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A Novel Investigation of the Hypothesis that Repeated Seizures (Kindling) Create a Functional Lesion.

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "A Novel Investigation of the Hypothesis that Repeated Seizures (Kindling) Create a Functional Lesion." submitted by Luke C. Henry in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Two studies were conducted to investigate the hypothesis that repeated seizures create a functional lesion in the affected structure. The first study examined alterations in motor behaviours in a cortically kindled group and an amygdala kindled group relative to a sham-control group. The results of the first study show that cortically kindled rats exhibited a consistent alteration in their ability to use their forelimbs as revealed by the rung-walking task and the single-pellet reaching task. The amygdala group showed one significant difference from sham controls on skilled motor behaviour. Furthermore, changes in anxiety level and memory function were ruled out as possible explanations for the change in skilled motor behaviour. The second study examined the differences between cortically kindled rats (kindled lesion) and focal devascularization lesioned rats (complete lesion) on motor tasks. The kindled lesion rats had a small, consistent range of error types on the single-pellet reaching task, while the complete lesion rats committed more errors with a larger range of error types on the single pellet reaching task. Overall, these studies suggest that repeated seizures create a functional lesion specific to behaviours mediated by the affected structure and that the lesion created by repeated seizures has different behavioural consequences than a complete lesion.

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INTRODUCTION

Overview

In this thesis two studies were conducted to test the theory that kindling results in a *functional* lesion. More specifically these studies investigate the effects of repeated seizures within the caudal forelimb area, a well-defined section of the rat sensorimotor cortex, on fine motor coordination, spatial memory, and anxiety. Functional lesions have two important distinctions relative to complete lesions. The first is that functional lesions alter behaviour because the tissue itself, while present, is distorted. The behavioural alterations are not necessarily defined by the absence of behaviours as functional lesions can also exaggerate behaviours, such as the increase in aggressive behaviour that occurs with kindling of the amygdala. The other way to distinguish functional lesions is by the tissue itself relative to complete lesion tissue. Functional lesions are local brain areas displaying distorted function that may include partial damage, whereas a lesion resulting from a stroke for example, is missing tissue and therefore not able to function (Lipton, 1999). The caudal forelimb area was chosen because kindling-associated changes in this area of the brain have been well documented at the ultrastructural, structural, and electrophysiological levels (Teskey et al., 1999; Teskey et al., 2002a; Goertzen et al., 2003; Teskey et al., 2005). Also, the functional organization of the caudal forelimb motor map exhibits plasticity (Kleim et al., 1998) and the proper use of the forelimb is dependent on the integrity of this structure (Whishaw and Pellis, 1990; Whishaw et al., 1993; Whishaw, 2000). Lastly, the CFA was chosen because it is necessary to choose the appropriate structure when investigating how an alteration in a particular behaviour is mediated by that structure. The rat was chosen because the reaching behaviour in this

species shows characteristics that are homologous, though not identical to the same behaviour in humans (Whishaw et al., 1992c). This introduction describes the interictal behavioural alterations in humans with epilepsy and how these alterations are modeled in kindled rats, a comparison of the organization of the motor cortex in primates and rats, and a description of the caudal forelimb area (CFA) and its plasticity. Finally, the objectives and hypotheses of the present work are outlined.

Historical Perspectives on Epilepsy

The epilepsies are likely as old as the true brain (including a spinal cord, cerebellum, cerebrum) itself. Ancient texts from 1700 b.c.e. China, 1600 b.c.e. Egypt, 1000 b.c.e. India, and 500 b.c.e. Babylon all anecdotally described the epilepsies in people (Engel and Pedley, 1998). The earliest full description of a secondarily generalized seizure in the human population was documented as early as 3000 years ago in a text found in Mesopotamia (modern day Iraq) written in Akkadian, the oldest written language (Engel and Pedley, 1998). The seizure described in the Akkadian text was attributed to the moon god (Engel and Pedley, 1998). Indeed, the attribution of seizures to supernatural forces still pervades thoughts and descriptions of the epilepsies throughout the world (Engel and Pedley, 1998). Scientific explanations of the disorder began to emerge around 460 b.c.e. in a book titled *On the sacred disease*, supposedly written by Hippocrates (1839–1861; Temkin, 1971). In the middle ages, Galen expounded on this work and theorized against the popular belief that seizures were the result of demon possession and instead proposed that seizures were caused by an imbalance of phlegm and bile in the ventricles (Engel and Pedley, 1998). Over the intervening centuries,

treatments for the epilepsies ranged from exorcism to mercury. It was not until the 19th century that neural anatomy and seizures were correlated to describe the epilepsies. John Hughlings Jackson localized seizures to areas of the brain by their clinical manifestations with confirmation by autopsy and also described the typical "march" of a motor seizure as it progressed through motor cortex (Kandel et al., 2000), Sommer reported that lesions in the hippocampus were the origin of epilepsy in 59 autopsies, while Bratz illustrated the microscopic characteristics of hippocampal sclerosis now commonly associated with temporal lobe epilepsy (Engel and Pedley, 1998). In the twentieth century, Hans Berger expanded on the discovery of spontaneous brain activity in animals by demonstrating the same in humans using his invention, the electroencephalogram (EEG) (Engel and Pedley, 1998; Kiernan, 2005b). Berger was able to show the characteristic spike and wave pattern now thought of as being synonymous with the epilepsies (Engel and Pedley, 1998). Since that time many questions about the causes and underlying mechanisms behind the epilepsies have been answered while many more questions remain to be answered.

Seizures and Epilepsy

Seizures are the result of hyper-synchronous, hyper-excitable firing patterns of neurons that are located predominantly in the cerebral cortex (Engel and Pedley, 1998; Teskey et al., 2005). The International League Against Epilepsy (1989) has developed a classification system allowing for distinctions between generalized and partial seizures. Those seizures where the initial discharge can be found in both cerebral hemispheres are termed generalized seizures, whereas those initial discharges that are only seen in one cerebral hemisphere are termed partial seizures. Partial seizures are further broken down

into subcategories that include simple, complex, and secondarily generalized. Simple seizures are necessarily those seizures in which consciousness (behavioural responsiveness) is not altered. Conversely, complex seizures represent those seizures in which consciousness is altered. Secondarily generalized seizures are seizures that spread from the initial focus to other brain areas that result in generalized tonic-clonic convulsions with bilateral motor manifestations.

Epilepsy on the other hand is a chronic condition that describes any one of various disorders marked by reoccurring seizures due to the excessive discharge of neurons. Epilepsy does not describe a single disorder, but is instead representative of a broad category of symptom complexes that can arise from one of several disordered brain functions, including cortical malformations or brain tumors (Engel and Pedley, 1998). The Commission on Classification and Terminology of the International League Against Epilepsy (1989) has dichotomized the epilepsies into localization-related epilepsies and generalized epilepsies. The chief utility of this classification is based on treatment efficacy as focal and generalized epilepsies respond differently to antiepileptic drugs and surgery. Focal epilepsies are hallmarked by focal seizures where focal seizures are brief temporary alterations in movement, sensation or autonomic nerve function in a localized area of the brain. Generalized epilepsy refers to those epilepsies that are marked by seizures with no focus. The epilepsies are then further subdivided into two broad categories: symptomatic and idiopathic. Symptomatic epilepsies are simply those epilepsies that are the consequence of a known lesion or other specific etiology such as head trauma, fever (febrile seizures), infection, genetic factors, and drugs to name a few. The idiopathic epilepsies refer to all other epilepsies where the origin is unknown, though

channelopathies (maladaptive changes in the structure and/or function of a channel) are suspected to be causal in part (Avoli et al., 2005).

The epilepsies have several ictal and interictal behavioural consequences associated with them. The term "ictal" refers to the seizures and epileptic discharges themselves, where "interictal" refers to all other periods between seizures and epileptic discharges. Ictal behaviours include seizures and auras, whereas impairment of memory, inappropriate social behaviour (over-emotionality, dissocial behaviour), and heightened levels of anxiety are often observed during the inter-ictal state (Bear and Fedio, 1977; Helmstaedter et al., 1996; Boro and Haut, 2003; Hommet et al., 2006). Many of the interictal behaviours have a significant impact on the treatment and quality of life of patients with epilepsy (Tellez-Zenteno et al., 2005). Analysis of the clinical characteristics of interictal behaviours and spontaneous seizures is an essential aspect of both psychosocial therapy and pre-surgical evaluation. The following sections describe interictal comorbidities (Tellez-Zenteno et al., 2005) including mood and anxiety disorders (Boone et al., 1988; Blumer et al., 1995; Brook et al., 1996; Kanner, 2004; Kanner and Dunn, 2004; Kanner et al., 2004; Jones et al., 2005a; Jones et al., 2005b), memory disturbances (Helmstaedter, 2002), reproductive dysfunction (Isojarvi, 2003; Isojarvi et al., 2004), and motor coordination alterations (Hernandez et al., 2002; Hommet et al., 2006) in people with epilepsy. Comorbidities are defined as the greater than coincidental co-occurrence of two or more conditions in the same individual (Feinstein, 1970).

Mood and Anxiety Disorders Associated with the Epilepsies

Psychiatric disorders like anxiety, depression, psychoses, and aggressive behaviour have a high co-occurrence with temporal lobe epilepsy (Kanner, 2004; Jones et al., 2005a). The high co-occurrence of anxiety with temporal lobe epilepsy allows the presence or absence of anxiety to be used as a diagnostic tool to differentiate between an epileptic disorder and different types of seizure disorders (Kanner et al., 2004). A study comparing patients who suffered from psychogenic pseudoepileptic seizures (seizures produced or caused by psychic or mental factors rather than organic factors that appear epileptic in nature, but are not epileptic) with patients suffering from epileptic seizures found a qualitative difference in the type of anxiety experienced between the respective groups (Owczarek, 2003). In two studies of the comorbidities of epilepsy with anxiety and depression, Nubupko and colleagues (2004a; 2004b) found that people with epilepsy had anxiety and depression scores on the Goldberg anxiety and depression scale nearly twice as high as the normal population. These studies also revealed a positive correlation between anxiety and depression scores with seizure frequency and lack of treatment. That is to say, those patients who received treatment had less anxiety and depression than their untreated counterparts, but still had higher levels when compared to the general population (2004a; 2004b). Depression and the epilepsies are significantly more prevalent in older patients who also suffer from more frequent focal epilepsies arising from the temporal lobe (Schmitz et al., 1999). The high rate of peri- and interictal depression amongst people with epilepsy has been reported to range between 17 and 60 percent, with the higher end of the scale representing those patients with intractable epilepsy. Again, seizure type (complex partial seizures) and seizure origin location

(temporal lobe, especially the left hemisphere) figure to be crucial elements in identifying depression in people with epilepsy (Kanemoto et al., 1996; Lambert and Robertson, 1999; Piazzini et al., 2001a, b; Piazzini and Canger, 2001; Jones et al., 2005a). Pre-operative differences between patients who undergo surgery make attributing changes in psychiatric symptoms to the surgery very difficult. This is because successful resection of epileptic brain tissue does not always eliminate anxiety and depression; however, experienced decreases in somatic anxiety and psychic anxiety are a likely post-surgical outcome (Mattsson et al., 2005).

People with temporal lobe epilepsy (TLE) and focal non-TLE do not differ in their rate of psychoses, but each of these groups was significantly more impaired than those with primary generalized epilepsy (Edeh and Toone, 1987). This suggests that the increased prevalence of interictal psychopathology commonly associated with TLE may also be a feature of other forms of focal epilepsy, and that the psychopathologies associated with epilepsy may vary with the location of the foci (Edeh and Toone, 1987). A retrospective study investigating the role of social and biological risk factors for the development of major depression and schizophreniform psychoses in persons with epilepsy revealed that people with schizophrenia had an earlier age of seizure onset and also had a more severe epilepsy distinguished by a history of status epilepticus, multiple seizure types, and greater severity of seizures compared to non-psychiatric controls (Roberts et al., 1990). Roberts and colleagues (1990) also concluded that while many of the epilepsies and associated symptoms are idiopathic, schizophrenia-like psychoses are not a random occurrence. They are significantly associated with lesions, particularly gangliogliomas (a type of tumor made up of mature gangliocytes and neuroglial cells)

that originate *in utero* or perinatally. They also affect neurons in the medial temporal lobe, with a disproportionate occurrence in the left hemisphere, and have an earlier age of onset (Roberts et al., 1990).

Memory Deficits Associated With the Epilepsies

People with epilepsy often experience impaired memory function (Thompson, 1991; Schwarcz and Witter, 2002; Andelman et al., 2004). Furthermore, the type of memory impairment can vary with seizure foci location. Bear and Fedio (1977) reported that TLE patients with left hemisphere lesions over-estimated their memory deficits, while the opposite was true of patients with right hemisphere lesions. This finding was confirmed by Andelman and colleagues (2004), who found a discrepancy between the self-estimated memory ability and performance on memory tests in patients with right hemisphere epileptogenic lesions when compared to patients with left hemisphere lesions and to demographically matched control participants. The effects of mesial temporal (MT) and cerebellar hypometabolism were studied using measures of verbal, visual, and motor skill learning with positron emission tomography revealed patients with more marked MT hypometabolism on the left had impaired delayed verbal memory compared to patients with more marked MT hypometabolism on the right who showed impaired spatial learning, but normal retention over delay (Harris et al., 2001). In a more ethologically valid test of memory, people with epilepsy were found to have significantly greater memory difficulties coupled with higher levels of anxiety and depression relative to control participants. This suggests that subjective perception of memory failure reflects objective memory impairment. However, the deficits in memory may still be confounded

to a degree as memory efficiency scores are also correlated with anxiety and depression levels (Giovagnoli et al., 1997).

The effects of repeated seizures on memory in people with temporal lobe epilepsy are memory-type specific. Episodic memory is typically not impaired in temporal lobe epileptics, while semantic memory is impaired. This impairment is even more prominent in temporal lobe epilepsies with a mesial temporal origin (Helmstaedter, 2002). The general consensus is that TLE patients present with deficits in declarative memory, (i.e. in their ability to acquire facts and events related to their personal past) and also show particularly pronounced deficiencies in the performance of spatial memory tasks. It is then surmised that because these memory processes require the integrity of the mesial temporal lobe, there is a causal link between specific brain lesions and the observed cognitive impairments (Abrahams et al., 1999; Ploner et al., 2000).

Mesial temporal lobe epilepsy (MTLE) is most often accompanied by damage to the hippocampal system resulting in some type of memory impairment. Alessio *et al.* (2004) sought to tease apart the effects of hippocampal atrophy from other potentially confounding variables like the type of initial precipitating injury and pathological substrate, effect of lesion lateralization, history of febrile seizures, status epilepticus, age of seizure onset, duration of epilepsy, seizure frequency, and use of antiepileptic drugs. They found that people with epilepsy who also had hippocampal atrophy, an earlier onset of seizures, longer duration of epilepsy, higher seizure frequency, and also used antiepileptic drugs had the most profound memory deficits. They also found a close relationship between deficits of verbal memory and left hippocampal atrophy. This finding is not surprising given the left hemisphere lateralization of language in the vast

majority of the population. Conversely, a similar deficit in visual memory was not seen in patients with right hippocampal atrophy, despite the notion that the right hemisphere is mainly responsible for visual memory (Alessio et al., 2004). Reminger and colleagues (2004) used hippocampal volume in people with temporal lobe epilepsy to predict memory performance. Consistent with the functional adequacy model, that states the epileptogenic hippocampus retains some degree of memory function despite potential structural damage, the combined volume of the left and right hippocampi was found to be the best predictor of objective verbal memory performance. In contrast, the best predictor of subjective ratings of cognitive functioning was the asymmetry between right and left hippocampal volume, where patients with left hippocampal sclerosis had lower ratings on cognitive performance, likely related to impaired verbal memory (Alessio et al., 2004). This is consistent with overall estimations of memory performance in patients with left hemisphere lesions and seizure foci (Andelman et al., 2004). The hippocampal atrophy is also most prominent in the CA1 field of the hippocampus in the proximal area bordering the subiculum, while granule cells remain relatively unscathed (Houser, 1990). Evidence from studies in humans and experimental animals also implicates the entorhinal cortex, as having a particularly important role in the declarative memory deficits of TLE patients. The perforant pathway connecting entorhinal cortex to hippocampal dentate granule cells and CA1 cells normally controls information flow to the hippocampus (Schwarcz and Witter, 2002). Expectedly, abnormalities in entorhinal cortex as seen in people with TLE, trigger discrete changes in the hippocampus resulting in structural and functional damage that mimics the effects of a lesion directly to the hippocampus (Heinemann et al., 2000).

Reproductive Dysfunction Associated with the Epilepsies

Both women and men with epilepsy have altered hypothalamic–pituitary–gonadal function resulting in interictal disruptions of sexual behaviour (Boro and Haut, 2003). This altered functioning has consequences for both arousal and conception rates (Edwards et al., 2000; Boro and Haut, 2003). Many women with epilepsy experience menstrual dysfunction resulting in anovulatory cycles and amenorrhea (Herzog et al., 1986a), both of which are symptoms of polycystic ovary syndrome. Polycystic ovary syndrome (PCOS) is linked to epilepsy via epileptiform discharges that disrupt normal hypothalamic function. The anovulatory cycles associated with PCOS are known to increase with seizure frequency (Boro and Haut, 2003). Women with epilepsy also have a significantly higher occurrence of hypogonadotropic (inadequate secretion of gonadotropins) hypogonadism (a condition resulting from abnormally decreased functional activity of the gonads) when compared with women from the general population (Herzog et al., 1986a). Reproductive endocrine disorders, such as hypothalamic amenorrhea (the absence, discontinuation or abnormal stoppage of the menstrual periods), premature menopause, and hyperprolactinemia (an increased level of the hormone prolactin) have also been reported to be more common in women with epilepsy (Isojarvi, 2003). Hemispheric differences also exert differential effects on the type of reproductive dysfunction that manifests. PCOS is predominantly associated with left-sided lateralization of interictal epileptic discharges. Right-sided interictal epileptic discharges are associated with hypogonadotropic hypogonadism. Hyposexuality, associated with low levels of leutenizing hormone, also occurs more often in women with predominantly right-sided interictal epileptic discharges (Herzog, 1993).

Men with generalized epilepsy have decreased potency and abnormal sperm structure resulting in reduced fertility (Taneja et al., 1994; Isojarvi et al., 2004). In a study of men with partial seizures of temporal lobe origin, 55% had diminished sexual interest or reduced potency (Herzog et al., 1986b). Another study of men with epilepsy found the patient base to be profoundly hyposexual with a high level of sexual dysfunction. Both free and serum testosterone levels were low, suggesting that the high level of sexual dysfunction and lack of sexual interest has a hormonal basis (Fenwick et al., 1985). This speculation was confirmed in another study by Fenwick and colleagues (1986) who found that men with epilepsy who had low free and serum testosterone levels also had significantly lower incidences of penile tumescence during sleep.

Alterations in Motor Coordination Associated with the Epilepsies

Motor partial seizures are among the most frequent manifestations of frontal lobe seizures suggesting that the epileptogenic area includes motor areas (Derambure et al., 1997). A study by Hernandez and colleagues (2002) investigating planning abilities, working memory, impulse control, attention, and certain aspects of motor coordination in 32 unresected children with epilepsy found differences relative to normal children depending on the type of epilepsy the child had (frontal lobe, temporal lobe, or generalized epilepsy). While the three epilepsy groups did not differ with respect to conceptual shift and recency memory, the children with frontal lobe epilepsy (FLE) did show deficits in planning and impulse control. Children with FLE also exhibited significantly more motor coordination problems and demonstrated greater rigidity than the other epilepsy groups on the tests of motor coordination (Purdue Pegboard test,

Thurstone's uni- and bimanual performance test, Luria's motor sequences). These problems were more pronounced in younger children with FLE (8-12 years). This same subgroup also showed impairment on verbal fluency measures. None of the differences observed could be attributed to gender, localization of the epileptic abnormality (unilateral versus bilateral) or medication (monotherapy versus polytherapy) (Hernandez et al., 2002). Consistent with this finding, Upton and Thompson (1997) found that children with FLE with early seizure onset (0-6 years) showed no differences in executive functioning when compared to children with either intermediate (7-12 years) or late (12-16 years) seizure onset. However, children with earlier seizure onset showed better performance in motor function tests than their intermediate to late seizure onset counterparts. Also of note, though not surprising given the distribution of handedness in humans, left hemisphere lesions across all age of onset groups resulted in decreased motor performance on motor tasks.

Derambue and colleagues (1997) investigated the spatiotemporal distribution of EEG μ rhythm desynchronization in people with partial epilepsy to determine whether frequent focal motor seizures could induce a change of cortical activation during the planning of voluntary movements. The μ rhythm is an EEG phenomenon considered to be a good indicator of cortical activation during the planning of a voluntary movement that has a specific spatial distribution over the cortex (Steriade et al., 1990; Lopes da Silva, 1991). Comparisons between people with FLE with frequent focal motor seizures, people with TLE with complex partial seizures but no ictal movement disorder, and control subjects of the same age revealed abnormal cortical activation in the planning of voluntary movements in the FLE group. They showed cortical desynchronization 500 ms

before the actual movement along with an increase in EEG amplitude of μ rhythm over the contralateral central region relative to the normal controls that showed cortical desynchronization 2000 ms before the onset of the movement. The shortened time course of the μ rhythm in people with FLE suggests that the planning of movements is temporally altered. The TLE group showed an increase in EEG amplitude as well, but no differences in the spatiotemporal pattern of event related desynchronization were observed. The change in the spatiotemporal pattern of event related desynchronization in FLE patients with focal motor seizures indicates that there is atypical cortical activation during the planning of a voluntary movement, perhaps in part explaining the results obtained by Hernendez *et al.* (2002). Kanovsky and colleagues (2003) investigated changes in the P3 (third positive going wave of an evoked potential) component of motor evoked potentials in people with epilepsy via intracortical recordings. The latency of the P3 component is thought of as being the chief parameter by which processing rate measurements of target stimuli are taken. Motor evoked potentials provide a non-invasive method of studying the neurophysiology of motor processes in the behaving human by indicating voltage changes in the electrical activity in the cortex. Using three different visual tasks, the occurrence of the P3 component, its latency and amplitude, and the dependency of the P3 component on task complexity were calculated. Data collected from medial parietal sites revealed a significant delay in P3 in the two tasks with a motor component relative to the task that did not include a motor component. Lateral sites all showed a latency increase in the two tasks with a motor component, particularly in frontal-parietal sites (Kanovsky et al., 2003). The lack of pronounced latency changes in the temporal lobe may indicate that there are not many functions associated with

movement planning and execution in this area, where changes in the frontal lobes indicate just the opposite (Kiernan, 2005b). The behavioural consequence to the shortened spatiotemporal characteristics of cortical desynchronization may manifest themselves as impaired motor coordination or response inhibition. A comparison between people with unresected TLE and FLE showed differential impairments in people with FLE on tasks assessing motor coordination and response inhibition (Helmstaedter et al., 1996). Matsuoka and colleagues (2000) examined the effects of higher mental activity on EEG. They found that mental activities involving use of the hands, including writing, written calculation, and spatial connection provoked the highest number of discharges among those patients who showed neuropsychological EEG activation. Changes in EEG activation either precipitating or inhibiting seizures should come as no surprise given the close relationship between seizures and ongoing brain activity (Brown and Fenwick, 1989; Fenwick and Brown, 1989; Fenwick, 1998).

Summary of the Interictal Behavioural Comorbidities Associated with the Epilepsies

To review, people with TLE have altered anxiety levels and mood disorders, namely depression. People with epilepsy also experience deficits in declarative memory, though this is somewhat linked to lateralization of the seizure focus as right hemisphere lesions tend to manifest as deficits in spatial memory. Hippocampal sclerosis and altered connections between entorhinal cortex and the hippocampus have also been implicated in the deficits in memory experienced by people with TLE. Sexual dysfunction in both women and men is prevalent in people with epilepsy. Both genders become hyposexual relative to the general population. Women experience a variety of changes in sexual

function. PCOS is particularly pervasive among women with left hemisphere lesions, while hypogonadotropic hypogonadism is more prevalent among women with lesions in the right hemisphere. Men with epilepsy have decreased potency, erectile dysfunction, and altered sperm structure, all of which translates into reduced fertility. Though people with TLE or generalized epilepsy do not experience any appreciable differences in motor coordination, people with FLE are generally less coordinated, showing rigid movements and altered electrophysiology with respect to the planning and execution of voluntary movements.

It is difficult to disambiguate the effects of seizures from the effects of antiepileptic drugs, social stigma, and individual differences in people with epilepsy. Indeed, it is nearly impossible to tell whether a patient's feelings of depression are related to the condition of having epilepsy and the social stigma attached to the disorder, or to the effects of the seizures themselves. Moreover, the effects of antiepileptic drugs are always a concern when studying behavioural comorbidities associated with the epilepsies. It is therefore necessary to have a model whereby the effects of repeated seizures can be studied in a relatively homogenous population without social stigma and drug effects. Kindling provides such a model. The following section describes the kindling model of epilepsy and details analogous interictal behavioural changes in kindled rats.

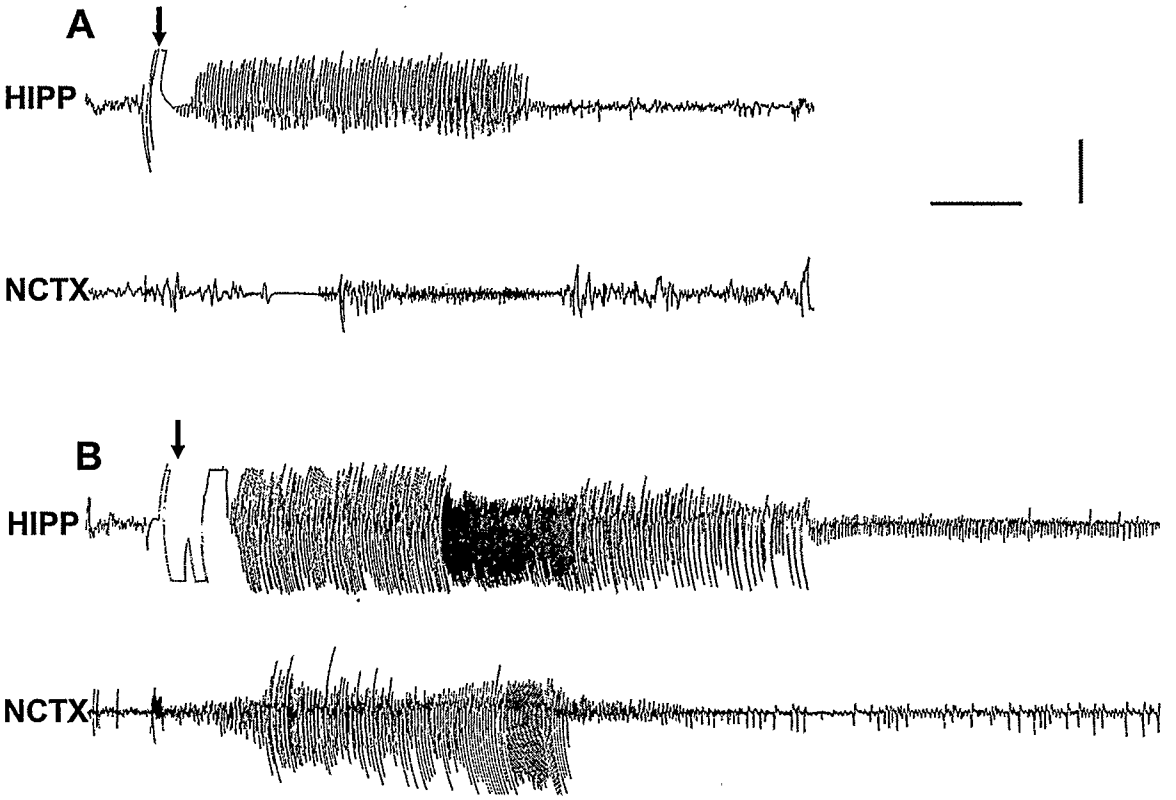
Kindling

Kindling is a process by which the repeated administration of an initially sub-convulsant electrical stimulation of certain brain areas leads to propagating hyper-

synchronous electrical activity (after discharge) that spreads throughout the brain resulting in behavioural seizures that increase in severity and duration (Goddard, 1967; Goddard et al., 1969). An afterdischarge (AD) is defined as the persistence of a hyper-synchronous, hyper-excitable response of neural tissue after the cessation of stimulation (Fig. 1). The phenomenon was initially discovered serendipitously in a study investigating the effects of repeated stimulation of the amygdala in rats. A subsequent series of experiments revealed that different brain sites differ in their respective kindling rates, the persistence of the effects, the stimulation intensity necessary to elicit a stimulation decreased with repeated stimulations, and that kindling was a trans-species phenomenon (Goddard, 1967; Goddard et al., 1969; Henke and Sullivan, 1985; Adamec and McKay, 1993). After the initial AD, subsequent ADs become longer and propagate more strongly with repeated stimulation occurring in concert with behavioural manifestations that begin with stereotyped jaw movements (Stage 1) progressing through to repetitive head nodding (Stage 2), unilateral forelimb clonus (Stage 3), bilateral forelimb clonus accompanied by rearing (Stage 4), ending in a convulsive response dominated by forelimb clonus, rearing, and falling (Stage 5) (Racine, 1972; Racine et al., 1975). Kindling is a model of seizures and the epilepsies because it provides strong homology to many clinical aspects of the aforementioned disorder. The creation of a seizure focus and the occurrence of a partial seizure and secondarily generalized seizures are important features that specifically model focal epilepsy (Racine, 1972). The progression in severity of seizures or the stability of seizures over time (Racine, 1972; Racine et al., 1973), the presence of multiple seizure generators

Figure 1: Seizure spread from initial afterdischarge (AD) in the hippocampus (HIPP) to motor neocortex (NCTX) after 40 kindling stimulations. Arrows indicate stimulation. A.) Initial AD elicited through stimulation of the hippocampus. The neocortex (NCTX) recording taken from motor neocortex shows minimal seizure activity during the initial AD, during which the animal had a stage 1 seizure. B.) AD elicited through stimulation of the hippocampus after 40 stimulations has spread to extrahippocampal areas resulting in an increase in subsequent seizure activity in motor neocortex , duration and severity (stage 5 seizure) (van Rooyen et al., 2006).

Figure 1 - 1: After Discharge and Seizure Spread



(Corcoran et al., 1975; Racine, 1975), pharmicoresistence (Loscher et al., 1986), and conditioned tolerance (Kim et al., 1995) all model important clinical features of the progression and stability of the epilepsies as well as subsequent treatment. Of particular importance to the current thesis are the interictal behavioural consequences of epileptogenesis modeled by kindling.

Anxiety and Psychotic Disorders Associated with the Epilepsies Modeled by Kindling

Research by Henke and Sullivan (1985) created a link between kindling in the centromedial amygdala and susceptibility to stress ulcers in rats. They showed that rats, even if only partially (non-convulsive) kindled showed more susceptibility to stress as shown through the extent of stomach pathology from stress-induced ulcers. In an investigation of the effects of long-term amygdala kindling on emotional behavior, rats received 99 basolateral amygdala, central amygdala, or sham stimulations (Kalynchuk et al., 1997). The long-term kindled rats in both amygdala stimulation groups displayed more resistance to capture in an open field indicating greater anxiety, but curiously showed more open-arm activity on an elevated plus maze, which has traditionally been interpreted as a measure of reduced anxiety. In a follow-up experiment, rats received a varying number of amygdala stimulations (20, 60, or 100). Kindled rats showed reduced exploratory behaviour in a novel open field, exhibited thigmotaxis, and showed resistance to capture, all measures indicating increased levels of anxiety. Again, the kindled rats in the 100 stimulations group showed the paradoxical behaviour of spending increased

amounts of time on the open arms of the elevated plus maze while kindled rats in the other two groups showed fewer open arm entries than the control group. The magnitude of these effects decreased with the number of stimulations and the effects persisted, though not to the same extent, up to one month after the last kindling session (Kalynchuk et al., 1998b). To explain the unexpected results from the elevated plus maze Kalynchuk and colleagues (1997; 1998b) argued that the 99 and 100 stimulation rats were extremely anxious and jumping off the open arms of the maze while also exhibiting extreme piloerection. Kalynchuk and colleagues (Wintink et al., 2003) also found that rats kindled in the amygdala, regardless of sex, showed increases in fearful behaviour on both the open field test and the forced swim task. Adamec and McKay (1993) noted that kindling of the medial amygdala produced lasting anxiogenic effects. Rats kindled in the anterior or posterior medial amygdala of the right hemisphere showed reduced levels of anxiety relative to implanted controls, but not to un-operated controls. Conversely, using the same stimulation parameters, rats kindled in the anterior medial amygdala showed increased levels of anxiety relative to both implanted and un-operated controls.

Similarly, Pinel and colleagues (1977) found that caudate-kindled rats showed no increase in aggressive behaviour, while rats kindled in temporal lobe structures (amygdala or hippocampus) did show an increase in aggressive behaviour lasting up to six weeks after the last seizure. Adamec (1976b) revealed that kindling of medial hypothalamic and ventromedial hypothalamic areas of the cat brain stopped the initiation of spontaneous predatory attack in cats, while stimulation of the mammillary bodies, was discovered to suppress attacking behaviour. Partial kindling of the amygdala of aggressive cats reduced the aggressive behaviour of the cats and increased their defensive

behaviour. Conversely partial kindling of the ventral hippocampus, an attack facilitating area, increased aggressive behaviours while defensive behaviours decreased. The opposite effects of kindling support the notion that kindling creates a functional lesion.

Memory Dysfunction Associated with the Epilepsies Modeled by Kindling

Kindling has also been shown to have effects on memory. Gilbert and colleagues (1996) assessed the spatial learning and memory of rats kindled in CA1 of the hippocampus. Using a protocol where the rats were kindled, then trained in the Morris water maze subsequent to kindling to assess acquisition, and the reverse in which animals were trained in the Morris water maze then kindled to assess retention. In both cases testing was done 24 hours after the last seizure. The two protocols allowed for the researchers to assess the possible anterograde and retrograde amnesiac effects respectively due to kindling. Acquisition testing revealed that rats kindled to secondarily generalized seizures in CA1 produced deficits in water maze performance, where partial kindling did not produce memory deficits. However, both secondarily generalized and partial seizures produced deficits in retention. In a similar protocol where the time between the last seizure and testing was changed from 24 hours to 25-45 after the seizure revealed differences in acquisition and retention regardless of whether the last seizure was fully generalized or partial (Gilbert et al., 2000). Assessment of the mnemonic effects of kindling using the 8-arm radial maze has revealed comparable results. Rats kindled in CA1 of the hippocampus to either a generalized state or to a pre-convulsive state showed deficits in radial maze performance, evaluated by correct arm entries in eight choices or total maze run (trial) time (Leung et al., 1990). Electrophysiological

correlates of the disruption in memory were confirmed by Leung and Shen (1991) who found that the average commissurally evoked potentials in CA1 were enhanced above levels obtained prior to kindling.

Rats kindled in the dorsal hippocampus assessed for changes in anxiety and memory across a variety of behavioural tests showed no behavioural alterations in anxious behaviours. However, memory deficits were observed in a delayed-match-to-place task in a water maze. This finding indicated that hippocampal kindling produces enduring and selective effects on hippocampal-mediated behaviours (Hannesson et al., 2001b). Similarly, rats kindled in the perforant path (entorhinal cortex to hippocampus), septum, or amygdala showed impairments in place learning, while showing no changes in visible platform training or swim speed (McNamara et al., 1992). While place learning remained unaffected in all groups, rats kindled in either the perforant path or the septum were impaired at learning the location of a reversed platform when seizures were triggered before training. These results suggest that hippocampally mediated behaviours were affected by limbic structure kindling, while extra-hippocampal behaviours remained unaffected. Memory performance in Wistar rats kindled in either the dorsal hippocampus or the ventral hippocampus show differential impairments, with ventral hippocampal kindling resulting in impaired active avoidance, while dorsal hippocampal kindling resulted in poorer performance on discrimination tasks (Becker et al., 1997). Kindling the hippocampal perforant path-dentate projection affected subsequent discrimination-reversal conditioning of the rabbit nictitating membrane response (Robinson et al., 1989). While kindling facilitated acquisition of the initial discriminative response, it impaired performance during reversal training. The initial acquisition was likely facilitated in a

manner highly similar to the reported effects of long-term potentiation by increasing the strength of the perforant path-dentate synapse. The learning deficit in the reversal phase is similar to deficits seen in hippocampal lesion studies. This paradoxical finding also lends credence to the notion that kindling produces a functional lesion (Robinson et al., 1989).

Sexual Dysfunction Associated with the Epilepsies Modeled by Kindling

Kindling in rats has revealed similar alterations in reproductive functioning to people with TLE. In adult female rats, generalized seizures disrupt normal ovarian cyclicity, while seizures evoked by repeated electroshock delayed the onset of puberty in juveniles. Adult female rats kindled in the right amygdala were acyclic, developed polycystic ovaries, and showed premature aging of the hypothalamic-pituitary neuroendocrine axis, leading to chronic anovulation and continuous estrogen exposure (Edwards et al., 2000). The effects of kindling on sexual function in female rats appear to be linked to the nature of the seizures themselves. Kindled rats showed the typical dysfunctions associated with seizures: arrested ovarian cyclicity, increased serum estradiol levels, increased pituitary weight, and PCOS. But in 93% of females, the dysfunctions began to manifest after the development of stage 5 motor seizures, when focal seizures had secondarily generalized indicating that sexual dysfunction was correlated with an increase in seizure behaviour severity.

Repeated electroshock seizures in adult male rats resulted in transient hypogonadism, distinguished by decreased levels of serum testosterone and lowered gonadal tissue weight. In contrast, kindling of the right amygdala resulted in increased

levels of serum testosterone and estradiol accompanied by an increase in gonadal weight. This contrasting effect lends itself to the idea that kindling creates a functional lesion (Edwards et al., 2000), where generalized seizures are modeled by repeated electroshock seizures (Fenwick et al., 1985; Edwards et al., 2000; Isojarvi et al., 2004). A study comparing kindled rats and intact controls revealed different results depending on the type of seizure and the hormonal profile of the rats. Kindling resulted in increase levels of serum testosterone, estradiol, and prolactin in males, accompanied by a significant increase in testis, epididymis, and pituitary weight, and a significant decrease in prostate weight (Edwards et al., 1999a).

Summary of the Interictal Behavioural Effects of Kindling

The amygdala is normally involved in mediating fearful behaviours, the hippocampus is involved in spatial memory and the hypothalamic/pituitary axis in sexual activities (Kolb and Whishaw, 2003). Thus it appears that kindling results in a functional lesion of the activated structures (Table 1). I can test this theory by kindling in a structure that specifically mediates a particular behaviour and then examine whether there is a deficit in the behaviour mediated by that structure. This theory can be investigated in a novel way by examining the effects of kindling on motor behaviour on behavioural tests sensitive to changes in motor coordination subsequent to kindling in the sensorimotor neocortex, specifically at the level of the caudal forelimb area.

While there has been substantial investigation of kindling in the hippocampus and the amygdala, there has been far less work done to investigate the effects of seizures kindled in the neocortex (Table 1). Seizure discharges in mesial temporal lobe structures

are often initiated from mesial limbic structures such as the hippocampus and the amygdala (Avoli et al., 2005). The mesial limbic structures from where the seizures originate are also subject to histopathological (alterations in cells and tissue at the microscopic level) changes. However, neocortical neurons in people with epilepsy that has a sub-gray matter focus do not display any remarkable histopathological changes, making the neocortex an important investigative system for assessing histology, cellular mechanisms, and behavioural changes associated with the epilepsies (Avoli et al., 2005). It is also important to investigate kindling in the neocortex as focal seizures differentially affect the areas from which they originate. Evidence of this can be seen when patients are examined after they have had the epileptic area resected. Kindling in the neocortex along the transcallosal pathway propagates seizures in both hemispheres, and therefore should not have disparate effects on forelimb preference, an important factor in motor behaviour testing. It is the aim of the current research to investigate the behavioural consequences on skilled learning as a result of the changes in the caudal forelimb area due to kindling.

Table 1: Comparison of the interictal behavioural comorbidities in people with epilepsy and the kindling model of epilepsy. The experimental data models the clinical data across several aspects of the epilepsies. The effects of mesial temporal lobe epilepsy are most commonly investigated as TLE represents the bulk of clinical cases. The current thesis adds to the strength of the kindling model by answering questions about behavioural changes associated with FLE.

Table 1

BRAIN STRUCTURE	EPILEPSY	KINDLING
HIPPOCAMPUS	• Left Hippocampus (sclerosis): verbal memory deficits • Right Hippocampus (sclerosis): spatial memory deficits	• Dorsal Hippocampus: spatial memory deficits/ discrimination tasks • Ventral Hippocampus: impaired active avoidance Increased anxiety
AMYGDALA	• Temporal lobe structures: increased anxiety • Depression	• Amygdala: increased anxiety/ fear • Defensive Aggression
HYPOTHALAMUS	• Hypogonadotropic hypogonadism (women, right hemisphere) • PCOS (women, left hemisphere) • Erectile dysfunction, sperm morphology (men) • Altered hormone levels	• PCOS (females), • Hypogonadism (males), • Altered hormone levels
NEOCORTEX (Frontal)	• Motor dysfunction • Altered executive functions	• This thesis

Organization of the Neocortex

The surface of the mammalian cerebral hemisphere is made up of the archicortex (hippocampal formation), the paleocortex (olfactory system) and the neocortex. The mammalian neocortex is laminated into six distinct layers and organized in columnar fashion. The thickness of the human neocortex varies depending on the location in question, with the thickest gray matter area being the primary motor cortex at a thickness of 4.5 mm and the thinnest gray matter area being located in the occipital lobes at a thickness of 1.5 mm. The six layers of the neocortex can be defined based on the cell types (spinous or smooth, dendritic and axonal morphology) and cell density within a given layer. The most superficial layer (layer I) is the molecular layer. It predominantly consists of terminal branches of dendrites and axons giving the layer its distinctive "molecular" appearance in nerve fiber stains. In essence the molecular layer is a synaptic field in the cortex. The external granular layer (layer II) contains many small pyramidal cells and interneurons. The external pyramidal layer (layer III) is primarily composed of pyramidal cells that increase in size from the external to the internal borders of the layer. The axons of these pyramidal cells project to other cortical areas as association and projection fibers. The internal granular layer (layer IV) is mainly composed of stellate cells with pyramidal cells and interneurons making up the rest of the layer. Of particular importance to the current thesis is the internal pyramidal layer (layer V). This layer contains large pyramidal cells (compared to layer III) and interneurons. Betz cells (giant pyramidal cells located in the motor cortex of the precentral gyrus of the primate brain) are located in this layer that in turn project into white matter to form the descending pyramidal (corticospinal) tract. Other layer V neurons project to subcortical targets like

the brain stem, the striatum, and the spinal cord. The multiform layer (layer VI) is typified by the presence of fusiform cells though pyramidal cells and interneurons are also present. The efferent fibers from this layer end in the thalamus and the claustrum.

The layering of the neocortex has functional significance. The neocortex receives inputs from the thalamus, cortico-cortical connections, and other sources and sends outputs to both ipsilateral and contralateral neocortical areas as well as to the basal ganglia, thalamus, pontine nuclei, and spinal cord. Because the different inputs are processed differently and the varying outputs arise from different populations of neurons the layering is essential to provide an effective method of systematizing the input-output relationships of neocortical neurons. Though the neocortex is organized in a laminar fashion the exact nature of these layers is not homogenous throughout the brain. For example, in the occipital lobe the neocortex has an extremely prominent layer IV that can be divided into sublayers whereas the precentral gyrus, functionally known as primary motor cortex, has a near-absent layer IV. This stark anatomical contrast makes perfect sense when considered functionally. The internal granule layer receives its main inputs from sensory areas. Primary visual cortex receives a large amount of well-organized information from the lateral geniculate nucleus of the thalamus and thus it is necessary to have an exaggerated layer IV to process the incoming information. Primary motor cortex is an output area and thus the need for sensory input is marginal when compared to primary visual cortex. However, the presence of basket cells (neurons with many small dendritic branches that enclose the cell bodies of adjacent Purkinje cells in a basket-like array) and the expanded internal pyramidal layer is necessary for the output of motor information.

Figure 1- 2: Cortical histology of the human brain as revealed by the Golgi staining method showing 1) Molecular layer. 2) External granular layer. 3) External pyramidal layer. 4). Internal granular layer. 5) Internal pyramidal layer. 6) Multiform layer (adapted from Kiernan, 2005).

Figure 1 - 2: Neocortical Histology of the Human Brain

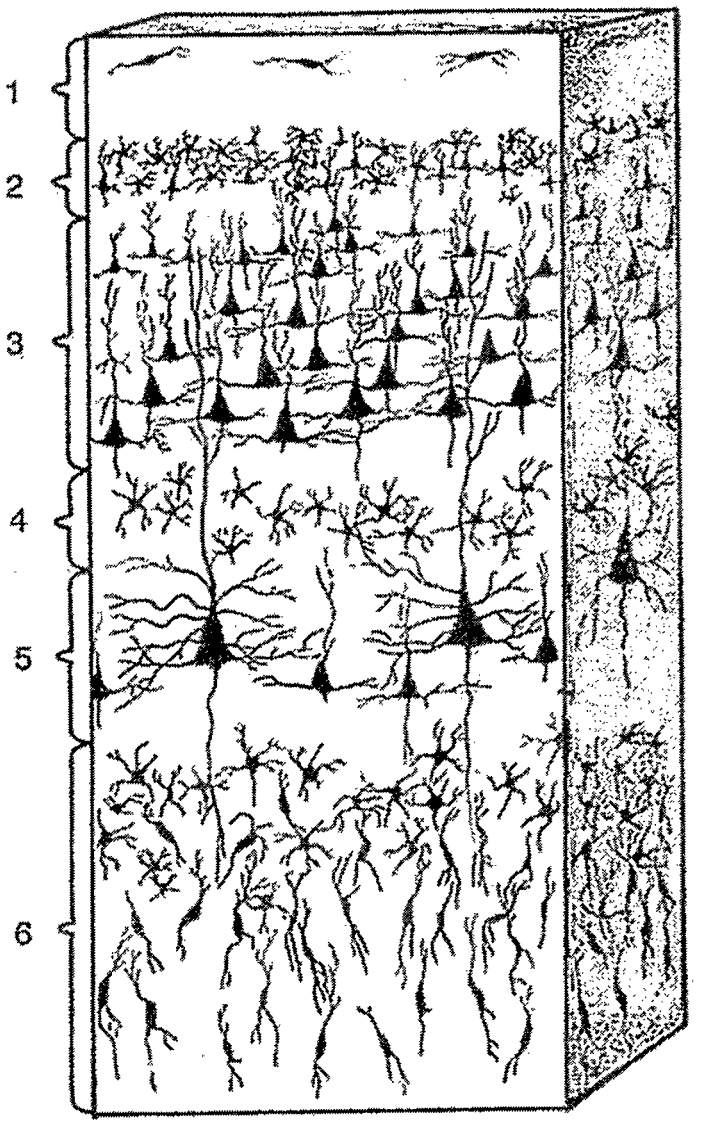
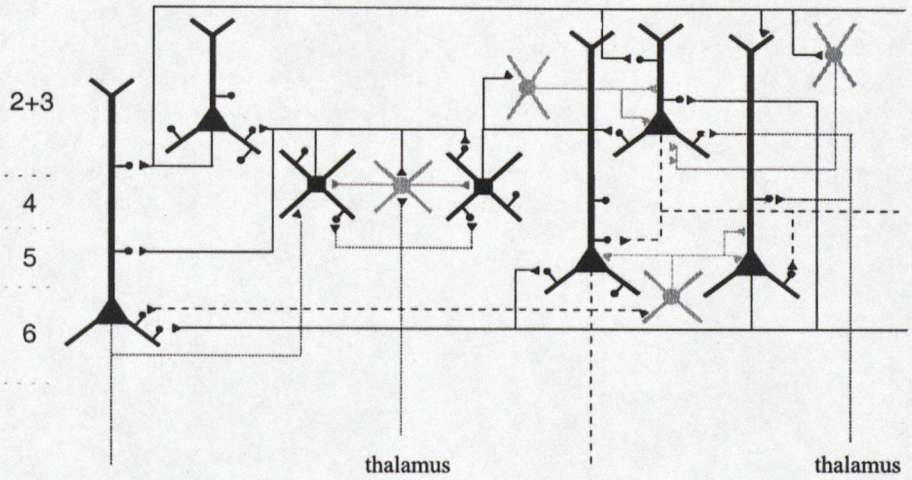


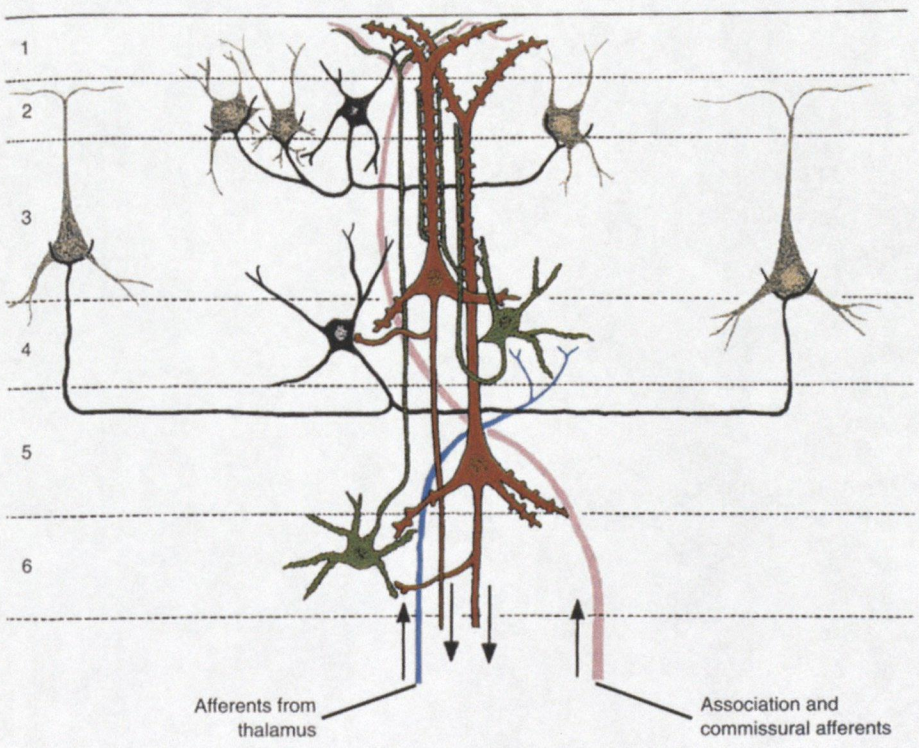
Figure 1 - 3: Corticocortical connections. The Basic circuit is shown in A.). Cortical layers as indicated. Gray indicates smooth GABAergic connections while black indicates spiny neurons and their connections (adapted from Shepard, 2004). Some intracortical connections are shown in B.) Thalamic afferents (blue) excite basal dendrites of pyramidal cells in layers 3 and 5 and stellate cells (green) in layers 4 and 6 which then excite pyramidal cells (red) in the same column. Branches of thalamic afferent and pyramidal cell axons excite basket cells (black) in layers 2 and 4 that inhibit pyramidal cells in adjacent columns (pink) (adapted from Kiernan, 2005).

Figure 1 - 3: Corticocortical Connections

A.



B.



There are two main types of neuronal cells in the neocortex: projection cells and interneurons. The principal projection cells in the neocortex are pyramidal cells. Pyramidal cells have a pyramid-shaped soma with an obvious apical dendrite emerging from the apex pointing toward the pial surface. The axon emerges from the base of the cell while sending out several collaterals before entering the sub-gray matter cortical white matter (Kiernan, 2005b). The excitatory nature of pyramidal cell axons supports evidence suggesting that glutamate is their primary neurotransmitter (Kiernan, 2005b). The axons of pyramidal cells connect with other cells in three ways. Projection neurons transmit impulses to sub-gray matter cortical locations like the corpus callosum and subcortical locations like the striatum, thalamus, brain stem, and spinal cord. Association neuron axons establish connections elsewhere within the same hemisphere, while the axons of commissural neurons make their connections in the contralateral hemisphere. Fusiform cells make up the remainder of the projection cells in the neocortex. They are far less abundant than their pyramidal counterparts. Though they are present in multiple cortical layers, they are predominant in the deepest cortical layer and as their name suggests, have spindle-shaped cell bodies.

The other main neuronal cell type, interneurons, can be readily identified based on their dendritic architecture. Stellate cells have dendritic spines and are located within the fourth neocortical layer. These small star-shaped cells are excitatory making their likely transmitter of choice glutamate. Virtually all other types of interneurons are inhibitory and therefore most likely use gamma-aminobutyric acid (GABA). Basket cells create horizontal connections with axons that connect directly to the cell bodies of pyramid cells. Cells of Martinotti are small multipolar nerve cells with short branching dendrites

scattered through various layers of the cerebral cortex with an ascending axon. Cajal-Retzius cells are small fusiform cell found in the superficial layer of the cerebral cortex with its long axis placed horizontally.

In addition to the laminar organization, the neocortex is also organized such that it forms minute functional vertical units known as columns that span from the most superficial to the deepest cortical layer. This organizational feature is particularly obvious in sensory areas (including visual cortex) and motor areas, as cells within a column respond similarly when stimulated because they are functionally a part of the same network. Though the number of cells in a given column is roughly the same across all neocortical areas, the cortical thickness of a given area of neocortex can vary when compared to another area in terms of the density of the cell packing. Primary visual cortex is perhaps the best example of this because not only are the cells twice as numerous when compared to other neocortical areas, but they are also packed much more densely, whereas an area as thick as motor cortex will invariably be less densely packed. When the differences in histology between different neocortical areas are considered, it becomes apparent that one cortical location cannot be studied in the same way as another cortical location. Thus it is important to the current thesis to explore motor neocortex in greater depth.

Primate Motor Neocortex

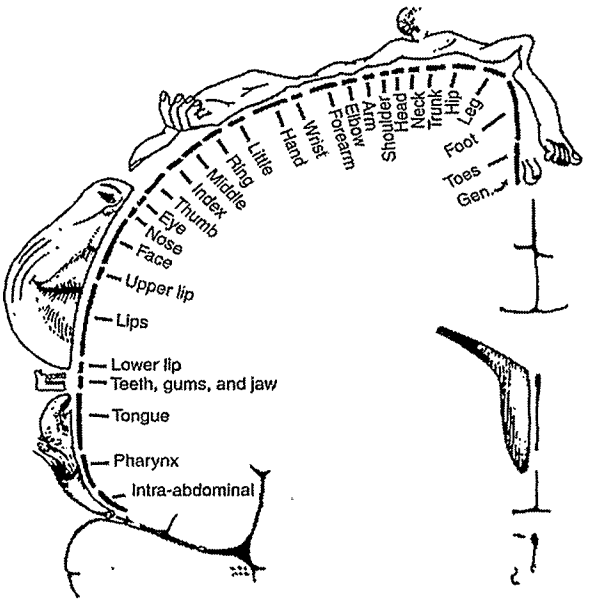
The primary motor area is defined based on the elicitation of motor responses at a low threshold of electrical stimulation. Though there are many areas of neocortex

dedicated to motor representation, primary motor cortex has the lowest threshold when electrically stimulated to elicit a response from skeletal muscle (Kandel et al., 2000; Kiernan, 2005b). In primates this area is located in the precentral gyrus including the anterior part of the paracentral lobule surface on the medial surface of the hemisphere and the anterior wall of the central sulcus. The defining histological feature of motor cortex is the Betz cells of layer V. The thin internal granular layer is primarily composed of cortico-cortical inputs and to a lesser extent includes input from the somesthetic cortex (pain, temperature, touch, pressure, position, and movement sensations) and the cerebellum via the posterior ventral lateral thalamus to layer IV. The Betz cells contribute 30,000 large, myelinated axons to the corticospinal tract of each side accounting for roughly 3% of the tract's axons. Stimulation of the primary motor cortex results in the contraction of muscles on the contralateral side of the body, with few ipsilateral exceptions such as most of the muscles of the head and axial muscles. The representation of the body along motor cortex is very stereotyped. The sequence of motor representation from the lateral edges of the cortex to midline starts with the pharynx, larynx, tongue and face followed by a small region comprised of muscles representing the neck. This is followed by an area representing the muscles of the hand disproportionate to the size of the hand, but functionally equivalent to the importance of manual dexterity in the primate. This is followed by smaller areas dedicated to the shoulder, trunk, and thigh; continuing on to the medial most cortical surface represented by the leg and foot respectively.

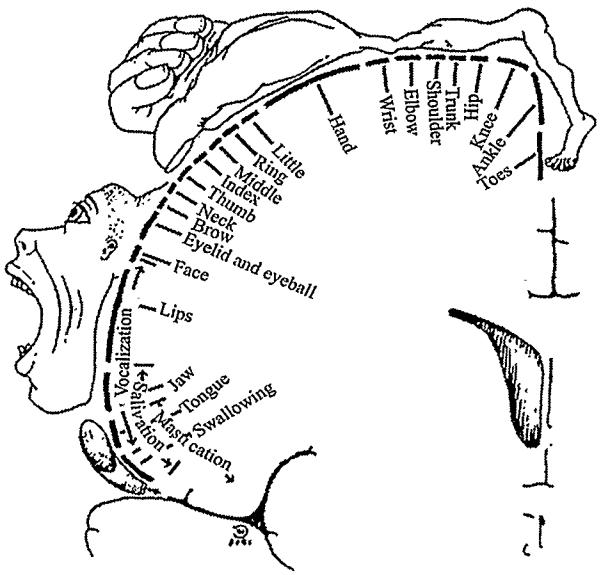
Figure 1 - 4: Human sensory (A) and motor (B) homunculus. Wilder Penfield did the original homunculus mapping (Penfield and Rasmussen, 1957), but made an error when he incorrectly mapped the human face upright, unlike all other primate species. In actuality humans are no different from other primate species in this respect (Servos et al., 1999). Penfield's error can be attributed to two factors: 1) the study participants were from a clinical population and may have undergone cortical reorganization. 2) The face representation is quite far down the side of the gyri in a very difficult location to map using a low resolution technique on the surface of the brain.

Figure 1 - 4: Human Sensory (A) and Motor (B) Homunculus

A.



B.

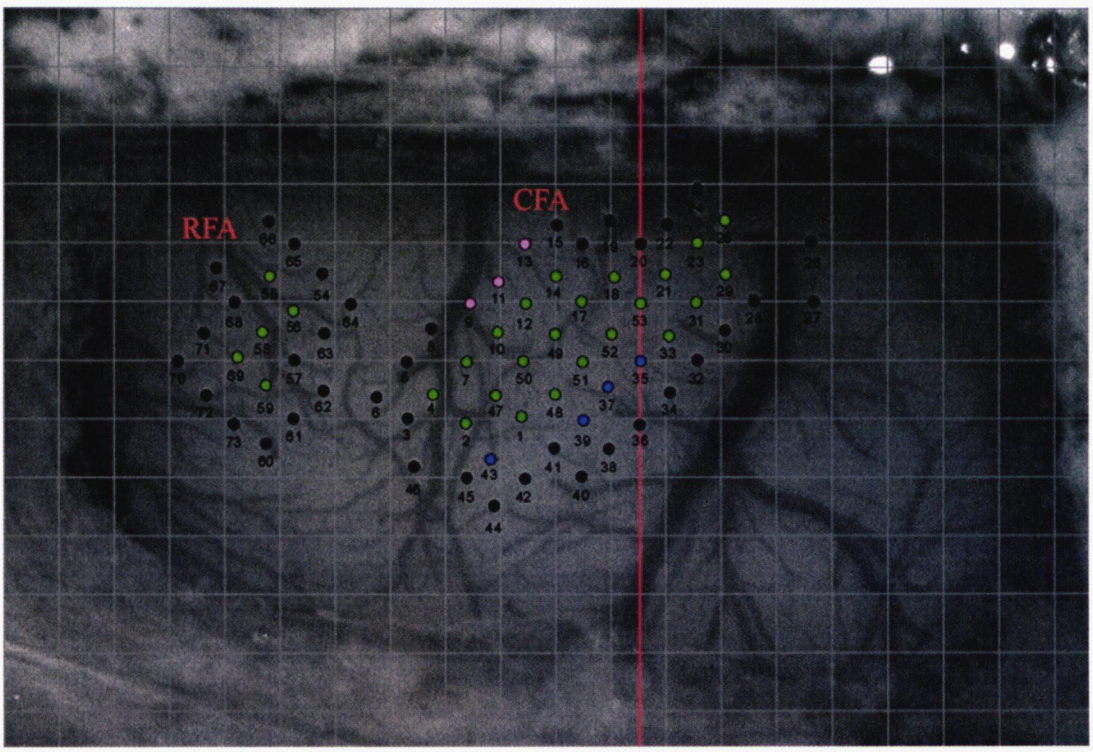


Rat Motor Neocortex

Whereas primates have well defined neocortical areas that function as either sensory or motor, rats have neocortical areas that are sensory, motor, or mixed. Much like their primate counterparts, layer IV is characteristically small in motor areas, but is much thicker in sensory areas. According to Zilles (1985) the motor cortex of the rat is made up of the agranular frontal area (Fr1, Fr2, Fr3), the forelimb area (FL) and the hindlimb area (HL). However, the mixed nature of sensory and motor areas in the rat makes defining motor cortex more difficult than this as there are many other areas of agranular cortex that can elicit movements (Donoghue et al., 1979; Sapienza et al., 1981; Neafsey et al., 1986). Others have argued that the differences in movement inducing threshold (granular areas require greater stimulation) are reflective of intracortical projections rather than somatotopical connections (Leong, 1983; Afsharpour, 1985; McGeorge and Faull, 1987). Motor cortex can be defined such that visceral, somatic, head, neck, tongue, and eye movements are included; nevertheless, the current thesis is only concerned with those areas of motor cortex that elicit shoulder, elbow, wrist, and digit movements that are the anatomical source of direct descending corticobulbar and corticospinal projections. The electrode locations in the current thesis are based on previous work using a methodology known as intracortical microstimulation (Stoney et al., 1968; Nudo et al., 1990; Monfils et al., 2004) where it was discovered that electrical stimulation of rat motor cortex resulted in plastic changes in the movement representation of the forelimb. The sub-portion of the motor cortex that upon stimulation yields a movement of the wrist, digits, elbow, and shoulder is defined as the forelimb areas. The topographic representation of the forelimb in the rat sensorimotor neocortex can be divided into functional areas that

Figure 1 - 5: Rostral (RFA) and caudal forelimb area (CFA) movement representations. Shade-coded topography of motor maps is represented from a sham control rat (green-wrist, blue-elbow, red- digits). The stimulating microelectrode was repeatedly lowered to layer 5 of left sensorimotor cortex, and a stimulation was applied (up to 60 μA) until a movement was elicited or no response was observed. External map boundaries (black-non-responsive, purple- neck, pink- whisker) were always defined as electrode penetrations that failed to elicit movement, or non-forelimb movements. A-P refers to the direction of the anterior-posterior axis. M-L refers to the direction of the medial-lateral axis. Horizontal and vertical bars represent 1mm. The red line marks Bregma.

Figure 1 - 5: RFA and CFA of Rat Sensorimotor Neocortex



are separated by a thin line of neck/whisker representations that include the caudal forelimb area (CFA) and the rostral forelimb area (RFA) (Kleim et al., 1998). The CFA is thought to be analogous to primary motor cortex in primates (Neafsey, 1990). It is also known to be more plastic than the RFA, and unlike the primary motor cortex of primates it is a mixture of motor and sensory functions (VandenBerg et al., 2002). Conversely, the RFA is thought of as being analogous to supplementary motor areas in primates (Neafsey, 1990), is less plastic than its caudal counterpart and is considered to be a strictly motor area (VandenBerg et al., 2002).

Karl Lashley's early studies on the sensorimotor neocortex of rats and monkeys yielded unexpected results. In several experiments Lashley ablated motor cortex in several rats and monkeys and found that over time, the general behaviour of the animals was unaffected. Lashley's experiments did not find an effect for two major reasons: first, his experiments were conducted on animals in their early infancy, and second, he was not assessing skilled behaviour, but was instead looking for gross impairments in locomotion and unskilled behaviour. In contrast, Castro (1972) found that cortical ablations did alter digit use in rats using an experimental design that tested their digital motor capabilities. Castro's work showed that despite their phylogenetically different position, rats do indeed possess a highly structured motor system. To further bolster Castro's findings more recent research has found that motor maps can be altered through either skilled motor learning in squirrel monkeys (Nudo and Milliken, 1996; Nudo et al., 1996) and rats or electrical stimulation in rats (Nudo et al., 1990; Flynn et al., 2002; Teskey et al., 2002a; Monfils et al., 2004). The anatomical layout of the descending tracts will now be described.

Descending Tracts

The descending tracts are an important link between brain and behaviour as they are the major projections from motor neocortex to the periphery. The descending tracts emerge from cortical white matter, and eventually go to the spinal cord and peripheral nervous system where activation of the tracts results in a net output of motor behaviour. Motor neurons in the lateral and ventral funiculi (nerve bundles) of the spinal cord respond to the descending tracts of the cerebral cortex, central nucleus of the reticular formation and the lateral vestibular nucleus. The lateral pathway is the chief output from the cortex to skeletal muscles and is thus subject to conscious or directed control. The ventral portion of the spinal cord falls under the control of the brain stem and is concerned with postural control and locomotion. Though the current thesis is primarily concerned with cortical stimulation and the ensuing effects on behaviour via the lateral pathways, the ventral pathway will also be expanded on due to its importance in cortically mediated movements, namely reaching.

The lateral pathway is composed of two major tracts: the corticopinal tract and the rubrospinal tract. Motor corticospinal fibers arise in primary and supplementary motor cortex and cingulated motor areas of the frontal lobe. The corticospinal pathway is both parallel with axons descending from all areas of motor cortex, and hierarchical with primary areas receiving fibers from association areas that in turn receives its information from prefrontal, parietal, and temporal association cortex. This hierarchy brings motor output under the influence of previously processed sensory information. Corticospinal

fibers pass through the white matter along with corticobulbar fibers and converge in the posterior limb of the internal capsule, which lies between the lentiform nucleus and the thalamus. At the reticular formation the corticospinal tract branches apart. Some of the fibers terminate at the red nucleus, others break up into fasciculi, while others still reconvene along with fibers from the corticobulbar tract at the caudal most portion of the pons on the ventral surface of the medulla to form the corticospinal tract. It is at the caudal end of the medulla that 85% of the corticospinal fibers decussate and enter the dorsal half of the lateral funiculus of the spinal cord to form the lateral corticospinal tract. The ventral corticospinal tract is comprised of the remaining 15% of the corticospinal fibers and descends ipsilaterally in the medial funiculus of the spinal cord. Corticospinal fibers terminate on interneurons in the dorsal portion of the internal gray matter of the spinal cord that influence motor neurons controlling skeletal muscles, while corticobulbar fibers mediate jaw and tongue movements. Much of what is understood about the corticospinal tract is gleaned from case studies and animal studies where lesions have occurred. In humans, lesions to this area result in a temporary contralateral flaccid hemiplegia and a permanent deficit in digit dexterity. Experimental lesions to both the corticopinal and rubrospinal pathways showed similar results in monkeys where hand and digit dexterity was lost, while postural control was maintained (Kiernan, 2005a).

The rubrospinal tract, a somatotopically organized fiber bundle, is much smaller than the corticospinal tract and in humans goes no further than the second cervical segment of the spinal cord. It originates in the magnocellular portion of the red nucleus in the midbrain where its fibers decussate and descend through the medulla to the dorsal part of the lateral column of the spinal cord at the ventral border of the lateral

corticospinal tract. It terminates in the zona intermedia of the spinal cord where its distribution coincides with that of the lateral corticospinal tract. In contrast to the latter it appears not to have direct connections with spinal motor neurons. Impulses conveyed by this tract indirectly increase flexor muscle tone. For rats, cats and monkeys the rubrospinal tract is largely responsible for the fine control of distal limb muscles used for the manipulation of objects, whereas in anthropoid apes and humans, the corticospinal tract largely assumes control of object manipulation.

The medial pathways of the spinal cord primarily mediate postural control. Phylogenetically older, these pathways include the vestibulospinal tracts (medial and lateral), reticulospinal tracts (medial and lateral) and the tectospinal tract. The vestibulospinal tracts are somatopically organized fiber bundles originating from the lateral vestibular nucleus (nucleus of Deiters) which descends uncrossed into the anterior funiculus of the spinal cord lateral to the anterior median fissure that extends throughout the length of the spinal cord, distributing fibers at all levels to the medial part of the anterior horn. Excitatory impulses conveyed by the vestibulospinal tract increase extensor muscle tone. The reticulospinal tract denotes a variety of fiber tracts descending to the spinal cord from the reticular formation of the pons and medulla. Part of these fibers conduct impulses from the neural mechanisms regulating autonomic functions to the corresponding somatic and visceral motor neurons of the spinal cord while others form links in nonpyramidal motor mechanisms affecting muscle tonus, reflex activity, and somatic movement. The tectospinal tract is a bundle of thick, heavily myelinated fibers originating in the deep layers of the superior colliculus, decussating at the dorsal tegmentum, descending along the median plane, between the medial longitudinal

fasciculus dorsally and the medial lemniscus ventrally, into the anterior funiculus of the spinal cord. The tract ends in the medial region of the anterior horn of the cervical spinal cord, and is involved in head movements during visual and auditory tracking. Throughout its course in the brainstem it is accompanied by fibers of the tectobulbar tract (Kandel et al., 2000). An understanding of the anatomy of the central nervous system areas involved in forelimb-related behaviours allows for a more thorough investigation underlying the behaviours themselves. The reaching behaviour in the rat and some of the work done on the functional reorganization of the CFA will now be reviewed.

Reaching Behaviour of the Rat

Rats have a highly stereotyped reaching behaviour that can be broken down into consistent and precise subcomponents. However, it was not always known that rats possessed fine coordination in their reaching behaviour. By comparing rats that had to reach for food in a trough and rats that had to dig for food, Bracha and colleagues (1990) proposed that the reaching behaviour of rats was merely an altered form of digging behaviour. Their results showed that the digging movement essentially resembled reaching except that the two forepaws alternated, the extension amplitude was shorter and grasping never terminated the digging movement. This idea was contradicted by Whishaw, Pellis, and Gorny (1992c) in a study that compared the reaching behavior of rats to that of humans. Not only was it discovered that rats reach in a highly stereotyped manner displaying several consistent subcomponents, but that the reaching behaviour of rats and primates were remarkably similar. Both species displayed more supination and lengthened grasping times when reaching for small as opposed to large objects; moreover, both species also moved the reaching limb medially using the proximal limb to

aim when they were required to reach through an aperture, but in a free reaching test only rats continued to aim. Human movements were far more variable, showed more independent digit use, and less distinct subcomponents than rats (Whishaw et al., 1992c). Primates and rats also differ in that primates guide their reaching using vision where rats use olfaction (Whishaw and Tomie, 1989). Despite obvious interspecies differences in reaching behaviour, the similarities in movements suggests that there is evidence for parallel development indicating that primates and rats may be more homologous than analogous with respect to this specific behaviour.

Subcomponents of the Reaching Behaviour in Long Evans Rats

Because different species of rats reach in different ways (Whishaw et al., 2003) only the reaching pattern and movements of the Long Evans rat will be described here as it is this species that was used in the current thesis. Movements were analyzed using a rating scale derived using the Eshkol-Wachman Movement Notation system. Eshkol-Wachman Movement Notation expresses relations and changes of relation between the parts of the body by treating the body as a system of articulated axes (i.e. body and limb segments). A limb is defined as any part of the body that either lies between two joints (shoulder to elbow) or has a joint and a free extremity (wrist to digits). Limbs are treated as straight lines (axes) of a constant length that move with one end fixed to the center of a sphere. Of note about the EWMN system is that the same movements are notated in several polar coordinate systems where the coordinates of each system are determined with reference to the surrounding environment, the animal's midline axis, and to the next proximal or distal limb/body segment. Comparing a behaviour that appears the same

along different axes elucidates consistencies within a behaviour that may be present along one set of coordinates, while remaining absent along another set of coordinates. Thus, a behavioural subcomponent may be invariant in relation to the animal's longitudinal axis in one instance where it will vary in relation to the next proximal or distal segment. The reaching behaviour and its subcomponents have been described in different instances with minor differences in their respective details (Whishaw et al., 1991; Whishaw et al., 2003). The current thesis used the exact criteria set out by Whishaw and colleagues in 2003 and are further described in the methods section of Study 1. Reaching behaviour in the rat is fairly consistent across different reaching tasks ranging from the single-pellet reaching task (Whishaw and Gorny, 1994) to free-feeding reaching from a tray (Whishaw et al., 1992b) to pasta reaching (Ballermann et al., 2000).

Reaching as a Movement Pattern

Reaching can be thought of as a movement pattern, where a movement pattern is defined as a kinematic sequence used by an animal that is distinctive and recognizable from one instance to another (adapted from Metz and Whishaw, 2000). Metz and Whishaw (2000) were able to illustrate this point using kinematic analysis on rats to assess changes in reaching behaviour where pellet size varied. Their results revealed rather consistent behaviour lending credence to the idea that reaching behaviour in the rat is a stereotyped pattern. It has been theorized that rats' reaching movement is ballistic and can only be adjusted by trial and error (Zhuravin and Bures, 1986). That is to say, rats do not use sensory feedback to make online corrections to an error, but instead make adjustments on the next attempt. Ballerman and colleagues (2000) showed that rats use

haptic (tactile) cues to aid in both locating and identifying a target that has not been examined. Rats trained on the pasta matrix reaching task were presented with either metal rods or pasta. The force with which the rats attempted to retrieve the target (either pasta or a metal rod) was recorded using a force transducer. Rats applied more force to retrieve the metal rods, indicating that haptic information is used to make discriminations between different targets. These results indicate that subcomponents of the reaching movement pattern such as postural orientation and limb transport trajectory were not modified as a function of target orientation; however, hand orientation and grasp force did vary as a function of the sensory qualities of the target object. Further investigation by Ballerman and colleagues (2001) showed a sensory alteration in rats with dorsal column lesions that were "unable to discriminate a food item from a tactually distinctive nonfood item as part of the reaching act," while their overall performance on the reaching task remained equal to that of the control group. This finding suggests that the dorsal columns are crucial for haptic discrimination, a contributor to the typical performance of the grasping subcomponent of the reaching behaviour. The use of haptic cues to reach suggests that the reaching behaviour of rats is not ballistic at all, but instead relies heavily on the ability of the brain to make online adjustments allowing for a high degree of modification.

Lesion Studies

Motor cortex lesions have been shown to cause impairments in the reaching success of rats (Whishaw et al., 1991; Teskey et al., 2003); moreover, the degree of impairment is dependent on the size of the lesion (Whishaw et al., 1991). Video analysis

and Eshkol-Wachman Movement Notation system analysis showed that the rats' impairments in reaching were due to (1) "an inability to pronate the [hand] over the food by abduction of the upper arm, and (2) an inability to supinate the [hand] at the wrist to orient the food to the mouth" (Whishaw et al., 1991). All of the lesioned rats showed mild impairments in lifting, aiming, and advancing the limb, regardless of lesion size. Moreover, rats with medium and large lesions also showed a loss of pronation and supination. These losses would affect the angle at which the hand contacts the target, and the angle at which the target is released relative to the mouth, which would in turn affect the overall reaching success of the rat. To compensate for the alterations in pronation and supination a variety of whole body movements, particularly involving the head and shoulders were employed by the rats. The ability of rats to reach after a lesion improves to near-baseline levels as time progresses after the insult (Whishaw et al., 1991). This suggests that the early impairment is due to the loss of the receptive fields of the cortical area that supports the movement patterns used for skilled movements (Whishaw, 2000). Furthermore, subsequent recovery in reaching success is not due to the recovery of typical movements, but instead reflects the use of compensatory movements to achieve the same end result independent of practice (Whishaw, 2000). Free-feeding rats with motor cortex lesions also show impairments in forelimb grasping (Whishaw et al., 1992b). When free-feeding, limb movements are typically bilaterally symmetrical, but when asymmetrical movements are necessary, the preferred forelimb initiates the movement. However, after unilateral motor cortex lesions to the forelimb area the unaffected (non-preferred) limb initiated lifting, positioning, and grasping movements. The ability to make appropriate adjustments to the size of the target of the digits of the

affected limb (bad limb) was impaired. When the target was missed, the bad limb remained extended with a closed-fist spastic posture and could not be repositioned independently (Whishaw et al., 1992b). Similarly, unilateral lesions to the corticospinal tract caused impairments on the single-pellet reaching task (Whishaw et al., 1993). Almost all of the reaching subcomponents, except those involved in digit opening to obtain the pellet were impaired, including lifting, aiming, pronating and supinating the limb, and releasing food. Reaching behaviour can be altered in ways independent of lesions. The CFA is also capable of reorganization in response to training and different kinds of stimulation that will be described in further detail in the following section.

Anatomical and Physiological Reorganization of the CFA

The CFA of rat sensorimotor cortex will be the focus in the proposed studies. This brain area was chosen because proper use of the forelimb is dependent on the integrity of the CFA (Whishaw, 2000) and plasticity of the CFA is observed following skilled learning (Nudo et al., 1996; Kleim et al., 1998) and kindling (Teskey et al., 2002; Goertzen et al., 2003). The functional reorganization of the CFA in response to training and stimulation can be seen at the structural level. Withers and Greenough (1985; 1989) have shown that reach training can induce dendritic hypertrophy within rat neocortex. It has also been shown that within the CFA the number of synapses per neuron shows an increase in layer V (Kleim et al., 2002). An increase that is conspicuously absent within the RFA and in hindlimb areas (Kleim et al., 2002). Changes in spine density can be indicative of the number of synapses as an increase in the number of dendrites implies the formation of more synapses. The changes in dendrite size and shape can occur through

the formation of new dendritic material through the lengthening of existing dendrites, an increase in the number of dendritic branches, or by increasing dendritic spine density (Horner, 1993). Electrical stimulation driven changes in dendritic morphology have also been reported (Teskey et al., 1999; Ivanco et al., 2000; Teskey et al., 2001; Monfils et al., 2004). Ivanco and colleagues reported that LTP induction increased the dendritic length in apical neurons and increased the spine density of basilar and apical layer III neurons (Ivanco et al., 2000) where Teskey and colleagues reported (1999; 2001) a decrease in spine density, dendritic length, and dendritic complexity due to kindling. These kindling induced changes went back to baseline levels upon a follow-up analysis of the morphological changes three weeks after the last seizure (Teskey et al., 2001; 2006a).

In addition to the changes seen in dendritic morphology, changes can also been seen in the shape and size of spinous synapses (Desmond and Levy, 1986a, b). Increases in the number of perforated synapses and more complex dendritic arborization (Greenough et al., 1973), and an increase in spine density of rats reared in complex environments has been reported (Greenough et al., 1973). Rats kindled along the transcallosal pathway also showed a change at the synaptic level. Though there was not a significant difference in synaptic density between the tissue of the kindled rats when compared to the tissue from non-kindled rats, there was a significant increase in the total number of synapses per mm^3 of the more efficacious perforated subtype that made axodendritic connections in layer V of the CFA from kindled as compared to non-kindled rats (Goertzen et al., 2003). In a powerful demonstration of the link between anatomical and functional changes within the CFA, Kleim and colleagues (2002) found that rats trained on the single-pellet reaching task had an increased number of synapses per neuron

within layer V of the CFA. These differences were not seen in either the RFA or the hindlimb areas. The functional changes seen in the CFA are detailed in the next section.

Functional Reorganization of the CFA Network

Both learning and electrical stimulation induce well-documented changes in the CFA and provide important insight into the plasticity of the rat neocortex. The functional changes associated with both learning and electrical stimulation on the CFA are also well documented. The functional changes in the CFA are investigated using a technique known as intracortical microstimulation. Briefly, intracortical microstimulation involves lowering a micro-electrode into motor cortex, then stimulating it and documenting the elicited movement. This process is repeated at several locations across the neocortex to create a topographical representation of motor cortex thus creating a map of the receptive fields within motor cortex (Fig. 5). In the rat, a typical motor map of the CFA is comprised of approximately 50 points and includes shoulder, elbow, wrist, and digit representations. Nudo and colleagues (1996) used intracortical microstimulation to map the motor cortex of squirrel monkeys trained on a skilled reaching task and found digit representation had increased at the expense of wrist representation. Showing the same distal bias, Kleim and colleagues (1998) demonstrated that rats trained on a skilled reaching task also show an increase in wrist and digit representations at the expense of elbow/shoulder representation when compared to rats trained on a simple bar-pressing task. To build on the anatomical changes previously described (Kleim et al., 2002), rats trained on a skilled reaching task exhibited an areal expansion of wrist and digit movement representations within the motor cortex. Again, no differences were seen in

either the RFA or the hindlimb representation, making this functional reorganization CFA specific, and parallel to the anatomical changes. The co-occurrence of functional and anatomical plasticity within the same cortical regions points to the notion that synaptic formation underlies learning dependent changes in cortical function.

Functional changes in the CFA can also be induced via electrical stimulation. Nudo *et al.* (1990) found that the electrical stimulation used in repeated intracortical microstimulation procedures alone was enough to induce changes in motor representations, even when the stimulation levels were below movement threshold. Using a much greater stimulation, Teskey and colleagues (2002a) found that kindling increased motor map representations of the CFA. The expansion seen was also distally biased, but the overall expansion was non-discriminate and bi-directional in terms of its effects on the forelimb from shoulder to digits. Much like Kleim's parallel findings between anatomical and functional changes, Teskey and colleagues (2002b; 2004) also found a parallel finding between kindling and LTP-induced map expansion and electrophysiological changes where it was discovered that the polysynaptic component of the evoked potential increased with map size. Long term depression was found to have opposing effects to LTP (Teskey et al., 2006b). Teskey and colleagues (2006b) found that rats that underwent long term depression had decreases in the number of perforated synapses, the size of the polysynaptic component of evoked potentials, and map size with an increase in the number of inhibitory synapses.

Table 2: Summary of the changes observed in the CFA due to kindling. Kindling in sensorimotor cortex of the rat brain leads to several specific changes ranging from the ultrastructural level to the network level. The current thesis will add to the multilevel analysis by examining changes at the behavioural level.

Table 2

LEVEL	CHANGES OBSERVED
Ultrastructural	↑ Perforated, Excitatory Synapses/ Neuron (Layer V)
Structural	↓ 30% Neurons (Layer V)
Dendrites	↓ Apical and Basilar Length ↓ Apical and Basilar Branching (Layer V)
Electrophysiology	↑ Polysynaptic Component Size of Evoked Potential (Layer V)
Network	↑ Overall Size, ↑ Distal Representation (Layer V)
Behaviour	This Thesis

Present Thesis: Objectives and Hypotheses

The present thesis had two major objectives. The first objective was to discover and describe the effects of repeated seizures on motor coordination and learning using an unskilled task assessing gross motor behaviour, and two skilled tasks assessing skilled motor coordination. The second objective was to measure and describe the types of changes in motor coordination relative to a sham control group, an amygdala kindled group, and a bilateral motor cortex lesion group.

Study 1

For Study 1, the effects of seizures (using the electrical kindling model) on gross and fine motor coordination and the possible mediation of those effects by anxiety and/or memory changes was investigated. This was an important experiment because it allowed for three distinct questions to be asked: 1) does kindling result in disrupted performance? 2) Do rats that have had repeated seizures show alterations in motor coordination? 3) Are these changes due to altered anxiety levels or memory impairments? Previous research has focused on other interictal behavioural alterations in kindled rats such as memory, anxiety, and sexual dysfunctions. While kindling does reliably model the aforementioned behavioural changes commonly seen in people with epilepsy, the effects of kindling on motor coordination have not yet been explored. Seizures were elicited in a cortically kindled group and an amygdala kindled group, and the gross and fine motor behaviours were assessed relative to a sham control group. This is the first experiment to investigate the consequences of seizures on motor coordination. Previous research found changes at the structural and ultrastructural levels (Goertzen et al., 2003), and at the network level

(Teskey et al., 2002a) due to kindling. Furthermore, skilled-training was also found to induce a functional reorganization of the caudal forelimb area (Kleim et al., 1998). **It was hypothesized that neither the cortically kindled nor the amygdala kindled rats would show changes in gross motor behaviour relative to sham controls as revealed by the cylinder task through wall touches and landings. However, it was hypothesized that cortical kindled rats would show alterations in skilled motor behaviour relative to sham controls as revealed by the rung walking task through the number of errors per step, and the severity of errors as well as by the single pellet reaching task through a decreased success rate and alterations in the kinematics of the reaching behaviour.**

Two different groups of rats (sham control and cortically kindled) underwent the same experimental manipulations but were assessed for memory dysfunction and altered levels of anxiety. This was an important study as it allowed us to properly attribute the results of the first experiment to either a functional lesion created by kindling, or to heightened levels of anxiety and/or memory dysfunction. Previous research has investigated changes in memory and anxiety due to kindling, but all of those studies kindled in structures known to mediate those particular behaviours and used different stimulation protocols. **It was hypothesized that the cortically kindled group would not show any changes in anxiety level as revealed by the elevated plus maze, or memory as revealed by the Morris water maze relative to a sham control group.**

Study 2

The effects of seizures on motor coordination (kindled lesion) were compared with the effects of a focal devascularization (complete lesion) on motor coordination. This was an important study because it allowed for the further clarification between lesion types and their ensuing behavioural effects. Previous research has focused on the effects of one lesion type or the other, but none of those studies has compared two distinct lesion types where one type of lesion (kindled lesions) was thought to be functional. **It was hypothesized that neither the cortically kindled nor the focal devascularization (complete) lesion rats would show changes in gross motor behaviour relative to each other and to sham controls as revealed by the cylinder task through wall touches and landings. It was further hypothesized that cortical kindled rats would show kinematic alterations in skilled motor behaviour relative to each other as well as to sham controls as revealed by the rung walking task through the number of errors per step, and the severity of errors as well as by the single pellet reaching task through a decreased success rate and alterations in the kinematics of the reaching behaviour and that these alterations would be more profound in the lesion group.**

Study 1: Repeated Seizures Create a *Functional* Lesion in Rat Sensorimotor Cortex.

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Running title: **Repeated Seizures in Sensorimotor Cortex**

First Author Contribution:
Electrode implantation surgery, Kindling, Behavioural testing and scoring. Manuscript Preparation – creation of written document including writing, statistical analysis and interpretation, and creation of figures.

Key words: epilepsy, motor coordination, motor cortex, skilled behaviour, learning

INTRODUCTION

Clinical reports suggest that epilepsy disorders have several interictal behavioural changes associated with them (Engel and Pedley, 1998; Tellez-Zenteno et al., 2005). People with epilepsy have been reported to have higher levels of anxiety and depression (Blumer et al., 1995; Giovagnoli et al., 1997; Nubukpo et al., 2004a; Nubukpo et al., 2004b), verbal and spatial memory deficits (Giovagnoli et al., 1997; Schwarcz and Witter, 2002; Alessio et al., 2004; Reminger et al., 2004; Crane and Milner, 2005; LoGalbo et al., 2005) as well as gender specific sexual dysfunctions (Souza et al., 2000; Isojarvi, 2003; Isojarvi et al., 2004). However, it is difficult to differentiate between the effects of repeated seizures from the effects of antiepileptic drugs, social stigma, and individual differences in people with epilepsy (Engel and Pedley, 1998; Wiebe et al., 2002). It is therefore necessary to have an animal model whereby the effects of repeated seizures can be studied in a relatively homogenous population that can rule out social stigma and drug effects (Engel and Pedley, 1998). Many of the interictal behavioural changes have been modeled using kindling (Becker et al., 1992; Kalynchuk et al., 1998b; Edwards et al., 2000; Hannesson and Corcoran, 2000). Kindled animals have been found to show altered aggression-related behaviours (Pinel et al., 1977; Kalynchuk et al., 1998b; Wintink et al., 2003), stress responses (Henke and Sullivan, 1985), spatial abilities (Gilbert et al., 2000; Hannesson et al., 2001b; Hannesson et al., 2001a; Hannesson et al., 2004; Hannesson et al., 2005), sex hormones and sexual function (Edwards et al., 1999a; Edwards et al., 1999b; Edwards et al., 1999c; Edwards et al., 2000; Edwards et al., 2002). Taken together the results from the clinical and kindling

studies suggest that repeated seizures result in a functional lesion of the involved structures.

In order to further test the hypothesis that repeated seizures result in local areas of the brain displaying distorted function we examined the caudal forelimb area (CFA) of the rat sensorimotor neocortex. The CFA of rat sensorimotor cortex is an ideal structure to examine because the proper use of the forelimb in skilled reaching behaviour is dependent on the integrity of the CFA (Whishaw, 2000), it can be kindled (Teskey et al., 2001; Flynn et al., 2002; Teskey et al., 2002a, b) and it is amenable to pre and postmortem analysis.

The effects of kindling in sensorimotor cortex have been examined extensively. Cortical areas play a role in seizure progression whether they are they focal site or not (Corcoran et al., 1975). Investigations at the cellular level revealed that after 25 kindling sessions, kindled rats showed a simultaneous cell loss and excitatory synapse increase in layer V (Goertzen et. al, 2003). Kindled rats also show altered dendritic morphology showing paradoxical decreases and increases in dendritic length and branching in layer III and layer V respectively at different time points (Teskey et al., 2001; Teskey et al., 2006a). Examination of the late component of evoked potentials in kindled rats has revealed that kindling leads to an increase in the size of evoked potentials (Racine et al., 1975; Teskey et al., 2001; Teskey et al., 2002a). Likewise, Teskey and colleagues (2002a; 2006) also looked at changes in motor map representation and found that kindling lead to an unprecedented doubling in the size of the anterior portion of the motor map representing the forelimb area (CFA).

The current study looked at the effects of kindling on motor behaviour following repeated seizures in the sensorimotor neocortex (specifically in the CFA) and the amygdala, and then examined the performance of the rats on behavioural tests sensitive to changes in motor coordination. The amygdala group was included to investigate alterations in motor coordination from repeated seizures originating from an extra-motor brain area. The cylinder task was employed to assess gross motor behaviours. It is hypothesized that the cylinder task will show no distinguishable differences between either of the kindled groups and sham control rats because the cylinder task measures unskilled behaviour. The rung walking task and the single pellet reaching tasks were used to assess skilled motor behaviour. It was hypothesized that cortically kindled rats would show greater impairment manifested in an increase in errors per step and in severity of errors on the rung walking task and an altered reaching behaviour on the single pellet reaching task. The elevated plus maze and the Morris water maze were also employed to evaluate the possibility that the altered motor behaviours may have been due to heightened anxiety levels or memory dysfunction.

METHODS AND MATERIALS

Experiment 1

Subjects

Twenty-four male Long-Evans rats, weighing between 331 and 426 g at the time of surgery, were used. The rats were obtained from the University of Calgary Breeding Colonies. All animals were housed individually in clear plastic cages in a colony room that was maintained on a 12h on/12h off light cycle. All experimentation was conducted

during the light phase. Rats were fed Lab Diet no. 5001 (PMI Nutrition International Inc, Brentwood, MO) and drank water *ad libitum*, except during reach training where the rats were food-restricted to 90% of their post-kindling weight. The rats were handled and cared for according to the Canadian Council on Animal Care guidelines.

Electrode Implantation

Rats were anesthetized with 58.53 mg/kg ketamine-xylazine (85:15) 1 ml/kg, injected intramuscularly. Lidocaine 2% (Austin, Joliette, QA) was administered subcutaneously at the incision site. Two bipolar electrodes were chronically implanted according to the stereotaxic coordinates of Swanson (Swanson, 1992). The stimulating electrode was implanted 1.0 mm anterior to bregma, on the midline, in the callosal matter. The recording electrode was implanted 1.0 mm anterior to bregma, and 4.0 mm lateral to midline, in the right frontal neocortex corresponding to the CFA. The CFA is defined as part of the area of sensorimotor neocortex in the rat that controls forelimb movements. Rats in the amygdala-kindled group received the same surgery with only the electrode locations differing: electrodes were bilaterally implanted 2.8 mm posterior to bregma, ±5.0 mm lateral to midline, and 8.5 mm ventral to surface in the basolateral amygdala.

The electrodes were Teflon-coated, stainless steel wire, 178 μm in diameter (A-M Systems, Everett, WA). One end of the electrodes was stripped of the Teflon coating and connected to gold-plated male amphenol pins. The two poles of the electrodes were separated by 1.0 mm for the recording electrode, and 0.5 mm for the stimulating electrode. In the case of the amygdala implants, both electrodes had a tip separation of 0.5 mm. Electrophysiological monitoring was performed during cortical electrode

implantation in order to adjust the dorsal-ventral placements to maximize evoked response amplitude. Histological analysis revealed that every rat in the callosal-kindling group had the stimulating electrode located in the corpus callosum, and the recording electrode located in the right CFA of motor cortex. The upper pole of the recording electrode was located in layer II/III, and the lower pole in deep layer V or upper layer VI. Of the six rats in the amygdala-kindled group, the electrodes were bilaterally implanted in the amygdalae of four of the rats while the other two had only one electrode correctly implanted in the amygdala. The gold-plated amphenol pins connected to the electrodes were inserted into a nine-pin McIntyre connector plug, and attached to the skull with four stainless steel screws in the top of the skull, a screw in each side of the skull, and dental cement. One of the stainless steel screws served as the ground reference. Experimental procedures commenced no earlier than seven days after surgery.

Treatment Groups

After surgery, each rat underwent baseline behavioural testing on the cylinder task and the rung walking task. Once baseline measures had been obtained the rats were divided into three groups: sham control, cortical kindled, and amygdala kindled. An afterdischarge threshold was determined for each rat in the cortical and amygdala kindling groups. The three groups received either 21 handling sessions (n= 9), callosal kindling stimulations (n= 8), or amygdala kindling stimulations (n=6) for 21 days. Twenty-four hours following the last stimulation or handling session early post-kindling measures were taken on the cylinder and ladder tasks. The rats then began the single-pellet reaching task. After 15 days of reaching, late post-kindling measures on the rung walking task were taken.

Kindling

The afterdischarge thresholds (ADT) were defined as the weakest current required to elicit an afterdischarge (AD). The current delivered commenced at 100 μA, increasing in steps of 100 μA, and was delivered at 60 s intervals until an afterdischarge of at least 4 s or longer was recorded. Kindling stimulations were delivered to awake, freely moving rats.

A once daily kindling stimulation was delivered through the electrode positioned in the callosal white matter or the first amygdala to yield an afterdischarge, depending on group designation. Stimulation consisted of a 1 s train of 60 Hz biphasic rectangular wave pulses, 1 ms in duration and separated by 1 ms, at an intensity 100 μA greater than ADT. A paper record of the resultant AD was obtained from both electrodes and the seizure behaviours were noted. The AD from the neocortical recording electrode or non-stimulated amygdala electrode was scored for duration (Teskey and Racine, 1993). The seizure behaviours were monitored and scored according to a five-stage scale (Racine, 1972).

Video recording

All behavioural testing was recorded using a Canon NTSC ZR60 digital video recorder (1/1000 of a second shutter speed). A Toshiba DR-1 DVD recorder was used for subsequent frame-by-frame analysis.

Cylinder Test Apparatus and Quantitative Analysis

Forelimb use during exploratory activity was assessed by placing rats in a transparent cylinder 20 cm in diameter and 30 cm in height (Schallert et al., 2000). The cylinder was placed over top a sheet of clear Plexiglas. A mirror was placed underneath the cylinder and Plexiglas sheet at a 45° angle to allow assessment of the rat's behaviour when it was not facing the camera. Each forelimb contact with the cylinder wall and Plexiglas floor was counted. The rats were individually placed in the cylinder for 5 minutes during each testing session (baseline, early post-kindling, and late post-kindling). A mean difference score was calculated by subtracting the number of hand placements counted when contacting the wall or landing after vertical exploration during the post kindling scores from the baseline scores.

Skilled Rung Walking Apparatus and Analysis

The ladder rung walking apparatus consisted of Plexiglas walls 50 cm in height and 1 m in length, and metal rungs 30 cm above the floor, 0.3 cm in diameter, separated by a distance of 1 cm. One end of the apparatus had an open starting platform, while the other end had a closed ending box. Rungs were removed randomly to form an irregular pattern where no more than two consecutive runs were removed allowing for a maximum gap length of 5 cm. The irregular pattern prevents the rats from learning the rung pattern in repeated test sessions.

The number of errors in each crossing was counted. Crossing the runway required that the rats accurately place their fore- and hindlimbs on the bars. In baseline testing sessions, all rats were trained over a minimum of two and a maximum of five trials to cross the horizontal ladder with all of the rungs in place. After the rat demonstrated

understanding of the task with the rungs in place (typically 1-3 trials), rungs were removed to test skilled walking and the rat's performance was video-recorded. From these recordings, the number of steps and the number of hand/foot faults (errors) for each fore- and hindlimb were counted respectively. The following rating system was used: 0 point was given when no placing error occurred, 1 point was given if a rat corrected hand/foot placement as the limb touched the rung, 2 points were given if a rat placed a hand/foot onto a rung, withdrew it and then replaced it either on the same rung or on a different rung, 3 points were given for a hand/foot slip after the limb was placed on a rung, and 4 points were given when a hand/foot completely missed a rung (Metz and Whishaw, 2002b).

Single Pellet Reaching Task Apparatus and Qualitative Analysis

Single pellet boxes with the dimensions 45 x 14 x 35 cm were made of clear Plexiglas. In the center of the front wall is a vertical slit 1 cm wide extending from 2 cm above the floor to a height of 15 cm. A shelf 2 cm deep mounted 3 cm from the floor on the outside of the front wall in front of the slit. Aligned with the edges of the slit, 2 cm from the inside wall on the shelf are two indentations where the pellets are placed for the rats to reach.

After kindling the rats were food-deprived to 90 % of free-feeding levels. Then they were placed in the reaching box for 10 minutes a day for training. They were trained to reach for a single food pellet (banana flavored) with their preferred hand, and to then return to the back of the reaching box. This ensured that each reaching attempt consisted of similar movements both prior to and during the actual reach. During the early trials,

the rats were rewarded with a banana pellet at the back of the reaching cage for each attempt. As training progressed, the rats were only rewarded at the back of the reaching apparatus for successful reaches. The rats had to return to the back of the reaching box between each attempt regardless of whether or not they had successfully obtained a pellet. Rats reached for as many pellets as they could for ten minutes daily over 15 days. A successful reach was one where the pellet was grasped with a hand from the shelf and transferred to the mouth without being dropped. The percent success was determined using the following formula:

Percent success = (number of successful reaches/ number of total reaches) x 100.

The qualitative ratings of each movement performance were obtained using frame-by-frame analysis of the video recordings. The first five successful reaches on the last day of reaching were analyzed and a mean score was calculated. The current study used the criteria set out by Whishaw and colleagues (2003). The subcomponents of the reaching behaviour are broken down into 10 discrete subcomponents. (1) *Digits to the midline.* Using mainly the upper arm, the reaching limb is lifted from the floor so that the tips of the digits are aligned with the midline of the body. (2) *Digits flexed.* As the limb is lifted, the digits are flexed, the hand is supinated, and the wrist is partially flexed. (3) *Elbow in.* 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. (4) *Advance.* The limb is advanced directly through the slot toward the food target. (5) *Digits extend.* During the advance, the digits extend so that the digit tips are pointing toward the target. (6) *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. (7) *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. (8) *Supination I.* As the limb is withdrawn, the hand supinates by almost 90°. (9) *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. (10) *Release.* The mouth contacts the hand and the hand opens to release the food. If the movement appears normal it is given a score of 0, if it is ambiguous it is given a score of 0.5, if it is impaired but recognizable it is given a score of 1, or a 2 if it is absent or completely unrecognizable (Whishaw et al., 2003). The first five unsuccessful reaches were also analyzed for qualitative descriptions. Historically only successful reaches are analyzed, but the current study included misses as an additional and separate measure to assess motor coordination.

Experiment 2

Subjects

Twenty-three male Long-Evans rats, weighing between 312 and 386 g at the time of surgery, were used. The rats were obtained from the University of Calgary Breeding Colonies. All animals were housed individually in clear plastic cages in a colony room that was maintained on a 12h on/12h off light cycle. All experimentation was conducted during the light phase. Rats were fed Lab Diet no. 5001 (PMI Nutrition International Inc, Brentwood, MO) and drank water *ad libitum*, except during Morris water maze testing where the rats were food-restricted to 90% of their post-surgery weight to ensure consistency with the rats in Experiment 1. The rats were handled and cared for according to the Canadian Council on Animal Care guidelines.

Treatment Groups

For Experiment 2, rats received callosal and neocortical electrodes using the above surgical procedure and were divided into sham control (n=11) and cortical kindled (n=12) groups after baseline measures on the elevated plus maze had been obtained. Once baseline behavioural measures had been obtained the rats received either 21 kindling simulations or handling sessions. The same kindling/handling protocols were followed as Experiment 1. After early post-kindling measures had been taken, at least 24 hours following the last stimulation or handling session, on the elevated plus maze rats began the Morris water maze. After seven days of learning and a visible platform trial, and seven days of relearning and a visible platform trial in the water maze late post-kindling measures on the elevated plus maze were taken.

Elevated Plus Maze Apparatus and Qualitative Analysis of Anxious Behaviour

The elevated plus maze was composed of 19-mm-thick painted plywood. The maze consisted of two sets of perpendicular interlocking arms 110 cm in length and 10 cm in width. The interlocking central region bisected the maze into two pairs of arms: one pair with 14-cm-high walls, the closed arms, and the other pair without walls, the open arms. The entire maze was elevated 45 cm above the ground.

Activity (i.e., exploratory behaviors) and anxiety related behaviours were assessed in an elevated plus maze. Rats were brought to the testing room and tested individually. Before each trial, the maze was cleaned thoroughly with a solution of 60% alcohol to remove odors. The trial began with the rat being placed in the closed arm of the maze facing the corner and continued for 5 min, at which point the rat was promptly removed

and returned to the housing colony. The following measures were taken: The time spent in each of the five regions of the maze (the two open arms, the two closed arms, and the central region) was recorded. Entries into each of the arms were recorded. For this purpose, the rat's entry to any of the four arms was counted each time all four limbs crossed from the central region into an arm. Finally, the total number of rears was recorded. Measures of activity included total number of arm entries, with higher values indicating higher levels of activity. Measures of anxiety included dwell ratio- the ratio of dwell time in the open arms to the dwell time in all four arms, and entry ratio- the ratio of open arm to closed arm entries, with lower values indicating higher levels of anxiety (Hannesson et al., 2001b).

Morris Water Maze Apparatus

The Morris water maze consisted of a circular pool 150 cm in diameter and 45 cm in height with a white inner surface. The pool was filled to a height of 25 cm of water at 20.5°C. The water was made opaque by the addition of powdered skimmed milk. The hidden escape platform (13 x 13 cm) was composed of clear plastic. It was submerged approximately 2 cm below the water surface so that it was invisible at water level.

Acquisition and Retention Phases and Quantitative Analysis

During acquisition and retention (as defined below), the submerged escape platform was located in the center of the northwest quadrant. All groups were given 4 trials daily for 7 consecutive days. On each trial, the rat was placed in the water facing

the pool wall at one of 4 starting locations (north, east, south, or west pole). The rat's swim path, distance (cm), and escape latency were measured with a video tracking system (HVS Image Water 2020). Once the rat found the platform, it was permitted to remain on the platform for 10 s; if the rat did not find the platform within 60 s, it was guided to the platform and permitted to remain on the platform for 10 s. After each trial, the rat was placed in a holding cage, for an inter-trial interval of approximately 15 min. After the last trial on the final day of acquisition or retention (day 7), a probe trial was given to assess the strength and accuracy of initial acquisition or retention. This procedure consisted of releasing the rats from the western pole and allowing them to swim for 30 s without an escape platform to measure whether the rats had learned the location of the platform. At the end of the 30 s the rat was removed from the pool and returned to its home cage. On the eighth day a visible platform trial was given to assess the rats' swimming behaviour to observe any potential differences in motor coordination.

Reversal Phase

To assess their proficiency at learning a new location, the rats were trained to locate the submerged platform in a different quadrant. Reversal training began on the day after the visible platform trial. The platform was relocated to the center of the quadrant diagonally opposite (southeast) to the previous location (northwest). As in the acquisition and retention phases, all rats were given 4 trials daily for 7 consecutive days, followed by a 30-s probe trial after the final trial on the last day and a visible platform trial the next day (Gilbert et al., 1996).

Statistics

Cylinder data, rung walking data, and reaching attempts and success were analyzed using two-tailed independent t-tests comparing each of the kindled groups to the sham control group. The elevated plus maze and the Morris water maze were analyzed using two-tailed independent t-tests comparing the cortical kindled rats to the sham controls.Analysis of the reaching subcomponents was done using a Mann-Whitney U test comparing each of the kindled groups to the sham control group. Planned comparison analyses were chosen as I was only interested in reporting differences in the kindled groups relative to the sham control group. All statistical procedures employed an *a priori* probability value of 0.05 and were performed using SPSS software (SPSS Inc., Chicago).

RESULTS

Experiment 1

Cylinder Task

Asymmetry in forelimb use was determined by counting the number of ipsi- and contralateral hand placements when rearing and contacting the wall of a cylinder, as well as limb contacts with the floor when landing from a rearing. A mean difference score was calculated by subtracting the number of hand placements counted during the post kindling scores from the baseline scores. There was not a significant difference between the sham control and the cortical kindled group on the mean use of the preferred hand ($t(16) = 0.582$, $p= 0.569$) the non-preferred hand ($t(16) = -1.16$, $p= 0.266$) or both hands

($t(16) = 1.39$, $p= 0.186$) for wall touches (Fig. 1A). Likewise, mean use of the preferred hand ($t(16) = 0.374$, $p= 0.714$), the non-preferred hand ($t(16) = 0.142$, $p= 0.889$) and both hands ($t(16) = 1.70$, $p= 0.111$) did not show significant differences in mean hand use during landing between the sham control and the cortical kindled groups (Fig. 1B).

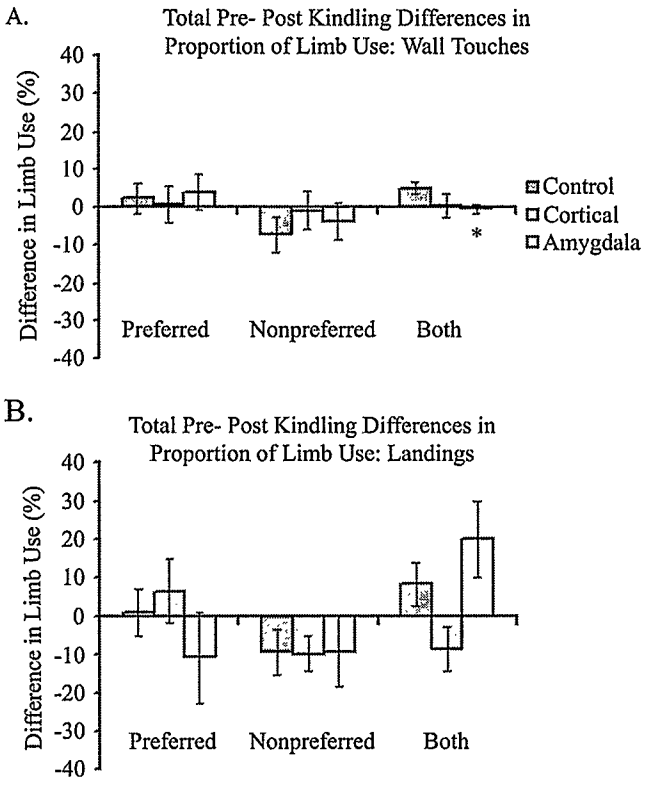
No significant differences were detected between the sham control and the amygdala kindled group on mean use of the preferred hand ($t(13) = 0.041$, $p= 0.968$) or the non-preferred hand ($t(13) = -0.816$, $p= 0.429$) for wall touches; however, there was a significant difference in the mean use of both hands between the sham control and amygdala kindled groups for wall touches ($t(13) = 2.39$, $p= 0.033$) (Fig. 1A). Analysis of the mean use of the preferred hand ($t(13) = 0.770$, $p= 0.455$), the non-preferred hand ($t(13) = -0.008$, $p= 0.994$) and both hands ($t(13) = -0.680$, $p= 0.508$) did not show significant differences in mean hand use during landings between the sham control and the amygdala groups (Fig. 1B).

Rung Walking Task

The number of errors per step and the type of errors were scored for fore- and hindlimbs. Analysis of the mean forelimb errors per step at both the early ($t(16)= -3.887$, $p= 0.001$) and the late post-kindle ($t(16) = -2.13$, $p= 0.049$) time points revealed significant differences between the sham control and cortical kindled groups (Fig. 2A). Cortically kindled rats also had significantly higher mean forelimb error scores at the early ($t(16) = -2.17$, $p= 0.045$) and late ($t(16) = -2.69$, $p= 0.017$) post kindling time points (Fig. 2B) indicating that in addition to committing a greater number of mean errors, the cortically

Figure 2-1: Mean percent difference between pre-kindled and post-kindled use of preferred, non-preferred, and simultaneous (both) forelimbs during exploratory activity in a cylinder (mean + SEM). **(A)** Total pre - post kindling differences in proportion of limb use: Wall touches. **(B)** Total pre - post kindling differences in proportion of limb use: Landings. The amygdala kindled group used both hands simultaneously significantly less than sham controls. Asterisk indicates significant differences relative to the sham control group * = $p \leq .05$.

Figure 2 - 1: Cylinder Task Proportion of Limb Use



kindled rats also committed more severe errors. Analysis of the number of hindlimb errors per step revealed no significant differences between the sham control and cortical kindled groups at either the early ($t(16) = 1.339$, $p= 0.200$) or late ($t(16) = 0.607$, $p= 0.553$) post-kindling time points. Hindlimb errors per step analysis did not reveal significant differences between the sham control and amygdala kindled groups at the early ($t(13) = 1.784$, $p= 0.098$) or late ($t(13) = 2.186$, $p= 0.055$) post-kindling time point (data not shown).

Comparisons between the sham control and amygdala kindled groups on the mean number of forelimb errors per step at both the early ($t(13) = -1.74$, $p= 0.105$) and the late post-kindling ($t(13) = 1.65$, $p= 0.124$) time points did not revealed significant differences (Fig. 2). Likewise, amygdala kindled rats did not significantly differ from sham controls on their mean forelimb error scores at the early ($t(13) = 0.525$, $p= 0.608$) and late ($t(13) = 0.294$, $p= 0.773$) post kindling time points (Fig. 2). Analysis of the hindlimb error scores revealed no significant differences between the sham control and cortical kindled groups at either the early ($t(16) = 0.766$, $p= 0.456$) or late ($t(16) = 0.748$, $p= 0.466$) post-kindling time points. Hindlimb error score analysis did not reveal significant differences between the sham control and amygdala kindled groups at the early ($t(13) = 1.603$, $p= 0.133$) or late ($t(13) = 0.409$, $p= 0.689$) post-kindling time point (data not shown).

Successful Single Pellet Reaching Task- Attempts and Success

The number of reach attempts was measured during every reaching session for 15 days. All groups increased the mean number of reach attempts throughout the 15 days. The sham control group made a mean 98.56 (± 4.84) reach attempts on day 15 while the cortical and amygdala kindled groups made 82.75 (± 7.66) (t (16) = 1.72, p= 0.107) and 66.67 (± 7.82) (t(13) = 3.674, p= 0.003) mean reach attempts on day 15 respectively (Fig. 3A).

Success rates of the preferred forelimb were measured during every reaching session for 15 days. The time course of mean reaching success rates is illustrated in Fig. 3B. All groups also showed improvement in reaching success, attaining asymptote on day 9. The sham control group had a mean success rate of 60.73% (± 4.61) on Day 15 while the cortical kindled group reached at a mean success rate of 46.87% (± 2.28) (t(16) = 2.51, p= 0.024) and the amygdala kindled group reached at a mean success rate of 49.04% (± 4.24) (t(13) = 1.718, p= 0.110).

Successful Single Pellet Reaching Task Kinematics

Video-recordings from Day 15 were analyzed for the kinematics of limb movements. Kinematic analysis revealed significant alterations in the reaching behaviour in two subcomponents: Elbow to Midline (III) and Supination II (IX). The cortical kindled group had significantly higher mean error scores on III (U= *1.000,* $p \leq$ 0.000) and IX (U= *0.000,* $p \leq$ 0.000), while the amygdala kindled group had statistical significance on IX (U= *10.500,* p= 0.045) (Fig. 4). The cortical kindled group's scores were obtained

mainly due to an altered or absent adduction and abduction of the elbow and an alteration in the ability to turn the wrist by 45° as seen in a typical successful reach. The cortical kindled rats additionally compensated for their altered forelimb movements with postural adjustments. They either oriented their body at an angle to the slot or aligned their body medially, but then leaned to the ipsilateral side to the reaching hand. All other subcomponents of the reaching behaviour appeared to be unaltered in the cortically kindled rats, but the full reaching behaviour appeared rigid and impulsive as the rats' movements appeared less fluid than those of sham controls'. The amygdala kindled group showed one statistically significant alterations in reaching behaviour. Qualitatively, the amygdala group was far more varied then either the sham controls or the cortical group. Though they only showed significant alterations on one subcomponent (IX), they also displayed nonsignificant alterations across a spectrum of subcomponents.

Qualitative Description of Unsuccessful Reaches

A qualitative analysis of five unsuccessful reach attempts on the last day of reaching revealed that all rats reached much the same way in unsuccessful attempts as they did in successful attempts differing mainly in the ability of all rats to advance to the pellet, display appropriate arpeggio, and grasp the pellet appropriately. The rats' main error on unsuccessful attempts was typically observed in their approach to the pellet, showing a tendency to over- or under-reach the pellet, resulting in either missing the pellet altogether in the case of an under-extension, or knocking the pellet off of the platform upon retraction of the forelimb in the case of an over-extension. All of the rats

Figure 2 - 2: Rung walking task. Comparison of sham control, cortical, and amygdala groups across two measures of skilled motor behaviour on the rung walking task. **(A)** Total forelimb errors per step over time. Graphical representation of the mean (+ SEM) number of total forelimb errors committed by sham control, cortical kindled and amygdala kindled rats across all error types at baseline, early post kindling (48 h after the last seizure) and late post kindling (20 d after the last seizure) on the rung walking task. A lower number reflects fewer errors per step. Cortically kindled rats committed a significantly greater number of forelimb errors per step relative to sham controls. Asterisks indicate significant differences relative to the sham control group * = $p \leq .05$, ** = $p \leq .01$.

(B) Total forelimb error score over time. Graphical representation of the mean (+ SEM) forelimb error scores added across error types at baseline, early post kindling (48 h after the last seizure) and late post kindling (20 d after the last seizure) rung walking task sessions for sham control, cortical kindled and amygdala kindled groups. A lower score reflects less severity in the types of errors committed. Cortically kindled rats committed more severe forelimb errors relative to sham controls. Asterisks indicate significant differences relative to the sham control group * = $p \leq .05$, ** = $p \leq .01$.

Figure 2 - 2: Rung Walking Task Forelimb Errors

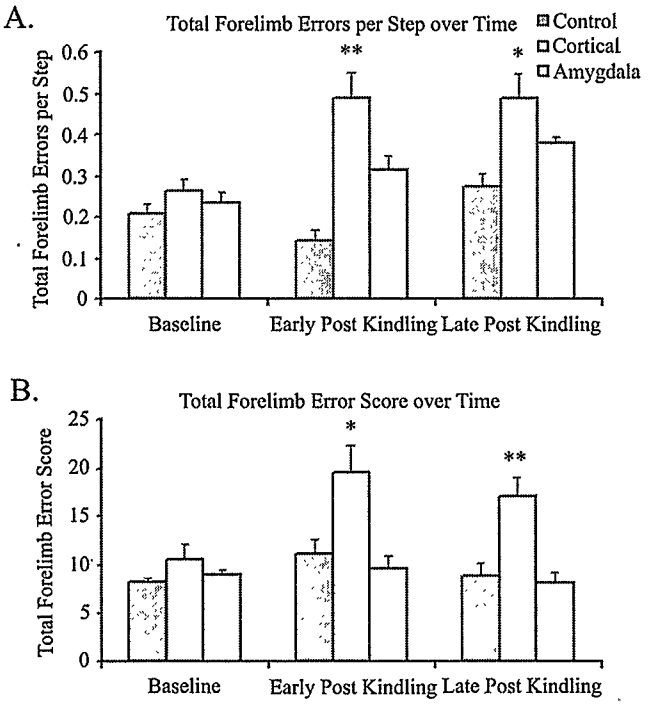
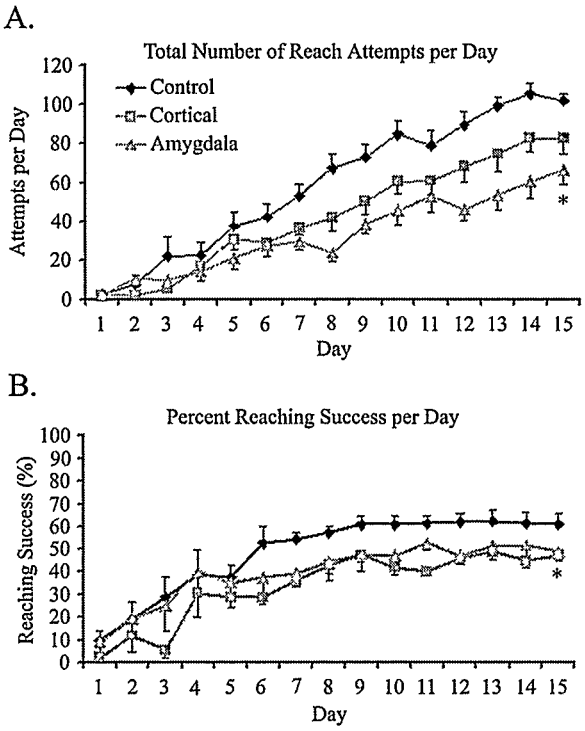


Figure 2 - 3: Single pellet reaching task attempts and success. Comparison of sham control, cortical and amygdala groups on the total number of reach attempts and the total number of reaching success over 15 days of reach training. **(A)** Total number of reach attempts per day. Graphical representation of the mean (+ or – SEM) number of reach attempts in the single pellet reaching task for sham control, cortical kindled, and amygdala kindled groups for reaching days 1 through 15. The amygdala kindled group was statistically different on day 15 from sham controls. Asterisks indicate significant differences relative to the sham control group * = $p \leq .05$. **(B)** Percent reaching success per day. Graphical representation of the mean (+ or – SEM) success per day in the single pellet reaching task (number of pellets successfully retrieved out of total number of attempts) for sham control, cortical kindled, and amygdala kindled groups for reaching days 1 through 15. Cortically kindled rats had a significantly smaller number of successful attempts relative to sham control group * = $p \leq .05$.

Figure 2 - 3: Total Number of Single Pellet Reach Attempts and Success per Day



displayed alterations in their ability to splay the hand over the pellet (arpeggio), though the two kindled groups exhibited this alteration more frequently than sham controls. The rats tended to show an impaired or absent arpeggio resulting in an unstable grasp of the pellet between the base of the hand and the fourth digit. The eventual grasp of the pellet in all rats was altered in the unsuccessful attempts, though the alterations in the preceding subcomponents can likely be attributed for the majority of unsuccessful grasping of the pellet.

Experiment 2

Elevated Plus Maze

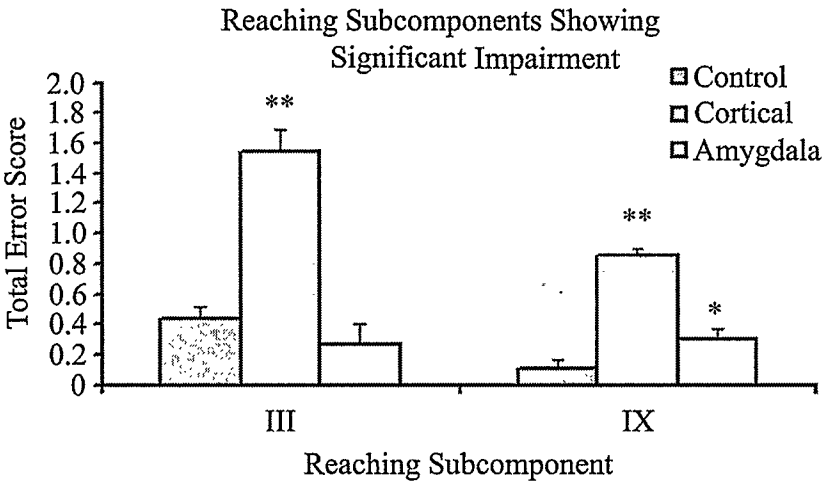
Cortical kindling did not significantly affect open arm entries, rearing, or time spent in the open arms in the elevated plus maze at either the early or the late post kindling time points. Sham control and cortical kindled rats exhibited a statistically nonsignificant mean proportion of total open arm entries ($t = 1.74$, $p= 0.097$) (Fig. 5A), and total rears ($t = -0.395$, $p= 0.697$) (fig. 5C) at the early post-kindling time point. Similar results for mean open arm entries ($t = 1.89$, $p= 0.071$) (fig. 5A) and rears ($t = -0.951$, $p= 0.353$) (Fig. 5C) were obtained at the late post-kindling time point. Sham control and cortical kindled rats spent a comparable amount of mean time spent in open arms at the early ($t = 1.54$, $p= 0.140$) and late ($t = 1.75$, $p= 0.095$) post-kindling time points (Fig. 5B).

Morris Water Maze: Acquisition

Rats in the cortical kindled group performed at statistically equal levels to sham control rats in learning the location of the submerged escape platform, as indicated by

Figure 2 - 4: Reaching subcomponents showing significant impairment. Graphical representation of the mean (+ SEM) qualitative scores of the two reaching components where statistical significance was found from five successful reaches on day 15 for sham control, cortical kindled and amygdala kindled groups. The cortical kindled rats showed significant impairment in the ability to adduct/abduct the elbow at subcomponent III and an inability to fully supinate the wrist at subcomponent IX while the amygdala group only showed an inability to fully supinate the wrist at subcomponent IX. Asterisks indicate significant differences relative to the sham control group * = $p = .05$, ** = $p \leq .01$.

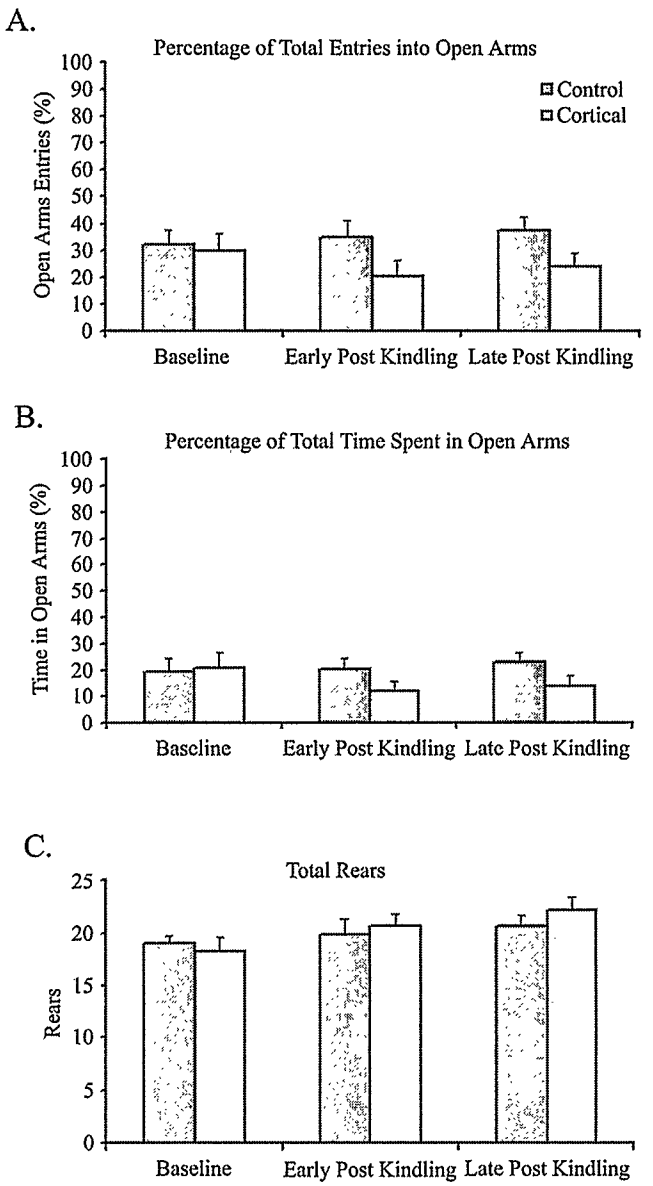
Figure 2 - 4: Reaching Subcomponents Showing Significant Impairment



their mean swim distances (t (22)= 0, p= 1.00) (data not shown) and mean latency to platform (t (22) = -1.13, p= 0.263) (Fig. 6A) on the last day of testing before the first probe trial as compared to sham controls. Cortically kindled rats spent equivalent mean amounts of time in the quadrant platform (t (22) = 0.946, p= 0.355) (Fig. 6B) and had a similar mean number of passes over the platform (t (22) = 0.793, p= 0.437) (Fig. 6D) on the probe trial.

Figure 2 - 5: Elevated plus maze open arm entries, time spent in open arms, and total rears. Comparison of sham control and cortical kindled rats on the elevated plus maze. **(A)** Percentage of total entries into open arms. Graphical representation of the mean (+ SEM) percentage of total arm entries into open arms of the elevated plus maze at baseline, early post kindling (48 h after the last seizure) and late post kindling (20 d after the last seizure) sessions for sham control and cortical kindled groups. Open arm entries are a measure of exploratory behaviour where a greater amount of activity is interpreted as lower anxiety. The groups were not statistically different across testing sessions. **(B)** Percentage of total time spent in open arms. Graphical representation of the mean (+ SEM) percentage of total time spent in open arms of the elevated plus maze at baseline, early post kindling (48 h after the last seizure) and late post kindling (20 d after the last seizure) sessions for sham control and cortical kindled groups. Time spent in open arms is a measure of anxiety where a greater amount of time spent in open arms is interpreted as lower anxiety. The groups were not statistically different across testing sessions. **(C)** Total Rears. Graphical representation of the mean (+ SEM) number of rears in a 5 m elevated plus maze session at baseline, early post kindling (48 h after the last seizure) and late post kindling (20 d after the last seizure) sessions for sham control and cortical kindled groups. Rears are a measure of exploratory behaviour where a greater amount of activity is interpreted as lower anxiety. The groups were not statistically different across testing sessions.

Figure 2 - 5: Elevated Plus Maze



There was also no difference between groups on a visible platform trial to assess gross motor behaviour ($t(22) = -0.101$, $p= 0.920$) (Fig. 6F).

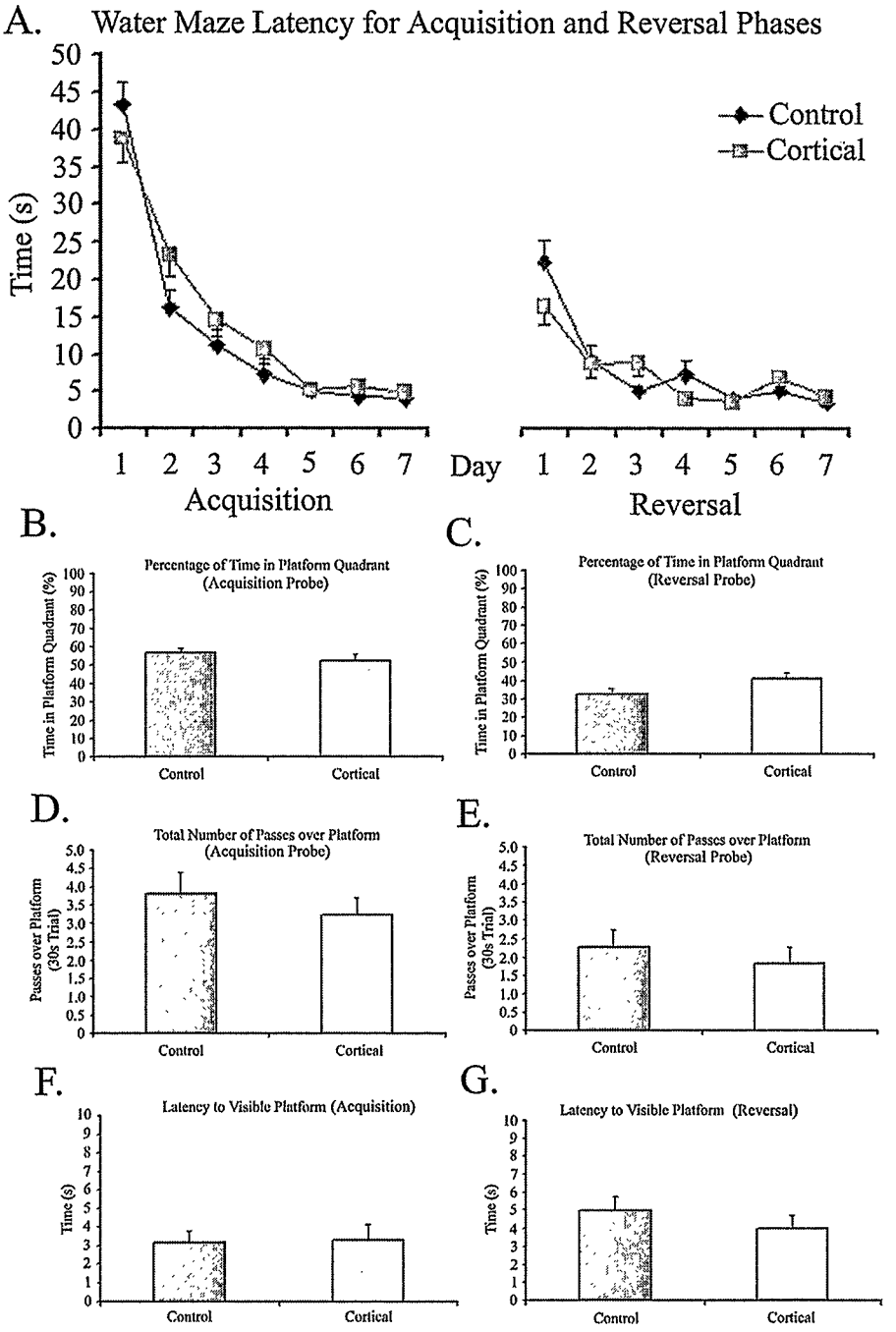
Morris Water Maze: Reversal

Rats in the cortical kindled group did not show impairment in learning the new location of the submerged platform when it was moved directly opposite to the previous quadrant as indicated by mean swim distance ($t(22) = 0.399$, $p= 0.690$) (data not shown) and mean latency to platform ($t(22) = -0.903$, $p= 0.369$) (Fig. 6A) on the least day of testing before the final probe trial compared to sham controls. On a second probe trial, the cortical kindled group spent a statistically equivalent mean amount of time in the new platform quadrant ($t(22) = -1.92$, $p= 0.068$) (Fig. 6C) and made an equivalent mean number of passes over the platform location ($t(22) = 0.683$, $p= 0.502$) (Fig. 6E) when compared to sham controls. There was also no difference between groups on a visible platform trial to assess gross motor behaviour (t (22) = 1.43, $p= 0.68$) (Fig. 6G).

Figure 2 - 6: Water maze acquisition and reversal phases. Comparison of sham control and cortical kindled rats on the Morris water maze across acquisition (left side of panel) and reversal phases (right side of panel). **(A)** Water maze latency for acquisition and reversal phases. Graphical representation of the mean (+ or – SEM) latency to platform from day 1 through 7 of water maze testing during the acquisition phase and the reversal phase for sham control and cortical kindled groups. Decreased latency over time indicates that the rat has learned and remembered the location of the platform. **(B)** Percentage of time in platform quadrant (acquisition probe). Graphical representation of the mean (+ SEM) percentage of time in a 30 s probe trial (no platform) spent in the northeast (platform) quadrant of the water maze for sham control and cortical kindled groups in the acquisition phase. **(C)** Percentage of time in platform quadrant (reversal probe). Graphical representation of the mean (+ SEM) percentage of time in a 30 s probe trial (no platform) spent in the southwest (platform) quadrant of the water maze for sham control and cortical kindled groups in the reversal phase. Less time spent in the platform quadrant would indicate a memory deficit. **(D)** Total number of passes over platform (learning probe). Graphical representation of the mean (+ SEM) number of passes over the location of the platform in a 30 s probe trial (no platform) for sham control and cortical kindled groups in the acquisition phase. **(E)** Total number of passes over platform (relearning probe). Graphical representation of the mean (+ SEM) number of passes over the location of the platform in a 30 s probe trial (no platform) for sham control and cortical kindled groups in the reversal phase. Fewer passes over the platform in a probe trial would indicate a memory deficit. **(F)** Latency to visible platform (acquisition probe). Graphical representation of the mean (+ SEM) amount of time taken to swim to a visible

platform where the platform is placed in the same location as it was for the learning phase for sham control and cortical kindled groups in the acquisition phase. (**G**) Latency to visible platform (reversal probe). Graphical representation of the mean (+ SEM) amount of time taken to swim to a visible platform where the platform is placed in the same location as it was for the reversal phase for sham control and cortical kindled groups in the reversal phase. A greater amount of time taken to swim to a visible platform would indicate a gross motor deficit.

Figure 2 - 6: Water Maze Acquisition and Retention



DISCUSSION

I examined the interictal behavioural comorbidities associated with repeated seizures by examining motor behaviours that required varying skill levels. To my knowledge this represents the first report of the effects of repeated seizures on fine motor coordination as revealed through reaching success and kinematic analysis of the forelimbs on the rung walking task and the single pellet reaching task. I found that repeated seizures elicited through stimulation of the corpus callosum at the level of the caudal forelimb area significantly altered skilled motor behaviour. Specifically, cortically kindled rats committed significantly more forelimb errors per step that were also greater in severity as evidenced by the higher forelimb error scores. Similarly, the cortically kindled rats also showed significant alterations in their reaching behaviour on two of the ten subcomponents that comprise the total reaching behaviour. The elevated plus maze failed to show a significant difference in behaviours supposed to indicated anxiety; moreover, there were no statistically significant differences in memory related behaviours found using the Morris water maze.

Cortically kindled rats showed reduced reaching success and altered kinematics relative to sham controls. Cortically kindled rats had lower success rates (Fig. 3B) than the sham controls that can be attributed to altered kinematics. The cortically kindled rats exhibited an inability to adduct/abduct the elbow, thus the reach appeared as more of a lateral swipe of the hand. In addition to the alterations in elbow coordination, the cortically kindled rats also showed alterations in their inability to supinate the wrist and employed postural compensations to successfully attain the pellet. The impairment of the preferred limb in the reaching task is only reflected in a subset of skilled movements

suggesting that the impairment in the limb is not a global deficit in function (Whishaw, 2000). The results of the kinematic analysis from the single pellet reaching task are comparable to the findings of Whishaw (2000) where control rats were compared with rats that had unilateral lesions of different sizes on the reaching task. However, there were appreciable differences in the results found by Whishaw (2000; Whishaw and Metz, 2002) and the cortical kindled group of the current study.

The results from the elevated plus maze indicated that the kindled rats were not exhibiting statistically different amounts of exploratory behaviour demonstrated by the percentage of open arm entries and rears, as well as nonsignificant differences in time spent on the open arms indicating comparable anxiety levels (Fig. 5). Though there have been many other studies documenting changes in anxiety related behaviour and kindling (Adamec, 1976a; Pinel et al., 1977; Henke and Sullivan, 1985; Adamec and McKay, 1993; Kalynchuk et al., 1998a; Kalynchuk et al., 1998b; Adamec and Shallow, 2000; Wintink et al., 2003), all of these findings are related to temporal lobe structures, namely the amygdala. The rats in the current study did not show statistically significant changes in anxiety level. This lack of anxiety-related change is not due to the kindling parameters. While some anxiety and kindling studies resort to a 99 stimulation protocol (Kalynchuk et al., 1997; Kalynchuk et al., 1998b; Wintink et al., 2003), other studies prescribe to a three or four consecutive stage-5 seizure cutoff (Adamec, 1976a; Adamec and McKay, 1993; Adamec and Shallow, 2000). The rats in the current study would have easily met the four consecutive stage-5 seizure cut off well before the last kindling stimulation in the current study, and were thus comparably kindled. Given the comparable kindling protocol it can then be inferred that the difference was not in the type or amount of

stimulation, but was instead the result of the site of stimulation. This notion is confirmed by Pinel *et al.* (1977) who found that amygdala kindled rats showed increased aggression, but caudate kindled rats did not.

Kindling has been shown to create memory deficits that become manifest in both the Morris water maze (Gilbert et al., 1996; Hannesson et al., 2001b; Hannesson et al., 2001a) and the radial arm maze (Leung et al., 1990; Leung and Shen, 1991; Leung et al., 1994) performance, but in these cases as in almost all others finding an effect of kindling on memory the stimulations were delivered at a temporal lobe structure, most notably the hippocampus. The cortically kindled group in Experiment 2 of the current study did not show statistically different memory (Fig. 6). This may be due in large part to the fact that the kindling site in the current study was not in the temporal lobe, thereby lessening any effects of repeated seizures on memory. While kindling has also been known to produce motor disturbances lasting up to four weeks after the last seizure (Ehlers and Koob, 1985; Caldecott-Hazard, 1988), the results from cylinder task (experiment 1) and the visible platform trial (experiment 2) in the current study suggest that there were no gross motor changes in the cortically kindled rats. The change in the use of both hands by the amygdala group can be explained by the unilateral nature of amygdala kindling. Because the stimulation is being delivered preferentially to one hemisphere it is not unreasonable to see a change affecting the simultaneous use of both hands. The results from the current study are confirmed by the findings of a slight gross motor impairment in amygdala kindled rats documented by Caldecott-Hazard (1988).

The results of the current study support the notion that focal seizures result in a functional lesion. Functional lesions have two important aspects to consider. The first

aspect is that the tissue is functioning, albeit in an altered manner. This is in contrast to ischemic tissue that is considered dead (Lipton, 1999). Evoked potentials can be recorded in kindled tissue (Racine et al., 1975; Teskey et al., 2001; Teskey et al., 2002a). Though the results of the current study are similar to lesion studies investigating similar behaviours (Whishaw, 2000; Metz et al., 2001; Metz and Whishaw, 2002a, b), the electrophysiological (Racine et al., 1975; Teskey et al., 2001; Teskey et al., 2002a) and motor map (Teskey et al., 2002a; van Rooyen et al., 2006) data suggest that the nature of kindled tissue is different than that of lesioned tissue. The second aspect of functional lesions to consider is that behaviour is disrupted by both over- and understating behaviours specific to the affected brain area. This aspect of functional lesions is demonstrated across several behavioural tasks. If the behavioural deficits associated with kindling were global then a deficit across all behaviours would be expected (Whishaw, 2000). However, only certain subcomponents of the reaching behaviour in the current study were affected. The results from the elevated plus maze and the Morris water maze confirm that the motor alterations were not due to a global alteration in anxiety or memory. If repeated neocortical seizures resulted in global alterations such that anxiety levels and memory were altered then the most parsimonious explanation of the alterations in kinematics on the rung-walking and single-pellet reaching tasks would be to attribute motor coordination alterations to changes in anxiety level and memory. However, the findings on these tasks reveal that the alterations in skilled motor behaviour are the result of a functional lesion in sensorimotor cortex created by repeated seizures.

People with temporal lobe epilepsy do not experience any appreciable differences in motor coordination compared to the normal population (Kolb and Milner, 1981;

Helmstaedter et al., 1996; Hernandez et al., 2002). Conversely, people with frontal lobe epilepsy are generally less coordinated, showing rigid movements and altered electrophysiology with respect to the planning and execution of voluntary movements (Helmstaedter et al., 1996; Matsuoka et al., 2000; Hernandez et al., 2002). Though motor disturbances have been documented with respect to frontal lobe epilepsy, the specific types of disturbances are not well described. The current study mirrors the clinical work by demonstrating altered motor behaviour in a measure of skilled behaviour documented in the reaching success of cortically kindled rats (Fig. 3B), but it also describes some of the types of motor changes associated with repeated seizures in the frontal lobes as is illustrated in the kinematic alterations on both the rung walking task (Fig. 2) and the single pellet reaching task (Fig. 4). The current study is further confirmed by the clinical data by showing less profound alterations in motor behaviour in the amygdala group (Fig. 4).

Not only do my results converge with aspects of interictal behaviours associated with epilepsy, but they also conform to ultrastructural, electrophysiological, and network level alterations associated with neocortical kindling. Goertzen and colleagues (2003) found that kindling of the corpus callosum at the level of the caudal forelimb area resulted in a 265 % increase of excitatory perforated synapses per remaining neuron in layer V. Perforated synapses are highly efficacious, and are hypothesized to be key in the hyper-synchronous firing associated with seizures (Teskey et al., 2005). Similarly, examination of the polysynaptic component of evoked potentials in kindled rats has revealed that kindling leads to a persistent increase in the polysynaptic component of an evoked potential that lasts at least 21 days after the last kindling stimulation (Racine et

al., 1975; Teskey et al., 2001; Teskey et al., 2002a). The larger late component of the evoked potential is reflective of polysynaptic activity and neuronal recruitment (Teskey and Valentine, 1998), and is a manifestation of strengthened neural communication (Teskey et al., 2005). The network level changes in kindled rats can be seen in the near doubling of the number of motor movement representations in the caudal forelimb area (Teskey et al., 2002a; van Rooyen et al., 2006) and are likely facilitated by an increase in glutamatergic transmission while simultaneously decreasing GABAergic transmission (Jacobs and Donoghue, 1991; Keller, 1993). Kindling augments glutamatergic transmission by enhancing and recruiting NMDA receptors (Mody and Heinemann, 1987; Mody et al., 1988; Behr et al., 2000), which are necessary for map expansion (Qiu et al., 1990). GABAergic transmission functions in the opposite way with respect to potentiation, where antagonism of GABAergic transmission has been shown to enhance synaptic potentiation (Hess et al., 1996) thereby enhancing motor map expansion (Jacobs and Donoghue, 1991). Flynn and colleagues (2002) found increased potentiation and map expansion in a rat strain known to express a decreased profile of $GABA_A$ receptor α1 subunits (the major subunit expressed in the adult brain) and an increased profile of α2, α3, and α5 subunits (subunits highly expressed during development when GABA has excitatory effects) relative to Long-Evans rats (Poulter et al., 1992; Poulter et al., 1999). Though it has been shown that learning increases the size of motor maps (Kleim et al., 1998), the motor maps of stimulated rats expanded in a non-specific multidirectional manner in contrast to the refinement within pre-existing borders seen in learning (Kleim et al., 1998). The altered kinematics likely reflect the increased synchrony and neural communication between neurons illustrated by an increase in perforated synapses, an

increase of the polysynaptic component of the evoked potential, and the expansion of the motor map during the interictal phase. It is possible that the increase in perforated synapses (Goertzen et al., 2003) allows for greater recruitment (larger evoked potentials) (Racine et al., 1975; Teskey et al., 2001; Teskey et al., 2002a) resulting in strengthened neural communication which in turn translates into larger maps on the network level (Teskey et al., 2002b). The larger maps have behavioural relevance in light of the current study as the distal bias in the maps' of kindled rats (Teskey et al., 2002a) is manifested in the alterations in the ability of cortically kindled rats to adduct/abduct the elbow (III) and fully supinate the wrist (IX).

The convergence of evidence from ultrastructural changes, increased electrophysiological responses, and network level expansion all support the results of the present study (Racine et al., 1975; Teskey et al., 2002a; Goertzen et al., 2003). Specifically, the idea that focal seizures create functional lesions whereby the affected brain area is still able to perform its designated task, but does so with modifications that are manifested in behavioural alterations is confirmed. Furthermore, the possibility that these motor alterations are caused by changes in anxiety level or memory is ruled out. Given that kindling is a form of long-term potentiation it is likely that long-term potentiation represents a common mechanism of learning, memory, and motor map reorganization, all of which in concert reveal themselves as behavioural changes operating within sensorimotor cortex.

Study 2: Kinematic Alterations Differ Between Rats with Kindled Lesions and Complete Lesions

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Running title: **Kindled and Complete Lesions**

First Author Contribution:
Electrode implantation surgery, Kindling, Behavioural testing and scoring. Manuscript Preparation – creation of written document including writing, statistical analysis and interpretation, and creation of figures.

Key words: lesion, motor coordination, motor cortex, skilled behaviour, learning

INTRODUCTION

Clinical reports have documented several motoric disturbances associated with damage to the cerebral cortex (Chan and Ross, 1997; Wittmann et al., 2001; Silverman et al., 2002) and frontal lobe epilepsy (Helmstaedter et al., 1996; Matsuoka et al., 2000; Hernandez et al., 2002). The experimental lesion data mirrors the clinical lesion data well across tasks that measure both unskilled (Schallert et al., 2000; Hua et al., 2002; Karhunen et al., 2003) and skilled behaviours (Whishaw et al., 1991; Whishaw et al., 1992a; Whishaw, 2000; Metz and Whishaw, 2002a; Teskey et al., 2003; Gharbawie et al., 2005). Though there have been many comparisons of the effects of different lesion locations and different types of lesion methodologies (Schallert et al., 2000; Whishaw, 2000; Metz and Whishaw, 2002a; Gonzalez and Kolb, 2003; Gharbawie et al., 2005), there is very little data comparing lesions where the tissue is absent to lesions where the tissue is still able to function.

We have previously shown that focal seizures result in a functional lesion (Study 1). A functional lesion is defined as a local brain area displaying distorted function that may include partial damage, whereas a complete lesion can be defined as any damage to the nervous system resulting from decreased oxygen to nervous tissue (Kolb and Whishaw, 2003) resulting from an acute insult that sets in motion a cascade of "highly interactive ionic and biochemical changes that cause cell death" (Lipton, 1999). Cell death is considered to be the elimination of the cell through either phagocytosis or disintegration (Lipton, 1999). Complete lesions and functional (kindled) lesions differ across two distinct aspects: The first aspect is that the tissue, though impaired, is operational in a kindled lesion, but it is necessarily dead and absent in a complete lesion.

Evoked potentials can be recorded in kindled tissue (Racine et al., 1975; Teskey et al., 2001; Teskey et al., 2002a) with the degree of kindling corresponding to the size of the evoked potential (Teskey et al., 2002a). Intracortical microstimulation movements can also be elicited at an equivalent intensity in kindled tissue relative to normal tissue (Teskey et al., 2002a; van Rooyen et al., 2006). This is in contrast to complete lesions where the tissue is considered dead and missing (Lipton, 1999), and no further effects can be seen upon repeated damage to previously lesioned tissue (Wishart et al., 1994). Though rats with kindled lesions show similar behavioural changes to rats that have had ischemic or occlusive lesions (Whishaw, 2000; Metz et al., 2001; Metz and Whishaw, 2002a, b), the electrophysiological data (Racine et al., 1975; Teskey et al., 2001; Teskey et al., 2002a) suggests that the nature of kindled tissue is different than that of complete lesioned tissue. The second differing aspect to functional lesions to consider is that behaviour is disrupted by either over- and understating behaviours specific to the affected brain area. My previous research demonstrated that kindled rats only showed a small, but consistent set of behavioural alterations, where Whishaw and colleagues (2000; 2005) have shown that lesioned rats display a more broad set of behavioural impairments.

In order to further test the hypothesis that kindled lesions result in different behavioural consequences than complete lesions I examined the caudal forelimb area (CFA) of the rat sensorimotor neocortex. The CFA of rat sensorimotor cortex is an ideal structure to examine because the proper use of the forelimb is necessary in skilled reaching behaviour, and is dependent on the integrity of the CFA (Whishaw, 2000), it can be kindled (Teskey et al., 1999, 2001; Flynn et al., 2002; Teskey et al., 2002a, b), and it is easily removed with focal devascularization. The current study will look at the effects of

kindling and bilateral focal devascularization on motor behaviour following repeated seizures in the cortical kindled group and motor cortex lesions in the lesion group in the sensorimotor neocortex (specifically in the CFA), and examine the performance of the rats on behavioural tests sensitive to changes in motor coordination relative to controls and to each other. The focal devascularization lesions were done bilaterally as kindling of the corpus callosum affects both hemispheres. The cylinder task (Schallert et al., 1986) has been employed to assess gross motor behaviours. It is hypothesized that the cylinder task will show no distinguishable differences between either the kindled group or the motor cortex lesion group and sham control rats because the cylinder task measures unskilled behaviour. Historically, the cylinder task is a good measure of measuring limb asymmetry in lesioned rats (Schallert et al., 2000; Hua et al., 2002; Karhunen et al., 2003), but the current study has employed a bilateral lesion, thus wiping out any preferential effects on one hemisphere over the other to approximate the bilateral effects of kindling the corpus callosum. The rung walking task (Metz and Whishaw, 2002a) and the single pellet reaching tasks have been used to assess skilled motor behaviour. It is hypothesized that both the cortically kindled rats and the lesioned rats will show more specific impairment manifested in an increase in errors per step and in severity of errors on the rung walking task; moreover, it is also hypothesized that both the cortically kindled rats and the lesion groups will show an altered reaching behaviour on the single pellet reaching task (Whishaw and Pellis, 1990).

METHODS AND MATERIALS

Experiment 1

Subjects

Twenty-three male Long-Evans rats, weighing between 312 and 407 g at the time of surgery, were used. The same sham control and cortically kindled rats used in Study 1 were used. The rats were obtained from the University of Calgary Breeding Colonies. All rats were housed individually in clear plastic cages in a colony room that was maintained on a 12h on/12h off light cycle. All experimentation was conducted during the light phase. Rats were fed Lab Diet no. 5001 (PMI Nutrition International Inc, Brentwood, MO) and drank water *ad libitum*, except during reach training where the rats were food-restricted to 90% of their post-kindling weight. The rats were handled and cared for according to the Canadian Council on Animal Care guidelines.

Neocortical Lesion

Each rat in the motor cortex lesion group received bilateral lesions to motor cortex. Rats were anesthetized with 58.53 mg/kg ketamine-xylazine (85:15) 1 ml/kg, injected intramuscularly. Lidocaine 2% (Austin, Joliette, QA) was administered subcutaneously at the incision site. After deep anesthesia was achieved a mid-saggital incision was made that extended from the eyes to the ears. The skull was exposed and bone removed bilaterally between 0.5 and 4.5 mm lateral to midline and between 1.0 mm posterior to bregma and 3.0 mm anterior to bregma. A 3.0 by 4.0 mm section of dura and pia mater was removed from the posterior part of the exposed area to deprive the

underlying neocortical area of blood supply. After all bleeding had ceased, the exposed area was packed with gel foam and the skull was covered with a thin veneer of dental acrylic attached to the skull with four stainless steel screws in the top of the skull to aid the healing process and prevent subsequent infection (Teskey et al., 2003). After the experiment the lesion rats were humanely euthanized, their brains were removed, and the lesion size was measured. The mean width (medial/lateral) of each hemispheric lesion was 2.439 (± 0.129) mm, while the mean length (A/P) of each hemispheric lesion was 4.005 (± 0.243) mm.

Treatment Groups

After surgery, each rat underwent baseline behavioural testing on the cylinder task and the rung walking task with the exception of the motor cortex lesion group which underwent surgery after baseline behavioural measures were obtained. Once baseline measures had been obtained the rats were divided into three groups: sham kindled, cortical kindled, and lesioned. An afterdischarge threshold was determined for each rat in the cortical and amygdala kindling groups. The three groups received either 21 handling sessions (n= 9), callosal kindling stimulations (n= 8) for 21 days or motor cortex lesion (n=5). Twenty-four hours following the last stimulation or handling session and subsequent early post-kindling measures were taken on the cylinder and ladder tasks, rats began the single-pellet reaching task with the exception of the lesion group which had early post-lesion follow-up measures one week post surgery. After 15 days of reaching, late post-kindling/ lesion measures on the rung walking task were taken.

Kindling

The same rats used in Study 1 were used in the current study.

Video recording

All behavioural testing was done in the same manner as described in Study 1.

Cylinder test apparatus and quantitative analysis

Forelimb use during exploratory activity was assessed done in the same manner as described in Study 1.

Skilled rung walking apparatus and analysis

The rung walking apparatus was identical to that described in Study 1. Behavioural assessment of the rung walking done in the same manner as described in Study 1.

Single Pellet Reaching Task Apparatus and Qualitative Analysis

Single pellet boxes were identical to that described in Study 1. The food deprivation and behavioural assessment of the single pellet reaching task were done in the same manner as described in Study 1.

Statistics

Cylinder data, rung walking data, and reaching attempts and success were analyzed using one-way two-tailed ANOVAs. The least squares difference post-hoc test was calculated for all cylinder, rung walking, reaching attempts, and reaching success. Analysis of the reaching subcomponents was done using a Kruskal-Wallis test. A Mann-Whitney U test was done post-hoc on all reaching subcomponents. All statistical procedures employed an *a priori* probability value of 0.05 and were performed using SPSS software (SPSS Inc., Chicago).

RESULTS

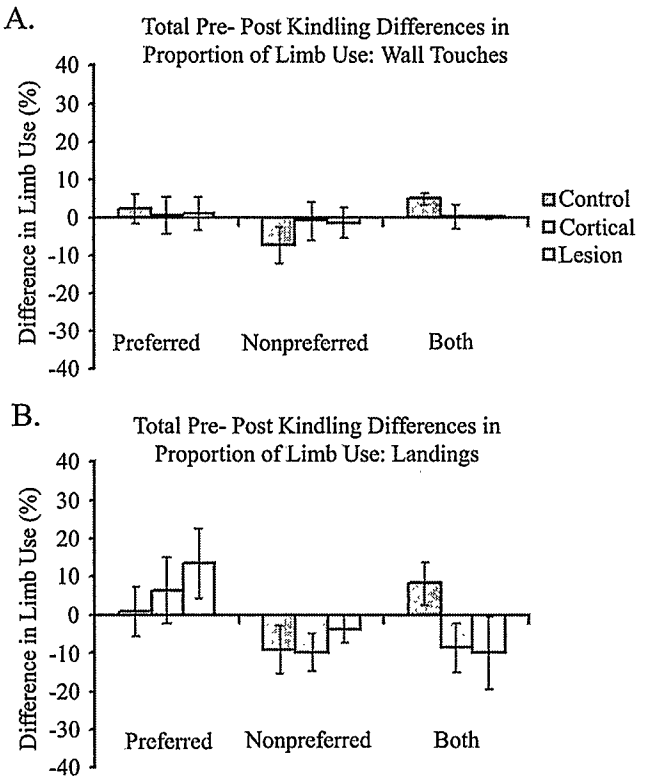
Experiment 1

Cylinder Task

Asymmetry in forelimb use was determined by counting the number of ipsi- and contralateral hand placements when rearing and contacting the wall of a cylinder, as well as limb contacts with the floor when landing from a rearing. A difference score was calculated by subtracting the number of hand placements counted during the post kindling scores from the baseline scores. There were no significant differences between the sham control, cortical kindled, and lesion groups on use of the preferred hand (F (2, 21) = 0.213, p= 0.810), the non-preferred hand (F (2, 21) = 0.936, p= 0.409), or both hands (F (2, 21) = 1.523, p= 0.244) for wall touches (Fig. 1A). Follow-up tests comparing the cortical kindled and lesion groups revealed no differences in use of the preferred (p= 0.476), non-preferred (p= 0.550), or both hands (p= 0.897). Likewise, use

Figure 3 - 1: Mean percent difference between pre-kindled and post-kindled use of preferred, non-preferred, and simultaneous (both) forelimbs during exploratory activity in a cylinder (mean + SEM). **(A)** Total pre - post kindling differences in proportion of limb use: Wall touches. **(B)** Total pre - post kindling differences in proportion of limb use: Landings. The lesion group used both hands simultaneously significantly less than sham controls. There were no significant between group differences.

Figure 3 - 1: Cylinder Task Proportion of Limb Use



of the preferred hand (F (2, 21) = 0.415, p= 0.666) the non-preferred hand (F (2, 21) = 0.284, p= 0.756) or both hands (F (2, 21) = 1.793, p= 0.193) for landings did not show significant differences in hand use during landing between the sham control, cortical kindled, and the lesion groups (Fig. 1B). Follow-up tests comparing the cortical kindled and lesion groups revealed no differences in use of the preferred (p= 0.941), non-preferred (p= 0.953), or both hands (p= 0.990).

Rung Walking Task

The number of errors per step and the type of errors were scored for fore- and hindlimbs. Analysis of the forelimb errors per step at both the early (F (2, 21) = 9.317, p= 0.002) and the late post-kindle (F (2, 21) = 2.004, p= 0.162) time points did not reveal significant differences between the cortical kindled and lesion groups at either the early (p= 0.190) or late post-kindling time points (p= 0.247). However, follow-up tests comparing the sham control group with the cortical kindled group revealed significant differences in the number of forelimb errors per step at the early post-kindling time point, (p= 0.000), but not at the late post-kindling time point (p= 0.063) (Fig. 2A). Follow-up comparison of the sham control and lesion groups revealed a significant difference at the early post-kindling time point (p= 0.031), but not the late post-kindling time point (p= 0.247) (Fig. 2A). Analysis of the hindlimb errors per step at the early post-kindling (F (2, 21) = 1.331, p= 0.288) time point did not reveal significant differences between sham control and cortical kindled groups (p= 0.255), the sham control and lesion groups (p= 0.554), or between the cortical kindled and lesion groups (p= 0.136). Analysis of the hindlimb errors per step did not reveal significant differences at the late post-kindling

time point (F (2, 21) = 0.653, p= 0.532) between sham control and cortical kindled groups (p= 0.294), the sham control and lesion groups (p= 0.921), or between the cortical kindled and lesion groups (p= 0.421) (data not shown).

Analysis of the forelimb errors scores at the early (F (2, 21) = 2.691, p= 0.094) and late post-kindle (F (2, 21) = 3.918, p= 0.038) time points did not reveal significant differences between the cortical kindled and lesion groups at either the early (p= 0.245) or late post-kindling time points (p= 0.076) (Fig. 2B). Follow-up tests comparing the sham control group with the cortical kindled group revealed significant differences in the forelimb error scores at the early post-kindling time point, (p= 0.032), and at the late post-kindling time point (p= 0.014) (Fig. 2A). Follow-up comparison between the sham control and lesion groups did not revealed significant differences at either the early post-kindling time point (p= 0.439), or the late post-kindling time point (p= 0.671) (Fig. 2A). Hindlimb error scores (F (2, 21) = 2.805, p= 0.086) did not reveal significant differences in the error scores at the late post-kindling time point between sham control or cortical kindled groups (p= 0.516), sham control and lesion groups (p= 0.087), but did reveal significant differences between the cortical kindled and lesion groups (p= 0.031) (data not shown).

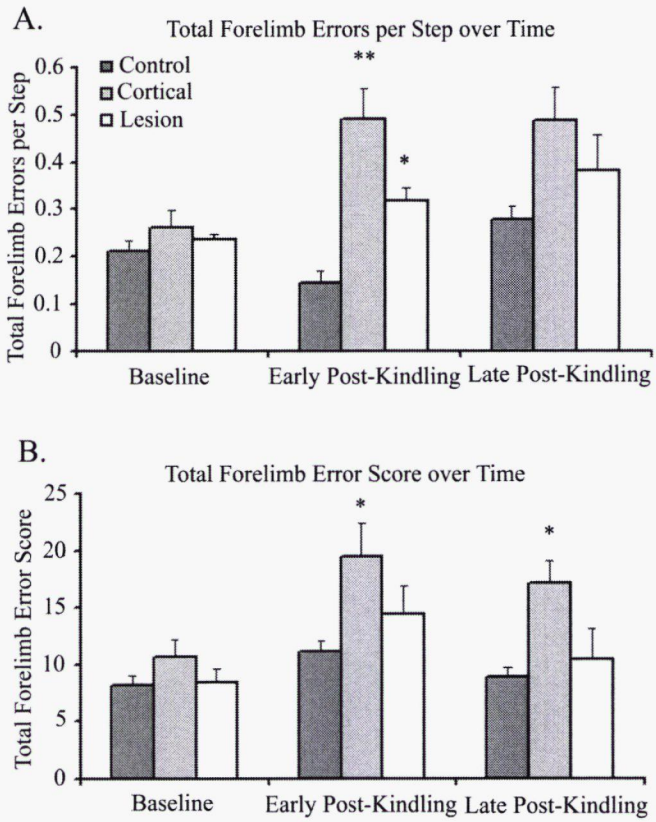
Successful Single Pellet Reaching Task- Attempts and Successes

The number of reach attempts was measured during every reaching session for 15 days. All groups increased the number of reach attempts throughout the 15 days. The

Figure 3 - 2: Rung walking task. Comparison of sham control, cortical, and amygdala groups across two measures of skilled motor behaviour on the rung walking task. **(A)** Total forelimb errors per step over time. Graphical representation of the mean (+ SEM) number of total forelimb errors committed by sham control, cortical kindled and amygdala kindled rats across all error types at baseline, early post kindling (48 h after the last seizure) and late post kindling (20 d after the last seizure) on the rung walking task. A lower number reflects fewer errors per step. Cortically kindled rats committed a significantly greater number of forelimb errors per step relative to sham controls at the early post-kindling time point. The lesion group also showed significant differences at the early post kindling time point. Asterisks indicate significant differences relative to the sham control group ** = $p \leq .01$.

(B) Total forelimb error score over time. Graphical representation of the mean (+ SEM) forelimb error scores added across error types at baseline, early post kindling (48 h after the last seizure) and late post kindling (20 d after the last seizure) rung walking task sessions for sham control, cortical kindled and lesion groups. A lower score reflects less severity in the types of errors committed. Cortically kindled rats committed more severe forelimb errors relative to sham controls. Asterisks indicate significant differences relative to the sham control group * = $p \leq .05$, ** = $p \leq .01$.

Figure 3 - 2: Rung Walking Task Forelimb Errors



sham control group made 98.56 (± 4.61) reach attempts on day 15 while the cortical kindled and lesion groups made 82.75 (± 2.28) and 55.2 (± 3.83) reach attempts on day 15 respectively (Fig. 3A). Analysis of the number of reach attempts on day 15 (F (2, 21) = 7.078, p= 0.005) revealed a significant group difference between the sham control and lesion groups (p= 0.001) and between cortical kindled and lesion groups (p= 0.030), but not between the sham control and cortical kindled groups (p= 0.132).

Success rates of the preferred forelimb were measured during every reaching session for 15 days. The time course of reaching success rates is illustrated in (Fig. 3B). All groups showed improvement in reaching success, achieving asymptote on Day 9. The sham control group had a success rate of 60.73% (± 4.84) on Day 15 while the cortical group reached at 46.87% (± 7.66) and the lesion group reached at 34.09% (± 11.67). Analysis of the number of successful reach attempts on day 15 (F (2, 21) = 10.251, p= 0.001) revealed a significant group difference between the sham control and cortical kindled groups (p= 0.017), the sham control and lesion groups (p= 0.000), and the cortical kindled and lesion groups (p= 0.049).

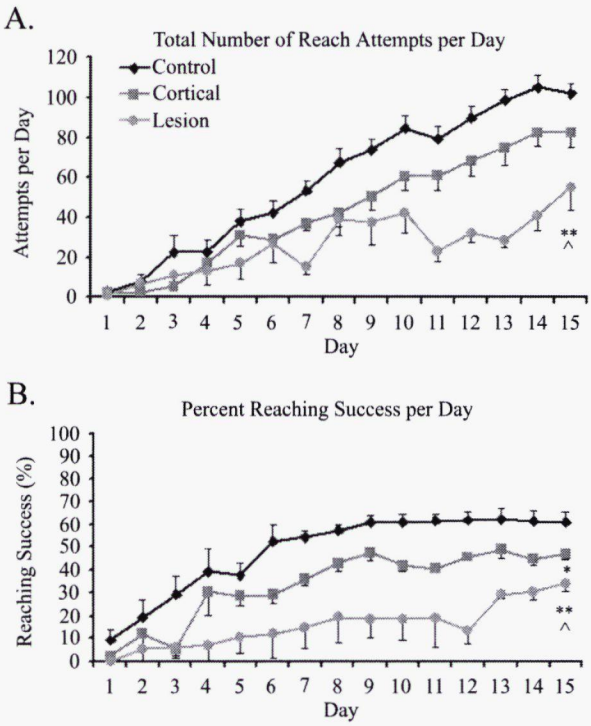
Successful Single Pellet Reaching Task- Kinematics

Video-recordings from Day 15 were analyzed for qualitative analysis of limb movements. Kinematic analysis revealed alterations in the reaching behaviour in five subcomponents: Elbow to Midline (III) (H= 14.692, p= 0.001), Advance (IV) (H= 19.029, p= 0.000), Apreggio (VI) (H= 8.342, p= 0.015), Supination I (VIII) (H= 5.023,

Figure 3 - 3: Single pellet reaching task attempts and success. Comparison of sham control, cortical and amygdala groups on the total number of reach attempts and the total number of reaching success over 15 days of reach training. **(A)** Total number of reach attempts per day. Graphical representation of the mean (+ or – SEM) number of reach attempts in the single pellet reaching task for sham control, cortical kindled, and amygdala kindled groups for reaching days 1 through 15. The lesion group was statistically different on day 15 from sham controls. Asterisks indicate significant differences relative to the sham control group * = $p \leq .05$. Cortical kindled rats also differed significantly from lesion rats ^ = $p \leq .05$.

(B) Percent reaching success per day. Graphical representation of the mean (+ or – SEM) success per day in the single pellet reaching task (number of pellets successfully retrieved out of total number of attempts) for sham control, cortical kindled, and amygdala kindled groups for reaching days 1 through 15. Cortically kindled and lesioned rats had a significantly smaller number of successful attempts relative to sham control group * = $p \leq .05$. Cortical kindled rats also differed significantly from lesion rats ^ = $p \leq .05$.

Figure 3 - 3: Total Number of Single Pellet Reach Attempts and Success per Day



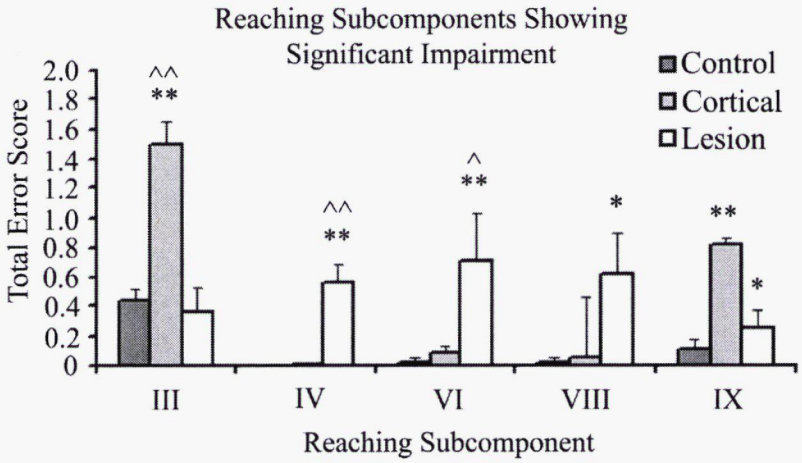
p= 0.081), and Supination II (IX) (H= 15.886, p= 0.000). Follow-up tests revealed significant differences between the sham control and cortical kindled groups on III (U= 1.000, p= 0.000) and IX (U= 0.000, p= 0.000). Sham controls differed significantly from lesion rats on IV (U= 0.000, p= 0.001), VI (U= 5.500, p= 0.019), VIII (U= 10.000, p= 0.037), and IX (U= 2.000, p= 0.004). Significant differences between the cortical kindled and lesion groups were revealed on III (U= 2.000, p= 0.006), IV (U= 0.000, p= 0.001), VI (U= 8.500, p= 0.051).

The cortical kindled groups' scores were obtained mainly due to an altered or absent adduction and abduction of the elbow (III) and an alteration in the ability to turn the wrist by 45° (IX) as seen in a typical reach. The cortical kindled rats additionally compensated for their altered forelimb movements with postural adjustments. They either oriented their body at an angle to the slot or aligned their body medially, but then leaned to the ipsilateral side to the reaching hand. All other subcomponents of the reaching behaviour appeared to be unaltered in the cortically kindled rats.

The lesion group's scores were obtained mainly due to an inability to properly navigate the hand toward the pellet (IV), and an inability to turn the wrist in a typical fashion at any point in the reach to properly cover the pellet (VI) and then subsequently withdraw the limb to consume the pellet (VIII and IX). The lesion rats additionally compensated for their altered forelimb movements with postural adjustments. They often oriented their reaching hand to the slot as opposed to aligning the midline of the body. Another common postural adjustment was a lowered body orientation at the reaching slot. Instead of approaching the pellet at a slight downward angle, the lesion rats were

Figure 3 - 4: Reaching subcomponents showing significant impairment. Graphical representation of the mean (+ SEM) qualitative scores of the two reaching components where statistical significance was found from five successful reaches on day 15 for sham control, cortical kindled and lesion groups. The cortical kindled rats showed significant impairment in the ability to adduct/abduct the elbow at subcomponent III relative to sham control and lesion rats as well as an inability to fully supinate the wrist at subcomponent IX relative to sham controls. Lesioned rats showed significant impairment in the ability to properly approach the pellet at subcomponent IV relative sham control and cortical kindled rats. Lesioned rats also showed impairment in the ability to place the hand over the pellet at subcomponent VI relative to sham control and cortical kindled rats, and an inability to supinate the wrist at subcomponents VIII and IX relative to sham controls. Asterisks indicate significant differences relative to the sham control group * = $p \leq .05$, ** = $p \leq .01$. Cortical kindled rats also differed significantly from lesion rats ^ = $p \leq .05$, ^^ = $p \leq .01$.

Figure 3 - 4: Reaching Components Showing Significant Impairment



almost parallel to the pellet in body angle. In some cases the lesion rats would also bring their mouth toward the hand in order to lessen the amount of limb movement in order to consume the pellet. The deficit in advance revealed a tendency for the lesioned rats to make straight path deviations to the pellet. The significantly lower number of reach attempts (Fig. 3A) may have been due in large part to the slow movement of the lesion rats to reset between reaches. Though no measurements were taken, the overall reaching behaviour of the lesion rats appeared to be more sporadic in nature.

DISCUSSION

We compared the interictal behavioural comorbidities associated with repeated seizures with post-lesion behavioural changes by examining motor behaviours that required varying skill levels. To my knowledge this represents the first report comparing the effects of repeated seizure and lesions on fine motor coordination using kinematic analysis. I found that both repeated seizures elicited through stimulation of the corpus callosum at the level of the caudal forelimb area and lesions in the same cortical area significantly altered skilled motor behaviour, but did so in different ways. Specifically, cortically kindled rats committed significantly more forelimb errors per step that were also greater in severity as evidenced by the higher forelimb error scores relative to sham controls. Lesioned rats had significantly higher hindlimb error scores (late post-kindling) relative to cortically kindled rats, but this is more suggestive that the devascularized area of cortex may have extended into hindlimb areas. However, the cortical kindled and lesion rats did not differ statistically on the rung walking task with respect to forelimb

kinematics, suggesting that kindled lesions and complete lesions do not exert differential effects on this measure.

The cortically kindled rats showed an altered reaching pattern, with significant alterations relative to sham controls in their reaching behaviour on two of the subcomponents that comprise the total reaching behaviour. These kinematic alterations manifested themselves in the decreased success of the cortical kindled rats on day 15. However, the reduced success of the cortical kindled rats was far less severe than the impairment seen in the reaching success of the lesion rats that differed significantly from both sham controls and cortical kindled rats. The lesion group also showed marked alterations in kinematics on four subcomponents of the reaching behaviour. The kinematic alterations of the lesion group extended to differences with the cortical kindled rats. The significant alteration of elbow adduction in the cortical kindled rats suggests a proximal impairment of the reaching limb relative to lesion rats. The inability of the lesion rats in the current study to advance to, and properly cover the pellet with the digits suggests difficulties in whole limb control (IV) and distal limb control (VI) relative to cortical kindled rats similar to those seen in previous research (Gharbawie et al., 2005; Gharbawie et al., 2006).

The results of the reaching task confirmed my hypothesis that kindled lesions have a different behavioural manifestation than complete lesions. The behavioural manifestation on motor function of lesions has been well documented in both experimental data (Schallert et al., 2000; Whishaw, 2000; Metz and Whishaw, 2002a; Whishaw et al., 2002; Teskey et al., 2003; Gharbawie et al., 2005; Gharbawie et al., 2006) and clinical data (Cirstea and Levin, 2000; Cirstea et al., 2003b; Cirstea et al.,

2003a; Jacobs et al., 2003; Roby-Brami et al., 2003b; Roby-Brami et al., 2003a; Michaelsen et al., 2004; Michaelsen and Levin, 2004; McCrea and Eng, 2005; McCrea et al., 2005). The performance of the lesion group relative to sham controls from the current study replicates both the previous experimental and clinical work with respect to the reaching data. Likewise, clinical data investigating motor alterations in people with epilepsy, particularly with frontal lobe foci (Helmstaedter et al., 1996; Matsuoka et al., 2000; Hernandez et al., 2002) confirm the results from the cortically kindled group relative to sham controls. Data from the current study suggests that the motor alterations resulting form a complete lesion are more severe than those resulting from a kindled lesion on a skilled learned task. This is confirmed in light of reports that severe focal devascularized tissue is considered dead and subsequently absent (Lipton, 1999) whereas evoked potentials can be recorded in kindled tissue (Racine et al., 1975; Teskey et al., 2001; Teskey et al., 2002a) and movements can be evoked using intracortical microstimulation (Teskey et al., 2002a; van Rooyen et al., 2006). Though the results of the current study are similar to lesion studies investigating similar behaviours (Whishaw, 2000; Metz et al., 2001; Metz and Whishaw, 2002a, b), the electrophysiological data (Racine et al., 1975; Teskey et al., 2001; Teskey et al., 2002a) and the behavioural data from the current study suggest that the nature of kindled tissue is different than that of lesioned tissue and that this difference has behavioural consequences.

The data from the rung walking task is not what I had hypothesized. Previous work has shown that rats with unilateral cortical lesions showed impairments on the rung walking task relative to controls (Metz and Whishaw, 2002a). The absence of a finding on the rung walking task in the current study is likely a reflection of one of two

possibilities: 1) the location of the lesions in the current study were not as posterior as in the previous study (Metz and Whishaw, 2002a) thus explaining the absence of a profound hindlimb difference (data not shown). 2) The smaller number of rats (due to unexpected mortality) and greater variability in step error severity within the lesion group may be washing out any statistical differences that would otherwise be present with respect to the forelimb errors per step and error scores.

Evidence from other lesion experiments (Lipton, 1999; Schallert et al., 2000; Whishaw, 2000; Metz and Whishaw, 2002a; Whishaw et al., 2002; Teskey et al., 2003; Gharbawie et al., 2005; Gharbawie et al., 2006) support the reaching results of the lesion group in the current study. While both the lesioned and cortical kindled rats showed motor disturbances, there was a clear difference in the type of disturbances scene in each group (Fig. 4). Histological analysis from lesion studies (Lipton, 1999) and kindling studies (Teskey et al., 1999, 2001; Goertzen et al., 2003; Teskey et al., 2006a) along with network analysis of both lesioned (Wishart et al., 1994) and kindled (Teskey et al., 2002a; van Rooyen et al., 2006) brains implies a fundamental difference in brain tissue that underlies the subsequent motor disturbances observed in complete lesioned and kindled lesioned rats. While it has been demonstrated that kindling does lead to cell (Goertzen et al., 2003) death in the order of 30% three weeks after the last seizure, complete lesions lead to the near total loss of cells in the core and penumbra of a permanent complete lesions that expands for up to seven days before the total infarct area is defined (Lipton, 1999). The malleability of tissue also differs between lesioned and kindled lesions. A positive correlation between brain damage and behaviour has been observed in lesioned rats (Wishart et al., 1994; Whishaw, 2000). Similarly, neuron loss

progresses with repeated seizures and number of seizures (Dam, 1980; Mathern et al., 1995). Neuron loss is also most prominent in the primary seizure focus (Babb et al., 1984b; Babb et al., 1984a; Cavazos and Sutula, 1990). Further brain-behaviour correlations have been observed in ictal behaviour (van Rooyen et al., 2006) and interictal behaviour (Kalynchuk et al., 1997) in kindled rats. However, there is a fundamental difference in the brain-behaviour correlations between lesioned and kindled rats, namely that once ischemic tissue is dead, no further damage to that area, and subsequently to behaviours mediated by that area, can be observed (Wishart et al., 1994) because the tissue is absent (Lipton, 1999). Kindled tissue can be manipulated such that network changes correspond with the amount of seizure spread to different brain areas (van Rooyen et al., 2006). Specifically, the idea that lesioned brain tissue is dead thus necessitating compensatory behaviours facilitated by adjacent brain areas (Whishaw, 2000) in the affected subject and that focal seizures create functional lesions whereby the affected brain area is still able to perform its designated task, but does so with modifications that are manifested in behavioural alterations is confirmed.

GENERAL DISCUSSION

The goal of the present thesis was to examine and describe interictal changes in fine motor skill after repeated seizures, and then to compare the changes precipitated by a kindling versus those of a complete lesion. Past research has thoroughly investigated changes in the kindled sensorimotor neocortex of the rat at the ultrastructural level, the anatomical level, the electrophysiological level, and the network level. The methodologies that were chosen were intended to answer behaviour level questions about the effects of sensorimotor kindling. Specifically, the current study attempted to bridge the findings from the aforementioned levels and describe the ensuing behavioural manifestations. Alterations in fine motor skill due to repeated seizures (kindling) were investigated and typified using two different behavioural tasks that required use of the affected brain tissue (CFA). The results indicated that cortically kindled rats do indeed show altered fine motor behaviour on a skilled task (rung walking) and a skilled learned task (single pellet reaching). Our results also showed that repeated seizures in a non-motor brain area, the amygdala, minimally affected motor coordination as only one subcomponent of the reaching behaviour showed alterations. The behavioural alterations in the cortically kindled rats were then compared with alterations evoked through focal devascularization that left the nature of the affected brain tissue profoundly different than the kindled tissue. The results from this experiment showed that while repeated seizures do alter motor behaviour, the alterations are different than the alterations seen in complete lesion rats. This finding supports other research that specifically investigated histology and electrophysiology. The work presented in this thesis has never previously been reported, making each aspect a new contribution to the scientific community.

Clinical Importance of Understanding Behavioural Alterations due to Repeated Seizures

Cortically kindled rats were shown to have altered fine motor coordination. This finding models the clinical data in people with FLE; however, the current thesis also describes the type of behavioural alterations. The clinical data does not describe the changes in motor behaviour in people with FLE except to say that it is "rigid" and "impulsive." It is therefore important for the clinical research to understand how motor coordination is altered to better understand the spread of seizures to different brain areas and to aid in the rehabilitation process. While rehabilitation may not result in total recovery of motor coordination, it may improve the efficiency of the compensatory strategies employed thereby improving the over all quality of life in both the immediate and long term future of people with epilepsy.

Physical therapy and rehabilitation are fairly homogenous across injury and pathology. That is to say, a sore knee is treated as a sore knee, where the cause of the soreness is diagnostic in nature, and not necessarily predictive of therapy. The results of Study 2 suggest that the etiology of motor alterations be a consideration when devising rehabilitation strategies. Specifically, arm rigidity and impulsivity of movements resulting from a stroke should perhaps be treated differently than similar symptoms in a person with epilepsy because the very nature of the brain insult is different. A difference in central pathology necessitates a difference in treatment of the periphery. To treat the peripheral alterations equally is to ignore the underlying source of the alterations lending the treatment itself to a lessened effect. By targeting alterations specific to their etiology

it is conceivable that better measurements of the deficits/ alterations will lead to more successful therapy and rehabilitation.

Limitations

While the alterations in motor behaviour were very consistent in the cortically kindled rats, a postural assessment of the rats in both the rung walking task and the single pellet reaching task would have been beneficial. Many of the kindled rats aligned themselves to the reaching slit along the midline of their bodies, but then leaned to the ipsilateral side of the preferred limb to reach and attain the pellet. This postural change necessitated the absence of bringing the elbow to midline as this would have thrown the aim of the limb off. Many other rats would also align their whole bodies at an angle to attain the pellet, again not requiring the alignment of the elbow to the midline. While the alterations observed in a single limb are important, the limb is attached to a body. These postural observations underscore the importance of looking at any particular motor behaviour as being whole-body activities, regardless of whether they chiefly involve only one limb or not. For example, a basketball player shooting a jump shot shoots the ball with his arms and hands, but the alignment of the hips, knees, and shoulders plays almost as big a factor in the outcome of the shot as the action of the arms and hands.

Unexpected mortality in the amygdala and motor cortex lesion groups decreased the sample sizes of those groups. The lower sample sizes in both the amygdala kindled group (experiment 1) and the lesion group (experiment 2) could be hiding potentially important changes in both of these groups. For example, the lesion group showed a trend toward significant impairment on the rung walking task, but due to the smaller sample

size, even a small outlier within this group biased both the mean and the standard error of the mean, washing out any statistical differences. Increasing the sample sizes in both of these groups would offer a more accurate picture into the differences in motor behaviour relative to the sham controls.

Another weakness of the current thesis is specific to the rung walking task. The actual apparatus is excellent at revealing forelimb behaviours, but due to the close proximity of the rungs does a poor job of revealing hindlimb errors. The feet of the rat are bigger than the gaps created by removing only one rung, possibly disguising more severe hindlimb errors as being either minor errors or from being recognized as errors at all making it very difficult to assess the actual coordination of the hindlimbs. This problem can be alleviated by removing consecutive rungs, bearing in mind that this will likely result in a sharp increase in forelimb errors.

Future Studies

The current thesis lends itself to many more questions than answers. The results of Study 1 show that kindling affected the kinematics of the reaching behaviour. Future studies should investigate whether pre-training on the single pellet reaching task results in higher success rates on the reaching task, and whether or not pre-training would effect the kinematic changes associated with kindling. Such a study may help to answer questions about how kindling affects behaviours that have already been learned, and if the kinematics of learned movements are resistant to kindling-induced changes. Within this potential study it would also be worthwhile to chart the kinematic changes in the pre-

trained paradigm across time since the last seizure to see if any potential kinematic changes normalize to pre-kindled status.

It has been shown in the cortical group that the persistence of the effects of the seizure on motor behaviours last at least 17 days. It is also known from other work that the map changes and changes in field potential last up to 21 days (Teskey et al., 2002a). These two findings indicate a high degree of persistence across levels of analysis. However, the status of the changes in the kindled brain is not well understood past three weeks. It would then be beneficial to investigate the changes in kindled tissue past the three week time point. Not only would a time course investigation answer questions about the permanence/ persistence of the kindling induced changes, but it would also answer questions about the ethological validity of the kindling model.

The current thesis made note of the impulsive and rigid nature of the kindled rats' reaching behaviour. A quantitative investigation into the differences in the force and velocity of kindled rats locomotive and reaching behaviour would help to further elucidate any differences from sham control rats along these dimensions. It is likely, given clinical accounts of similar behaviour in people with both temporal and frontal lobe epilepsy, that this added level of kinematic analysis would reveal changes common to all kindled rats, regardless of foci, but that there may still be differences between the two groups along these measures. One might predict that cortically kindled rats would show greater changes from sham controls on both force and velocity. Future studies may consider investigating kinematic alterations, including force and velocity, by kindling in the hippocampus and over-kindling (30+ stimulations) in sensorimotor cortex. Because the hippocampus kindles slowly it is an ideal structure to correlate seizure

severity/duration with kinematic alterations. An over-kindling experimental group would also provide a simple means of comparing seizure severity/duration with kinematic alterations as the number of kindling stimulations increases, so to does the probability of a rat having consecutive stage 5 seizures.

FAST rats (epileptogenically prone) and SLOW rats (epileptogenically resistant) represent a unique opportunity to investigate possible genetic contributions to altered kinematics. Replicating Study 1 and conducting a study in which the rats are pre-trained on the single pellet reaching task prior to kindling with FAST and SLOW rats would allow one to disambiguate between kinematic alterations that occur after seizures and kinematic alterations that are pre-existing. It may be that being epileptogenically prone has behavioural consequences that pre-date any post-seizure alterations. Such a finding could then be used as a predictive measure of epileptogenicity in humans where there is a positive family history for epilepsy.

Clinical research lags far behind experimental work in terms of the complexity and relevance of behavioural tasks used to assess motor coordination. While there are several tasks that do assess motor coordination, like Thurstone's Uni- and Bimanual Performance Test and the Purdue pegboard test to name two, these tasks do not include kinematic norms that allow for the assessment of movements, except for gross performance measures. Introducing a task analogous to the single pellet reaching task or the rung walking task would allow for a greater degree of detection of alterations in motor behaviours. More sensitive tests will allow for a better description and understanding of any potential alterations in motor coordination, and the functional consequences of those alterations.

One of the greatest challenges of clinical research is the heterogeneity of the patient population. It is difficult, if not impossible to account for and match participants across every variable; however, the current thesis has demonstrated that repeated seizures in one brain area do not necessarily amount to the same behavioural consequences as repeated seizure in another brain area. The discoveries in the experimental and clinical realms then beg for better group selection and delineation in clinical research. While this will inevitably lead to smaller sample sizes and a subsequent loss of statistical power, it will lead to greater precision and specificity in understanding the behavioural effects of epilepsy in different brain areas.

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