UNIVERSITY OF CALGARY

Skilled Behaviour and Bilateral Motor Map Expression

by

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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN PSYCHOLOGY
CALGARY, ALBERTA

OCTOBER, 2015

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Abstract

Multiple seizures cause changes to both cortical movement representations (motor maps) and behavioural impairment. The contribution of seizures to motor map alterations and impaired skilled forelimb movement was studied using the electrical kindling model and short-duration intracortical microstimulation (SD-ICMS). Forelimb motor map movements changed considerably from contralateral in sham-kindled rats to ipsilateral and bilateral in rats kindled for over 5 sessions. Furthermore, bilateral movement representations were related to impairments in skilled forelimb subcomponents. Finally, skilled forelimb impairment was also related to the number of bilateral seizures experienced as well as the duration of bilateral seizures.

Next, the role of the ipsilateral hemisphere in bilateral movement representations was examined using SD-ICMS and reversible cooling inactivation. Bilateral representations were produced using electrical kindling as well as pharmacological HCN channel blocker ZD7288. Inactivating the cortex ipsilateral to the skilled forelimb completely eliminated ipsilateral and bilateral movement, indicating its role in bilateral movement representations.
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<tr>
<td>µA</td>
<td>microamp</td>
<td></td>
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<tr>
<td>aCSF</td>
<td>Artificial Cerebrospinal Fluid</td>
<td></td>
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<tr>
<td>AD</td>
<td>Afterdischarge</td>
<td></td>
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<tr>
<td>ADD</td>
<td>Afterdischarge Duration</td>
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<tr>
<td>ADT</td>
<td>Afterdischarge Threshold</td>
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<tr>
<td>AMPA</td>
<td>2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid</td>
<td></td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ASD</td>
<td>Anti-seizure Drug</td>
<td></td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CST</td>
<td>Corticospinal Tract</td>
<td></td>
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<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
<td></td>
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<tr>
<td>FLE</td>
<td>Frontal Lobe Epilepsy</td>
<td></td>
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<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
<td></td>
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<tr>
<td>GABA</td>
<td>Gamma-amino-butyric Acid</td>
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<tr>
<td>HCN</td>
<td>Hyperpolarization-activated Cyclic Nucleotide-gated Channel</td>
<td></td>
</tr>
<tr>
<td>HCN1</td>
<td>Hyperpolarization-activated Cyclic Nucleotide-gated Channel 1</td>
<td></td>
</tr>
<tr>
<td>HCN2</td>
<td>Hyperpolarization Activated Cyclic Nucleotide-gated Channel 2</td>
<td></td>
</tr>
<tr>
<td>HFS</td>
<td>High Frequency Stimulation</td>
<td></td>
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<tr>
<td>ICMS</td>
<td>Intracortical Microstimulation</td>
<td></td>
</tr>
<tr>
<td>Ih</td>
<td>Hyperpolarization-activated mixed cation current</td>
<td></td>
</tr>
<tr>
<td>ILAE</td>
<td>International League Against Epilepsy</td>
<td></td>
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<tr>
<td>IPSP</td>
<td>Inhibitory Postsynaptic Potential</td>
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K+  Potassium Ion
KA  Kainic Acid
KIP  Kindling-induced Potential
KO  Genetic Knockout
LD-ICMS  Long Duration Intracortical Microstimulation
LFS  Low Frequency Stimulation
NMDA  N-methyl-D-aspartate
PET  Positron Emission Tomography
SA  Stimulation Artifact
SEM  Standard Error of the Mean
SD-ICMS  Short Duration Intracortical Microstimulation
TLE  Temporal Lobe Epilepsy
ZD 7288  Bradycardic Agent 4-(N-ethyl-N-phenylamino)-1,2-Dimethyl-6-(methylamino) Pyrimidinium Chloride
CHAPTER 1: GENERAL INTRODUCTION

1.1 A History of the Motor Cortex

A critical function of the motor cortex is the control of voluntary movement. The motor cortex can be defined as the area of the cerebral cortex in which motor movement arises in response to reasonable levels of electrical current application. In the eighteenth century, the cerebral cortex was thought of as a protective rind to the midbrain with no mental function (Gross, 2007). The midbrain or striatum were thought to be responsible for motor control, as the cerebral cortex was believed to be unexcitable. In 1849 Robert Bentley Todd made post mortem examinations of patients with epilepsy and found that many had damage to their cerebral cortex (Todd, 1849). Furthermore, patients with seizures localized to one side of the body had damage to the other side of their cerebral cortex, suggesting that motor control was represented contralaterally in the cerebral cortex. Twenty years later John Hughlings Jackson examined the progression of seizures in patients with epilepsy, and made key observations about the cerebral organization of motor control (Jackson, 1870). Firstly, Jackson described partial seizures as a series of convulsions that consistently spread from one body part to the next. For example, a seizure with convulsions beginning in the left hand would spread up the left limb and upwards toward the left side of the face. This progression of a focal seizure is now referred to as a Jacksonian March. Based on his observations, Jackson proposed motor control as being represented topographically in the brain. In addition, Jackson noted that seizures usually began in commonly used areas of the body such as the arm, and from within these areas seizures usually originated in body parts that are more commonly used still, such as the
index fingers of the hand. From this observation, he hypothesised areas of the body that are most often used in movement as well as in complex movements have physically larger representations in the brain. However, Jackson did not experimentally determine where in the brain these movement representations reside.

Early research by Fritsch and Hitzig (1870) confirmed John Hughlings Jackson’s hypothesis that the motor cortex is somatotopically organized, meaning that specific areas of the motor cortex are involved in the motor control of different parts of the body. Using dogs, Fritsch and Hitzig stimulated different areas of the cortex, which elicited different movements such as an elbow flexion or hindlimb movement. This research demonstrated that motor function is localized to a region of the cerebral cortex and that the cortex is electrically excitable. Wilder Penfield was the first to describe the topographical organization of the human motor cortex by using surface electrical stimulation (Penfield & Rasmussen, 1950). Penfield created a map of the movement representations of the motor cortex, which is now the well-known motor homunculi (see figure 1.1). The topographical organization of the motor homunculi represents disproportionate sizes of body parts, with larger areas such as fingers and the mouth representing more precise motor control or the involvement of more muscles and gross smaller areas controlling gross motor movements such as the trunk.
Following Penfield's discovery, Asanuma and Sakata (1967) developed a refined stimulation technique for deriving motor maps called intracortical microstimulation (ICMS). Instead of stimulating the surface of the neocortex, a small microelectrode was inserted allowing for the focal stimulation (<40 ms) of cortical neurons. This technique requires 1/100th of the current that would otherwise have been needed with surface stimulation to elicit a motor movement (see figure 1.2). The optimal stimulation parameters for ICMS induced motor movements in rats are similar to that of other mammals such as cats and monkeys (Young et al., 2011). The lowest movement thresholds required to elicit a movement are found at an electrode depth of cortical layer V which includes predominantly large pyramidal neurons (Young et al., 2011). A lower threshold is needed to activate pyramidal tract cells in layer V of the motor cortex because of the closer proximity of the electrode to these corticospinal cells (Coombs et al., 1955). ICMS therefore stimulates a smaller area of cortex than surface stimulation, which means that stimulation points can be closer together, creating motor maps with a much higher degree of spatial resolution than surface stimulation.

Asanuma’s stimulation protocol, termed short duration ICMS (SD-ICMS), gave rise to simple single joint movements. Asanuma postulated that the motor cortex was organized as single columns, each connected to a single muscle (Asanuma, 1975). The stimulation of a column would therefore result in the flexion or extension of a single joint. This hypothesis suggests that the cortex controls each muscle individually and electrical stimulation activates simple single joint movements. In this protocol, the stimulation threshold of a movement is detected by reducing the stimulating current until a movement is just barely detectable. The
limitation to this technique is that behaviourally useful movements usually involve the use of more than one muscle. In addition, some muscles may be more active than others in a particular movement. Thus, Asanuma may have only been observing the “tip of the iceberg” (Graziano, 2009). A single stimulation site may involve multiple muscles or movements, each with its own threshold that corresponds to how active it is within the movement (Jankowska et al., 1975; Cheney and Fetz., 1985). Cheney and Fetz (1985) confirmed that when a single neuron in the motor cortex is active, it communicates to a group of muscles. These experiments recorded the naturally occurring activity of single neurons in the motor cortex of monkeys as well as several muscles in the wrist and fingers. Each neuron was found to communicate various degrees of excitation or inhibition to a set of muscles. Interestingly, following the creation of the homunculus, Wilder Penfield stated that his somatotopic depiction of motor movements in the motor cortex was not meant to be a perfectly accurate portrayal of motor movements derived by electrical stimulation but instead a teaching model representing a general trend. He noted that brief surface stimulation would often result in multiple movements (movement of more than one joint), rather than just a single movement (Penfield and Boldrey, 1937). A more ethologically relevant neocortical organization can be revealed using long-duration (500 ms) ICMS (LD-ICMS). Using LD-ICMS elicits species relevant movements such as bringing the hand to the mouth, climbing and leaping in primates (Graziano, 2009; see figure 1.3), and walking and grasping movements in rats (Brown and Teskey, 2014). Although the conventional SD-ICMS only looks at “the tip of the iceberg”, it is
valuable as it allows a researcher to determine the excitability of the cortex by evaluating changes in movement thresholds.

1.2 Anatomy of the Motor Cortex

At the beginning of the thesis, I defined the motor cortex as the area of cerebral cortex in which motor movement arises in response to reasonable levels of electrical current. This definition is based on the methodology (SD-ICMS) that was used in this thesis, resulting in a map of the motor cortex representing motor output. However, the resultant topographical map of motor movements produced from electrical stimulation varies depending on the stimulation protocol used. For example, SD-ICMS (40 ms) results in simple single joint movements while LD-ICMS (500 ms) yields complex multi-joint movements. Therefore, definitions based on methodology vary depending on the methodology employed. A more comprehensive definition of the motor cortex is that it is any of the six layers of the neocortex that contain neurons that project their axons directly to the spinal cord (corticospinal neurons; Teskey & Kolb, 2011) or to the medulla via the corticobulbar tract for head and neck movement.

Each of the six layers of the neocortex consists of a distinct distribution of neuronal cell types (see figure 1.4). Layer I, the molecular layer, contains a scarce number of neurons and glial cells. The external pyramidal layer, layer II, consists of small pyramidal neurons as well as stellate neurons. Layer III is identified as the external pyramidal layer and contains small and medium pyramidal neurons. Non-pyramidal neurons with vertically oriented intracortical axons
are also present in layer III. This layer is the main source of intra-hemispheric corticocortical efferents. Layers I to III are the primary targets of interhemispheric corticocortical afferents. Layer IV, termed the internal granular layer, is mainly an input layer. This layer is the primary target of thalamocortical afferents from the thalamus and intra-hemispheric corticocortical afferents. One of the functions of the thalamus is to integrate and regulate the transfer of sensory and motor information. Stellate and pyramidal neurons make up the majority of this layer. Layer V is the internal pyramidal layer and includes large pyramidal neurons and Betz cells that project their axons via the corticospinal tract. Originally, it was thought that these considerably large Betz cells were responsible for the majority of motor output to the spinal cord due to the fact that they project to the spinal cord (Betz., 1874). This hypothesis was later found to be incorrect, as Betz cells were found to only account for 2-3 percent of projections to the spinal cord (Lassek., 1941). Layer V is often termed the output layer of the motor neocortex (Teskey & Kolb, 2011) as it is the most effective for ICMS, because it is where the majority of large pyramidal cell bodies and dendrites reside. Layer VI consists of small spindle-like pyramidal neurons that send excitatory as well as inhibitory efferent fibers to the thalamus.

Although the layers of the neocortex are defined as the presence of certain cells and exclusion of others, this definition is mainly seen as a guideline as there is some overlap between layers and the size of the layers can vary. Often the motor cortex will be defined in a stereotaxic atlas as the presence of a large layer V and small layer IV, separate from the sensory cortex. There are however corticospinal neurons present in the human sensory cortex. In fact, 30-40 percent of corticospinal neurons arise from what is defined as the sensory cortex (Brazis
et al., 2007). Functionally, this overlap between “sensory” and “motor” areas makes sense as performance on a skilled motor task undoubtedly relies on sensory input. This overlap is also present in the rat cortex; therefore the term “sensorimotor cortex” is used. In summary, it is the networks of interconnected neurons within the motor cortex combined with subcortical structures such as the thalamus that allow for complex motor behaviours (Teskey & Kolb, 2011).

1.3 Anatomy of the Corticospinal Tract (CST)

The CST mainly consists of the axons of pyramidal cells with their cell bodies residing in the cortex (see figure 1.5). These axons travel through the cortex, brainstem, anterior medulla, and into the spinal cord. At the pyramidal decussation in the medulla, approximately 80 percent of the axons cross over midline to the opposite side from which they originated, forming the lateral CST (Kandel et al., 2012). The remaining axons form the anterior CST. It is because of this crossover that a hemisphere of the brain generally controls the contralateral side of the body. Once CST axons meet the vertebral level of the muscle they innervate, they synapse onto lower motor neurons either directly or indirectly through interneurons in the ventral horn (Alstermark and Isa., 2002; Lemon, 2008). Lower motor neurons innervate sets of muscle groups and consist of two types. The first type, alpha motor neurons, innervate extrafusal muscle fibres and are involved in muscle contraction, while the second type, gamma motor neurons, innervate intrafusal muscle fibres and are responsible for muscle tautness as well as proprioception (Isa et al., 2007; Kanning., 2010).
1.4 Motor Cortex Plasticity

The functional organization of the motor cortex is highly plastic and can change in response to a variety of situations such as stroke recovery (Sawaki et al., 2008), skilled reaching (Kleim et al., 2004), and sensory deprivation (Florence et al., 1998). Skilled motor tasks are dependent on the motor cortex and there is a resultant reorganization of movement representations following injury or experience. For example, following skilled motor learning in rats, the area of the cortex representing the skilled movements increases (Kleim et al., 1998). In monkeys, Nudo et al. (1996) revealed that after the acquisition of a skilled motor task combining the use of two arm joints, the number of ICMS sites involving both joints (i.e. a dual movement) also increased. Furthermore, throughout development as kittens gain more motor experience and learn to perform multi-jointed motor tasks, the neocortical somatotopy transforms from mainly single joint representations to the adult’s more multi-jointed representations. Synapse formation (Kleim et al., 2002), protein synthesis (Kleim et al., 2003), and synaptic efficacy (Monfils and Teskey, 2004) facilitate the functional reorganization of movement representations following skilled motor learning. Pathological changes to motor map topography have also been demonstrated in conditions such as Parkinson's disease (Brown et al., 2009), stroke (Kleim et al., 2003), and seizures (Henderson et al., 2011; Teskey et al., 2002; for review see Teskey et al., 2008).
1.5 Seizures, Epilepsy and Interictal Disturbances

A seizure is a transient occurrence of signs and or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2014). Seizures can include both positive and negative sensory or motor symptoms. Positive sensory symptoms can include a perceptual experience that occurs in the absence of the required external stimuli, known as auras. Auras may include the perception of flashing lights, a specific odour, déjà vu or a sense of fear. An example of a positive motor symptom would be the jerking of an arm. Impairments of brain function such as transient blindness, impairment of consciousness, or temporary paralysis are all negative sensory and motor symptoms of seizures.

Seizures are classified into two categories: focal and generalized. Focal seizures are defined by originating in a small group of neurons called a seizure focus. The initial manifestation of a focal seizure often occurs as an aura, with the nature of the aura depending on the area of the brain in which the focus resides. For instance, a visual aura would have a seizure focus within the occipital lobe. The excessive neuronal activity that occurs within the seizure focus can spread to neighbouring regions. A focal motor seizure for example, begins in the motor cortex and may progress to a tonic phase in which the individual falls to the ground and extends all limbs in a rigid manner. A tonic phase may in turn be followed by the convulsing of all extremities, termed the clonic phase. If a focal seizure becomes tonic-clonic, it has become secondarily generalized. Generalized seizures involve both hemispheres from the onset. They are further divided into non-convulsive and convulsive (tonic and/or clonic).
The International League Against Epilepsy (ILAE) defines epilepsy as “a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological, and social consequences of this condition” (Fisher et al., 2005). It is important to note that epilepsy requires a pathologic predisposition to generate epileptic seizures. A seizure provoked by a transient factor such as a photic stimulus in a healthy brain does not contribute towards epilepsy (Fisher et al., 2005). In 2005, the ILAE indicated that the operational definition of epilepsy requires that two unprovoked seizures occur at least 24 hours apart from one another (Fisher, 2005). Although two seizures are often needed to confirm a predisposition, conceptually, epilepsy is determined to exist following at least one unprovoked seizure if the patient has a lower threshold for subsequent seizures (Fisher et al., 2005). Following an individual’s first unprovoked seizure the general recurrence risk is 40-52 percent (Berg & Shinnar, 1991), compared to a lifetime risk of 10 percent for only a single unprovoked seizure (WHO, 2012). The general recurrence risk following two such seizures is 60 percent (Berg & Shinnar, 1991).

The emergence of epilepsy can be due to genetic factors or acquired pathologies that result in hyperexcitable and hypersynchronous neural activity and the likelihood of developing epilepsy depends on the pathology involved. A patient who experienced a single unprovoked seizure following an isolated brain injury such as a stroke, CNS infection, or trauma has the same risk for a second unprovoked seizure as a patient who has had two such seizures. In 2014, the ILAE updated their operational definition of epilepsy to accommodate the multifaceted and complex nature of developing the disease. In addition to their previous definition, they added
epilepsy is a disease of the brain if one unprovoked seizure occurs and the probability of further seizures is similar to the general recurrence risk following two unprovoked seizures (at least 60%), occurring over the next 10 years.

Patients with epilepsy can have disturbances in behavioural and cognitive functions between seizures (Swinkels et al., 2005). Interictal disturbances experienced by people with epilepsy can include anxiety and depression (Giovagnoli et al., 1997), poor verbal and spatial memory (Reminger et al., 2004), sexual dysfunction (Isojarvi et al., 2003), as well as motor disturbances (Hernandez et al., 2002). These behavioural disturbances are dependent on the function of the brain region at the seizure focus or the areas in which seizure activity spreads. Epileptic activity itself contributes to interictal impairments above and beyond the impairments that arise from the underlying pathology alone, and this can worsen over time (Berg et al., 2010). This process is termed epileptic encephalopathy and closely resembles the kindling animal model that will be described shortly.

1.6 Seizures and Motor Cortex

While Penfield noted that brief surface stimulation would often result in multiple movements (movement of more than one joint), he also observed movements that combined both sides of the body. It is important to note that Penfield’s subjects were a population of people with epilepsy. Remarkably, the homunculus has since been discovered to contain an error in the orientation of the face (Servos et al., 1999). Penfield’s face is depicted as being right side up when it is actually represented as upside down in the motor cortex (Teskey et al., 2008).
Many of the multiple movements and bilateral movements that Penfield saw may be the result of a reorganization of the motor cortex due to seizures. For instance, Cisek et al. (2003) have shown that in individuals with epilepsy bilateral activation of the motor cortex can occur during unilateral forelimb movements. Following seizures, this bilateral cortical activation can be potentiated (Stoeckel et al., 2002). Several studies have demonstrated atypically organized motor maps in people with frontal lobe epilepsy (FLE) (Uematsu et al., 1992; Lado et al., 2002; Branco et al., 2003; Chlebus et al., 2004; Labyt et al., 2007; Lee et al., 2009). In addition, individuals with FLE can exhibit deficits in skilled motor tasks (Lewis et al., 1992; Helmstaedter et al., 1996; Matsuoka et al., 2000; Hernandez, et al., 2002). Hernandez and colleagues discovered that children with FLE showed deficits on motor coordination tasks, while children with temporal lobe epilepsy (TLE) and generalized seizures did not. Patients with FLE are more likely to exhibit motor deficits than patients with TLE because the motor cortex is found in the frontal lobe and is therefore more likely to have seizure activity propagate to it. There is still much to learn about the relationship between seizures, motor maps, and behavior. This thesis will investigate the relationship between atypically organized motor maps and impairments on skilled motor tasks following multiple seizures affecting the neocortex using an animal model.

1.7 Bilateral Movement Representations Following Seizures

It has been established that during unilateral forelimb movements in humans, bilateral activation of the motor cortex can occur (Cisek et al., 2003). Following seizures, this bilateral cortical activation can be potentiated (Stoeckel et al., 2002). In an individual suffering from
medically intractable partial seizures, Stoeckel and colleagues (2002), using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), discovered that abnormal bilateral activation of the motor cortex occurred during unilateral finger movements.

Unilateral intracortical microstimulation of the naive rat motor cortex elicits contralateral forelimb movements; however, bilateral movements can appear at increased current intensities (Kartje-Tillotson et al., 1985; Liang, 1993; Brus-Ramer, 2009). A lesion of the corpus callosum results in a 32.7% increase in the ICMS current threshold required to evoke an ipsilateral forelimb movement, while the current required to elicit a contralateral movement remains the same (Brus-Ramer et al, 2009), suggesting that interhemispheric signalling through callosal fibres plays a role in bilateral movements. While an increase in ipsilateral cortical activation during motor movements following seizures has been demonstrated, the role that interhemispheric interactions play in motor movement is unclear.

1.8 Kindling Phenomena and Technique

Kindling is a process in which the epileptiform response is strengthened following repeated invariant electrical stimulation to a particular brain area (Goddard et al. 1969, Racine et al., 1991). In electrical kindling, a chronically implanted electrode provides a brief electrical stimulation to a brain site and initiates a seizure. Initially, the stimulation results in highly synchronous EEG activity that outlasts the initial stimulus but may not produce a behavioural seizure (Racine, 1972). This synchronous EEG activity following stimulation is referred to as an afterdischarge (AD; see figure 1.6). As kindling stimulation is repeated daily, seizure severity
increases and the AD duration (ADD) lengthens (Racine, 1972). In addition, the threshold required for an afterdischarge is reduced but this is due to the repeated application of current (Flynn and Teskey, 2007). The kindling phenomenon is defined by this increase in seizure severity and ADD (Teskey et al., 2005).
Figure 1.6. Electroencephalography (EEG) of seizure activity in the rat. The afterdischarge begins at the end of the stimulation artefact (SA) and terminates at the last hypersynchronous spike.
The kindling phenomenon varies depending on the brain structure that is being stimulated (Mohapel et al., 1996). For example, seizure severity progresses much slower via hippocampal kindling than callosal kindling. This is in part due to the functional connectivity between brain structures. Electrical kindling has a number of benefits compared to other methods of inducing seizures. For instance, there are no pharmacological side effects in electrical kindling, unlike in the use of convulsant drugs. Furthermore, the stimulating electrode mimics an epileptic seizure focus and avoids the occurrence of spontaneous seizures. The lack of spontaneous seizures allows researchers to have more control over their experimental design and eliminates the issue of spontaneous seizures interfering with behavioural testing. The limitation of this model, however, is that it does not mimic the spontaneous seizures seen in those with epilepsy.

Kindling was discovered serendipitously by Goddard and colleagues (1969) while examining the effects of conditioning on rats via amygdala electrical stimulation. Accidentally, high-intensity stimulation was repeatedly administered to the amygdala of multiple rats. One rat developed seizures following the stimulations and continued to have seizures following subsequent stimulation. Following Goddard’s discovery, Racine (1972) examined kindling and EEG activity and concluded that the stimulation must trigger an AD in order for the kindling phenomenon to occur. In addition, Racine confirmed that repeated stimulation of the amygdala and hippocampus lowers the threshold required for an AD, and this lower threshold lasts over six weeks without stimulation. It is the seizures themselves and not just the lower seizure threshold associated with kindling that result in the reorganization of motor maps and
behavioural impairment (Flynn et al., 2010). Seizures that originate in a distant brain region such as the hippocampus, can cause reorganization of motor representations if the afterdischarge propagates to the frontal neocortex (Van Rooyen et al., 2006). Both convulsant (pilocarpine) (Young et al., 2009) and electrical induced seizures can also reorganize motor representations.

Experimental manipulations in non-humans have provided essential insight into the ways in which motor representations change in response to recurrent seizure activity. Teskey and colleagues (2002) discovered that following repeated seizures in rats, the area devoted to forelimb movement was twice as large as in naive rats. Since this discovery, it has also been demonstrated that the area of non-forelimb movement representations such as whiskers, jaw, trunk, and tail increase as well (Henderson et al., 2011). In addition to an increase in ICMS movements following kindling, there is a vast increase in the number of dual movements (Henderson et al., 2011).

Previous research has demonstrated deficits in the ability of rats to learn a skilled reaching task following repeated seizures elicited from the corpus callosum (Henry et al., 2008; Flynn et al., 2010). Following callosal kindling, rats have lower reaching success as well as fewer reach attempts than sham-kindled controls throughout the acquisition of the single-pellet reaching task (Henry, 2008). Once skilled reaching is learned (reaching success plateaus), rats kindled prior to learning the task remain at a lower reaching success than sham-kindled rats. In addition to lower reaching success, rats show altered kinematics in their successful reaches following callosal kindling (Henry et al., 2008; Flynn et al., 2010). While seizures have been
shown to cause a deficit in skilled motor behaviour as well as changes in motor map
topography, there is still much to learn about this relationship.

1.9 Mechanism of Kindling

There have been three explanations of the potential mechanisms responsible for the
kindling phenomenon (Teskey et al., 2005): 1) kindling-induced potentiation (KIP), 2) A loss of
synaptic GABAergic inhibitory drive, and 3) the development of a kindling-induced burst
response. A balance of these three hypotheses provides a close approximation to the
underlying neural mechanism responsible for the kindling phenomenon.

1) Kindling-induced Potentiation (KIP).

KIP is defined as the increase in synaptic efficacy between excitatory neurons after
kindling (Teskey et al., 2005). Following kindling, the current threshold required to elicit a
movement is decreased, unmasking latent ICMS sites, resulting in an increase in the number of
responsive ICMS sites as well as an increase in the proportion of sites producing dual
movements (Henderson et al., 2011). A likely reason for this kindling induced change is synaptic
potentiation in neocortical layer V (Teskey et al., 2008). Kindling via the corpus callosum causes
an increase in the number of perforated synapses in the neocortex producing more highly
efficacious synapses (Henry et al., 2008). Simply strengthening polysynaptic efficacy via high
frequency stimulation (HFS) of layer V of the motor cortex results in an increase in ICMS
movement representations (Monfils et al., 2004). HFS produces long-term potentiation (LTP),
which is defined as the strengthening of synaptic efficacy and plays a critical role in the development of neural circuitry and learning (Chen and Tonegawa, 1997). Moreover, low-frequency stimulation (LFS) can induce long-term depression (LTD), the weakening of synaptic efficacy (Christie et al., 1994). LFS has been shown to decrease the number of detectable ICMS sites in both sham-kindled controls and kindled rats (Teskey et al., 2007; Ozen et al., 2008). Both KIP and LTP appear to be mediated by the Hebb Rule, which states “neurons that fire together, wire together” (Hebb, 1949). In other words, when a presynaptic neuron repeatedly excites a postsynaptic neuron, the synaptic strength between the two neurons increases. It is however, important to note a distinct difference between KIP and LTP. While KIP requires an AD following stimulation, LTP does not (Cain, 1989).

Following kindling, the minimum current threshold required to elicit an ICMS movement decreases after both kindling and motor experience (Henderson et al., 2011; Young et al., 2012), suggesting synaptic potentiation. Prior to kindling, a movement may require a current intensity above the stimulation parameters and is thus not seen. However, following kindling the threshold required for movements may decrease to a level that is within ICMS stimulation parameters causing the “expansion” in motor maps and increase in dual sites.

2) Disinhibition.

The kindling technique may also reduce GABA mediated inhibition. For example, following focal injections of GABAergic blockers, intracellular recordings of neocortical neurons portray an electrophysiological pattern similar to what is seen at the time of a seizure
(Timofeev and Steriade, 2004). Furthermore, in the hippocampus, AD thresholds are reduced and the frequency and extent of hippocampal AD is increased following a GABA blockade, thus increasing seizure susceptibility (Leung et al., 2005).

3) Bursting.

Neuronal bursting consists of a periotic group of action potentials with a large amplitude (20-30 mv) and prolonged (50-100 msec) membrane depolarization (Matsumoto and Ajmone-Marsan, 1964; Matsumoto, 1964). Bursting occurs interictally as well as during an AD in people with epilepsy and also occurs during the kindling process (Teskey et al., 2005). The propagation of bursting activity is prevented by hyperpolarization as well as via surrounding inhibitory neurons. However, excessive, repetitive discharges causes an increase in extracellular potassium, diminishing the hyperpolarizing effect of outward flowing potassium, allowing the depolarization of surrounding neurons and the propagation of epileptiform neuronal activity (Teskey et al., 2005).

1.10 Hyperpolarization Activated Cyclic Nucleotide-gated (HCN) Channels

HCN channels are ion channels found in the brain as well as the heart (DiFrancesco et al., 1993). These channels are structurally similar to K+ channels and are partially permeable to K+ as well as Na+ (Pape, 1996). HCN channels and their mixed cation current Ih regulate synaptic integration important in the control of chronic pain (Tibbs et al., 2013), spatial working memory (Nolan et al., 2004), as well as cerebellar motor learning (Nolan et al., 2003). HCN1
channels are most prevalent in the cortex and hippocampus, with their key feature being their ability to “leak” current. Specifically, HCN channels are open at a neuron’s resting potential resulting in a continuous leakage current, Ih, that can be modulated up or down by the neuron to modulate its excitability (Noam et al., 2007; see figure 1.7). In pyramidal neurons, HCN channels are primarily localized to dendrites, giving the neuron the ability to control the flow of excitatory input to the cell body (Lorinez et al., 2002).

HCN1 channels are highly expressed in layer V cortical pyramidal neurons in the motor cortex, suggesting that they may play a role in regulating cortical movement representations. In the Teskey lab, Boychuk and Colleagues (2014) discovered that following the cortical application of the Ih blocker ZD7288 in rats, dual and bilateral ICMS representations increase by over 800 percent. In addition, mice lacking HCN1 channels (HCN1 KOs) also exhibited an increase in dual and bilateral ICMS representations relative to wild-type controls. Furthermore, HCN1 KO mice demonstrated a reduction in skilled reaching accuracy and coordination. These results suggest that HCN channels play an important role in separating movement representations and that this separation in encoding is important for skilled motor movements. Altering the intensity of ICMS, such as with LD-ICMS can elicit separate types of movements at single ICMS sites (Teskey & Kolb, 2011) indicating that cellular networks are indeed overlapping and complexly organized in the motor cortex. HCN channels may provide a form of organization or gating within cellular networks that assists in generating motor behaviour.

The aforementioned increase in dual and bilateral ICMS movement representations in callosally kindled rats may in part be the result of a decrease in HCN channel function. Not only
is a reduction in cortical Ih found in animal models of epilepsy (Albertson et al., 2011), it is also upregulated by several anti-seizure drugs (ASDs; Postea and Biel,. 2011 ). HCN1 KOs also experience epileptogenesis at a rate six times faster than controls (Huang et al., 2009; Santro 2010). Using voltage clamp recordings, Boychuk and colleagues have discovered that pyramidal cells in callosal-kindled rats display reduced Ih (Boychuck et al., 2011). These researchers also demonstrated an increase in dual contralateral and bilateral movement representations in young, male Long-Evans rats following the application of ZD 7288 to the cortex prior to ICMS (Boychuck et al., 2011). The application of ZD 7288 does not however, have the same effect as kindling. For instance, the size of the motor map does not increase as motor maps do following kindling. This thesis seeks to determine to what extent interhemispheric interactions are involved in bilateral movements following the cortical application of ZD 7288 and how this may differ to the kindling model.

1.11 Cortical Deactivation

Many neurological studies rely on inactivation techniques to determine the function of specific brain regions. Many forms of cortical inactivation are permanent and can include techniques such as tissue removal, chemical inactivation, and electrolytic lesions (Lomber, 1999). While these techniques successfully remove the function of specific brain regions, they can also have several undesirable effects such as allowing for functional compensation to occur over time, compromising blood flow to other areas of the brain, and effecting fibres of passage (Lomber et al., 1999). Reversible inactivation such as cortical cooling allows one to acutely
inactivate an area of the brain without these undesirable effects. Once cooling ceases, the brain region is once again fully functional (Lomber et al., 1999).

1.12 Hypotheses and Objectives

1.12.1 Experiment one.

Repeated seizures result in both changes to the expression of neocortical motor maps and behavioural impairment, but the relationship between these two phenomena has not yet been determined. Furthermore, while the effect of kindling on the learning of a skilled forelimb task has been investigated, the effects of kindling on a skilled forelimb task learned prior to kindling has not been explored. This experiment examined the effect of the number of seizures on changes to neocortical forelimb motor representations and skilled reaching performance. I hypothesized that there would be a dose dependent increase in ICMS-derived forelimb movement representations following seizures, and of these representations, there will be an increase in bilateral and dual contralateral movements.

1.12.2 Experiment two.

I have previously demonstrated that following multiple seizures via callosal-kindling, a drastic increase in bilateral ICMS derived forelimb motor movements occurs (Rodych et al., 2011). Also following repeated seizures, bilateral cortical activation can occur during the stimulation of a single forelimb (Vuong et al., 2009). It is fascinating that the stimulation of the motor cortex in one hemisphere following seizures can result in the movement of both sides of
the body. This experiment further investigated the neurological underpinnings of bilateral movement representations and explored the role interhemispheric interactions via the corpus callosum may play in this atypical representation. I hypothesized that during SD-ICMS, the sensorimotor cortex contralateral to ICMS is responsible for ipsilateral forelimb movement representations. Therefore, inactivating the hemisphere contralateral to ICMS will rid all ipsilateral forelimb movements from the motor map.

The purpose of this study in its entirety is to investigate the neural network changes that arise in the motor cortex following multiple seizures and their underlying mechanisms.
CHAPTER 2: EMPIRICAL PAPER

Skilled Behaviour and Bilateral Motor Map Expression. An Intracortical Microstimulation and Reversible Inactivation Study.

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Justin Rodych designed and constructed all cryoloops, kindled all rats, performed dual-hemisphere craniotomies, map/cooling remap anesthetics and ICMS procedure for ZD 7288 rats and twice a day kindled rats, data analyses, statistical analyses, creation of motor maps, and contributed to the experimental design for experiment two. He also performed the qualitative skilled-reaching analysis and statistical analyses for experiment one and wrote the manuscript.

Ryan McCarthy and Justin Rodych performed head cap surgeries, reach trained rats, video recorded rats, kindled rats, performed single-hemisphere craniotomies, anesthetics and ICMS procedures, and data analyses for experiment one.

Ryan McCarthy contributed to the experimental design for experiment one.

Jeffrey Grab contributed to reach training rats and calling ICMS.

Cam Teskey conceived and funded the project and edited the manuscript.
2.1 Introduction

Individuals with epilepsy often have interictal disturbances in behavioural and cognitive functions (Swinkels et al., 2005). Moreover, recurrent seizures contribute to these interictal impairments above and beyond the impairments that arise from the underlying pathology alone (Berg et al., 2010). In patients with uncontrolled seizures, the prevalence of depression ranges from 30 to 55 percent (Gilliam et al., 2004) and after controlling for several confounding variables including the location of the seizure focus, depression still presents itself as a serious issue in epilepsy (Hermann et al., 1996); the exacerbating factors of interictal disturbances likely play a role in this depression. It is important to keep in mind that while epilepsy is a condition defined by epileptic seizures and cognitive symptoms, it is also defined by the enduring psychological and social consequences that arise (Fisher et al., 2005).

Individuals with FLE often exhibit interictal deficits in skilled motor tasks (Lewis et al., 1992; Helmstaedter et al., 1996; Matsuoka et al., 2000; Hernandez, et al., 2002) and several studies have also demonstrated atypically organized motor maps in people with FLE (Uematsu et al., 1992; Lado et al., 2002; Branco et al., 2003; Chlebus et al., 2004; Labyt et al., 2007; Lee et al., 2009). Using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), patients with epilepsy have displayed increased bilateral activity within the motor cortex (Stoeckel et al., 2002).

Electrical kindling provides the opportunity to examine the progression of a model of epileptogenesis and its effect on the neocortex and behaviour. Kindling lowers seizure thresholds, however it is the seizures themselves that result in atypical motor maps and
behavioural impairment (Flynn et al., 2010). SD-ICMS allows a detailed insight as to how kindling alters motor maps as a whole as well as how individual movement representations are affected. Following kindling, motor maps derived using SD-ICMS increase in the number of responsive stimulation sites (Teskey et al., 2002; van Rooyen et al., 2006; Ozen et al., 2008; Young et al., 2009) and the number of sites that generate bilateral and ipsilateral movement when stimulated (Henderson et al., 2011).

The first experiment in this thesis explored the relationship between seizures affecting the motor cortex, behavioural impairment, and atypically organized forelimb motor representations. In order to determine whether seizures affect the neocortex and skilled forelimb use in a dose dependant manner, rats were trained on a skilled reaching task and then subjected to one seizure a day for either 5, 10, 20, or 30 days. Subsequent to kindling rats were re-evaluated on the skilled forelimb task and then had their motor map derived using SD-ICMS. This is the first time that someone has determined to what extent the neocortex and behaviour are altered at varying stages of epileptogenesis.

Experiment two will focus on bilateral and ipsilateral movement representations and explore the role interhemispheric interactions via the corpus callosum may play in these representations that proliferate following kindling or the application of an Ih channel blocker. Kindling, as well as a reduction of Ih current result in similar neocortical and behavioural characteristics. In experiment two, rats will either be kindled or have ZD 7288 applied to the neocortex. Following these procedures, SD-ICMS will be used to derive a motor map. Once an
initial motor map is complete, the hemisphere contralateral to ICMS will be inactivated via cooling while second motor map is derived.

2.2 Methods and Procedures

2.2.1 Experiment One.

2.2.1.1 Subjects.

42 Adult male Wistar rats weighing between 250-400 g, were obtained from Charles River (Charles River Laboratory International, St-Constant, PQ, Canada). Rats were housed in groups of two or individually, in clear plastic cages under a 12-h light/dark cycle. All experiments were conducted during the light cycle. Twelve hours prior to surgery rats were food deprived and had water available ad libitum. All handling and housing were in accordance with the Canadian Council Animal Care guidelines.

2.2.1.2 Groups.

Rats were trained on the single-pellet reaching task until proficient. The same rats were then divided into kindle and sham-kindle groups of 5, 10, 20 or 30 stimulation sessions. Reaching kinematics were analyzed prior to kindling as well as following the last kindling session. Lastly, short-duration ICMS was performed to generate a motor map.

2.2.1.3 Handedness.

Before electrode implantation, the handedness of the subjects was determined. Food-
deprived rats were exposed to banana-flavoured dustless pellet F0059 (Bio-Serv, Frenchtown, NJ, USA) for 24 hours prior to handedness determination. Subjects were placed in the reaching apparatus, with a pellet on either side, until several reasonable reach attempts and at least one successful attempt was observed. Once rats learned to consistently reach with one forepaw (within 1 to 5 days), they were caged individually and allowed food ad libitum until electrode implantation.

2.2.1.4 Electrode Implantation.

Rats were chronically implanted with a stimulating electrode. Twisted wire bipolar electrodes were constructed from Teflon-coated, stainless steel wire, 178 μm in diameter (A-M Systems, Everett, WA, USA). The ends of the wire were stripped of Teflon coating and connected to gold-plated male amphenol pins. The poles of the electrodes were separated by 0.5 mm. Rats were anaesthetized with isoflurane (5% induction, 2% maintenance) and mounted into a stereotaxic frame. An injection of lidocaine (2%) was administered subcutaneously at the incision site. The electrode was then chronically implanted in the corpus callosum (1.0 mm anterior to bregma, 0.5 mm lateral to midline, and 3.5-4.5 mm ventral from brain surface) according to the stereotaxic coordinates of Swanson (1992). The electrode was then cemented to one anchoring screw and one ground screw with dental acrylic. The rats recovered for a minimum of 7 days before continuing experimentation.
2.2.1.5 Skilled Reach Training.

Rats were food-deprived to 85-90% free-feeding levels and maintained at that level for the duration of the experiment. Rats spent 10 consecutive minutes in the reach-training chamber once a day until proficient. Rats were trained to reach with their preferred forelimb (Peterson and Devine, 1963; Whishaw, 1992). A single banana-flavoured dustless pellet F0059 (Bio-Serv, Frenchtown, NJ, USA) was placed on a shelf, 5.5 cm high and 1.5 cm from the inside of outside front wall of the Plexiglas reaching chamber. The rat was given a single chance to reach the pellet through a 1.3 cm vertical in the reaching chamber. The reach is considered a valid attempt if the rat extends its forelimb to the pellet and displaces it in any direction. A successful reach was defined as a single attempt in which the rat grasped the pellet and placed it in its mouth. If the rat failed after a single attempt, the pellet was removed from the shelf. For the first five days of training, regardless of success, the rat was given a pellet at the other end of the chamber to ensure that the rat moves away from the slot, thus resetting its reaching position for the next attempt. This approach discouraged multiple frantic reach attempts and reduced the number of reaches from an inappropriate initial location and posture (Whishaw, 2000). After the rat had moved to the back of the apparatus, the pellet was replaced. As training progressed (> day 5) rats were rewarded at the back of the chamber only for successful reaches, but were still required to run to the back of the cage between attempts. Reaching performance was assessed daily by calculating the percentage of success (number of successful reaches / total number of attempts x 100). Subjects were considered graduated from reach training if they have completed a minimum of 10 days of training and achieve a mean of 30%
success and a minimum of 20 reaches per day in their last three days of reach training. Rats that
fail to meet these criteria continued reach training until proficient. The final day of training was
video-recorded for within-subject comparison post-kindling or post-sham-kindling.

2.2.1.6 Electrical Kindling.

On day one of kindling, the afterdischarge threshold (ADT) was determined. The ADT
was defined as the weakest current required to elicit an afterdischarge of ≥ 4 s. Current was
delivered through the callosal electrode and recordings were made from the same electrode.
Stimulation consisted of a 1-s train of 60-Hz biphasic rectangular pulses, 1-ms in duration and
separated by 1-ms. The current intensity for ADT began at 100 μA and was increased by 50 μA
increments in 60-s intervals until an ADT was determined. Kindling stimulation was delivered at
an intensity 100 μA above that required to elicit an ADT. Kindling stimulation that elicited an
afterdischarge was delivered once daily. Electroencephalography (EEG) was recorded from the
stimulating electrode in the corpus callosum to determine the afterdischarge duration. Seizure
stages was scored based on a five stage behavioural scale (Racine, 1972): (0) freezing
accompanied by electrographic seizure; (1) motor arrest accompanied by facial automatisms
such as vibrissae twitching; (2) head nodding and chewing; (3) unilateral forelimb clonus; (4)
rearing on hindlimbs and bilateral forelimb clonus; (5) rearing, clonus of all four limbs, and
falling.
2.2.1.7 Kinematic Assessment.

One day after the final session of kindling or sham-kindling rats were placed in the reaching apparatus and video-recorded for reach assessment. Percent success was determined over a period of 10 minutes. Rats that failed to make 20 attempts were evaluated again the next day. No rats required more than two days of kinematic assessment. A reach is composed of 10 components (Whishaw et al., 2003):

(i) Digits to the midline. Using mainly the upper forelimb, the reaching limb is lifted from the floor so that the tips of the digits are aligned with the midline of the body. (ii) Digits flexed. As the limb is lifted, the digits are flexed, the hand is supinated and the wrist is partially flexed.

(iii) Elbow to midline. Using an upper arm movement, the elbow is adducted to the midline while the tips of the digits retain their alignment with the midline of the body. (iv) Advance. The limb is advanced directly through the slot toward the food target. (v) Digits extend. During the advance, the digits extend so that the digit tips are pointing toward the target. (vi) Arpeggio. When the hand is over the target, the hand pronates from digit 5 (the outer digit) through to digit 2 while the hand simultaneously opens. (vii) Grasp. The digits close and flex over the food, with the hand remaining in place, and the wrist is slightly extended to lift the food. (viii) Supination I. As the limb is withdrawn, the hand supinates by almost 90°. (ix) Supination II. Once the hand is withdrawn from the slot to the mouth, the hand further supinates by about 45° to place the food in the mouth. (x) Release. The hand contacts the mouth and opens to release the food.

A normal (free of error) reach component was given a score of 0. When it was
ambiguous whether or not an error occurred it was given a score of 0.5. When the component was impaired but recognizable it was given a score of 1. When the component was absent or completely unrecognizable it was given a score of 2. Five successful reaches by each rat were scored to determine the rat’s average ordinal error score for each component of reaching.

2.2.1.8 Short Duration Intracortical Microstimulation (SD-ICMS).

A detailed map of the forelimb motor neocortex was generated using SD-ICMS according to the methodology of Young et al. (2011). Rats were food deprived 24 hours prior to surgery and given water *ad libitum*. Before surgery rats were injected with ketamine (100 mg/kg). and xylazine (5 mg/kg). A supplement of ketamine (25 mg/kg), or a cocktail of ketamine (17 mg/kg) and xylazine (2 mg/kg) was injected as required throughout surgery to maintain a constant level of anesthesia. Anaesthetic levels were monitored by assessment of breathing rate, whisker movements, and withdrawal reflex from a light pinch to the foot. An 8 x 5-mm craniotomy window was made in both hemispheres to expose the underlying sensorimotor cortex. The windows extended 4 mm anterior of bregma, 3 mm posterior, and 5 mm lateral of midline. The cisterna magna was then punctured with an 18-gauge needle to reduce pressure from edema and the dura matter was carefully removed. Silicone liquid (Factor II, Inc., Lakeside, AZ, USA) warmed to body temperature (37-38 °C) was placed on the exposed cortex. Using a digital camera (Canon Canada Inc., Mississauga, ON, Canada) and Stemi 2000-C stereomicroscope (Carl Zeiss, Thornwood, NY, USA) a photograph magnified 32x was taken and displayed on a personal computer. A grid composed of 500 μm squares was then overlaid on
the image. The intersections of these gridlines and centre points of each square depict points of penetration for the ICMS microelectrode, except when located over a blood vessel, allowing for no less than 353 μm between penetration points. From the surface of the brain, the electrode was lowered to a depth of , which corresponds to layer V of the cortex. Stimulation consisted of 13 monophasic 200 μs cathodal pulses at 250 Hz (Young et al., 2011).

Rodents were maintained in the prone position, with their forelimb supported by a finger, elevating the limb for a closer inspection of possible movements. A threshold was determined at each penetration site. This was done by quickly increasing the current from 0 μA towards 60 μA until a movement was noted. The current was then decreased until the movement ceased. The movement threshold is defined as the minimal ICMS current able to elicit a movement. If the maximal current of 60 μA was unable to elicit a movement, then the penetration site was considered non-responsive.

2.2.2 Experiment Two.

2.2.2.1 Subjects.

16 male Long Evans rats aged P38-P43 and adult male Wistar rats weighing 250–400g were obtained from Charles River (Charles River Laboratory International, St-Constant, PQ, Canada). Rats were housed in groups of two or individually, in clear plastic cages under a 12-h light/dark cycle. All experiments were conducted during the light cycle. Twelve hours prior to surgery rats were food deprived and had water available ad libitum. All handling and housing were in accordance with the Canadian Council Animal Care guidelines.
2.2.2.2 Groups.

Experiment two consisted of two groups. In group 1, adult male Wistar rats underwent the same electrode implantation as rats in experiment one. Rats were then either kindled or sham-kindled twice a day for 10 days. Following kindling the final kindling session, ICMS was performed to obtain a motor map. A remap was then created while the hemisphere contralateral to ICMS was inactivated via cooling (see figure 2.1). Group one rats (P38-P43) were mapped following the cortical application of ZD7288 or aCSF. After the first map, a remap was done while the hemisphere contralateral to ICMS was inactivated via cortical cooling.

2.2.2.3. Electrical Kindling.

The determination of ADTs, seizure severity, and stimulation parameters follow the kindling methods outlined in the skilled behaviour, seizures and SD-ICMS experiment, however kindling stimulation eliciting an afterdischarge was delivered twice daily for 10 days with at least 4 hours separating each stimulation session.

2.2.2.4. Application of ZD 7288.

In the ZD 7288 condition, before beginning ICMS, 50 μL of 30 μM ZD 7288 (Tocris Bioscience Ellisville, Missouri, USA) dissolved in sterile saline was applied to the exposed cortex with a pipette. The solution was allowed to diffuse into the cortex for a duration of 10 minutes. ICMS was then used to derive a motor map. Once all forelimb, non-forelimb responsive points
and non-responsive points were determined using ICMS, all ICMS points were then revisited while the hemisphere contralateral to ICMS was inactivated via cortical cooling.

2.2.2.5. Short Duration Intracortical Microstimulation (SD-ICMS) Map/remap.

The first map of the forelimb motor neocortex was generated using the SD-ICMS methodology outlined in the skilled behaviour, seizures and SD-ICMS experiment however a double craniotomy (craniotomy over each hemisphere) instead of a single craniotomy was performed, leaving midline over venous sinus intact. A 6 x 4 mm cryoloop was placed on the neocortical surface of the hemisphere opposite of the hemisphere ICMS was to be performed on (see figure 2.2). A microthermocouple was attached to the union of the cryoloop with silver solder following the application of flux to the tubing. Dental acrylic was used to coat the silver solder and attach the microthermocouple to the cryoloop. Once all forelimb, non-forelimb responsive points and non-responsive points were determined using SD-ICMS, all ICMS points were revisited while the opposite hemisphere was inactivated using cortical cooling. Chilled methanol was pumped through hypodermic tubing and through the cryoloop during cooling. Room temperature methanol was drawn from a synchronous rotating and reciprocating piston pump (Fluid Metering Inc., Oyster Bay, NY; with low flow isolation adapter) through 3.0 mm O.D. x 1.5 mm I.D. Teflon tubing (Varian Associates, Walnut Creek, CA). The methanol exited through 2 m of 1.6 mm O.D. x 0.5 mm I.D. Teflon tubing (Varian Associates, Walnut Creek, CA; Part # AL35668) was coiled and immersed in a bath of methanol and dry-ice, cooling the methanol within the tubing to -75 degrees Celsius. The chilled methanol was then pumped
through the cryoloop and back into the methanol reservoir. Cryoloop temperature was monitored using a digital thermometer (Omega Engineering, Stamford, CT; Model #HH25TC) connected to the microthermocouple and the flow rate adjusted to sustain a temperature of 4 degrees Celsius. Cooling to a surface temperature of 4 degrees is optimal as it lowers the temperature of the neocortex to between 15 and 20 degrees, a temperature necessary to inactivate the cortex (Lombar et al., 1999; see figure 2.3).
Figure 2.2. Cryoloop used in experiment two. A cryoloop was used to inactivate the motor cortex contralateral to SD-ICMS during the SD-ICMS remap.
Figure 2.3. Cryoloop distance–temperature profile. A temperature probe was lowered to layer V of the sensorimotor cortex at locations A-F surrounding the cryoloop. Cooling was initiated at zero minutes and was terminated at six minutes. Throughout cooling the cryoloop was regularly adjusted to maintain a loop temperature of -4 °C +/- 1 °C. Temperature was recorded every 20 seconds for a duration of 12 minutes. The dotted line represents the 20-degree threshold required for cortical inactivation.
2.2.3 Statistical Analysis

Linear regression was used to examine the number of reach attempts made over the training period. Multiple and single regressions were used to determine coefficients of determination ($R^2$) for within-subject analysis. Differences in pre and post-kindled reaching performance were analyzed using paired two-tailed t-tests for each group. The Kruskal-Wallis test, a non-parametric test, was used to analyze reaching kinematics that was scored on an ordinal scale. To determine differences in motor maps between kindled groups and the sham-kindled group, two-way ANOVAs with Bonferroni post hoc comparisons were used. Pearson bivariate correlations were used to determine correlation coefficients ($r$) for within-subject analysis. All map/re-map comparisons were analysed using two-tailed paired t-tests. All statistical analyses were conducted using GraphPad Prism 6.

2.3 Results

2.3.1 Experiment 1.

Pre-Kindle Reach Training

Throughout training sessions 1-9 the number of successful reaches increases and levels off at session 10 (final session of training), representing the transition from increase in reaching proficiency to maintenance of skill proficiency (Monfils and Teskey, 2004; see figure 2.4A). Percent of successful reaches was determined by dividing the number of successful reach attempts by the sum of successful and unsuccessful attempts for each session. The number of
reach attempts made over the ten training sessions was examined by linear regression and increased steadily throughout the course of training (see figure 2.4B, F(1,8)=2352.00, p<0.0001).
Figure 2.4. Performance on the single-pellet reaching task. (A) Mean number of reach attempts and (B) mean percent of successful reaches over the course of 10 training sessions. The number of reach attempts increased significantly over ten days of training ($F(1,8)=2352.00, p<0.0001$). Error bars represent the standard error of the mean.
Kindling Progression is Related To Seizure Stage and Afterdischarge Duration

The progression of seizure stage and afterdischarge duration for 5, 10, 20 and 30 session kindling groups. The first session of kindling consisted of stimulation at an intensity that elicited an ADD ≥ 4 seconds. Following the first session, kindling stimulation was delivered at an intensity 100 μA higher than the first session for all remaining sessions. As kindling progressed behavioural seizures became more intense, increasing in seizure stage and length of ADD (see figure 2.5). The number of kindling sessions accounts for a large proportion of the variance in seizure stage ($R^2=0.6324$) and a smaller proportion of variance in ADD ($R^2=0.2709$; see figure 2.6).
Figure 2.5. Progression of seizure activity in 5, 10, 20 and 30 session kindling groups. (A) Mean afterdischarge duration (ADD) and (B) Mean percent of successful reaches. The duration of seizures (ADD) as well as seizure stage (severity) increased as kindling sessions progressed.
Figure 2.6. The kindling phenomenon across 5, 10, 20 and 30 day kindling groups. (A) Mean ADD increase in duration and (B) mean seizure stage increases during the kindling process. Each point on the graph represents a specific kindling session and the average ADD (A) or seizure stage (B) between all kindled groups (5, 10, 20, and 30 session groups). The red line indicates the line of best fit.
Skilled Reaching Performance Following Kindling

The mean change in percent success was determined by calculating the difference between pre-kindled percent success (the average of the last three training sessions) and post-kindled percent success. In the last three training sessions, the sham-kindled group achieved an average of 51.22 ± 2.77 % success prior to sham-kindling and 49.38 ± 4.41 % success following sham-kindling ($t(17) = 0.595, p = 0.595$). The 5-day kindled group achieved 52.06 ± 5.49 % success prior to kindling and 57.55 ± 6.46 % success post kindling ($t(5) = 0.991, p = 0.367$). The 10-day kindled group significantly decreased from a pre-kindled 52.89 ± 4.21 % success to 40.37 ± 5.88 % post-kindled ($t(6) = 0.2.977, p = 0.0247$). The 20-day kindled group experienced the largest decrease in performance, decreasing from 58.43 ± 5.76 % success to 39.27 ± 4.65 % success ($t(7) = 3.291, p = 0.0133$). The 30-day kindled group achieved an average of 42.35 ± 2.67 % success prior to sham-kindling and 36.85 ± 5.80 % success following sham-kindling ($t(3) = 1.064, p = 0.3655$). The 10-day and 20-day kindled groups had a significant decrease in reaching performance (see figure 2.7A) and an analysis of variance (ANOVA) between each kindled group and the sham-kindled group indicated that only the 20-day kindled group was found to have a significant change in percent success compared to the sham-kindled group ($t(38) = 2.911, p = 0.0240$; see figure 2.7B).
Figure 2.7. Performance on the single-pellet reaching task. (A) The mean percent of successful reaches averaged over the last three reach-training sessions compared to post-kindling mean percent success. (B) The mean change in percent success represents the difference between pre-kindle percent success (the average of the last three training sessions) and post-kindle percent success. Error bars represent the standard error of the mean (*p<0.05).
**Kinematics**

On the skilled-reaching task, only the group kindled for 30 sessions had significantly higher error scores than the sham-kindled group on some of the reaching subcomponents (see figure 2.8). Specifically, in rats kindled for 30 sessions, mean reach component errors on components i (Digits to Midline; 0.350 +/- 0.149), iii (Elbow to Midline; 0.325 +/- 0.083), and vi (Arpeggio; 0.250 +/- 0.067) were significantly higher than sham-kindled rats who did not exhibit any errors in the corresponding components (H = 17.58, p = 0.0015; H = 9.829, p = 0.0434; H = 28.64, p < 0.0001, respectively). In addition to analyzing reaching subcomponent errors, total error scores were also analyzed. Rats kindled for 20 and 30 days had a significantly higher total error score (0.871 +/- 0.189 and 1.250 +/- 0.166, respectively) than sham-kindled rats (0.09 +/- 0.048; H = 19.56, p = 0.0006). On components i (Digits to Midline), ii (Digits Semi-Flexed), iii (Elbow to midline), vi (Arpeggio) and x (release) only kindled rats displayed errors.
Figure 2.8. Mean ordinal error scores on the single-pellet reaching task. Rats were scored on five successful reaches (see 2.2.17 Kinematic Assessment; Wishaw et al., 2003). Total error represents the mean sum of error scores on all reaching subcomponents. Rats kindled for 30 days had significantly higher error scores than sham-kindled rats on certain subcomponents. Error bars represent the standard error of the mean († p<0.05, ‡ p<0.01, § p<0.001).
SD-ICMS Multiple Movements Following Kindling

The vast majority of SD-ICMS derived forelimb movements in sham-kindled rats consist of single contralateral movements (92 +/- 1.86%). Throughout kindling, the proportion of single contralateral movements decreases as the proportion of bilateral movements increase (see figure 2.9A). The percentage of multiple forelimb movements and the total area of multiple forelimb movements were both analyzed in a two-way ANOVA. Results indicated a significant difference in multiple forelimb movements between kindled groups and the sham kindled group in multiple forelimb movements for both the percentage of movements (F(3, 144)=222.7, p<0.0001) and the total area of movements (F(3, 144)=72.13, p<0.0001). Following five sessions of kindling, the percentage of bilateral forelimb area significantly increases in rats kindled for 10, 20 and 30 sessions (39 +/- 4.77%, p<0.0001; 71 +/- 5.79%, p < 0.0001; and 51 +/- 4.40%, p<0.0001 respectively) while the percentage of single contralateral movements significantly decrease (51 +/- 6.26%, p<0.0001; 19 +/- 7.09%, p<0.0001; and 40 +/- 4.26%, p<0.0001 respectively) compared to sham-kindled rats. Total bilateral forelimb area was significantly higher than sham-kindled rats in 10, 20, and 30 session kindled rats (5.80 +/- 0.67 mm², p<0.0001; 13.55 +/- 1.90 mm², p<0.0001; and 7.77 +/- 0.79 mm², p<0.0001 respectively), while total single contralateral forelimb area was only significantly less in rats kindled for 20 sessions (3.75 +/- 0.98 mm², p<0.05).
Figure 2.9A. Representative motor maps from (A) Sham-kindled, (B) 5-day kindled, (C) 10-day kindled, (D) 20-day kindled and (E) 30-day kindled Wistar rats. Rats were selected that had SD-ICMS movements that were representative of the group mean. Motor maps are bordered by non-forelimb or non-responsive SD-ICMS sites. Blank areas represent a site made inaccessible by skull or blood vessels.
Figure 2.9. The effect of kindling on multiple ICMS-derived movements represented as (A) the proportion of ICMS forelimb sites and (B) the number of ICMS forelimb sites. Some histobars may appear higher than 100 percent, as dual-bilateral sites were calculated both as dual and bilateral sites. Error bars represent the standard error of the mean (* p<0.05).
The Relationship Between Kindling and Bilateral Motor Map Expression

The sum of bilateral ADDs predicted expression of bilateral ICMS derived movements ($R^2 = 0.718$, $P < 0.001$; see figure 1.6A). The number of bilateral seizures experienced also predicted expression of bilateral ICMS sites ($R^2 = 0.718$, $P < 0.001$).
Figure 2.10. Percent of forelimb map area that is represented as bilateral movements predicted by (A) the sum of bilateral seizure ADDs and (B) Total number of bilateral seizures experienced. Bilateral seizures were defined as stage 4 (rearing on hindlimbs and bilateral forelimb clonus) or stage 5 (rearing, clonus of all four limbs, and falling) seizures.
**The Relationship Between Skilled Reaching Errors, Kindling, and Motor Map Expression**

Mean error scores by each rat on each of the 10 kinematic reach components was compared against kindling data (see figure 2.11), as well as SD-ICMS data (see figure 2.12) using the Pearson bivariate correlation test.

Total number of seizures, total number of bilateral seizures and the total bilateral ADD all significantly correlated with errors on components i (digits to midline; \( r=0.566, p<0.001; r=0.560, p<0.001; r=0.503, p<0.01 \), respectively) and vi (Arpeggio; \( r=0.647, p<0.001; r=0.631, p<0.001; r=0.410, p<0.05 \), respectively). Total number of seizures and total number of bilateral seizures also significantly correlated with errors on component iii (digits to midline; \( r=0.429, p<0.05; r=0.415, p<0.05 \), respectively). The total bilateral ADD significantly correlated with component ix (supination II; \( r=0.430, p<0.05 \)).

The total number of bilateral SD-ICMS sites significantly correlated with errors on reaching components ii (digits semi-flexed; \( r=0.450, p<0.05 \)) and ix (supination II; \( r=0.411, p<0.05 \)). The total number of shoulder SD-ICMS sites significantly correlated with errors on reaching component iii (elbow to midline; \( r=0.475, p<0.01 \)). Component iii relies on the use of the upper arm, including the shoulder. The total number of wrist SD-ICMS sites significantly correlated with errors on reaching components vii (grasp; \( r=0.361, p<0.05 \)) and ix (supination II; \( r=0.376, p<0.05 \)). Component vii requires the use of the digits and wrist and component ix relies solely on rotary movement of the wrist.
Figure 2.11. The relationship between kindling and errors on the skilled-reaching task. Total seizures, total bilateral seizures and the sum of bilateral ADDs were correlated with error scores on the kinematic assessment of the ten reaching sub-components of skilled reaching (†p<0.05, ‡p<0.01, ‡‡p<0.001)
Figure 2.12. The relationship between SD-ICMS movements and errors on the skilled-reaching task. Forelimb movements were correlated with error scores on the kinematic assessment of the ten reaching sub-components of skilled reaching (†p<0.05, ‡p<0.01)
2.3.2 Experiment 2.

Inactivating the sensorimotor cortex contralateral to SD-ICMS by cooling it to 10-20 degrees Celsius in kindled rats blocks the kindle-induced increase in ipsilateral movement previously evoked by SD-ICMS.

In order to determine the extent of involvement the neocortex contralateral to ICMS has in kindle-induced movements involving the ipsilateral forelimb, three sham-kindled and four kindled adult male Wistar rats underwent a standard ICMS procedure producing a motor map, followed by a re-map while the sensorimotor cortex contralateral to ICMS was inactivated. The vast majority of sham-kindled SD-ICMS forelimb sites in the initial map were single movements on the contralateral side in which SD-ICMS took place (88.60 +/- 8.70 %); cooling the hemisphere contralateral to ICMS did not significantly change the mean (+/- SEM) proportion of single contralateral forelimb movements (92.21 +/- 4.85 %; paired two-tailed t-test, t=1.816, df=2, p=0.2111). In the initial map, only one sham-kindled rat displayed an ipsilateral (0.88 +/- 1.23 %) and bilateral (0.88 +/- 1.23 %) site within the group. These sites were eliminated in the re-map (paired two-tailed t-test, t=1.000, df=2, p=0.4226). The proportion of sham-kindled forelimb SD-ICMS sites that were originally dual (9.65 +/- 6.43 %) did not significantly change in the re-map (7.79 +/- 4.85 %; paired two-tailed t-test, t=1.890, df=2, p=0.1994).

Inactivating the sensorimotor hemisphere contralateral to ICMS in kindled rats significantly and completely eliminated the percentage of bilateral (55.60 +/- 6.32 to 0.00 +/- 0.00; paired two-tailed t-test, t= 8.609, df=3, p=0.0033) and ipsilateral (19.40 +/- 2.38 to 0.00
+/- 0.00; paired two-tailed t-test, \( t=7.723, \text{df}=3, p=0.0045 \) forelimb movements that were present during the original map. The percent of single contralateral forelimb sites in the original map (20.90 +/- 7.98) significantly increased during the re-map (88.33 +/- 2.32; paired two-tailed t-test, \( t=7.122, \text{df}=3, p=0.0057 \)), as many bilateral movements in the original map turned into single contralateral movements during the re-map. The percentage of kindled forelimb SD-ICMS sites that were originally dual (10.82 +/- 3.88 %) did not significantly change in the re-map (11.67 +/- 2.32 %; paired two-tailed t-test, \( t=0.7340, \text{df}=3, p=0.5161 \)).
Figure 2.13. Representative motor map and re-map while the hemisphere contralateral to ICMS was cooled in kindled rats. A representative rat with SD-ICMS movements that were representative of the group mean was selected. Motor maps are bordered by non-forelimb or non-responsive SD-ICMS sites. Blank areas represent a site made inaccessible by skull or blood vessels.
Figure 2.14. The mean percent of forelimb movements represented as dual, ipsilateral, bilateral or single contralateral evoked using SD-ICMS in a standard motor map followed by a re-map while the hemisphere contralateral to ICMS was cooled in (A) Sham Kindled and (B) Kindled rats. Map/re-map forelimb movements of the kindled rat are also represented as area (C).

**p<.01
Inactivating the sensorimotor cortex contralateral to SD-ICMS by cooling it to within 10-20 degrees Celsius following the cortical application of ZD7288 blocks ipsilateral movement previously evoked by SD-ICMS.

In order to determine the extent of involvement the neocortex contralateral to ICMS has in ipsilateral and bilateral movements that arise when HCN channel activity is reduced, rats had either aCSF or Ih current blocker ZD7288 applied to the surface of the neocortex. Both groups then underwent a standard ICMS procedure producing a motor map, followed by a re-map while the sensorimotor cortex contralateral to ICMS was inactivated. The vast majority of aCSF SD-ICMS forelimb sites in the initial map were single movements on the contralateral side in which SD-ICMS took place (88.00 +/- 5.56%); cooling the hemisphere contralateral to ICMS resulted in single contralateral forelimb movements being the only type of forelimb movements (100.00 +/- 0.00%; paired two-tailed t-test, t=2.000, df=2, p=0.1835). No aCSF rats displayed ipsilateral or bilateral sites within the original map or re-map. The proportion of aCSF rat forelimb sites that were originally dual (12.00 +/- 5.56%) disappeared in the re-map (0.00 +/- 0.00%; paired two-tailed t-test, t=2.00, df=2, p=0.1835).

Inactivating the sensorimotor cortex contralateral to ICMS in rats with ZD7288 applied to the surface of the neocortex eliminated the percentage of bilateral forelimb movements that were present during the original map (7.34 +/- 5.06%), although this did not reach statistical significance (paired two-tailed t-test, t=1.715, df=3, p=0.1849). The percent of single contralateral forelimb sites in the original map (62.39 +/- 4.21%) significantly increased during the re-map (82.95 +/- 8.28%; paired two-tailed t-test, t=9.937, df=3, p=0.0022). The percentage
of ZD7288 forelimb sites that were originally dual (30.28 +/- 8.48%) did not significantly change in the re-map (18.18 +/- 7.57%; paired two-tailed t-test, t=1.709, df=3, p=0.1860).
Figure 2.15. Representative motor map and re-map while the hemisphere contralateral to ICMS was cooled in rats with cortically applied ZD 7288. A representative rat with SD-ICMS movements that were representative of the group mean was selected. Motor maps are bordered by non-forelimb or non-responsive SD-ICMS sites. Blank areas represent a site made inaccessible by skull or blood vessels.
Figure 2.16. The mean percent of forelimb movements represented as dual, ipsilateral, bilateral or single contralateral evoked using SD-ICMS in a standard motor map followed by a re-map while the hemisphere contralateral to ICMS was cooled in rats with cortically applied (A) aCSF and (B) ZD7288. Map/re-map forelimb movements of the rats with cortically applied ZD7288 are also represented as area (C). **p<.01
Cooling the sensorimotor cortex contralateral to SD-ICMS to within 10-20 degrees Celsius raises the average threshold for SD-ICMS sites in kindled rats as well as rats with cortically applied ZD7288.

The mean threshold of forelimb SD-ICMS movements in kindled rats (30 +/- 1µA) significantly increased while the hemisphere contralateral to ICMS was cooled (35.25 +/- 2.93µA; paired one-tailed t-test, t=2.504, df=3, p=0.0437). The mean threshold of forelimb movements also increased significantly in rats with ZD7288 applied to the cortex (32 +/- 1µA to 37 +/- 3µA; paired one-tailed t-test, t=2.970, df=3, p=0.0295).
Figure 2.17. Mean SD-ICMS forelimb threshold for the standard motor map versus re-map while the hemisphere contralateral to ICMS is cooled. Both Kindled and rats with cortically applied ZD7288 are shown here. *p<.05
2.4 Discussion

The present study used electrophysiological, pharmacological and behavioural techniques to further elucidate the underlying mechanisms at play in the brain and behavior following seizures. In experiment one, I confirm rats that are callosally-kindled following training on a skilled forelimb task perform atypically on several subcomponents of the forelimb reaching movement. Rats that experience more bilateral seizures and longer bilateral ADDs throughout kindling are more likely to perform abnormally on several reaching subcomponents as well as have a higher proportion of cortical sites that elicit bilateral forelimb movement. This increase in bilateral movement representation is associated with a higher likelihood of rats performing atypically on subcomponents ii (digits semi-flexed) and ix (supination II). This investigation shines a new light on seizure-induced alterations in the sensorimotor cortex and the role bilateral movement representations may play in motor deficits.

Due to the dramatic kindling-induced shift from single contralateral to bilateral movement representations and their relationship to irregular skilled-reaching kinematics, experiment two sought to investigate the mechanistic underpinnings of this bilateral sensorimotor activity. Experiment two’s innovative sensorimotor inactivation technique revealed the entirely novel finding that bilateral and ipsilateral movement representations following kindling or pharmacological reduction of HCN channel function are completely eliminated while the hemisphere contralateral to ICMS is inactivated via cooling. Moreover, while both kindled rats and rats with a reduction in HCN channel function exhibit a decrease in SD-ICMS forelimb movement thresholds, these thresholds are raised during cortical cooling.
Seizures and Skilled Forelimb Behaviour

Using the kindling model, this research sought to determine to what extent seizures alter the sensorimotor cortex and affect skilled forelimb use. Groups undergoing 0, 5, 10, 20, or 30 kindling sessions were used to investigate if these alterations occur in a dose dependent manner. The 10 and 20 session groups had a significant reduction in skilled-reaching success while the sham, 5 and 30 session groups did not. However, although percent success of skilled reaching prior to kindling varied, post-kindling percent success is similarly low in the 10, 20 and 30 session groups (40.37 ± 5.88 %, 39.27 ± 4.65 %, and 36.85 ± 5.80 % respectively) compared to sham and 5 sessions (49.38 ± 4.41 % and 57.55 ± 6.46 % respectively). It is possible that following 10 kindling sessions, skilled-reaching success can only be lowered to a certain threshold until other compensatory behaviours are used to accommodate reaching deficits. It may also be the case that the 30 session group did not achieve a high enough percent success pre-kindling to have properly acquired the task and make robust changes to the sensorimotor cortex (that are in turn altered by kindling). It is also important to note the group kindled for 30 sessions exhibited noticeably shorter ADDs as well as having a slower progression towards stage five seizures during kindling. I suspect that the differences seen in this kindled group is due to the fact that the rats were ordered separately from the other rats used in the experiment. Because of the differences in this group, I would suggest replicating the experiment with a group kindled for 30 days in order to confirm the validity of the original 30 session group. In order to further investigate the effect of seizures on skilled forelimb movement it would be valuable to use additional behavioural tests such as vermicelli pasta
handling. Atypical handling patterns as well as adjustments of either forelimb are assessed in this test to determine impairment (Allred et al., 2008). The pasta-handling task would benefit this thesis since both forelimbs are used and can be evaluated for impairment.

Finally, I revealed that following callosal-kindling, an increase in wrist movement representations is associated with atypical reaching behaviours on two reaching subcomponents that involve wrist manipulation - vii (grasp) and ix (supination II). Similarly, an increase in shoulder movement representation is associated with atypical reaching behaviour on subcomponent iii (elbow to midline), which relies on manipulation of the shoulder.

**Seizures and Interhemispheric Communication**

Following kindling, a shift from single contralateral SD-ICMS movements to bilateral SD-ICMS movements was observed. This is the first study to analyze bilateral representations and their relation to kindling and behavioural impairment. In kindled rats, the sums of bilateral (seizure stage 4 or 5) ADDs as well as the total number of bilateral seizures account for a large portion of variance in the proportion of bilateral forelimb representations seen in motor maps. It is likely that this relationship is the result of the kindling phenomena potentiating interhemispheric projections between the motor cortex of both hemispheres. The complex combination of the sensorimotor cortices in both hemispheres being affected by kindling and a stronger callosal connection between the two, is a fitting explanation for the appearance of bilateral representations in this thesis.
Due to the synchronous and repetitive nature of seizures, strong neuronal networks are created along their path of propagation (Spencer., 2002). In the sensorimotor cortex, these networks contain a high number of perforated synapses, highly efficacious in facilitating synaptic transmission (Henry et al., 2008). Perforated synapses contain considerably more glutamate receptors (AMPA and NMDA), involved in excitatory synaptic transmission (Ganeshina et al., 2004). In a cortical kindling experiment, Henderson and colleagues showed that the mean ADD from both the corpus callosum and the amygdala increased in duration over 20 sessions. This is important because it demonstrates the corpus callosum is also reliably kindled, probably potentiating interhemispheric pathways. Transcallosal connections between sensorimotor cortices are thought to be glutamatergic pathways synapsing with pyramidal tract neurons (Reis et al., 2008). This connection is likely affected by repeated seizures, as kindling has been demonstrated to enhance glutamatergic transmission (Van Rooyen et al., 2006).

Although not fully understood, transcallosal pathways appear to be rather precise. Connections between the distal forelimb representation of the motor cortices have been discovered in monkeys and cats (Rouiller et al., 1994; Jenny, 1979; Asanuma and Okuda, 1962). Contingent on the stimulation parameters, transcranial magnetic stimulation (TMS) studies have shown direct inhibitory and, to a lesser extent, excitatory influences between the two motor cortices (Ferbet et al., 1992). Though direct evidence is lacking, research suggests that these interhemispheric interactions are comprised of excitatory transcallosal projections onto excitatory or inhibitory networks in the homologous motor cortex (Reis et al., 2008). In callosal-kindling, early stages often result in unilateral (focal) seizures (stages 0-3) and become
generalized (stages 4 and 5) fairly quickly as kindling progresses. The reason seizures begin focally is due to the stimulating electrode residing in callosal matter 0.5 mm lateral to midline in the hemisphere contralateral of the dominant forelimb. Although not directly investigated in this thesis, an essential structure for the bilateralization of seizures is the corpus callosum, specifically the anterior half (Wong et al., 2006; Wada., 1995).

While much is still to be discovered about the roles of interhemispheric projections between primary motor cortices, it is known that fluctuations in interhemispheric excitation and inhibition are involved in normal motor function (Meyer et al., 1995; Reis., 2008). The communication between motor cortices is kept in balance in a healthy brain, however in epilepsy this balance can be disrupted (Widjaja et al., 2013; Woodward et al., 2014a; Woodward et al., 2014b). Interestingly, patients with temporal and frontal lobe epilepsy can display an increase in diffusivity of the corpus callosum, especially in regions connecting homotopic cortical structures (O’Dwyer et al., 2010).

Several studies have shown SD-ICMS stimulation sites that evoke bilateral movement are as common as sites that evoke contralateral movement, although higher thresholds are needed for the ipsilateral movement (Brus-Ramer et al., 2009; Liang et al., 1993; O’Donoghue et al., 1986; Kartje-Tillotson et al., 1985). These studies used variations of the SD-ICMS protocol in this thesis, applying up to 40 percent more current. The existence of latent bilateral connections suggest that just as kindling unmask latent contralateral SD-ICMS sites by lowering the threshold within stimulation parameters (Henderson et al., 2011), it also unmask latent bilateral SD-ICMS sites. Further research is needed to substantiate my claim that callosal-
kindling strengthens interhemispheric excitation through kindling-induced potentiation and disinhibition. Such research should include the examination of kindled corpus callosum tissue for elevated levels of excitatory receptors such as AMPA and NMDA receptors. In addition, callosal axon tracing studies would confirm changes in interhemispheric pathway efficacy between motor cortices. Finally, while kindling other structures such as the hippocampus can lead to changes in the sensorimotor cortex, the alterations to the corpus callosum have yet to be discovered (van Rooyen et al., 2006).

**HCN Channels and Epileptogenicity**

Similar to kindling, the reduction in HCN channel function dramatically increases the proportion of dual contralateral and bilateral movements evoked by SD-ICMS. In addition, HCN1KO mice also exhibit atypical reaching behaviours (Boychuck et al., 2011). While there are many similarities seen in the sensorimotor cortex subsequent to kindling and the reduction of Ih, there are also some differences. For example, reducing HCN channel function does not alter the number of forelimb movements in a motor map (Boychuk et al., 2011). In experiment two, I used both the kindling model and the pharmacological application of Ih blocker ZD7288 to investigate the involvement of the hemisphere contralateral to ICMS in bilateral movement representations. For the first time, I showed that bilateral and ipsilateral movement representations in both groups are completely eliminated while the sensorimotor cortex contralateral to ICMS is inactivated via cooling.
Several animal models of reduced or eliminated HCN channel function have exhibited increased cortical excitability and susceptibility to seizures (Phillips et al., 2014; Huang et al., 2009; Nolan et al., 2004; Ludwig et al., 2003). For example, Huang et al (2009) showed that HCN1\(^{KO}\) mice only required half the concentration of kainic acid to induce seizures. Following the termination of these seizures, HCN1\(^{KO}\) mice had spontaneous recurring seizures approximately 72 hours later, compared to two weeks later in wild-type controls. The same HCN1\(^{KO}\) mice also showed heightened spontaneous HCN1 -/- neural activity. In 2011, DiFrancesco and colleagues discovered a loss-of-function point mutation in the gene coding for the HCN2 channel in an individual with generalized epilepsy. Intriguingly, rats transfected with this mutation as newborns show increased cell excitability and lower thresholds for action potentials. Comprehensive evidence of increased neural excitability and lower seizure thresholds in humans is however, lacking.

Experiment two of this thesis showed an increase in dual contralateral and bilateral forelimb movements evoked by SD-ICMS following kindling or the reduction of cortical Ih, supporting past research by Boychuk (2011) in the Teskey lab. Adding to this research, I revealed that similar to kindling, reduced HCN channel function leads to lower SD-ICMS movement thresholds. Moreover, during cooling of the sensorimotor cortex contralateral to ICMS, thresholds increase and the ipsilateral forelimb is no longer active. These results along with previous HCN channel research suggest that reduced Ih plays an important role in the facilitation of motor representations in the motor cortex and that this facilitation is not only unilateral, but bilateral as well. It also indicates that the dysfunction of HCN channel activity
resulting in increased seizure susceptibility may play a role in epilepsy. More research is needed however, to determine the extent of involvement of reduced Ih in epilepsy as well as the exact function of Ih in the motor cortex.

**Future Directions**

While this research elaborated on the vulnerability of the motor cortex to seizure-induced alterations, there are many potential studies that could build upon this thesis’ novel findings. First, reversible inactivation allows for the temporary inactivation of an area of the brain. Using this technique, researchers can conduct multiple trials with the same animal, comparing its behaviour with a fully functional brain to its behaviour during inactivation. Cooling the hemisphere contralateral to ICMS raised low thresholds and eliminated bilateral movements that resulted from kindling as well as reduced Ih. It would be interesting to observe if a chronically implanted cryoloop placed over the sensorimotor cortex ipsilateral to the skilled forelimb would improve reaching behaviour in kindled or reduced Ih rats. The inactivation, or partial inactivation, may reduce interhemispheric communication to a functional level, restoring skilled reaching performance. An additional study might examine the effect of kindling as well as reduced Ih on LD-ICMS derived motor maps. LD-ICMS elicits species relevant movements that use multiple joints at single ICMS sites. LD-ICMS allows for the closer examination of the overlapping and complexly organized cellular networks in the motor cortex that HCN channels are thought to facilitate. Changes in these movement representations would further elucidate alterations that take place following kindling or reduced Ih. The innovative
techniques and novel findings in this thesis provide a launchpad for exciting new research in the field of motor maps and epilepsy.

2.5 Conclusion

The present study revealed the contribution of seizures to motor map alterations and impaired skilled forelimb movement. Not only did the research demonstrate that forelimb movement representations in the sensorimotor cortex dramatically shift from contralateral representations to bilateral and ipsilateral representations following electrical kindling, but for the first time this increase in bilateral representations was shown to be related to skilled forelimb impairment. A completely novel cooling technique used to reversibly inactivate the hemisphere contralateral to the hemisphere responsible for the skilled forelimb revealed that following kindling or HCN channel impairment, the resultant bilateral movement representations are reliant on the sensorimotor cortex contralateral to ICMS for the ipsilateral portion of bilateral SD-ICMS induced forelimb movement. Moreover, inactivating the sensorimotor cortex contralateral to ICMS can eliminate this ipsilateral movement. Taken together, this research demonstrates that an increase in bilateral SD-ICMS induced movement representations following repeated seizures may play a critical role in motor impairment and a reduction in interhemispheric communication between the motor cortices may allow for motor recovery.
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