERVENTION

The repetitive rehabilitation training consisted of 25 beam traversals and 3 x 1 min of treadmill walking per day for five days a week. The amount of training was a tradeoff between enabling a large number of repetitions while assuring consistency in the quality of the movement to induce *hebbian plasticity* (T. H. Murphy & Corbett, 2009; Woldag & Hummelsheim, 2002). As such, the level of training was chosen based on observations from pilot experiments where the performance declined when the training was increased beyond the amount described above.

## 4.5. DATA ACQUISITION

### 4.5.1. STUDIES I-III

#### IC recordings

To assess the neural network functionality, a train of 240 electrical stimulation pulses was delivered to the cuff electrode (frequency = 2 Hz, pulse width = 0.1 ms, amplitude = 3 mA), and the evoked sensorimotor cortex responses were simultaneously recorded by the IC MEA.

### 4.5.2. STUDY IV

#### IC microstimulation mapping

IC microstimulation mapping was performed to identify the IC MEA electrodes that were related to hindlimb function. Each electrode of the IC MEA was consecutively stimulated (frequency = 100 Hz, pulse width = 200 μs, amplitude range = 100 - 500 μA, amplitude increment = 50 μA), and the motor responses were visually assessed.

#### Behavioral function

For each recording session, 20 beam traversals were conducted to quantify the rats' ability to perform a skilled movement, and 4 x 30 s of treadmill walking was performed to analyze the kinematics of the affected hindlimb.

### IC recordings

To assess the neural network functionality, the neural activity of the sensorimotor cortex was recorded by the IC MEA during the treadmill walking.

## 4.6. DATA ANALYSIS

### 4.6.1. STUDIES I-III

### IC recordings

The data analysis algorithms translated the neural activities obtained from the individual electrodes into 2D maps describing the activity at various locations within the IC MEA recording area according to the metrics of distance. A set of global and focal features were extracted from the maps to characterize the progression of the electrophysiological changes over time and space. The global features were designed to provide information about the overall state of the neural tissue, and the focal features were designed to provide information about spatio-temporal changes within the neural tissue (see Fig. 4-5).

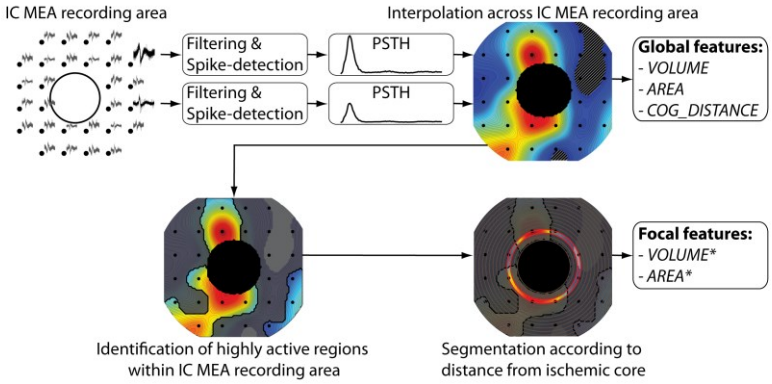


*Figure 4-5. Steps of the IC recording data analysis that was conducted in studies I-III. '*' Focal features VOLUME and AREA were computed for the highly active areas located at various distances from the site of the photothrombosis intervention.*

### 4.6.2. STUDY IV

### IC microstimulation mapping

The motor responses were grouped into *hindlimb*, *forelimb*, and *no-motor* responses based on visual observations while performing the mapping.

### Behavioral function

The average number of foot drops per beam traversal was computed to quantify the rats' ability to perform a skilled movement. The rat was placed on the broad end of a ledged beam, and it traversed the beam to reach a shelter on the narrow end of the

beam. The ledged beam was based on the one described by Schallert et al. (Schallert, Woodlee, & Fleming, 2002). For similar purposes, the centers of gravity (COG) for the *toe*, *ankle joint*, and *knee joint markers* were computed from video recordings of the rat walking on the treadmill. An *abdomen marker* was used as a point of reference during the calculation.

### IC recordings

The data analysis algorithm grouped the neural activities obtained from the individual electrodes during the treadmill walking. The procedure was based on observations from the IC microstimulation mapping and resulted in three groups: **1)** electrodes that only elicited a hindlimb motor response prior to photothrombosis, **2)** electrodes that elicited a hindlimb motor response both prior to and post photothrombosis, and **3)** electrodes that only elicited a hindlimb motor response post photothrombosis. Each group of electrodes was analyzed individually to investigate trends within the neural responses (see Fig. 4-6).

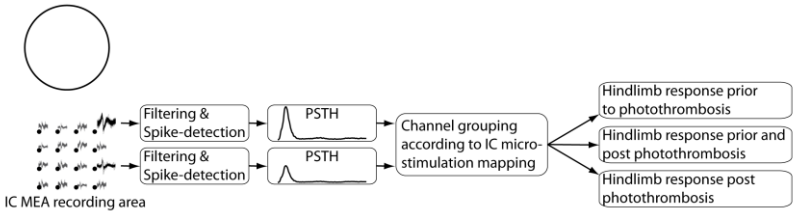


*Figure 4-6. Steps of the IC recording data analysis that was conducted in study IV.*

# CHAPTER 5. SUMMARY OF MAIN FINDINGS

This thesis investigated the potential of MEAs to monitor electrophysiological signals with the aim of investigating ischemic stroke and developing novel approaches for neuroprotection and rehabilitation interventions. An overview of the objectives and main findings of the four studies are summarized in Table 5-1 and will be discussed in Chapter 6.

*Table 5-1 Overview of the objectives and main findings of the four studies*

| | Objective | Main findings |
|---|---|---|
| **Study I** | To develop and test a novel MEA and data analysis techniques for use in the ischemic stroke model. | **1.** The MEA and analysis methods allowed for detailed spatial and temporal characterization of the neural responses within the sensorimotor cortex.<br><br>**2.** The MEA design did not allow for recording from the *ischemic core*. |
| **Study II**<br>**Study III** | To investigate the neuroprotective effect of lf-ES applied to the ischemic brain in the acute phase following ischemic stroke. | **1.** Acute hyperexcitability occurred within the *penumbra* and/or lesser-affected neural networks following ischemic stroke. The hyperexcitability spread along a complex path influenced by time, space and neural connectivity.<br><br>**2.** Lf-ES minimized the overall amount of hyperexcitability and restricted its spread through the ischemia-affected neural networks.<br><br>**3.** Lf-ES may potentially delay and reduce the spread of the progressively expanding *ischemic core*. |
| **Study IV** | To explore how the timing of rehabilitation onset affects the recovery of ischemia-affected neural networks and behavioral function within the subacute-chronic phase. | **1.** Rehabilitation initiated seven days after ischemic stroke resulted in improved motor function and neural activity patterns with a large similarity to the healthy state compared to initiating rehabilitation on day one after ischemic stroke.<br><br>**2.** The findings supported the existence of a *critical period* where rehabilitation may be more effective and facilitates a higher degree of recovery.<br><br>**3.** Initiating rehabilitation prior to the *critical period* may potentially exacerbate the ischemic injury. |

# CHAPTER 6. DISCUSSION

## 6.1. METHODOLOGICAL CONSIDERATIONS

In general, experimental and clinical studies differ on various aspects (see Table 6-1). The failure of especially many neuroprotection interventions is attributed to the large diversity and heterogeneity in all aspects of clinical studies compared to experimental studies (Fisher et al., 2009; STAIR, 1999; Traystman, 2003).

*Table 6-1 Overview of differences between experimental and clinical studies. Adapted from* (Moretti et al., 2015; Sutherland et al., 2012; Willing, 2009)

| **Experimental studies (animal)** | **Clinical studies (human)** |
|---|---|
| *1) Investigated population* | |
| Highly controlled, homogeneous population | Variable, heterogeneous population |
| Younger animals | Older patients |
| Limited comorbidities | Numerous comorbidities |
| Previously untreated with drugs | Previously treated with drugs |
| Gender: mostly male | Gender: male and female |
| *2) Nature of ischemic stroke* | |
| Induced onset of ischemic stroke | Spontaneous onset of ischemic stroke |
| Uniform etiology | Variable etiology |
| Ischemic territory originating from *MCAO* | Ischemic territory not restricted to *MCA* |
| Controlled occlusion duration | Variable occlusion duration |
| *3) Time window of intervention* | |
| Control over intervention time window (usually early onset in studies on neuroprotection) | Less control over intervention time window (usually delayed onsets of both neuroprotection and rehabilitation) |
| *4) Outcome measurements* | |
| Infarct volume as primary outcome (in neuroprotective studies) | Behavioral function as primary outcome |
| Behavioral function | Long follow-up time |
| Short follow-up time | |

In the present work, many of the characteristic differences between experimental and clinical studies were present. Because the focus of the work was on the design and testing of novel monitoring methods and ischemic stroke interventions, it was attempted to control as many parameters as possible. As such, it is believed that the present model can provide insight into injury and recovery mechanisms, but the clinical potential of the findings is still debatable, and therefore they should be investigated further by broadening the model. The following will elaborate on the methods that were used in the model and how they may be improved.

### 6.1.1. THE RAT AS A MODEL OF ISCHEMIC STROKE

Aging has been related to reduced *angiogenesis* and poor recovery of behavioral function following ischemic stroke in rats (Zhang et al., 2005), but contradictory

results have been reported when the infarct volume has been investigated (Durukan & Tatlisumak, 2007). For example, Duverger and MacKenzie (Duverger & MacKenzie, 1988) and Wang et al. (Wang, Wang, & Yang, 2003) found no correlation between the age and infarct volume in rats, whereas Davis et al. (Davis, Mendelow, Perry, Chambers, & James, 1995) found that the infarct volume was larger in older rats, and Shapira et al. (Shapira, Sapir, Wengier, Grauer, & Kadar, 2002) oppositely found that younger rats had a larger infarct volume. Studies in female rats have shown that ovarian hormones have a neuroprotective effect (Hoffman, Merchenthaler, & Zup, 2006), and hypertensive rats develop larger and less variable infarcts when subjected to intraluminal occlusion (Coyle, 1986).

In the present work, male Sprague-Dawley rats were used in all experiments. The age of the rats ranged from 10 to 14 weeks, which corresponds to young adulthood when compared to human age (Sengupta, 2013), and at this age, the cortex of the rat has reached its adult size (Kolb & Tees, 1990). The rats did not have any underlying chronic diseases or genetic predisposition to certain diseases. To further test the results of the present work, the experiments should be replicated while including both female rats of varying ages and hypertensive rats. Furthermore, the *Stroke Therapy Academic Industry Roundtable* (*STAIR*) (Fisher et al., 2009; STAIR, 1999) emphasizes that at least one other species (preferably a gyrencephalic species) should also be used.

### 6.1.2. INDUCTION OF ISCHEMIC STROKE

Several methods for the induction of ischemic stroke exist, and each has its individual advantages and disadvantages (see Sec. 2.4.1). In the present work, the photothrombosis method was used due to its high reproducibility. The method results in a well-demarcated infarct with a small penumbral region that rapidly reaches its full extent as microvascular occlusions occur throughout the illuminated area (Dietrich, Ginsberg, Busto, & Watson, 1986; Watson et al., 1985).

**Study I** showed that the MEA did not record from the *ischemic core* or severely affected *penumbra*, as it was observed that the neural networks did not turn functionally silent as the ischemic stroke progressed (Astrup et al., 1981, 1977). This observation indicates that the penumbral region may have been small and did not extend far from the site of the photothrombosis intervention. This may be a problem, as the *penumbra* is the main target of neuroprotection interventions to salvage neural tissue (STAIR, 1999). A modified version of the photothrombosis methods may help to circumvent this problem by inducing a ring-shaped ischemic infarct with a *'region-at-risk'* situated in the center of the ring (Wester, Watson, Prado, & Dietrich, 1995). Within this region, spontaneous reperfusion (Hilger, Blunk, Hoehn, Mies, & Wester, 2004) and *PIDs* have been observed (Risher et al., 2010), and the pathological mechanisms more closely resemble those of clinical stroke with a larger *penumbra*. Thus, applying this photothrombosis method to the current ischemic stroke

model may help to improve the clinical relevance of the model and allow for electrophysiological monitoring of the neural networks that are directly damaged by the ischemic stroke (i.e., the neural tissue in the *ischemic core* and the *penumbra*).

Furthermore, *STAIR* (Fisher et al., 2009; STAIR, 1999) emphasizes that both permanent and transient ischemic stroke models should be used for the evaluation of the neuroprotective potential of an intervention. A transient ischemia cannot be obtained with the photothrombosis method (see Sec. 2.4.1), and therefore another stroke induction method should be used when testing lf-ES in transient ischemia models. However, specific precautions should be taken when inducing transient ischemia, as the secondary damage following prompt reperfusion (e.g., as with the intraluminal or ligation stroke methods) does not resemble the secondary damage following gradually and prolonged reperfusion that may occur spontaneously or following *recombinant tissue plasminogen activator* (*rtPA*) intervention in the clinical setting (Hossmann, 2012). Consequently, the endothelin-1 method may be more suitable for use in a transient ischemia model, as it results in gradual reperfusion (see Sec. 2.4.1).

### 6.1.3. THE USE OF MICROELECTRODE ARRAYS

MEAs may be custom-designed to meet the needs of a specific experiment. A different electrode layout and configuration of the MEA was used in **Studies I-III** compared to those in **Study IV**. These differences in MEA design reflected the availability of recording equipment and the progression in the expertise and knowledge of electrode manufacturing that was obtained during the work. **Study IV** was performed first, at a point in time where a recording system allowing for the recording of only 16 channels was available, whereas for **Studies I-III,** a recording system that allowed for the recording of more channels was available.

However, one general disadvantage was that the *guiding tube* covered the area situated directly beneath it, thereby making it impossible to record from the neural tissue in this region (see Fig. 4-3 and Fig. 4-4). Consequently, it was not possible to establish the distribution of the hindlimb motor and/or sensory function within this sensorimotor cortex region, and it was also impossible to monitor the *ischemic core* and directly estimate the severity of the insult. The *guiding tube* of the MEA is, however, believed to have been located in the center of the *hindlimb area*, as it was carefully placed by the use of a micromanipulator based on knowledge from prior microelectrode stimulation/recording studies (Neafsey et al., 1986; Welker, 1976), transdural stimulation mapping studies (Fonoff et al., 2009), and histological studies (Paxinos & Watson, 2008). As described in **Study I**, it may be possible to both record and induce ischemic stroke within the same neural tissue if using a *clear-transparent UV-curing biocompatible resin* (e.g., *Freeprint® splint, Detax GmbH & Co, Ettlingen, Germany*) as a base for the MEA cast.

### 6.1.4. DATA ANALYSIS OF ELECTROPHYSIOLOGICAL RECORDINGS

MEAs render it possible to utilize various data analysis methods to infer on the state of the brain. The most basic data analysis focuses on single-electrode data, but as brain functions (e.g., motor functions) are encoded by large populations of neurons, this approach means that information may be lost (Georgopoulos, Schwartz, & Kettner, 1986; M. A. Nicolelis, Ghazanfar, Faggin, Votaw, & Oliveira, 1997).

In the present work, it was sought to characterize the changes within the entire recorded region of the sensorimotor cortex rather than to investigate highly local changes on a single-electrode basis. The focal measures, which were developed and used in **Study I** and **Study III** (see Sec. 4.6.1), are believed to hold the largest potential to characterize the progression of the ischemic stroke because these features provide information not only on the area-wise size and the magnitude of the neural response but also on the distance between the responding network and the *ischemic core*. As such, the results from the focal measures showed that the hyperexcitability progressed outward from the *ischemic core* over time. However, the focal measures did not provide any indication on whether the outward spread of the hyperexcitability progressed along a specific direction away from the *ischemic core* (e.g., rostrally or laterally) or had an inclination towards occurring within another functional subregion of the sensorimotor cortex (e.g., occurring within the *forelimb area* after injury in the *hindlimb area*). To further improve the data analysis, it would be valuable to map the recorded region of the sensorimotor cortex prior to induction of the ischemic stroke by, for example, recording the evoked responses to tactile stimulation applied via a piezoelectric device, as described by Sigler et al. (Sigler, Mohajerani, & Murphy, 2009). This information may then be overlaid on the 2D map that describes the neural activity within the MEA recording area (see Sec. 4.6.1). In this way, it may be possible to investigate if the electrophysiological changes had an inclination towards occurring within another specific subregion of the sensorimotor cortex.

## 6.2. MONITORING ISCHEMIC STROKE BY USE OF MICROELECTRODE ARRAYS

MEAs make it possible to simultaneously record or stimulate large populations of excitable cells over long periods of time (i.e., up to several months) (Flint, Wright, Scheid, & Slutzky, 2013; Hofmann & Bading, 2006; M. D. Murphy, Guggenmos, Bundy, & Nudo, 2015; Spira & Hai, 2013; Vetter et al., 2004). They have frequently been used to investigate the cell functionality and plasticity as well as to decode brain activity patterns (Laubach, Wessberg, & Nicolelis, 2000; Lebedev & Nicolelis, 2006; M. A. Nicolelis et al., 1997; M. A. L. Nicolelis, 2009). MEAs have, however, not been extensively used to investigate the effect of ischemic stroke on the neural tissue (Jensen et al., 2006).

In the present work, the use of MEAs made it possible to investigate the electrophysiological changes following ischemic stroke with high spatial and temporal resolution during the entire course of the ischemic stroke (acute to chronic phase). The obtained results are in line with what has previously been documented concerning the progression of ischemic stroke. **Study I** showed that hyperexcitability occurred within the neural networks following ischemic stroke and that the spread of hyperexcitability could be closely monitored. Previously, hyperexcitability in the acute phase of ischemic stroke has been documented by Sigler et al. (Sigler et al., 2009) using VSDI and Fujioka et al. (Fujioka, Kaneko, Suzuki, & Mabuchi, 2004) using cortical surface electrodes. The results in the present work additionally revealed that the neural networks changed their response patterns. As such, more temporally diffuse neural responses were observed in some neural networks, whereas in others, the neural responses became more focused.

**Study IV** showed that the neural response patterns changed and functional reorganization occurred as recovery took place during the subacute and chronic phases. Similar reorganization has been documented by Nudo et al. (Randolph J Nudo et al., 1996) in adult squirrel monkeys that were subjected to rehabilitative training.

## 6.3. LOW-FREQUENCY ELECTRICAL STIMULATION AS A NEUROPROTECTION INTERVENTION

Lf-ES applied to the ischemia-affected brain has recently been suggested as a novel approach for inducing neuroprotection in the acute phase of ischemic stroke (Plow & Machado, 2013). Baba et al. (Baba et al., 2009) exposed rats to epidural electrical stimulation (frequency = 2 Hz, amplitude = 100 μA, pulse width = 1 ms square-wave pulses) directly following a transient ischemic stroke. The stimulation was maintained for up to one week and showed that lf-ES may trigger a myriad of neuroprotective mechanisms, thereby improving behavioral function, reducing cell death (both necrotic and apoptotic), and facilitating *angiogenesis*.

**Studies II-III** showed that the hyperexcitability that spread throughout the sensorimotor cortex could be minimized by lf-ES. As such, it is suggested that the application of lf-ES may have caused the neurons to become less sensitive to the large amount of neurotransmitters within the extracellular space which, in turn, could result in the minimization of *excitotoxicity* and *PIDs* (Collingridge, Peineau, Howland, & Wang, 2010; Harvey & Nudo, 2007; Luscher & Malenka, 2012). This suggestion is based on previous research, where similar stimulation paradigms have been used to induce *LTD* in healthy tissue (Bliss & Cooke, 2011; Hess & Donoghue, 1996; Luscher & Malenka, 2012). Recalling that the IC recordings are most likely obtained from neural networks situated within the lesser-affected regions of the *penumbra* or in healthier tissue (see Sec. 6.1.2 and Sec. 6.1.3), it may also be the case that the decreased hyperexcitability instead reflects a reversal of *diaschisis*. Consequently, further investigation of the effect of lf-ES is needed to establish the

specific cause of the reduction in hyperexcitability. Finally, lf-ES should be tested after delaying the intervention (e.g., 2 – 3 hours) to investigate the robustness of the intervention in relation to the treatment time (see Sec. 2.3.1).

## 6.4. THE EXISTENCE OF A CRITICAL PERIOD OF INCREASED REHABILITATION EFFICIENCY

The existence of a *critical period* where rehabilitation may be particularly efficient in promoting recovery has been postulated to exist in both rats and humans (see Sec. 2.3.3). In rats, this period has been proposed to span from day 5 to day 14 after the ischemic insult, and initiating rehabilitation or forcing the subject to use the impaired limb earlier than day 5 has been shown to result in less recovery and an enlargement of the ischemic territory (Bland et al., 2000; Humm et al., 1998; Kozlowski et al., 1996).

**Study IV** showed that the initiation of rehabilitation prior to or within the *critical period* resulted in a similar degree of recovery of skilled movement at the termination of the experiment. As such, both groups of animals recovered to the degree at which the beam traversal performance was similar to that of healthy state. On the contrary, the hindlimb movement pattern during treadmill walking was significantly different from that of the healthy state for both groups. Initiating rehabilitation within the *critical period*, though, resulted in hindlimb movement patterns that were more similar to those of the healthy state at the termination of the experiment.

The assessment of the electrophysiological characteristics showed that the *hindlimb area* expanded and that the neural activity patterns were significantly altered when initiating rehabilitation prior to the *critical period*. An expansion of the *hindlimb area* was not observed when initiating rehabilitation within the *critical period*, and the neural activity patterns were more similar to those healthy state. Previously, Kozlowski et al. (Kozlowski et al., 1996) found that the very early use of the impaired limb resulted in increased tissue loss. It is believed that this may also have occurred in the present study and that the larger degree of cortical reorganization and electrophysiological changes reflected an increased demand of the brain to compensate for the exacerbated injury. This may also be reflected in the large alteration in the movement pattern of the hindlimb.

## 6.5. LIMITATIONS AND PERSPECTIVES OF THE ISCHEMIC STROKE MODEL

As described in Section 6.1.3, the *ischemic core* and *penumbra* could not be verified by the electrophysiological recordings in the present work. Therefore, the MEA should be redesigned and at the same time used in connection with histological techniques to provide a second estimate of the infarct size. Utilizing histological techniques, though, raises an issue in reproducing **Studies I-III**, as it is difficult to

calculate the infarction volume very soon after the ischemic insult. This is due to the ischemic changes taking up to 24 hours to mature into a well-demarcated infarct that may be observed on the histological exhibits (Liu et al., 2009). Thus, the experiment should be prolonged to enable the ischemic changes to mature and could with benefit be extended into the chronic phase to accommodate the guidelines of *STAIR* (Fisher et al., 2009; STAIR, 1999), which suggest that monitoring of ischemic stroke and recovery should be performed from the acute to the chronic phase to properly assess the effects of interventions.

In the present work, the electrophysiological characteristics of the ischemic brain were investigated by analyzing the sensorimotor cortex responses to hindlimb-related sensory inputs (**Studies I-III**) and the sensorimotor cortex activity during walking on a motorized treadmill (**Study IV**). In future work, the spontaneous activity of the ischemia-affected neural networks may be recorded, as it would be of high value to verify the occurrence of *PIDs* and investigate whether lf-ES reduces these. *PIDs* have previously been recorded in mice by Risher et al. (Risher et al., 2010) with a glass microelectrode inserted within layer I of the sensorimotor cortex. Further, it would be of relevance to record from the *rostral motor area* (which has a resemblance to the *supplementary motor area* and *premotor area* of primates) or the contralateral homotopic regions because the electrophysiological properties of these areas change as a consequence of the ischemic stroke and subsequent recovery (Dancause & Nudo, 2011; Dancause et al., 2006; Eisner-Janowicz et al., 2008; Kopp et al., 1999; Mohajerani et al., 2011).

Finally, to improve the clinical relevance of future research, it may be beneficial to translate the model onto an animal with a higher degree of physiological similarity to humans, such as pigs (Fisher et al., 2009; STAIR, 1999). The pig has an gyrencephalic brain and has previously been used in acute ischemic stroke experiments by Sakoh et al. (Sakoh, Rohl, et al., 2000) and Røhl et al. (Røhl et al., 2001) to investigate cerebral blood flow and the *penumbra* by use of MRI.

# CHAPTER 7. CONCLUSIONS

The present thesis hypothesized that utilizing microelectrode arrays to monitor intracortical electrophysiological signals would provide novel insight into ischemic stroke and can be a useful tool for testing novel approaches for neuroprotection and rehabilitation interventions. The hypothesis was tested in a rat model of ischemic stroke.

**Study I** sought to establish novel methods and techniques for the use of MEAs. **Studies II-III** utilized these novelties to investigate the neuroprotective effect of lf-ES, and **Study IV** investigated the existence of a *critical period* in which the initiation of rehabilitation is especially efficient in promoting recovery, whereas initiating rehabilitation prior to this period may exacerbate injury.

The thesis showed that the use of MEAs allowed for monitoring the electrophysiological properties of the ischemia-affected brain in high spatial and temporal resolution (**Study I**). This property is believed to be of high importance because it renders it possible to closely monitor the evolving ischemic stroke and can provide detailed information on the effects of neuroprotection and rehabilitation interventions. Lf-ES was found to be a promising neuroprotection intervention because it reduced the hyperexcitability within the *penumbra* and/or lesser-affected neural networks following ischemic stroke (**Studies II-III**). Finally, the existence of a *critical period* where rehabilitation may be especially efficient in promoting recovery was supported, and it was observed that initiating rehabilitation prior to this period may exacerbate injury (**Study IV**).

Thus, the thesis has demonstrated the novel and innovative use of MEAs in ischemic stroke research and has shown beneficial effects of novel approaches for neuroprotection and rehabilitation interventions. The work is believed to have a high potential as a basis for further studies on the underlying mechanisms of ischemic stroke and may assist in the design of more effective neuroprotection and rehabilitation interventions in the future.

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