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The Role of Ipsilesional Forelimb Experience on Functional Recovery After Unilateral Sensorimotor Cortex Damage In Rats

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The Role of Ipsilesional Forelimb Experience on Functional Recovery After Unilateral Sensorimotor Cortex Damage In Rats

by

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To all the lab rats that made this possible
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The Role of Ipsilesional Forelimb Experience on Functional Recovery
After Unilateral Sensorimotor Cortex Damage In Rats

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Following unilateral stroke there is significant loss of function in the body side contralateral to the damage and a robust degenerative-regenerative cascade of events in both hemispheres. It is natural to compensate for loss of function by relying more on the less-affected body side to accomplish everyday living tasks (e.g. brushing teeth, drinking coffee). This is accompanied by a “learned disuse” of the impaired side thought to occur due to repeated experience with its ineptness. However, as investigated in these studies, it may also be due to brain changes instigated by experience with the intact body side.

The central hypothesis of these dissertation studies is that experience with the intact forelimb, after unilateral sensorimotor cortex (SMC) damage, disrupts functional recovery with the impaired forelimb and interferes with peri-lesion neural plasticity.
Following unilateral ischemic lesions, rats were trained on a skilled reaching task with their intact (less-affected) forelimb or received control procedures. The impaired forelimb was then trained and tested on the same skilled reaching task. Intact forelimb experience worsened performance with the impaired forelimb even when initiated at a more delayed time point following lesions. Intact forelimb training also reduced peri-lesion expression of FosB/ΔFosB, a marker of neuronal activation, and caudal forelimb motor map areas compared to animals without intact forelimb training. It was further established that it is focused training of the intact forelimb and not experience with this limb per se, as animals trained with both forelimbs in an alternating fashion did not exhibit this effect. Transections of the corpus callosum blocked the maladaptive effect of intact forelimb experience on impaired forelimb recovery, suggesting a disruptive influence of the intact hemisphere onto the lesion hemisphere that is mediated by experience.

Together these dissertation studies provide insight into how experience with the less-affected, intact body side, can influence peri-lesion neural plasticity and recovery of function with the impaired forelimb. The findings from these studies suggest that compensatory use of the less-affected (intact) body side following unilateral brain damage is not advantageous if the ultimate goal is to improve function in the impaired body side.
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Chapter 1
Introduction

1.1. Overview

Unilateral cortical damage in rats can result in bilateral behavioral changes, including impairments in the contralateral-to-lesion (contralesional) forelimb and an increased reliance on the ipsilesional, “intact” forelimb. Compensatory reliance on the intact forelimb is an adaptive strategy that enables animals to perform many normal behaviors despite their impairments. However, recently we have found that behavioral experience with the intact forelimb may come at a cost of worsening recovery of function of the impaired forelimb. The general hypothesis underlying this dissertation research is that, following unilateral ischemic sensorimotor cortex (SMC) damage, behavioral experiences with the intact forelimb can interfere with neuroplastic processes that could otherwise be driven to mediate functional recovery of the impaired forelimb.

In the human population, most early post-stroke rehabilitation programs, as well as self-taught coping strategies, focus on compensatory movements with the less-affected body side; which, according to our findings, could ultimately be detrimental for functional recovery of the impaired body side. Findings from these studies could provide valuable insight into human post-stroke recovery processes and lead to new ways in which rehabilitation in the human population is carried out.

It is now well established that unilateral injury can also cause some deficits in the ipsilesional forelimb (e.g., O’Bryant, Bernier, & Jones, 2007; Gonzalez et al., 2004), and
hence this limb is sometimes referred to as the “less-affected” or “less-impaired” limb; but for the purposes of these dissertation studies, the ipsilesional forelimb will be referred to as the “intact” forelimb and the contralesional forelimb will be referred to as the “impaired” forelimb.

The central hypothesis of these dissertation studies is that experience with the intact forelimb after unilateral SMC damage disrupts functional recovery with the impaired forelimb and interferes with peri-lesion neural plasticity. This hypothesis was tested in a series of experiments that manipulated post-lesion behavioral experience with the intact forelimb. The first study (chapter 3) tested the effects of intact forelimb training on impaired forelimb function. A second study (chapter 4) tested whether the effects of intact training required lateralized focused training versus bilateral training. A third study (chapter 5) tested whether there is a post-lesion time-sensitive window when experience with the intact forelimb influences impaired forelimb recovery. A fourth study (chapter 6) examined the role of the corpus callosum and the contralesional hemisphere in mediating the effect of intact forelimb experience.

The remainder of this chapter focuses on background research that supports the approach adopted for these studies. First, rats are considered as a model for stroke induced impairments. Next, the behavioral and neural effects of motor learning and unilateral brain damage are considered. This section is then concluded with discussions of the effects of rehabilitation after brain damage and the anatomy of the rat sensorimotor cortex. These topics set up the basis for the questions asked in these dissertation studies.
1.2. Rats make good animal models for stroke research

Frequently following stroke, humans lose the ability to accurately shape their paretic hand for grasping objects and show deficits in the capacity to extend the fingers and thumb (Lang, DeJong, & Beebe, 2009). The movements required to complete a reach-to-target task are similar between rats and humans (Whishaw, 1992; Sacrey, Alaverdashvili, & Whishaw, 2009). Some deficits following stroke can be modeled in rats, which have very skillful use of the forepaw. There are established sensorimotor tests that measure deficits incurred from brain damage (e.g., see Kleim, Boychuk, & Adkins, 2007; Cenci, Whishaw, & Schallert, 2002), including the single pellet retrieval task (a reach-to-grasp task). Recovery of function can be linked with resultant changes in neural activity and connectivity. A recent review from Cramer (2008) details findings that suggest that there are similarities in the ways rats and humans recover after stroke. For example, reorganization in the peri-infarct zone in rats and humans is a major contributor to recovery of motor function in both species.

1.3. Skilled reach training drives motor cortical plasticity

A skilled learning task was used in these dissertation studies as both an outcome measure and as a behavioral manipulation. Motor skills training in intact animals induces plastic changes in the motor cortex contralateral to the trained forelimb, including increases in dendritic arborization (Greenough, Larson, & Withers, 1985; Withers & Greenough, 1989; Allred & Jones, 2004) and synapses (Kleim et al., 2004) and an
expansion of motor representations devoted to reaching movements (e.g., digit and wrist, Kleim, Barbay, & Nudo, 1998; Nudo, Milliken, Jenkins, & Merzenich, 1996a). It has been further established that practice with a skilled motor task (and not a simple bar pressing task) is necessary to induce motor map reorganization (Kleim et al., 1998). Practice with skilled reaching in humans has also been shown to be associated with changes in motor cortical movement representations, as measured using transcranial magnetic stimulation (TMS, Muillbacher, Ziemann, Boroojerdi, Cohen, & Hallett, 2001; Pascual-Leone et al., 1995) and functional magnetic resonance imaging (fMRI, Perez et al., 2007).

Mechanisms involved in learning a motor skills task may share some similarities with those involved in long-term potentiation (LTP), an artificial means of inducing synaptic strengthening. Motor skills training results in LTP-like effects in the cortex opposite the trained forelimb (e.g., Rioult-Pedotti, Friedman, & Donoghue, 2000; Monfils & Teskey, 2004). Tetanic stimulation delivered to layer II/III pyramidal neurons in motor cortex can induce LTP in vitro (e.g., Monfils, Plautz, & Kleim, 2005; Rioult-Pedotti et al., 2000; Hodgson, Standish, Boyd-Hodgson, Henderson, & Racine, 2005). LTP can also be induced in motor cortex in awake animals (e.g., Monfils & Teskey, 2004). LTP is thought to occur via strengthening of horizontal connections in the stimulated hemisphere (Hess, 1994) and is dependent on protein synthesis (Luft et al., 2004). Inhibiting protein synthesis blocks LTP (Scharf et al., 2002) and has also been shown to disrupt motor learning (e.g., Luft et al., 2004). An infusion of anisomycin (a protein synthesis inhibitor) into sensorimotor cortex, but not into parietal cortex,
disrupted performance on a learned task (Hsu, Donlan, & Jones, 2007; Kliem et al., 2003). This deterioration in performance recovered to baseline levels within 48 hours. Anisomycin application can also disrupt motor map plasticity. Kleim and colleagues (2003) have shown that, following protein synthesis inhibition, reorganized motor map representations are eliminated and synapses are lost in the same region, effects which were replicated by Hsu and colleagues (2007). The extent to which intact forelimb training can influence peri-lesion motor map plasticity was unknown and was therefore examined in the study described in chapter 5.

1.4. Behavioral and cortical changes following unilateral sensorimotor cortex damage

Unilateral SMC damage has been shown to result in profound sensory and motor impairments in the contralesional forelimb, including deficits in skilled reaching (Whishaw, 2000; Gilmour et al., 2004), delayed responsiveness to tactile-stimulation (Napieralski et al., 1998), decreased use of this limb in tests of postural support (Napieralski et al., 1998; Adkins et al., 2004; Allred & Jones, 2004; Hsu & Jones, 2005) and increased errors in measures of coordinated limb use (Bury & Jones, 2002; Adkins et al., 2004). Following SMC damage, a deficit in skilled reaching performance has been found to occur in rats (e.g. Whishaw, 2000; Gilmour et al., 2004; see also Gharbawie, Auer, & Whishaw, 2006), in monkeys (Friel & Nudo, 1998; Nudo, Wise, SiFuentes, & Milliken, 1996) and in humans (Green, 2003).
Unilateral damage to the SMC results both in degenerative events in the area surrounding and connected to the lesion, including neurotoxic and excitotoxic effects (e.g., Doyle, Simon, & Stenzel-Poore, 2008), and in increased neuroplastic events in the contralesional cortex, including increased dendritic arborization (Jones & Schallert, 1992; Jones, Kliem, & Greenough, 1996), synapse number per neuron (Jones 1999; Jones et al., 1996; Luke, Allred, & Jones, 2003), and synapse- and growth-associated proteins (Cheng, Tong, & McNeill, 1998; McNeill, Mori, & Cheng, 1999; Stroemer, Kent, & Hulsebosch, 1995). Both unilateral electrolytic and unilateral ischemic SMC lesions have previously been shown to increase the density of dendritic processes immunoreactive (IR) for microtubule associated protein 2 (MAP2) in layer V of contralesional, homotopic cortex of rats (Bury & Jones, 2002; Allred & Jones, 2004).

Following focal cortical lesions, NMDA receptor binding has been shown to increase in both hemispheres (Que, Schiene, Witte, & Zilles, 1999; Redecker, Wang, Fritschy, & Witte, 2002). Middle cerebral artery occlusion has been shown to result in an increased paired-pulse excitation (Reinecke et al., 1999) and an enhancement in neuronal calcium currents (Bruehl, Neumann-Haefelin, & Witte, 2000) in the contralesional hemisphere of rats. Recent human studies using TMS approaches have also shown contra-to-stroke hemispheric changes, including increased disinhibition and increased excitability (Liepert et al., 2000; Shimizu et al., 2002, see also Murase, Duque, Mazzocchio & Cohen, 2004).

In the rat, these contralesional neuroplastic changes may facilitate compensatory use of the ipsilesional forelimb. We have previously shown in male and female rats,
following unilateral SMC damage, an enhanced ability to acquire a skilled reaching task with the ipsilesional forelimb compared to intact animals (Bury & Jones, 2002; Luke et al., 2004; Allred & Jones, 2004; Hsu & Jones, 2005). Denervation of transcallosal fibers through transection of the corpus callosum (CCX) also facilitates acquisition of a skilled reaching task with one forelimb compared to intact animals (Bury & Jones, 2004).

As previously mentioned, learning a skilled reaching task is associated with growth of dendrites in primary motor cortex opposite the trained limb (Greenough et al., 1985; Withers & Greenough, 1989), and this effect is increased when training the forelimb ipsilesional to a unilateral SMC lesion (Bury & Jones, 2002; Allred & Jones, 2004). These findings indicate that both lesion-induced and behaviorally-induced neural plasticity are necessary for contralesional cortical changes. There is an exaggerated responsiveness of the motor cortex to learning with the ipsilesional forelimb. A question is whether this coincides with, and possibly leads to, exaggerated inhibitory or confounding influences of the “intact” hemisphere over the lesion hemisphere.

1.5. **Compensatory use of the less-affected body side**

For many stroke survivors, physical therapy is necessary to improve behavioral function. An ongoing issue in the clinical literature is how chronic stroke therapy should be managed for optimal recovery. A naturally occurring event following stroke is the development of compensatory use of the intact limb. Compensatory use of this limb occurs as a self-taught behavior (Allred & Jones, 2008a) and is also a focus of rehabilitation programs (Dobkin, 2006). However, excessive reliance on the intact body
side may lead to learned non-use, where the impaired limb may have the potential to function, but this potential is not realized due to increased reliance on the intact limb (Mark & Taub, 2002). One way to overcome learned non-use is through constraint induced movement therapy (CIMT), where most of the waking hours are spent with the intact limb placed in a sling in combination with a rehabilitation program (reviewed in Mark & Taub, 2002). CIMT has previously been shown to be a beneficial means of increasing chronically impaired limb function (e.g. Miltner, Bauder, Sommer Dettmers, & Taub, 1999; Taub & Morris, 2001; Wolf et al., 2006).

In rats, however, forcing overuse of the impaired limb early after damage has been shown to exacerbate lesion size and worsen functional outcome compared to control animals (DeBow, Davies, Clarke, & Colbourne, 2003; Humm, Kozlowski, James, Gotts, Schallert, 1998; Kozlowski, James, & Schallert, 1996; Bland et al., 2000). In rats it is easy to manipulate forelimb experience to test its effect on post-stroke recovery processes. By examining the influences of intact forelimb experience on recovery of function of the impaired forelimb and neural plasticity in peri-lesion cortex, these studies will contribute to the understanding of how post-injury forelimb experience can influence functional outcome.

1.6. Rehabilitation following brain damage

After unilateral brain damage, some functional improvements in the impaired limb have been found following practice on a skilled reaching task (e.g. Whishaw, 2000; Gharbawie & Whishaw, 2005; Allred, Maldonado, Hsu, & Jones, 2005; Nudo et al.,
and following rehabilitative training using a similar, but less skilled task (i.e., the tray reaching task where animals reach for multiple pellets at a time; Maldonado, Allred, Felhauser, & Jones, 2005; O’Bryant et al., 2005). However, training-induced improvements do not normalize the movement sequences used to accomplish the task (Whishaw et al., 2002; Gharbawie, Gonzalez, & Whishaw, 2005). This suggests that improvements in function are linked, at least in large part, to development of compensatory ways of moving the impaired forelimb (Alaverdashvili, Foroud, Lim, & Whishaw, 2008). Exercise following unilateral lesions did not result in improvement in impaired forelimb skill (Maldonado et al., 2005), which suggests that improvements result from practice on a motor skill task, not simply from activity.

As previously noted, in intact rats, experience with a skilled reaching task (reaching and grasping for food) results in an increase in pyramidal neuron dendritic arborization (Greenough et al., 1985; Withers and Greenough, 1989) as well as in changes in motor map representations, including an increase in distal forelimb (i.e. digit and wrist) representations and a decrease in proximal forelimb (elbow/shoulder) representations (e.g. Remple, Bruneau, VandenBerg, Goertzen, & Kleim, 2001; Kleim et al., 1998). Following motor cortex damage, depending on lesion size and location, training with the impaired limb has been found to induce motor map representational changes in monkeys (Friel & Nudo, 1998; reviewed in Nudo, 2003), rats (Castro-Alamancos & Borrell, 1995) and humans (Liepert et al., 2000; Weiller, Ramsay, Friston, & Frackowiak, 1993; Cramer, Moore, Finklestein, & Rosen, 2000; Green, 2003). Deficits can be reinstated if the reorganized cortex is ablated (Castro-Alamancos & Borrel, 1995), inactivated by
disruptive TMS (Fridman et al., 2004), or disrupted with anisomyocin infusions (Hsu et al., 2007). These data suggest that the peri-lesion cortex is vulnerable to post-injury experience, and plasticity in this region is an important contributor to recovery. Peri-lesion motor map reorganization has been suggested as playing a large role in mediating recovery (see Liepert et al., 2000; Green 2003; Nudo, 2003).

It is not known what effect intact forelimb experience has on peri-lesion motor map plasticity. We have previously shown that intact forelimb experience can influence the expression pattern in peri-lesion cortex of FosB/ΔFosB, an immediate early gene that accumulates chronically and is an indicator of neural plasticity (McClung et al., 2004). The ΔFosB isoform (which is lacking amino acids at the C-terminus) has been shown to upregulate a variety of genes in mice that over-express ΔFosB, including GluR2 (an AMPA receptor subunit), and microtubule associated protein-2 (MAP2, a dendritic cytoskeletal protein) (McClung & Nestler, 2003). We used immunolabeling for this protein to assay its effects on cortical neuronal activity, and we used intracortical microstimulation (ICMS) procedures to understand its effects on motor cortical maps.

Experience with the intact body side following stroke is not always detrimental to recovery. One type of frequently employed training is assisted bilateral training. In this treatment, the stroke-affected arm is held by the intact arm and the two arms move in synchrony to accomplish tasks (Stoykov & Corcos, 2009). Previous clinical studies have shown beneficial effects of bilateral training, such as bilateral arm training with rhythmic auditory cueing (BATRACT). BATRACT, where the patient pushes and pulls T-bar handles either in synchrony or in alternation has been shown to improve impaired arm
function and to induce contralesional cortical reorganization (Luft et al., 2004). Bilateral isokinematic training or BIT, which involves several reach-to-target tasks, has also been shown to result in enduring improvements in affected limb performance (Mudie & Matyas, 2000). Other types of bilateral training, including mirror therapy, device driven bilateral training and bilateral priming (where the motor cortex is stimulated prior to movements) have also been shown to be efficacious in promoting paretic arm recovery (Stoykov & Corcos, 2009). It has been suggested that the mechanism mediating recovery following training with both limbs is a restoration of balance of interhemispheric activity (Stinear, Barber, Coxon, Fleming, & Byblow, 2008). These findings led us to ask how bilateral forelimb training (training both limbs in an alternating fashion) would influence impaired forelimb function in chapter 4.

1.7. **Interhemispheric connectivity of the SMC**

The SMCs of the two hemispheres interact via transcallosal inputs, which mediate interhemispheric inhibition and excitation (Lee, Gunraj, & Chen, 2007). Transcallosal inputs make excitatory connections onto pyramidal neurons of the opposite cortex (Chapin, Sadeq, & Guise, 1987; Karayannis, Huerta-Ocampo & Capogna 2007, Carr & Sesak, 1998; Cisse, Grenier, Timofeev, & Steriade, 2003) and also project onto GABAergic inhibitory interneurons (Perez & Cohen, 2008; Innocenti, Clarke, & Kraftsik, 1986;). Bilateral and intercortical activity occur as a response to unilateral sensory stimulation (Liepert, Haevernick, Weiller, & Barzel, 2006) and movement (Cisek, Crammond & Kalaska, 2003; Brus-Ramer, Carmel & Martin, 2009). Previously,
the SMC region damaged in these studies was shown to receive transcallosal input from the opposite cortex, as reflected in biodextrine-amine (BDA) labeled axons (Bury & Jones, 2004). Layer V pyramidal neurons also make synaptic connections with ipsilateral and contralateral striatums (Cospito & Kutlas-Ilinkshy, 1981).

There is converging evidence that the two cerebral hemispheres have a balance of activity that can be disrupted following unilateral brain damage. For example, following induction of visual neglect in cats there is an increase in activity in the contralesional hemisphere, which returns to baseline levels with transient lesions of the contralesional hemisphere (Rushmore, Valero-Cabre, Lomber, Hilgetag, & Payne, 2006). Chronic suppression of barrel field cortex (BFC) leads to a disinhibition of contralateral BFC (Rema & Ebner, 2003). In humans, following stroke there is an increased inhibitory drive (as measured using a paired pulse TMS paradigm) from the contralesional to the lesion cortex (Duque et al., 2005; Perez & Cohen, 2009). This has also been shown to be correlated with a reduction in reaction time on a finger tapping task (Murase et al., 2004).

Callosal lesions and agenesis of the corpus callosum in humans results in a loss of interhemispheric inhibition (e.g., Meyer, Rörich, Gräfin von Einsiedel, Kruggel, & Weindl, 1995), which strongly suggests that interhemispheric activity is mediated through the corpus callosum. A disruption of interhemispheric activity following unilateral damage may become exaggerated with behavioral experience, and it stands to reason that if this unbalance is blocked, e.g., by callosal transections (chapter 6), this would prevent activity in the intact hemisphere from disrupting the ability of the lesion cortex to recover.
1.8. Anatomy of the rat sensorimotor cortex

1.8.1. General organization

The forelimb area of the rat sensorimotor cortex (SMC) is partially overlapped between primary motor (M1) and primary sensory (S1) cortices (Hall & Lindholm, 1974; Wise & Jones, 1977; Donoghue & Wise, 1982). S1, which is posterior and lateral to M1, receives sensory input primarily in layer IV, the granular region, and is characterized by receptive fields that respond to sensory stimuli. M1, which lacks a clearly defined granular layer, contains large pyramidal neurons in layer V and is defined based on populations of neurons that, when stimulated, elicit distinct body movements (Wise & Jones, 1977; Donoghue & Wise, 1982; Welker, Sanderson, & Shames, 1984; Sanderson, Welker, & Shambes, 1984). This is the basis of the motor maps revealed using ICMS procedures (chapter 5). Layer V pyramidal neurons within the overlap zone synapse on spinal motor neurons to elicit forelimb movements (Valverde, 1966; Hicks & D’Amato, 1977). The caudal and rostral forelimb areas are distinct from each other and are distinguishable based on characteristics of their movement representations. The SMC lesions used in these dissertation studies are aimed at the caudal forelimb area. The caudal forelimb representation area, and not the rostral area, may be the more important contributor to skilled motor learning (e.g., Gharbawie, Karl, & Whishaw, 2007). It receives input from S1 (Sievert & Neafsey, 1986) and is the targeted region for the lesions induced in these dissertation studies. Damage to this region in rats has been shown to result in pronounced deficits in the forelimb contralateral to the lesion (Allred & Jones, 2004; Hsu & Jones, 2005; Maldonado et al., 2005).
1.8.2. Ascending and Descending Projections

S1 receives thalamic input from the ventral posterior nucleus (Donaldson, Hand, & Morrison, 1975; Wise & Jones, 1978) and the posterior thalamic complex, which terminates mainly in layer IV and lower layer III (Koralek, Jensen, & Killackey, 1988; Killackey, 1973; Killackey & Sherman, 2003). M1 also receives input from the ventral posterior thalamus, which mainly terminates in layer III (Killackey, 1973; Killackey & Sherman, 2003). The overlapping region of M1/S1 receives extensive input from the ventrolateral thalamus with projections terminating in layers II/III and V (Donoghue & Parham, 1983). The ventrolateral thalamus receives input from several regions, including the spinal cord, cerebellum, and basal ganglia (Donoghue & Parham, 1983). Projections originating in S1 terminate in areas involved in motor behavior, including the striatum and pontine nuclei (Wise & Jones, 1977; Donoghue & Parham, 1983; Mihailoff, Lee, Watt, & Yates, 1985). Layer V neurons originating in M1 then project to the spinal cord (Leong, 1983; Bates & Killackey, 1984; Miller, 1987) red nucleus, and pons (Legg et al., 1989; Mihailoff et al., 1985), bilaterally to striatum (Donogue & Kitai, 1981), and also to the reticular formation (Valverde, 1966).

The corticospinal tract is mostly a crossed pathway, but approximately 5-10% of this pathway is estimated to be ipsilateral (Brosamle & Schwab). Layer V motor neurons project to the spinal cord via corticospinal projections. Damage to the corticospinal tract (CST) can result in deficits in motor tasks, though the medial and lateral portions of the CST appear to contribute to different aspects of motor performance. The SMC
contributes to both pathways. Rats with lateral CST lesions (aimed at C5 of the spinal cord) have more enduring deficits on a skilled reaching task compared to medial CST lesion animals, though the medial CST appears to play a larger role in mediating grip strength (Anderson, Gunawan, & Steward, 2007).

1.9. Conclusions

Following unilateral damage there are bilateral cortical changes. It has been demonstrated that rats have an enhanced ability to acquire a skilled reaching task with the intact forelimb following unilateral damage. The extent to which experience with the intact forelimb influences motor performance with the impaired forelimb was unknown. The purpose of these dissertation studies was to examine the effects of motor learning with the intact forelimb on 1) impaired forelimb function, 2) peri-lesion expression of FosB/ΔFosB, 3) peri-lesion motor map plasticity, 4) and also to test the role of interhemispheric connections in mediating this effect.
Chapter 2

General Methods used in Dissertation Studies

2.1. Single Pellet Retrieval Task

The single pellet-retrieval test (McKenna & Whishaw, 1999; Miklyaeva & Whishaw, 1996; Peterson & Devine, 1963; Withers & Greenough, 1989) was performed in a Plexiglas chamber with a tall narrow window in the center of the front wall (see Figure 2.1). Animals were placed on scheduled feeding (16-19g rat chow/one time per day) beginning two days before experiments began to motivate reaching behavior. All animals were given banana flavored food pellets (45 mg, Bioserve, Inc.) in their home cages for approximately two weeks before the start of reaching behavior to reduce neophobic responses to unknown food. Animals were shaped over several days on the single pellet retrieval task whereby a limb of preference (dominant limb for the task) was established (more than 50% reach attempts with same limb over a 10 minute period). After a dominant limb was established, animals were trained on the task to a proficient level (> 50% success/reach attempt) with this forelimb. A wall was placed 1.5 cm from the center window to discourage use of the non-trained limb. Pre-operative training consisted of 30 trials in which, on each trial, rats could make up to 5 reach attempts for a banana pellet located in a shallow well (1 cm from the window). A trial ended when greater than 5 reach attempts (extension of the forepaw through the window) were made or the pellet was knocked from its well (failures), the pellet was dropped inside the chamber before consumption (drop), or when the pellet was successfully retrieved from
its well and eaten. A short pause preceded each trial such that the animal was distracted from, or turned away from, the center window (by tapping on the side of the chamber, or by dropping a pellet in the chamber) while a new pellet was placed in the well. During control procedures, rats were placed into a reaching chamber without a wall and were given food pellets on the chamber floor at approximately the same rate as a yoked, trained animal.

![Figure 2.1 Reach training apparatus.](image)

Rats were trained to reach for banana flavored food pellets placed 1 cm from the window. A wall was placed ipsilateral to the animal’s reaching
forelimb such that they could not make successful retrievals with their non-trained forelimb.

### 2.2. Surgical Methods

Unilateral ischemic sensorimotor cortex (SMC) lesions were induced through topical application onto the cortical surface of endothelin-1 (ET-1), a vasoconstricting peptide. Topical application of ET-1 has previously been shown to induce reliable damage to the area of interest and to cause forelimb behavioral impairments (Adkins et al., 2004). Animals were anesthetized with ketamine/xylazine or Equithesin prior to surgical procedures. A craniectomy was made using stereotaxic coordinates over the area corresponding to the caudal forelimb region of the dominant (for the reaching task) forelimb of the SMC and dura was removed. ET-1 was applied to the cortical surface and the skin was sutured 10 minutes later. Sham operated animals did not have craniectomies because Adams and colleagues (1994) have shown that behavioral asymmetries can result from skull removal. Skilled behavioral measures began 5 days after lesions.

### 2.3 Histology

At the conclusion of each experiment, animals were given an overdose of sodium pentobarbital and were transcardially perfused with fixative (0.1M sodium phosphate buffer followed by 4% paraformaldehyde in the same buffer) or fixative (0.1M sodium phosphate buffer followed by 2% paraformaldehyde/2.5% glutaraldehyde in the same
buffer). Brains were removed and sectioned with a vibratome. Tissue processing was either compatible with immunohistochemistry (six rostral-caudal sets of 50µm thick coronal sections were taken throughout the brain) or for electron microscopy (4 rostral-caudal sets of alternating micron thickness 200, 100, 50, and 50). One set of 50 µm thick sections was Nissl stained and used for volume measurements and lesion reconstructions. Tissue was stored in cryoprotectant before being processed for immunocytochemistry.

2.4. Immunohistochemistry

Tissue was processed for FosB/ΔFosB, a transcription factor, in a free-floating section protocol. FosB/ΔFosB has been found to accumulate over time in response to neural activation (McClung et al., 2004) and has previously been found in cortex following hippocampal lesions (Kurushima et al., 2005). The antibody used in these studies does not distinguish between the two isoforms of FosB. ΔFosB is lacking amino acids in the C-terminus and unlike FosB, which is rapidly and transiently induced, ΔFosB has been shown to accumulate over time (McClung et al., 2004). It is hypothesized in these dissertation studies that the presence of immunoreactivity for FosB/ΔFosB reflects an accumulation of the ΔFosB product over time in response to motor skills learning. There was a 24 hour period of no-training before animals were euthanized to minimize the contribution of the FosB isoform and to more sensitively assay the effects of the cumulatively expressed ΔFosB isoform.
Relatively thick 50µm sections were used because they are most suitable for the optical disector method used to quantify neuronal density (Harding, Halliday, & Cullen, 1994). Briefly, five, 50 µm thick sections throughout the sensorimotor cortex (between +1.2 and -0.3 mm to Bregma) were rinsed in 10mM phosphate buffer saline (PBS). Sections were then inactivated for endogenous proteases in 0.3% hydrogen peroxide in 10mM PBS for 30 minutes. Following several washes in 10 mM PBS, sections were incubated in block solution for 2 hours followed by 48 hours of primary incubation at 4°C. The primary antibody was FosB/ΔFosB sc-48 (1:400, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The secondary antibody (Sigma) was antirabbit IgG made in goat (1:300). After primary incubation, sections were rinsed several times in 10mM PBS followed by secondary antibody incubation. Sections were then rinsed, incubated in ABC solution (Vector Laboratories, Burlingame, CA). Immunoreactivity was then visualized using DAB and NAS. No primary control tissue was run at the same time to verify staining specific reactivity. Tissue reacted for FosB/ΔFosB was counterstained with Methylene blue/azure II (a Nissl stain). Slides were coded prior to quantification such that the experimenter was blinded to behavioral conditions.

2.5. Quantification of FosB/ΔFosB immunolabeled neurons

Neuronal density of FosB/ΔFosB was quantified using the optical disector method (e.g. Harding et al., 1994; Korbo et al., 1990). Positive (brown) and negative (blue) neurons were then counted with a 100X oil objective in layers II/III and V of the sensorimotor cortex and other related brain regions. Neuronal density (Nv) was...
calculated using the equation: \( N_v = \frac{\Sigma Q}{\Sigma v(\text{frame})} \), where \( \Sigma Q \) is the sum of all FosB/ΔFosB neurons counted per brain and \( \Sigma v(\text{frame}) \) is the total sample volume. Figure 2.2 diagrams sampling strategies for brain regions quantified in these dissertation experiments.

**Figure 2.2 FosB/ΔFosB Sampling Strategy.**

Immunoreactivity for FosB/ΔFosB was quantified in, sensorimotor cortex, dorsolateral striatum, and nucleus accumbens. The square boxes on each brain template correspond with sampling areas.

### 2.6 Cortical Volume Assessment

In lesion brains, volume measurements of remaining cortex were used as an indirect measure of lesion size. Using Neurolucida perimeter tracing software (Microbrightfield Inc) at a magnification of 17X, six to seven, 50μm thick Nissl stained coronal sections within the caudal forelimb area of the SMC were traced, beginning approximately 2.2 mm anterior and ending approximately 0.80 mm posterior to bregma.
The volume of remaining cortex in the lesion hemisphere and volume of the contralateral hemisphere was calculated using the Cavalieri method (Gundersen et al., 1988) as the product of the distance between sections (600μm) and the summed cortical areas. (A slightly different method of volume estimation of SMC was used in chapter 6.) Lesion extent and placement were then assessed through reconstructions onto schematic templates of coronal sections.

2.7. Commonly Used Abbreviations

Sensorimotor cortex (SMC), corticospinal tract (CST), endothelin-1 (ET-1), IntactT (lesion groups receiving training with their intact forelimb), Cont (lesion groups receiving control procedures), non-dominant (ND).
Chapter 3

Motor learning with the intact forelimb after unilateral sensorimotor cortex damage

3.1. Abstract

Unilateral lesions of the sensorimotor cortex (SMC) in adult rats cause major behavioral changes in the ipsilesional, intact forelimb. An increase in function and reliance on this forelimb can aid compensation for contralesional impairments but might also promote disuse and reduced functionality of the impaired forelimb. We hypothesized that training focused on the intact forelimb following a unilateral SMC lesions would reduce the efficacy of later motor rehabilitative training of the impaired forelimb. Rats with ischemic SMC lesions were trained on a skilled reaching task with the intact forelimb (IntactT) or received control procedures (Cont) for 10 days. Both groups were then trained with the impaired forelimb on the same reaching task for 10 days. In contrast to Cont, IntactT rats had little improvement on the reaching task with the impaired forelimb and had a more enduring disuse of the impaired forelimb for postural support behaviors. Lesion sizes were similar between groups. Thus, behavioral experience with the intact forelimb early after unilateral SMC lesions has the potential to increase disuse and dysfunction of the impaired forelimb. These findings are suggestive of competitive processes in experience-dependent neural restructuring after
brain damage and may have major implications for post-stroke rehabilitative training approaches.

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3.2. Introduction

Stroke remains one of the leading causes of disability in the United States (Heart Disease and Stroke Statistics, 2004) and, for many people with strokes, physical therapy is necessary to improve behavioral function. Although there have been major advances in recent years in understanding how rehabilitative training can be used to promote better function and restorative plasticity after brain damage (for reviews see Johannson, 2003; Nudo, 2003), an ongoing issue for unilateral strokes affecting the upper extremities is how the two limbs should be treated in order to promote the most effective level of functioning (Taub, 2004; Rose & Winstein, 2004, review).

In rats, unilateral lesions in the caudal forelimb representation of the sensorimotor cortex (SMC) cause pronounced sensorimotor impairments in the contralesional forelimb (e.g., Jones & Schallert, 1992; Adkins, Voorhies, & Jones, 2004). Rats adapt behaviorally by using the intact (ipsilesional) forelimb more for postural support behaviors and in compensatory ways, as revealed in tests that require coordinated forelimb movements (Bury & Jones, 2002; Schallert et al., 1997, review). The degenerative effects of SMC lesions also appear to create a fertile environment for experience-dependent plasticity in the contralesional motor cortex (Jones et al., 2003;
Jones et al., 1996), which increases in excitability (Witte et al., 2000), NMDA receptor protein, dendrites and synapses (Adkins et al., 2004; Jones et al., 2003). These changes may contribute, in part, to a hyperfunctionality in the intact forelimb that can be observed in skilled motor tests. When required to learn a novel reaching task, rats with unilateral SMC lesions perform better with the intact forelimb compared to sham-operated rats (Bury & Jones, 2002; Luke, Allred & Jones, 2004; Allred & Jones, 2004; Hsu & Jones, 2004). We have previously hypothesized that the enhanced functionality of the intact forelimb, although good for development of compensatory ways of using the intact body side, might occur at the expense of creating greater disuse and dysfunction of the impaired forelimb (Bury & Jones, 2002).

The purpose of the present study was to test the hypothesis that training focused on the intact forelimb after SMC ischemic infarcts would interfere with later functional improvements of the impaired forelimb.

3.3. Materials and methods

3.3.1. Animals

Three to 4 month old male Long-Evans rats were given pre-operative shaping procedures on a skilled reaching task, the single pellet retrieval task (see Section 2.1). A limb of preference was established after the animal reached 15 of 20 reaches with 1 limb. Animal protocols were approved by the University of Texas at Austin Animal Care and Use Committee.
3.3.2. Surgical procedures

Endothelin-1 (ET-1, Fuxe et al., 1997; Adkins, et al., 2004) lesions were induced in the SMC opposite the animal’s dominant for reaching forelimb. Lesions were created by removing the skull and dura over the SMC (coordinates relative to bregma: 0.5 mm posterior to 1.5 mm anterior, 3.0 to 4.5 mm lateral) and applying 1.2µl of ET-1 (96 pmol) to the pial surface.

3.3.3. Behavioral procedures

Single Pellet Retrieval Task

Five days after lesions rats were trained on the reaching task for 10 days with the intact forelimb (IntactT group, n = 8), while another group (Cont group; n=8) received control procedures. All animals were then trained on the same skilled reaching task with the impaired (preferred) forelimb for 10 days. As described in more detail previously (e.g., Allred & Jones, 2004), daily reach training consisted of training animals for 10 minutes or 30 trials, whichever came first. Animals were trained each day with a Plexiglas wall inserted into the reaching chamber ipsilateral to the animal’s reaching limb. The Plexiglas wall prevented animals from using their untrained forelimb. For each trial, a palatable food pellet was placed on a 3 cm high shelf outside of an animal enclosure, and rats were permitted to make up to 5 reaches with the trained limb through a narrow window to retrieve it. A successful retrieval consisted of the rat grasping,
retrieving and eating the pellet. Unsuccessful attempts included dropping the pellet or "misses", i.e., failing to grasp the pellet or knocking the pellet from its well. Data are reported as the percentage of successful reaches per reach attempt. Cont rats received food pellets placed inside of the enclosure. (See Section 2.1 for more detail.)

The Schallert Cylinder Test

The Schallert cylinder test (Schallert et al., 1997; 2000) was used as an assay of lesion-induced asymmetries in forelimb use for upright postural-support behavior. Animals were filmed in a Plexiglas cylinder (19 cm diameter) for 2 min pre-surgery and at four post-surgery time-points. From slow-motion playbacks of each session, the first 30 instances of sole use of either forelimb (ipsilateral or contralateral to the lesion) or simultaneous bilateral forelimb use for upright support against the cylinder wall were recorded. The forelimb asymmetry score was calculated using the formula: (total ipsilateral limb use + 1/2 bilateral)/total limb use *100.

3.3.4. Histology and volume of remaining SMC

Twenty-two days after surgeries rats were transcardially perfused with fixative, brains were removed and vibratome sectioned coronally and 50 µm thick sections were stained with Toluidine blue.

The volume of remaining cortex in the SMC region of the infarcted cortex was obtained by measuring the area of remaining non-necrotic/non-gliotic cortex with Neurolucida software (Microbrightfield Inc.) at a final magnification of X17, as
described previously (Allred & Jones, 2004). The Cavalieri method (Gundersen et al., 1988) was used to calculate remaining SMC region volume, i.e., the product of the summed areas by the distance between section planes (600µm). Lesion extent and placement were assessed by reconstruction of lesions onto schematic coronal templates.

3.3.5. Statistics

Data were analyzed using repeated-measures analysis of variance (ANOVA), one-way ANOVAs or bivariate correlations with SPSS statistical software package (SPSS, Inc.). Post-hoc analyses were performed using SAS (SAS Institute Inc.) general linear models procedure for Scheffe's test.

3.4. Results

3.4.1. Prior training with the intact forelimb after unilateral SMC damage prevents acquisition of a skilled reaching task with the impaired forelimb

As shown in Figure 1, IntactT rats performed significantly worse with their impaired forelimb on the reaching task compared to animals without prior training. There was a significant effect of Group (F(1,14) = 5.41, p < .05), Day (F(9,126) = 2.71, p < .01) and a Group by Day interaction effect (F(9,126) = 2.49, p < .05), reflecting the greater improvement in reaching performance of the Cont group over days of training the impaired forelimb compared to the IntactT group. The reduction in successful retrievals in the IntactT group compared to the Cont group was due to a significant increase in the percentage of missed reach attempts (F(1,14) = 5.31, p < .05), in which the pellet was not retrieved after 5 attempts or in which the pellet was knocked from its well, and not due to
an increase in the percentage of dropped pellets, in which the pellet was successfully
grabbed but dropped inside the chamber before the animal could eat it (F(1,14) = 0.34, p
> .05).

Figure 3.1 Prior training with the intact forelimb prevents post-lesion
acquisition of the skilled reaching task with the impaired forelimb.
The percentage of successful reaches with the impaired forelimb on the
skilled reaching task after unilateral SMC lesions. Rats that had received
prior training of the intact forelimb (IntactT) performed significantly worse
(*p < .05) with their impaired forelimb compared to control (Cont) rats.
Data are means ± S.E.M.
3.4.2. Training the intact forelimb increased reliance on the intact forelimb during exploratory behavior

All animals had increased asymmetrical forelimb use following the lesions (Figure 2), resulting from an increased reliance on the ipsilesional forelimb and decreased use of the impaired forelimb. Additionally, animals in the IntactT group had a significantly greater asymmetry score during the time-period corresponding to impaired limb training (D18 and D24) compared to the Cont group. A repeated-measures ANOVA revealed a significant effect of Group (F(1,14) = 4.77, p < .05), but no Day or Group by Day interaction effects (p’s > .05), during the impaired limb training period. There were no significant group differences in the asymmetry score in the time periods preceding the onset of impaired forelimb training.
Figure 3.2  Training the intact forelimb after unilateral SMC lesions increases reliance on this forelimb in a test of postural support.

Forelimb asymmetries in postural support behaviors as measured in the Schallert cylinder test. In both groups, SMC lesions resulted in increased use of the ipsilesional forelimb and disuse of the impaired forelimb. IntactT rats continued to show significantly more (p < .05) reliance on their intact forelimb compared to Cont rats during the impaired forelimb training period (D18 and D24). Data are means ± S.E.M.
3.4.3. Training the intact forelimb does not increase lesion size

All lesions produced damage to the caudal forelimb representation of the SMC (see Figure 3) and lesion placement and extent was similar between groups as evidenced in lesion reconstructions. One-way ANOVA revealed no significant difference in remaining cortical volume between the two groups (means ± SEM volume in mm$^3$: IntactT = 86.07 ± 1.47, Cont = 87.81 ± 1.77, F(1,15) = .17, p > .05). In the Cont group, there was a significant, and in the IntactT group a marginally significant, positive correlation between average reaching performance with the impaired limb and remaining cortical volume, (Cont: r = .76, p < .05; IntactT: r = .60, p = .059, 1-tailed,) revealing that animals with larger lesions tended to perform worse with their impaired limb compared to animals with smaller lesions.
Figure 3.3 Intact forelimb training did not cause an increase in lesion size.

Nissl stained coronal sections depicting representative lesions in each group. Scale bar = 1 mm.
3.5. Discussion

In summary, rats with ischemic SMC lesions that were trained on a skilled reaching task with the intact forelimb later performed significantly worse with the impaired forelimb compared to animals without prior training. The IntactT group also demonstrated a more enduring decreased use of the impaired forelimb in postural support behaviors compared to animals in the Cont group, as was evident during the time-period of impaired forelimb training. Training focused on the intact forelimb may therefore contribute to greater disuse of the impaired forelimb. The greater disuse and reduced reaching ability of the impaired forelimb in IntactT animals can not be explained by lesion size effects as both groups had similar volumes of remaining cortex in the SMC region. These data indicate that training focused on the intact forelimb early after a SMC lesion results in detrimental effects to recovery of function of the impaired forelimb on a skilled reaching task.

Other studies have demonstrated that forced overuse of the impaired forelimb after unilateral SMC lesions worsens behavioral outcome of the impaired forelimb (Kozlowski et al., 1996; Humm et al., 1998; Leasure & Schallert, 2004). This effect was linked to a use-dependent exaggeration of tissue loss from the lesion. In the present study, there was no difference between groups in the amount of tissue loss; therefore the mechanisms underlying the exaggeration of impairment resulting from overuse of the non-impaired forelimb are likely to be fundamentally different than the effects of overusing the impaired forelimb.
One issue is the contribution of motor learning interference effects to the present results. The single pellet retrieval task used in this study is not a truly unilateral task, because, while animals are reaching with one forelimb, the other forelimb is used for postural support. It could be posited that learning to use the impaired forelimb for postural support during training reduces the ability for rats to learn to use it for reaching. This, however, seems unlikely given that rats in the IntactT group actually relied less on their impaired forelimb for postural support behaviors, as measured in the Schallert cylinder test. The configuration of the reaching apparatus used in this study also minimizes the postural support requirements of the non-trained forelimb (Miklyaeva & Whishaw., 1996). Preliminary unpublished data also indicate that, in intact animals, reach training with one forelimb (the non-preferred limb) does not interfere with later reaching performance of the other forelimb.

Many issues remain to be addressed in future studies. The inclusion of sham-operates in this experimental design would more clearly identify any motor learning interference effects due to sequential training of the 2 forelimbs. The lesions in this study were rather small and, in the Cont group, produced relatively transient forelimb asymmetries in the Schallert cylinder test. It remains to be determined whether the effect found in this study will generalize to rats recovering from more substantial motor system damage, such as that producing detectable ipsilesional impairments. It is unknown whether there are sensitive post-lesion time windows for the effects of training that focuses on the unimpaired forelimb. It also remains to be determined if the
worsening of function of the impaired forelimb can be prevented using prior bilateral, rather than focused ipsilesional, forelimb training.

While several issues remain to be addressed, this study provides insight into problems associated with focusing rehabilitative training efforts after unilateral motor system injury. Animals, including humans, spontaneously learn to use the intact body side to compensate for lesion-induced impairments. We have previously found that, after small unilateral cortical lesions, learning with the unimpaired forelimb can even be facilitated relative to intact animals, presumably as a result of degeneration-triggered growth promoting processes that facilitate the synaptic changes underlying learning (Bury & Jones, 2002; Luke et al., 2004; Allred & Jones, 2004; Hsu & Jones, 2004, Jones et al., 2004). Although the development of this compensation with the intact forelimb appears to be functionally adaptive, the present results indicate that making this limb the focus of rehabilitative training efforts can be detrimental to later functional recovery of the impaired forelimb. The neural bases of these effects are as yet unknown, but learning a skilled reaching task involves plasticity of synaptic activity and structure in the motor cortex opposite the trained forelimb (Kleim et al., 1998; Monfils & Teskey, 2004; Nudo, 2003), and there is some evidence to support a bilateral contribution to skilled reaching with one forelimb (Gonzalez et al., 2004). It is possible that early training focused on the intact forelimb confiscates neural circuits in the contralesional and/or infarcted hemisphere that could be used to mediate better recovery of the impaired forelimb.
Chapter 4
Maladaptive effects of learning with the less-affected forelimb after focal cortical infarcts in rats

4.1. Abstract

It is common following stroke to focus early rehabilitation efforts on developing compensatory use of the less-affected body side. Here we used a rat model of focal cortical infarct to examine how motor skill acquisition with the less-affected ("intact") forelimb influences sensorimotor function of the infarct-impaired forelimb and neural activity in peri-infarct cortex. Rats proficient in skilled reaching with one forelimb were given focal ischemic lesions in the contralateral sensorimotor cortex (SMC). Recovery in this forelimb was tested following a period of reach training focused on the intact forelimb or control procedures. Quantitative measures of the cumulatively expressed transcription factor, FosB/ΔFosB, were used to assay intact forelimb training effects on neuronal activity in remaining SMC of the infarcted hemisphere. Intact forelimb training worsened behavioral recovery in the impaired forelimb following unilateral focal ischemia. Furthermore, it decreased neuronal FosB/ΔFosB expression in layer II/III of peri-infarct SMC. These effects were not found in sham-operated rats trained sequentially with both forelimbs or in animals receiving bilateral forelimb training after unilateral infarcts. Thus, focused use of the intact forelimb has detrimental effects on recovery of impaired forelimb function following a focal ischemic injury, and this is linked to reduced neuronal activation in remaining cortex. These results suggest that
peri-infarct cortex becomes vulnerable to early post-stroke experience with the less-
affected forelimb and that this experience may drive neural plasticity here in a direction
that is maladaptive for functional outcome.

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Maladaptive effects of learning with the less-affected forelimb after focal cortical infarcts

4.2. Introduction

Following unilateral cerebral damage, humans and other animals develop a
compensatory hyper-reliance on the less-affected body side ipsilateral to the injury
(Schallert, 2006). The development of compensatory behaviors is an advantageous
strategy in that it permits performance of daily activities despite impairments. The
cortex contralateral and homotopic to unilateral sensorimotor cortex (SMC) damage has
increased neuroplasticity, e.g., dendritic growth (Jones, 1999; Jones, Kleim, &
Greenough, 1996), which appears to facilitate the ability to acquire new skills with the
less-affected body side (Bury & Jones, 2002; Allred & Jones, 2004; Hsu & Jones, 2006).
Furthermore, it is typical in human stroke patients to focus early rehabilitation on
compensatory use of the less-affected body side (Dobkin, 2005). However, such
compensation may contribute to learned disuse of the impaired body side (Taub et al.,
2006A). Furthermore, recent studies in humans and animal models suggest that the
intact hemisphere develops an exaggerated disruptive influence over the infarcted
hemisphere that is linked with worsened outcome (reviewed in Ward & Cohen, 2004).
For example, after visual cortex lesions in cats, reversal of visual hemineglect was
accomplished by cooling regions of the contralesional hemisphere (Rushmore et al., 2006). During movements of the paretic hand in humans with strokes, there is an abnormal interhemispheric inhibitory influence arising from the intact motor cortex (Murase et al., 2004; Duque et al., 2005). Anesthetization of the intact upper extremity in both humans (Voller et al., 2006) and rats (O'Bryant et al., 2007) transiently improves function in the stroke-impaired limb. These and related findings suggest that the intact hemisphere can negatively impact function of the impaired body side. It seems possible that behavioral experience with the intact body side is a major contributor to these effects.

We recently found that, following unilateral ischemic SMC lesions, training behaviorally naive rats on a reaching task for 10 days with their intact forelimb worsened their ability to learn the same reaching task with their impaired forelimb compared to those without the training (Allred et al., 2005). However, it was unknown whether this behavioral experience would also influence recovery of preexisting (before the infarct) motor skills in the impaired forelimb and whether this effect requires focused training of the intact limb. Furthermore, the neural basis of this effect had previously been unexplored.

The present study was designed to test the behavioral and neural consequences of early intact forelimb experience using a focal model of cortical stoke in rats. Cortical damage in humans and rats can result in major deficits in distal forelimb behaviors (e.g., Desrosiers et al., 2003; Gharbawie et al., 2005). Given that impairments in hand and arm sensorimotor function and compensation with the other hand are typical after stroke,
a skilled forelimb reach-to-grasp task in rats seemed ideal for studying bilateral behavioral experience effects on recovery of function. Rats readily learn to reach for and grasp palatable food pellets, mimicking similar movements performed by humans (Iwaniuk & Whishaw, 2000; Whishaw, 2003). The single pellet retrieval test, a skilled reaching test, was therefore used as a sensitive measure of distal forelimb impairments (Whishaw & Gorny, 1994) and as an experiential manipulation of forelimb experience.

To assay functional activation of remaining cortex, we took advantage of a transcription factor, ΔFosB, which is cumulatively and persistently expressed in response to repeated neuronal activation (McClung et al., 2004), making it suitable for investigations of practice-dependent plasticity, such as that required for motor skill acquisition and motor “re-learning”. Immunocytochemistry for FosB/ΔFosB was used. Experimental designs are summarized in Figure 4.1.
Experiment 1 was designed to test the hypothesis that training the intact forelimb worsens functional recovery in the infarct-impaired forelimb. After training to proficiency with the dominant (for the task) forelimb on a skilled reaching task, the single pellet retrieval task, rats underwent either an ischemic sensorimotor cortex (SMC) lesion or a sham operation.
in the hemisphere contralateral to this limb. Beginning on postoperative day 5 (D5), rats received either 15 days of training (60 trials/day) with their intact, non-dominant forelimb (IntactT and Sham ND) or control procedures (Cont, Sham Cont). All animals were then tested for 13 days (30 trials/day) with their impaired and/or dominant forelimb and then sacrificed to assay neuronal activation in peri-infarct cortex (n's = 10/group). Experiment 2 was designed to test whether focused intact forelimb training is necessary to influence recovery of impaired forelimb function. Animals were trained pre-operatively as in Experiment 1. All animals then received an SMC lesion followed by 12 days of training (30 trials/day) with the intact forelimb (IntactT) both forelimbs (BiT) or control procedures (Cont). All rats were then tested with their impaired forelimb as in Experiment 1 followed by 5 days of intact forelimb retesting (n's = 9/group).
4.3. Materials and Methods

4.3.1. Animals

Well-handled rats were pair housed with standardized housing supplementation (a PVC pipe piece and small wooden objects) on a 12:12 light/dark cycle. Animals were maintained on scheduled feeding (15-17g/day) to motivate reaching performance. Animal use was in accordance with a protocol approved by the University of Texas at Austin Animal Care and Use Committee.

4.3.2. Focal Ischemic Stroke Model

Unilateral SMC lesions were made in the caudal forelimb representation area opposite the animals’ dominant-for-reaching forelimb. Rats were anesthetized with ketamine (10mg/kg) and xylazine (120mg/kg), Exp. 1, or Equithesin (150 mg/kg choral hydrate; 37mg/kg sodium pentobarbital, Exp. 2). A craniectomy (-1.0/+2.0 A/P; 2.0/4.5 M/L) was made, dura was removed and 2.5 µl (200 pmol) of endothelin-1 (ET-1), a vasoconstricting peptide, were applied to the pial surface, as previously described (Allred & Jones, 2004). Sham-operated rats received procedures up to the craniectomy.

Adams et al. (1994) have previously shown that skull removal can result in forelimb behavioral and neurochemical asymmetries and, in this study, it was important for these rats to serve as intact controls.
4.3.3. Behavioral Procedures

Skilled Reach Training

The single pellet retrieval task (Miklaeva & Whishaw, 1996) was used as an assay and manipulation of behavioral function. A design that forces rats to use the experimenter-designated forelimb(s) for reaching was used. Rats reached through a narrow window for 45 mg banana flavored food pellets (Bioserve Inc.) from a shallow well in a shelf, as previously described (Hsu & Jones, 2006; Allred et al., 2005). Wall(s) were placed inside the reaching chamber (1.5 cm from window) ipsilaterally to the trained forelimb. In experiment 2, to permit training of one or both forelimbs, there were two reaching windows on opposite sides of the chamber which rats reached through in alternating trials and partial walls (in the form of corners) next to each window spanning ~ ¼ of the length of the chamber. For each trial, rats were permitted up to 5 reach attempts to successfully retrieve the pellet. Each extension of the forepaw through the window was counted as an attempt. Successful retrievals/reach attempt x 100 are reported.

Pre-operatively, all rats were trained to 60% success rate (approximately asymptotic performance) on the reaching task with the dominant-for-reaching forelimb (the to-be-impaired limb). Early post-lesion animals underwent control procedures or were trained with their intact (non-dominant) limb for 15 days (60 trials/day; Experiment 1) or 12 days (30 trials/day; Experiment 2) (Fig. 4.1). Bilaterally trained animals received 15 trials with each forelimb in an alternating fashion. Following this, impaired forelimb recovery was assessed. Experiment 2 rats then underwent an additional period
of re-testing the intact forelimb. Controls received pellets on the chamber floor at approximately the same rate as trained animals retrieved them.

**Footfault test**

The footfault test measures coordinated locomotor forelimb use (Barth, Grant, & Schallert, 1990). Animals traversed an elevated grid-platform for 2 min. From slow motion playback, instances of slipping through the grid with either forelimb were recorded. In experiment 2, at the last time-point, the intact forelimb was anesthetized with lidocaine (0.20 cc of 1.0% lidocaine into the bicep muscle) to test its contribution to performance. Performance was recorded as slips/step x 100.

**Schallert cylinder test**

Lesion-induced forelimb asymmetry was measured using the Schallert cylinder test (Schallert et al., 1997), which encourages upright postural exploratory movements. Animals were filmed in a Plexiglas cylinder (19 cm diameter) for 2 min and the first 30 instances of sole (impaired or intact) or simultaneous forelimb use for support against the cylinder wall were recorded using slow-motion playbacks of each session. The percent impaired forelimb use was calculated as (impaired use/total forelimb use) X 100. The post-lesion difference in use of the impaired limb (postoperative - preoperative % impaired limb use) was then calculated.
4.3.4. Immunohistochemistry and Quantitative Microscopy

At the conclusion of each experiment, animals were overdosed with sodium pentobarbital (150mg/kg) and transcardially perfused with 0.1M phosphate buffer followed by 4% paraformaldehyde in the same buffer. Brains were removed and 50µm thick coronal sections of cerebrum were produced using a vibratome. Archived tissue from a previous experiment (chapter 3) was also used to test the reproducibility of Experiment 1 results. Immunoreactivity for FosB/ΔFosB was visualized using 3-3’ diaminobenzidine tetrahydrochloride with nickel ammonium sulfate and a free-floating section method, as previously described (section 2.3.1).

The remaining SMC (inclusive of agranular and granular cortex medial and lateral to the lesion) in the rostral extent of the lesion territory was chosen for the quantitative analysis because this region contains remaining forelimb representation zones. Layer II/III was chosen because it has previously been found to undergo neuroplastic changes as a result of motor skill training in intact animals (e.g., Bury & Jones, 2002; Kleim, Barbay, & Nudo, 1998). Layer V was chosen because it is the major source of corticofugal projections and it has also been found to undergo synaptogenesis as a result of motor skill training (Jones et al., 1999). Four sample sites per layer in each of four sections were analyzed using a 100X oil immersion objective with a final magnification of X1250. FosB/ΔFosB neuronal expression was also examined in layers II/III and V of the non-infarcted cortex; however, there were no consistent effects of differential training between and within experiments and therefore these data are not shown. Granular insular cortex, responsive to thermal stimulation of the tongue, served as a control region.
Immunostained sections were counterstained with methylene-blue azure II, a Nissl stain. The density of FosB/ΔFosB positive neurons (brown) and FosB/ΔFosB negative neurons (blue) were estimated using the optical disector method (Korbo et al., 1990). \( N_v \) was calculated using the formula: \( N_v = \frac{\sum Q}{\sum v(frame)} \), where \( \sum Q \) is the sum of all FosB/ΔFosB neurons counted per brain and \( \sum v(frame) \) is the total sample volume. Slides were coded prior to quantification such that the experimenter was blinded to behavioral conditions.

**Volume of Remaining SMC and estimate of lesion size and placement**

Cortical volume measurements in the SMC region of both hemispheres were used as an indirect measure of lesion size. At 17X magnification, seven 50µm thick Nissl stained coronal sections within the caudal forelimb area of the SMC (between 2.2 mm anterior to and 0.80 mm posterior to Bregma) were traced using Neurolucida perimeter tracing software (Microbrightfield Inc). The Cavalieri method (Gundersen et al., 1988), the product of the distance between section planes and the summed cortical areas, was used to calculate volume. Lesion extent and placement were assessed by reconstructing lesions onto coronal schematic templates relative to cytoarchitectural and macrostructural landmarks (as previously described in Allred & Jones, 2004).

**4.3.5. Statistical Analyses**

Repeated measures analysis of variance (ANOVA), using planned comparisons, were carried out for behavioral tests. One-way ANOVAs or t-tests were used for within
group and between 2 group comparisons. Statistics were performed using SPSS (SPSS, Inc) and GraphPad Prism (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA). Descriptive results are shown as means ± SEM. Results were considered significant at p < .05.

4.4. Results

4.4.1. Training the intact forelimb interferes with recovery of skilled reaching with the impaired forelimb

Intact forelimb training in the early post-infarct time period interfered with subsequent skilled reaching with the impaired forelimb (Figs. 4.2-4.3). Rats trained with their intact forelimb (IntactT) performed significantly worse during the impaired forelimb training period compared to Cont (F(1,18) = 7.21, p < .05, Fig. 2). In experiment 2 (in which rats received less intense intact forelimb training, 30 trials/day instead of 60), there was a significant group by day interaction (F(9,144) = 3.03, p < .01), but the main effect of group failed to reach significance (F(1,16) = 3.58, p = .08, Fig. 3). In post-hoc analyses, IntactT animals were significantly more impaired than Cont on Days 1, 2, 4 and 6 (p's< 0.05). There were no group differences in the number of trials completed during each training session.

During the early training period, IntactT rats performed better with their intact forelimb compared to sham operated controls (Figure 4.2A; F(1, 18) = 8.96, p < .01). This was expected because these lesions facilitate acquisition of new skills in this forelimb, even in the presence of subtle impairments (e.g., Allred & Jones, 2004; Hsu &
Jones, 2006). As expected, lesions also reduced reaching performance in the impaired forelimb compared to sham-operates (Fig. 4.2B).

4.4.2. Training the non-dominant forelimb in sham-operates does not impair the other forelimb

Sequentially training the forelimbs in the same order in sham-operates did not reproduce effects found after SMC lesions (Fig. 4.2B). Training the non-dominant-for-reaching forelimb after sham operations (Sham ND) did not significantly change subsequent success rates with the preferred forelimb compared to Sham Cont (F(1,18) = 2.86, p > .05, Figure 2B). However there was a tendency for Sham ND to achieve lower asymptotic performance compared with Sham Cont in later days of testing.
Figure 4.2 Training the intact forelimb worsens subsequent performance of the impaired forelimb in lesion animals, an effect not seen with sequential forelimb training in sham animals.

Training the intact forelimb in the skilled reaching task early after unilateral SMC lesions worsened subsequent performance in the impaired forelimb.

A: Intact training period of Experiment 1. Lesion rats trained with their intact forelimb (IntactT) performed significantly better than sham (Sham_ND) rats, p < .01. B: Impaired testing period. Sham animals performed significantly better than lesion animals (p < .01). IntactT rats performed significantly worse with their impaired forelimb compared to Cont rats (p < .05). The shaded forelimb indicates the trained/tested forelimb. Data are means ± SEM.

4.4.3. Training both forelimbs together does not worsen impaired forelimb performance

Rats trained bilaterally (BiT, Exp. 2) did not show a decrease in reaching performance with their impaired forelimb compared to Cont (F(1,16) = 1.49, p > .05), and this group performed significantly better than IntactT rats (F(1,16) = 13.73, p < .01, Fig 4.3).
Figure 4.3 Training both forelimbs after unilateral lesions does not worsen subsequent performance with the impaired forelimb.

Training both forelimbs in skilled reaching early after the lesions did not reproduce the effects of focused intact limb training. IntactT rats performed significantly worse with their impaired forelimb compared to BiT rats (p < .01) and performed worse compared to Cont dependent on day (p < .01). Training with both forelimbs did not significantly change impaired forelimb performance compared to control animals (p > .05).

These results are from Experiment 2. Data are means ± SEM. Note the difference in scale between Figure 4.2 and Figure 4.3.
4.4.4. Intact forelimb training results in abnormal reliance on this forelimb for coordinated forelimb behaviors

In the footfault task, rats traverse a grid floor by coordinated placement of their paws on the rungs of the grid. Recovery on this task after unilateral SMC lesions depends, in part, on compensatory ways of using the intact forelimb (Bury & Jones, 2002; Schallert et al., 2000). Rats compensating with their intact forelimb have increased errors with their impaired forelimb when the intact forelimb is anesthetized (the "lidocaine challenge"). In contrast, sham-operated animals show no impairment in the unanesthetized forelimb when the other limb is anesthetized (Bury & Jones, 2002). Rats trained with their intact forelimb early after SMC lesions, had increased misses (slipping through the grid) with their impaired forelimb during the lidocaine challenge compared to pre-lidocaine tests (t(8) = -2.39, p < .05) and compared to Cont rats (F(1,16) = 5.25, p < .05, Fig 4.4). BiT rats were not significantly different from Cont (F(1,16) = .01, p > .05).
Reach training focused on the intact forelimb increased reliance on this limb in a measure of coordinated forelimb placement during locomotion (the footfault test). Unilateral lesions resulted in impairments in the contralateral forelimb on the footfault test, which recovered by Day 31. IntactT rats had increased slips with their impaired forelimb during the lidocaine challenge test compared to Cont (*p < .05). Data are means ± SEM.
As shown in Table 4.1, lesions reduced use of the impaired forelimb for postural support behavior as measured in the cylinder test during the early post-operative period. During the impaired limb training period, IntactT rats, but not Cont, remained significantly impaired in the cylinder test relative to the training-matched shams (Sham, p < 0.05). However, the IntactT results failed to reach significance compared with lesion controls (Cont) in experiment 1 (F(1, 18) = 2.56, p = .13) and 2 (F(1, 16) = 3.78, p = .07).

**Table 4.1** Intact training effects on use of the impaired forelimb in postural support behaviors.

<table>
<thead>
<tr>
<th>Group</th>
<th>Early Post-operative</th>
<th>Impaired Forelimb Training</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>-9.18 ± 5.55% *</td>
<td>-4.46 ± 4.03%</td>
</tr>
<tr>
<td>IntactT</td>
<td>-12.16 ± 6.66% (p = .06)</td>
<td>-17.69 ± 6.24% *</td>
</tr>
<tr>
<td>Sham Cont</td>
<td>7.00 ± 2.56%</td>
<td>7.00 ± 6.24%</td>
</tr>
<tr>
<td>Sham ND</td>
<td>5.33 ± 5.49%</td>
<td>9.29 ± 8.93%</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>-16.40 ± 6.73%</td>
<td>-14.48 ± 4.71%</td>
</tr>
<tr>
<td>IntactT</td>
<td>-26.23 ± 7.35%</td>
<td>-30.94 ± 7.03%</td>
</tr>
<tr>
<td>BiT</td>
<td>-18.57 ± 3.76%</td>
<td>-21.85 ± 5.96%</td>
</tr>
</tbody>
</table>

Data are means ± SEM post-operative – pre-operative % impaired limb use. * p < .05 compared to Sham of the same training condition.
4.4.5. Training the impaired forelimb does not worsen performance in the intact forelimb

After the end of the impaired forelimb training period, Experiment 2 rats received tests of intact forelimb reaching. Compared with their asymptotic performance levels during the earlier training period, there was no significant change in the performance of the intact forelimb in the IntactT rats (mean ± SEM retest % success = 62.25 ± 2.20 versus 58.35 ± 6.30, t(8) = -0.733, p > .05) and BiT rats (52.73 ± 2.77 versus 48.82 ± 3.22, t(8) = -1.57, p > .05). In Cont rats, which were being tested for the first time on this forelimb, performance was similar to performance of IntactT and BiT rats during their initial acquisition (e.g., Cont = 37.74 ± 3.61 versus 34.78 ± 2.99 in the first 5 days of early training in IntactT).

4.4.6. Behavioral manipulations did not significantly influence lesion size

Ischemic lesions produced damage to the caudal forelimb representation area of the SMC, as assessed using reconstructions of the lesion placement relative to cytoarchitectural and macrostructural landmarks. Lesions damaged most of the overlap zone of the caudal forelimb representation area of the SMC and considerable non-overlapping motor (lateral agranular) cortex, but spared most of the medial agranular cortex and much of the granular cortex lateral to the lesion. No striatal damage was evident, though approximately half of all lesion animals had at least subtle damage to the corpus callosum. Lesion placement and extent were similar between groups as evidenced in these reconstructions. Intact forelimb training did not increase infarct size
as measured by remaining cortical volume (Table 4.2). Figure 4.5 shows coronal reconstructions of SMC lesions from Experiment 1. Rats in Experiment 2 tended to have smaller lesions than those in Experiment 1 (as evidenced by greater remaining cortical volume), but there was no differential training effect on this measure.

Table 4.2. Volume of remaining infarcted sensorimotor cortex.

<table>
<thead>
<tr>
<th>Group</th>
<th>Remaining SMC volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>82.12 ± 2.24</td>
</tr>
<tr>
<td>IntactT</td>
<td>83.20 ± 1.96</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>91.41 ± 2.33</td>
</tr>
<tr>
<td>IntactT</td>
<td>90.08 ± 2.37</td>
</tr>
<tr>
<td>BiT</td>
<td>95.70 ± 2.83</td>
</tr>
</tbody>
</table>

Data are means ± SEM.
4.4.7. Training the intact forelimb decreases FosB/ΔFosB expression in peri-infarct cortex

As shown in Figure 4.6, lesions increased FosB/ΔFosB in remaining SMC, as assessed after the period of impaired forelimb training (Exp. 1). Intact forelimb training decreased expression of FosB/ΔFosB in layer II/III compared to Cont (F(1,19) = 5.92, p < .03). In contrast, in layer V, where FosB/ΔFosB neuronal expression was relatively low, both lesion groups had increased expression compared with sham-operates and there was no significant difference between lesion groups (p > .05). To assess the reproducibility of the intact training effect in layer II/III, FosB/ΔFosB neuronal
expression was measured in peri-infarct cortex of archived tissue from a previous study (Allred et al., 2005). The experimental design was similar to that of IntactT and Cont groups of Experiment 1 (Fig. 1A), with the exception that rats had no pre-operative reaching experience. Rats trained with their intact forelimb after unilateral SMC lesions also had decreased FosB/ΔFosB neuronal density in peri-infarct cortex compared to Cont (F(1,14) = 6.97, p < .02).

In sham-operates, there was no significant decrease of FosB/ΔFosB expressing neurons as a result of the prior forelimb training (F(1,19) = 1.29, p > .05) in layer II/III or in layer V (F(1,19) = 1.58, p > .05). FosB/ΔFosB neuronal expression in layer V of granular insular cortex (GIC), a control region, was not significantly different between lesion groups (F(1,14) = 2.79, p > .05).

Rats in Experiment 2 had a training period focused on the intact forelimb before being sacrificed, and this creates a potential confound to the interpretation of early intact training effects on peri-infarct cortex. Nevertheless, the FosB/ΔFosB results showed a pattern of change consistent with that found in Experiment 1. The ratio between FosB/ΔFosB positive and negative neurons was significantly reduced in IntactT compared to Cont (F(1,16) = 27.18, p < .001) in layer II/III of peri-infarct cortex. BiT rats were not significantly different from either Cont (p = .95) or IntactT (p = .13).
Figure 4.6 Training the intact forelimb after unilateral SMC lesions results in a reduction of FosB/ΔFosB expression in peri-lesion cortex.

FosB/ΔFosB representative photomicrographs from layer II/III peri-infarct cortex (A,B, scale bars = 10 µm) and quantitative results (C) from Experiment 1. Unilateral lesions increased FosB/ΔFosB neuronal density in remaining SMC compared to sham-operates. Intact forelimb training significantly decreased FosB/ΔFosB neuronal density in layer II/III compared to Cont rats. A representative lesion is shown in panel A. Scale bar = 500µm. Data are means ± SEM. *p < .05.
4.4.8. There was no effect of lesions or forelimb experience on FosB/ΔFosB expression in the nucleus accumbens

The expression pattern of FosB/ΔFosB was also examined in the nucleus accumbens bilaterally (see Figure 2.2). There were no differences in the pattern of staining between the nucleus accumbens shell and core and therefore these data were combined. There was no differential training effect on expression of FosB/ΔFosB in either hemisphere (Appendix A, Table A1). There was also no differential effect of the lesion in either hemisphere in any of the groups.

4.4.9. There was no effect of lesions or forelimb experience on FosB/ΔFosB expression in the dorsolateral striatum

There was no difference in FosB/ΔFosB expression pattern in the dorsolateral striatum of the lesion cortex in animals with intact forelimb experience compared to animals that received control procedures (Cont) and animals that had training with both forelimbs (BiT) (Appendix A, Table A2). The dorsolateral striatum receives projections from the motor cortex and is involved in learning motor skills (Schallert, Kozlowski, Humm, & Cocke, 1997). It is logical to think that following impaired forelimb training all animals would have a relative increase in neural activation in this region.
4.5. Discussion

Training the less-affected, "intact" forelimb in skilled reaching following unilateral SMC ischemic lesions increased deficits in reaching with the impaired forelimb. Additionally, this training reduced expression of a marker of persistent neuronal activation, FosB/ΔFosB, in remaining motor cortex. It also increased reliance on the intact forelimb in tests of coordinated forelimb movements. In human stroke patients, it is common to focus post-stroke therapy on the execution of everyday tasks. This is frequently accomplished through compensatory use of the less-affected hand and arm. However, this may contribute to the phenomenon of learned nonuse, where disuse of the impaired arm is believed to further limit its recovery (Taub et al., 2006B). Our results suggest this lack of recovery may stem, not only from disuse, but also from disruptive influences of behavioral experience with the less-affected forelimb.

Our results indicate that peri-infarct cortex is vulnerable to early post-injury intact forelimb experience and suggest that neural activity in this region is disrupted by experience with the intact body side. ΔFosB, a splice variant of the FosB gene lacking 101 amino acids from the C-terminus (Nakabeppu & Nathans, 1991), is present for days after exposure to a stimulus, and its activation is believed to initiate neuronal structural plasticity (McClung et al., 2004). In the present study, unilateral lesions increased FosB/ΔFosB neuronal density in layer II/III of remaining SMC near the infarct after impaired forelimb training; however, early intact forelimb training reduced this expression. This is significant because the remaining cortex has been strongly implicated...
as a mediator of sensorimotor functional recovery in the impaired limb after unilateral cerebral damage (Nudo, 2003).

The remaining cortex near an ischemic infarct undergoes a time-dependent cascade of degenerative and regenerative changes (Carmichael, 2006). Inhibition of peri-lesion cortex dramatically impairs recovery from cortical lesions in rats (Hernandez & Schallert, 1990). In humans, transient transcranial stimulation-induced “virtual lesions” of peri-infarct motor cortex worsens paretic hand function (Werhahn et al., 2003). Functional magnetic resonance imaging (fMRI) studies indicate that restoration of cortical activity in the infarcted hemisphere is positively correlated with functional recovery in rats (Dijkhuizen et al., 2003) and humans (Johansen-Berg, et al., 2002). Facilitative stimulation of peri-infarct motor cortex in rats (Adkins-Muir & Jones, 2003; Kleim et al., 2003; Teskey et al., 2002) and squirrel monkeys (Plautz et al., 2003) further improves motor function and increases synaptic density in stimulated cortex (Hsu, Adkins, & Jones, 2006). Thus, there is a wealth of evidence to indicate that after unilateral cerebral damage, remaining cortical regions can mediate, or be driven to mediate, functional recovery. The present findings indicate that prior experience with the intact forelimb limits neuronal activation in remaining motor cortex, possibly suppressing or interfering with activity-dependent plasticity that could have mediated better recovery in the impaired forelimb.

Skilled reaching has been linked with lateralized neural plasticity in the motor cortex opposite the trained forelimb, including increased dendritic arborization, (Allred & Jones, 2004; Withers & Greenough, 1989), greater forelimb movement representations
Pyramidal neurons of layer II/III of rat sensorimotor cortex are particularly responsive to motor learning (reviewed in Nudo, 1999), and changes here may be reflective of mechanisms related to long-term potentiation (e.g., Hess, Aizenman, & Donoghue, 1996). In contrast to FosB/ΔFosB expression in layer II/III, neuronal labeling in layer V was not sensitive to the effects of intact forelimb training, though it was increased by the lesions alone. Both layers receive callosal projections (Donoghue & Parham, 1983), but dendritic spines in layer II/III are particularly responsive to manipulations of forelimb experience in intact rats (Adkins, Bury, & Jones, 2002). It may be that a greater duration of intact forelimb training is needed to influence FosB/ΔFosB expression in layer V. In intact adult rats, 28 days of training on a complex motor skills task, the acrobatic task, increased layer V synapses per neuron (Jones et al., 1999). Given differences in baseline levels of FosB/ΔFosB labeling between layers, these laminar differences should also be addressed using other measures of neuronal activation. Furthermore, the molecular mechanisms of these differential training effects and their relationship to synaptic structural plasticity require further investigation.

Following intact forelimb experience there is an induction of FosB/ΔFosB that may be more site specific to the cortex. Though FosB/ΔFosB has been shown to accumulate in the nucleus accumbens in response to rewarding stimuli (McClung, Perotti, etc.), these data suggest that the nucleus accumbens is not a brain region sensitive to enhancement of neural activation in response to motor skills training nor is it sensitive to lesion-induced changes. It is not possible, however, to rule out that training alone in
intact animals may induce greater neural activation in this brain region. There was also no differential training effect on FosB/ΔFosB expression in the dorsolateral striatum, though, as with above, it cannot be ruled out that motor training alone in intact animals would result in an increased expression of this protein in this brain region.

Constraint-induced movement therapy improves stroke-affected hand function (Mark & Taub, 2002; Wolf et al., 2006). However, extreme and early forced use of the impaired forelimb in rats increases lesion size and exacerbates behavioral deficits (Kozlowski, James, & Schallert, 1996; Schallert et al., 2000). Thus, there may be maladaptive consequences of overuse of either forelimb after unilateral brain damage. Our experiments used a challenging motor skills task, but this manipulation is subtle compared to behavioral manipulations found to exaggerate infarct size, such as forced use (Kozlowski, James, & Schallert, 1996). Furthermore, all rats with lesions increased reliance on the less-affected forelimb for postural support behavior, which presumably reflects asymmetrical forelimb use in the home cage. This raises the possibility that it is lateralized skill acquisition with the less-affected forelimb, rather than simply use of this limb, that alters behavioral outcome with the impaired forelimb. Following cerebral insult, animals have to “re-learn” new ways of using the impaired forelimb (Schallert, 2006; Shubring-Giese et al., 2007), and it is possible that this is especially sensitive to experience with the less-affected forelimb. Previously, we found a large effect on impaired forelimb function following intact forelimb training in rats with little reaching experience prior to the infarct (Allred et al., 2005). Intact training effects may therefore be particularly maladaptive for learning new motor skills with the impaired forelimb.
Rats trained early after lesions with both forelimbs were not more impaired and tended to perform better than controls. Humans trained to use both arms to push and pull T-bar handles (following strokes) had increased activity in the affected hemisphere and better functional outcome with the paretic limb (Luft et al., 2004). Function in the paretic limb also improved following training of both limbs on reach-to-target tasks (Mudie & Matyas, 2000). It is important to note that BiT rats received half the intact training trials (15) compared to IntactT rats (30), and it is possible that the experience with the intact limb was simply too low in intensity to worsen function in the impaired forelimb in this group. Consistent with the possibility that training intensity is important; rats in Experiment 1 received 2.5 times as many intact training trials as rats in Experiment 2 and had more persistent worsened function in the impaired forelimb. However, this difference between experiments may also be related to infarct size, as rats in Experiment 2 tended to have smaller lesions. Differential stress cannot be ruled out as a contributor to the present results, though this seems somewhat unlikely given that neither bilateral training nor focused impaired limb training (e.g., Maldonado et al., in press) worsen function in the impaired limb on this reaching task.

In contrast to rats with infarcts, sequential forelimb training did not worsen behavioral performance with the preferred forelimb in intact animals. However, there was a non-significant tendency for training one limb to worsen function in the other limb in sham-operates. This is consistent with there being subtle, interhemispheric motor learning interference effects that become exaggerated in rats with unilateral SMC lesions while re-learning to use the impaired forelimb. In both intact humans (Ward & Cohen,
reduction in ipsilateral sensory activity has been found to improve function in the other body side. Sensory input from ipsilateral whiskers has been shown to result in a strong constraining influence on the receptive fields in the barrel field cortex (Glazewski, Benedetti, & Barth, 2007). Anesthetization of the less-affected hand improves performance of a finger-tapping task with the paretic hand in stroke patients (Floel et al., 2004) and improves reaching function in the impaired forelimb in rats (O’Bryant, Bernier, & Jones, 2007). Moreover, the contralesional hemisphere can have an exaggerated inhibitory influence over the stroke-affected hemisphere during movements of the paretic hand compared with healthy subjects (Murase et al., 2004; Duque et al., 2005). Our findings suggest that these exaggerated interhemispheric effects are likely to be mediated, in part, by behavioral experiences with the intact body side.

Approximately 5% of the corticospinal tract descends ipsilaterally in rats (Brosamle & Schwab, 1997), and it is possible that this pathway contributes to the maladaptive effects of training the intact forelimb in the present study. The ipsilateral corticospinal tract has not been found to be a significant contributor to skilled forelimb function in rats (Whishaw & Metz, 2002), but this does not rule out a potential role in the exaggeration of forelimb impairments seen after training the intact forelimb.

It remains to be determined whether the post-injury time of onset of the training is an important variable in these effects. Furthermore, it is unknown whether the effects of learning with the intact forelimb extend to other types and modalities of function, such as less-skilled use of the forelimb, use of other body parts, and sensory functions. The
behavioral and environmental experiences of the animals in this study were likely less intense and varied than the experiences of human stroke survivors. It is reasonable to hypothesize that training rats with their intact limb on motor skills of greater complexity (e.g., the pasta matrix task, Ballerman et al., 2001) or of greater variety (e.g., such as handling different kinds of pasta, Whishaw & Coles, 1996) would result in an even greater detriment in behavioral recovery of the impaired forelimb. It is also important to determine whether experiences with the less-affected body side influence behavior after infarcts in other brain regions and after other types of brain damage, including traumatic brain injury and Parkinson’s disease, which frequently manifests asymmetrically.

The present results indicate that experiences with the less-affected forelimb can disrupt neuronal activity in the infarcted hemisphere in a manner linked to greater injury-induced impairments. Additional research is needed to address the timing and persistence of intact forelimb training effects, the causality of its relationship with peri-infarct neural activity, its molecular mechanisms, and the involvement of inter-hemispheric communication and corticospinal tract in these effects. There is also a need to assess the generalizability of reaching experience to other types of behavioral experience, including exercise. A better understanding of these effects could be of major relevance for rehabilitation approaches in humans surviving stroke, and, possibly, other types of brain damage.
Chapter 5

Effects of early and delayed intact forelimb training on recovery of impaired forelimb function and peri-lesion motor map plasticity after SMC lesions

5.1. Abstract

Following unilateral sensorimotor cortex (SMC) damage in rats, the contralateral homotopic cortex develops a highly plastic environment that is linked with a facilitation of learning a motor skills task with the “intact” (ipsilesional) forelimb. However, training the intact forelimb further debilitates function in the impaired forelimb and reduces expression of a marker of neuronal activity in peri-lesion cortex compared to animals without intact forelimb experience. The purpose of the present study was to determine 1) if delaying intact forelimb training after lesions would still convey the same maladaptive effect on impaired forelimb recovery and 2) how intact forelimb training influences motor map reorganization in both hemispheres. It was hypothesized that peri-lesion forelimb motor map representations would be smaller in animals trained with the intact forelimb compared to those receiving control procedures. Furthermore it was hypothesized that earlier intact forelimb training after lesions would be more detrimental than delayed training. Intact forelimb training significantly reduced peri-lesion forelimb motor map area and increased deficits in impaired forelimb skilled reaching. Early and delayed onsets of intact forelimb training had similar effects. These results indicate that intact forelimb training disrupts the organization of movement maps in peri-lesion cortex.
and further support that this type of experience is detrimental to impaired forelimb function even after a longer recovery period.

5.2. Introduction

Though deaths resulting from cardiovascular disease are declining, stroke is still a leading cause of long-term disability in the United States (Lloyd-Jones et al., 2009). It is increasingly important to refine post-stroke therapies for regaining lost function. A promising therapy is constraint induced movement therapy (CIMT) where the less-affected arm is restrained and the stroke-affected arm is used exclusively for most waking hours (Taub & Morris, 2001; Taub et al., 2006). However, following unilateral damage there is a decrease in the use of the impaired forelimb and an increased reliance on the intact forelimb (Bury & Jones, 2002; Allred & Jones, 2004; Luke et al., 2004). Humans, and other animals, naturally develop compensatory use of the “intact” body side following unilateral damage so that daily living tasks can still be accomplished (for review see Allred & Jones, 2008).

Following unilateral SMC damage, there is a time course in which neuroplastic changes occur in the contralateral homotopic cortex, including an increase in dendrites and synapses (Jones et al., 1996). These plastic changes represent an injury-induced facilitation of behavioral change with their less-affected (intact) forelimb which is reflected in a better ability to acquire a skilled reaching task with this limb (e.g., Bury & Jones, 2002; Allred & Jones, 2004; Luke et al., 2004; Hsu & Jones, 2005). However, this learning comes at a cost of a further deterioration in impaired forelimb performance.
Animals with unilateral SMC lesions that are trained with their intact forelimb have a significant worsening of impaired forelimb function compared to control rats and have a more enduring reliance on the intact forelimb in postural support behaviors (Allred et al., 2005; Allred & Jones, 2008).

Following unilateral lesions, rehabilitative training focused on the impaired forelimb can aid performance recovery (e.g., Maldonado et al.; Hsu et al., 2006; Biernaskie & Corbett, 2001) and can expand forelimb motor maps (Kleim et al., 1998; Nudo et al., 1996). If the reorganized motor cortex is inactivated following rehabilitation, there is a subsequent loss of the recovered skill (e.g., Castro-Alamancos & Borrel, 1995). The reorganization of motor maps is proposed to be the last step in a cascade of events (which include synaptic strengthening, e.g., long term potentiation) leading to the long term maintenance of a newly acquired motor skill (see Monfils, Plautz, & Kleim, 2005).

On the behavioral level, animals with intact forelimb training have a weakened response to rehabilitative training of the impaired forelimb (Allred & Jones, 2008). The extent to which this experience affects peri-lesion motor maps was unknown. It was also unknown if there is a time-sensitive window following unilateral damage when experience with the intact forelimb augments impaired forelimb deficits. The purpose of the present studies was to determine the effects of intact forelimb training, beginning 6 and 20 days post-operatively, on impaired forelimb recovery of function and peri-lesion motor map reorganization in rats. The facilitation of learning with the intact forelimb is time sensitive (Hsu & Jones, 2005) and the peri-lesion cortex has been found to be sensitive to other experiences in a time-sensitive manner (Humm et al., 1998). It was
therefore hypothesized that animals with intact forelimb training beginning 20 days after stroke would not show the same decrement in impaired forelimb function or disruption of peri-lesion motor maps as rats with earlier onset (6 days after stroke).

5.3. Materials and Methods

5.3.1. Animals

Forty adult male Long-Evans rats were trained to a proficient level on the single pellet retrieval task (see Methods below and also section 2.1) and were then given ischemic (endothelin-1 induced, 240 pmol) unilateral sensorimotor cortex (SMC) lesions opposite their preferred-for-reaching (dominant) forelimb. Animals were divided into four groups matched for lesion-induced reaching deficits as measured on day 5 post-lesion: Early intact training (IntactT_Early, n = 14), early control (Cont_Early, n = 14), delayed intact training (IntactT_Delayed, n = 9) and delayed control (Cont_Delayed, n = 9). Animals in the early groups were trained or underwent control procedures for 15 days followed by 10 days of impaired forelimb training. Animals in the delayed groups were re-assessed (for 15 trials) with their impaired limb 19 days post-lesion to monitor spontaneous recovery effects. Animals in the delayed groups were then trained with the intact forelimb or underwent control procedures for 15 days (beginning on day 20 post-operative) followed by 10 days of impaired forelimb training. See Figure 5.1 for the experimental time lines. During days 6-18 post-lesion, delayed groups were maintained on a restricted food diet and given banana pellets several times per week in their home cage, but were otherwise left undisturbed.
Figure 5.1 Timeline of experimental procedures.

All animals were pre-operatively trained with their dominant (for the task) forelimb and then received unilateral SMC lesions opposite their dominant forelimb. Lesion induced reaching deficits were assayed 5 days after the lesion, and animals were matched into groups. Animals in the early groups underwent procedures as previously described (chapter 4). Animals in the delayed groups underwent the same procedures with intact forelimb training initiated at a delayed (two weeks) time point. At the conclusion of the experiment all animals underwent intracortical microstimulation mapping procedures.
5.3.2. Surgical Procedures

Ischemic Lesions

All animals were anesthetized with ketamine (10mg/kg) and xylazine (120mg/kg) and then given a unilateral ischemic lesion in the SMC opposite their preferred-for-reaching forelimb (coordinates from bregma: A/P: -1.0, +2.0, M/L: 2.0, 4.5). 3.0 µl (240pmol total) of endothelin-1 (ET-1; American Peptide, Inc.), a vasoconstricting peptide, were placed directly onto the cortical surface after removal of dura mater. The cortex was left undisturbed for 10 minutes. The craniectomy was then covered with gel foam (source, n = 34) or gel film (source, n = 6) and dental cement. (The addition of gel foam/gel film to this ischemic lesion protocol was intended to reduce cortical tissue distortion outside of the lesion cavity, an effect frequently seen after ET-1 lesions, see figure 3.3.). The wound was then sutured. Animals were administered s.c. buprenorphine (10mg/kg) post-lesion as an analgesic and were given 4 days to recover before behavioral training commenced.

Intracortical Microstimulation Mapping

At the end of impaired forelimb training, rats were anesthetized with a ketamine (12mg/kg), xylazine (60mg/kg) cocktail. Plane of anesthesia was monitored throughout the mapping procedure through response to tail pinch, breathing patterns, and whisking behavior. Ketamine and isoflurane gas (1-2% in oxygen) were administered when animals showed signs of waking. After placing rats in a stereotax, lidocaine (2mg/kg) was injected s.c. into the scalp and a midline incision was made. At this time, the cisterna
magna was punctured to drain CSF to prevent swelling of the cerebral cortex. Skull and dura of the sensorimotor cortex of both hemispheres were removed. Silicone oil (37°C) was applied to the cortical surface to prevent drying.

A platinum wire was inserted into a glass microelectrode (20-25 µm diameter) filled with 3.5M saline, and caudal and rostral areas of the forelimb were established in a systematic manner. Peri-lesion cortex was mapped first, followed by contralesion cortex. Intracortical penetrations in 500 µm intervals were made at a depth of 1550 µm (and 2000 µm in the direct lesion territory to compensate for potential tissue distortion effects), corresponding to layer V pyramidal neurons. Trains of thirteen, 200 µs monophasic, cathodal pulses were delivered at a rate of 350 Hz from a constant current stimulator. Stimulation was gradually increased at each site up to 60 µA until a visible movement was evoked. In the peri-lesion cortex stimulation was further increased up to 100 µA to determine if effects varied greatly with stimulation amplitude. If no movement was evoked at this threshold, the site was determined to be non-responsive. Canvas software (ACD systems) was used to obtain area representations. While one experimenter controlled the micropositioner another experimenter supported the rat’s forepaw opposite the stimulated cortex to better enable movement detection. This latter experimenter called the movements and was blind to cortical position. Both experimenters were blind to behavioral groups. The extent of the rostral and caudal forelimb areas were mapped in each hemisphere. The entire jaw representation was additionally mapped. Maps from 9 animals (IntactT_Early, n = 4; Cont_Early, n = 4, IntactT_Delayed, n = 1) were not used in ICMS analyses because of technical difficulties with mapping equipment. Peri-lesion
cortex was always mapped before contralesion cortex. Many sites in peri-lesion cortex were non-responsive. To increase power in statistical analyses, control rats (both early and delayed) were combined and IntactT rats (both early and delayed) were combined for ICMS between-group analyses after determining that the behavioral effects were not significantly different between these conditions (early and delayed). At the conclusion of mapping procedures, animals were overdosed with sodium pentobarbital.

5.3.3. Behavioral Methods

Single Pellet Retrieval Task

The single pellet retrieval task was carried out as previously described (section 2.1). Post-operatively, animals in the intact training groups were trained with this limb for 5 days for 60 trials/day and for an additional 10 days for 120 trials/day. All animals were trained to reach with their limb for 5 days for 30 trials/day with the pellet in the close position (1 cm from window) and for an additional 5 days for 60 trials/day, 30 close and 30 remote (2 cm from window). Performance on this test was quantified as % successful retrievals ((drops + successes)/total reach attempts).

5.3.4. Histology

Following training, all animals underwent intracortical microstimulation mapping (see Methods above) and were then overdosed with sodium pentobarbital and trans-cardially perfused with 4M phosphate buffer with .05g/L heparin salt followed by 2% paraformaldehyde/2.5% glutaraldehyde in the same buffer. Brains were removed and
sliced on a vibratome rostral to caudal throughout the cerebrum. Fifty µm thick sections were Nissl stained and used for lesion verification and area tracing. Two hundred µm thick sections were processed for electron microscopy for future analyses.

5.3.4.1. Lesion Reconstruction and Volume Estimation

50 µm thick Nissl stained sections were used to assess lesion extent and placement. Nissl stained sections were scanned into Adobe Photoshop (Adobe software). Seven sections containing sensorimotor cortex between 2.7 and -0.3 mm relative to bregma from both hemispheres were outlined. Image J software was then used to obtain area measurements. Volume was calculated based on ΣA * section thickness (Gunderson, 1988). This method was found to result in similar volume measurements compared to that obtained when using Neurolucida perimeter tracing software. As both hemispheres underwent mapping procedures, to control for potential ICMS-induced area changes, the volume measurement used was intact – lesion in mm³. Lesions were reconstructed onto coronal templates as previously reported (chapter 2). Evident damage e.g., electrode damage, that may have resulted from the ICMS procedure was not included in area tracing or lesion reconstructions.

5.3.5. Statistical Analyses

All statistical analyses were carried out using SPSS statistical software (SPSS, Inc) with a priori planned comparisons. For the impaired forelimb training period the following comparisons were made: 1) Cont_Early vs. IntactT_Early, 2) Cont_Delayed vs.
IntactT_Delayed. For intact forelimb training IntactT_Early was compared with
IntactT_Delayed. Behavioral analyses were performed with repeated-measures
ANOVAs and student’s t tests. Sensorimotor cortex volume for both hemispheres and the
difference between the hemispheres was analyzed using one-way ANOVAs or student’s t
tests. ICMS analyses for control groups combined (Cont) and intact trained groups
combined (IntactT) were performed with one-way ANOVAs or student’s t tests. SPSS
bivariate correlations were also used. All data are expressed as mean ± SEM. Effects
were considered significant at p < .05.

5.4. Results

5.4.1. Intact forelimb training at early and delayed post-lesion time points worsens
behavioral performance with the impaired forelimb

Unilateral SMC lesions resulted in profound impairments in the contralateral
forelimb as measured on post-lesion day 5 (Figure 5.2) and were still evident on day 19,
before intact forelimb training in the delayed groups. Training the intact forelimb resulted
in a significant worsening of behavioral performance with the impaired forelimb in both
the early (F(1,20) = 16.24, p < .01) and delayed (F(1,16) = 26.81, p < .001) training
paradigms (Figure 5.2). A similar performance pattern was seen on a more difficult task
(when the pellet was in the remote position, Figure 5.3). Animals trained with the intact
forelimb performed significantly worse with their impaired forelimb compared to control
animals (IntactT_Early vs. Cont_Early = (F(1,20) = 10.94, p < .01; IntactT_Delayed vs.
Cont_Delayed = (F(1,16) = 5.59, p < .05).
Figure 5.2 Intact forelimb training worsens performance of the impaired forelimb.

A. Animals with intact forelimb training had a significant worsening of performance with the impaired forelimb compared to control animals at the early time point (p < .01). B. Intact forelimb training, even if initiated at a later time point, significantly reduces impaired forelimb performance compared to time matched animals that received control procedures (p < .001).
Figure 5.3 Training the intact forelimb worsens performance of the impaired forelimb on a more difficult task.

Animals with intact forelimb training performed significantly worse with their impaired forelimb on a more difficult task compared to control animals. **A.** Animals trained early after lesions with their intact forelimb later performed worse with their impaired forelimb on remote trials ($p < .01$). **B.** Animals trained with their intact forelimb initiated at a more delayed time point also performed worse with their impaired forelimb on remote trials compared to time matched control animals ($p < .05$).

5.4.2. Intact forelimb training induces perseveration of reach attempts with the intact forelimb during later impaired forelimb training

Animals with intact forelimb training consistently made reach attempts with this forelimb during the impaired forelimb training period (IntactT_Early = 16.85 ± 2.42 reaches; IntactT_Delayed = 17.32 ± 3.59 reaches on average over impaired forelimb training days) even though the task was configured such that these reaches were unsuccessful. In contrast, reach attempts with the intact forelimb in control groups were minimal on the first few days and absent after day 3 of impaired forelimb training. Animals that had been trained with their intact forelimb continued to make reach attempts with this limb throughout the impaired forelimb training period. On day 10 of impaired forelimb training, 7 of 11 rats in the IntactT_Early group, and 9 out of 9 animals in the
IntactT_Delayed group, were still making reach attempts with this limb. In animals with intact forelimb training there was a significant correlation between the number of intact limb reaches (across all days of impaired forelimb training) and impaired forelimb reaching performance (IntactT_Early, r = -.67, p < .01; IntactT_Delayed, r = -.72, p < .02, Fig. 5.3). However, intact forelimb training did not reduce activity with the impaired forelimb. There was no difference in the number of completed trials with the impaired forelimb per day between groups (IntactT_Early = 29.01 ± 1.05; Cont_Early = 29.44 ± 0.39; IntactT_Delayed = 29.67 ± 0.22; Cont_Delayed = 29.89 ± 0.08).
Figure 5.4 Perseverative reaching with the intact forelimb negatively correlates with impaired forelimb reaching success.

There was a significant inverse relationship between the number of intact forelimb reach attempts during the impaired forelimb training period and impaired successful retrieval. This occurred after both early and delayed (relative to the post-lesion time point) intact forelimb reaching experience.

5.4.3. Intact forelimb training blocks spontaneous recovery

Spontaneous recovery was assayed in delayed groups by comparing the two post-operative time points (day 5 vs. day 19) before the onset of any intact forelimb training (see Figure 5.1). In the early groups, this time span was the period of intact forelimb
training and control procedures. During this time, the delayed groups received no behavioral manipulations. Experience with the intact forelimb blocked spontaneous recovery effects in the impaired forelimb. As noted previously, after 15 days of training the intact forelimb, IntactT animals had a significantly poorer performance on the single pellet retrieval task with their impaired forelimb compared to control rats (Figure 5.5). The two delayed groups performed better than the IntactT_Early group, but not as well as the Cont_Early group, suggesting that control procedures may facilitate impaired forelimb recovery. This was further supported by evidence of spontaneous recovery in Cont_Delayed animals after 15 days of control procedures. In contrast, in animals with delayed initiation of intact forelimb training (IntactT_Delayed) there was a significant reduction in impaired forelimb performance after 15 days of experience with the intact forelimb.
Figure 5.5 Experience with the intact forelimb disrupts impaired forelimb spontaneous recovery.

Following a period of intact forelimb training, spontaneous recovery of the impaired forelimb was reduced compared to control animals (p < .05, Cont_Early vs. IntactT_Early). Control procedures facilitated spontaneous recovery compared to animals which had two weeks of home-cage experience (p < .01; Cont_Early vs. Delayed groups). After control procedures, animals in the delayed group had a facilitation of spontaneous recovery (similar level to Cont_Early) compared to IntactT_Delayed (p < .05) animals, which had a reinstatement of early post-lesion impaired forelimb reaching deficits. Note that this graph includes a subset of data shown in Figures 5.2A and 5.2B but is presented
here so that recovery relative to day 5 post-lesion could be compared between the early and delayed conditions. Day 21 and 35 correspond to impaired limb training day 1 in Figures 5.2A and 5.2B, respectively.

5.4.4. There is little effect of delayed initiation of intact forelimb training on acquisition of the skilled reaching task with this forelimb

There was no difference in asymptotic performance of the skilled motor task with the intact forelimb between early and delayed groups (F(1, 20) = 0.95, p > .05) though there was a significant group x day interaction effect (F(14, 280 = 2.25, p < .01), reflecting the greater initial success levels in rats trained earlier after the lesions. IntactT_Delayed animals performed worse than IntactT_Early rats on days 1 and 3 of intact forelimb training (Figure 5.5). The slightly better performance with the intact forelimb at earlier versus later time points is consistent with previous findings (Hsu & Jones, 2005).
Figure 5.6 Final performance with the intact forelimb was similar between early and delayed groups.

Though earlier trained rats were initially better on the skilled reaching task, there was no difference in asymptotic performance with the intact forelimb compared to the early groups.
5.4.5. Lesion placement was similar between groups

All lesions resulted in damage to the caudal forelimb representation area of the sensorimotor cortex between 2.2 and 0.2 mm relative to bregma (Figure 5.6 depicts a representative lesion). One animal in the IntactT_Delayed group had a more posterior lesion compared to other animals and was not used in data analyses (and is not included in the overall N reported in section 6.3.1). Animals which had nonusable maps (see 5.3.3.2) were not included in reconstruction of lesions (but were included in all behavioral analyses).
Lesion placement was similar between groups

Representative lesions from each group were reconstructed onto schematic templates. Coordinates are mm relative to bregma.
5.4.6. Intact forelimb training did not exacerbate lesion size

There was no difference between groups in volume difference between the intact and lesion hemispheres (IntactT_Early = 13.56 ± 2.51 %; Cont_Early = 14.05 ± 3.07 %; IntactT_Delayed = 17.63 ± 1.51 %; Cont_Delayed = 18.09 ± 1.38 %).

5.4.7. Experience with the intact forelimb results in a loss of peri-lesion caudal forelimb representation cortex

The results below are the combined ICMS data from intact trained (IntactT_Early + IntactT_Delayed) and control animals (Cont_Early + Cont_Delayed). These data were combined to increase power of statistical analyses between groups. This was also warranted by the similarity in behavioral effects across the time points. Animals with intact forelimb experience had significantly smaller caudal forelimb maps in peri-lesion sensorimotor cortex (SMC) compared to control animals (p < .05). There was no difference between groups in the rostral forelimb area.

IntactT animals tended to have larger jaw representations (IntactT = 1.49 ± 0.14 mm²; Cont = 1.16 ± 0.19 mm²) though this failed to reach significance (p = .08). IntactT animals also tended to have movements clustered more medially compared to control rats (Fig. 5.7). In all animals, jaw representations tended to be lateral to forelimb areas.

For a site to be considered responsive, movements had to be elicited at or below 60 µA of stimulation. Of the sites in which responses were found, thresholds required to elicit a movement were not significantly different between groups (IntactT = 39.82 ± 2.59 µA; Cont = 41.14 ± 2.05 µA). The standard maximum intensity of stimulation is set at 91
the 60 µA level because higher levels tend to elicit multiple movements in normal maps. However, to determine whether the observed group differences were due to different thresholds needed to elicit movements in the peri-lesion cortex, we also tested the effects of stimulating up to 100 µA at any site that was non-responsive at ≤ 60 µA. If the effects were due to the higher thresholds in IntactT maps, then the higher stimulation would be expected to reveal more forelimb sites in this group than in controls. However there was no difference between intact forelimb trained and control animals in the number of sites in which forelimb movements could be evoked only at this higher intensity level (Intact T = 1.26 ± 0.39; Cont = 0.93 ± 0.26).
Figure 5.8 Intact forelimb training reduces peri-lesion caudal forelimb movement representations.

Rats with intact forelimb training had a significantly smaller area of peri-lesion motor cortex devoted to the caudal forelimb representation area compared to control rats (p < .05). There was no difference between intact forelimb trained and control rats in area devoted to forelimb movements in the rostral forelimb area.
Figure 5.9 Representative peri-lesion motor maps.

A representative peri-lesion map from a control animal (left) and an animal with intact forelimb training (right) relative to a typically placed lesion. B, bregma, A, anterior, P, posterior, L, lateral,
**Figure 5.10 Surface plots of peri-lesion motor maps.**

The top panel is from control animals and the bottom panel is from intact forelimb trained animals. Medial to lateral relative to bregma is depicted on the x-axis. Anterior to posterior relative to bregma is depicted on the y-axis. The legends refer to the percentage of animals with forelimb movements at those sites.

### 5.4.8. Intact forelimb experience expands forelimb motor maps opposite the trained forelimb

Animals with intact forelimb experience tended to have larger contralesional forelimb motor maps than control animals, though this effect did not reach significance (IntactT = 4.74 ± 0.48 mm²; Cont = 3.98 ± 0.39 mm², p = .09).

### 5.5. Discussion

Training focused on the intact forelimb, whether commenced at 6 days or 20 days post-lesion, resulted in a worsening of behavioral function in the impaired forelimb. This indicates that even at a delayed time point after unilateral damage (i.e., after a prolonged recovery period) experience with the intact forelimb can disrupt recovery of the impaired forelimb. Intact forelimb training also significantly reduced forelimb motor map area. These results indicate that experience with the intact forelimb after unilateral
lesions can have a profound detrimental effect on impaired forelimb function and perilesion motor map plasticity.

It has long been thought that boundaries between cortical representations are actively maintained via competitive processes (Jacobs & Donoghue, 1991; Hickmott & Merzenich, 1998). For example, Jacobs and Donoghue (1991) found that local infusions of GABA antagonists cause an expansion of the local map into surrounding territories, such that multiple movements can be evoked at the same site. Following a forced use rehabilitation therapy in stroke patients, a shift in motor representations in the infarcted motor cortex occurred toward the direction where pre-therapy inhibition was lowest, as measured using paired pulse TMS protocols (Liepert et al., 2006). These authors postulated that this may have occurred through unmasking of circuits that are normally inhibited by GABA activity. Additionally, Weiller and colleagues (1993) found that arm and hand motor representations in patients with good motor recovery tended to move into areas previously occupied by face as measured using positron emission tomography. In adult owl monkeys with extensive exposure to tactile stimulation of the digits there was a lateral movement of digit representations into areas previously occupied by face (Jenkins, Merzenich, Ochs, Allard, & Guíc-Robles, 1990).

All animals in the present study tended to have jaw representations located laterally to forelimb movement representations. Animals with intact forelimb experience also tended to have a larger area devoted to the jaw representation compared to control animals. It may be that with additional impaired forelimb training in IntactT rats, motor cortex devoted to jaw movements could be driven to control forelimb movements. This
idea is further supported by the tendency of forelimb movements in IntactT rats to be clustered more medially than in Cont animals. It may that in control rats the preservation or re-emergence of peri-lesion forelimb movement representations occurs at the expense of jaw representations, and that this was blocked or slowed in the presence of intact forelimb experience. Although the present results are suggestive of there being post-lesion reorganization near the jaw-forelimb border, this can’t be determined conclusively without comparison to pre-injury baseline maps or controls. The contralesional cortex cannot serve as an internal control because it tended to be changed by the intact forelimb training. Intact forelimb trained animals tended to have larger forelimb movement representations in this cortex compared to controls.

All animals with intact forelimb experience made reach attempts with this forelimb during impaired forelimb training, an effect not seen in control animals. This finding was correlated with impaired forelimb performance, suggestive of a potential motor learning interference effect. It is possible that the greater the intensity of intact forelimb experience the longer it takes to extinguish intact forelimb behavior, leading to a prolonged unbalance in interhemispheric activity. The perseverance of intact forelimb reaching in intact forelimb trained groups is likely related to learned disuse of the impaired forelimb, though there was not a reduction in the use of the impaired forelimb during impaired forelimb training. It may be that animals trained with their intact forelimb have a greater disuse of the impaired forelimb in home cage experiences. Consistent with this, we found a greater disuse of this limb for postural support and coordinated forelimb stepping in previous studies (chapters 3 and 4). Previously Humm
and colleagues (1998) found that unilateral lesions increase reliance on the intact forelimb in the home cage. Intact forelimb training may further capitalize on this effect leading to an exaggerated disuse of the impaired forelimb during most waking hours. Learned disuse is a phenomenon where experience (i.e., practice on a skilled reaching task) with the intact body side leads to neglect of use of the impaired body side (Taub, Uswatte, Mark, & Morris, 2006), a phenomenon demonstrated in humans (Taub et al., 2006) and in rats (see Alaverdashvili et al., 2008).

In animals with exaggerated learned disuse of the impaired forelimb (e.g., following focused experience with the intact forelimb), it may take longer to relearn how to use the impaired forelimb in an effective manner. Following unilateral lesions, animals receiving impaired forelimb rehabilitative training tend not to plateau in skilled reaching performance until at least 20 days of training have occurred (e.g., Maldonado et al., 2005). Visual neglect, induced through monocular deprivation in cats, recovered with visual experience, though the maximum level of regained acuity did not occur until greater than 70 days after post-deprivation reversal (Iny, Heynen, Sklar, & Bear, 2006). It is conceivable that the maladaptive effect of intact forelimb experience could be overcome with more intense rehabilitative training, though overuse of the impaired forelimb early after unilateral lesions in rats also impaires motor recovery and exacerbates lesion size (Kozlowski et al., 1996; Humm et al., 1998). Thus increasing intensity of impaired forelimb training should be considered with caution.

It has been proposed that learning a motor skills task involves, at least in early stages, mechanisms similar to long-term potentation (LTP, Monfils & Teskey, 2004).
There is a decrease in the ability to induce LTP and an increase in the ability to induce long term depression in motor cortex opposite a reach trained forelimb (Monfils & Teskey, 2004), suggesting that LTP-like mechanisms have been involved. Strengthening of synaptic connections through motor skills learning is thought to permit motor map reorganization (e.g., the expansion of distal forelimb movement representations with skilled reaching, Kleim et al., 1998) and synaptogenesis (Kleim et al., 2002). A possible mechanism mediating the maladaptive effect of intact forelimb training might be a reduced ability to induce LTP in peri-lesion cortex following experience with this limb. Even tasks that require use of only one body side activate motor cortex bilaterally (e.g., Sehm, Perez, Xu, Hidler, & Cohen, 2009). An induction of LTP in intact cortex through intact forelimb motor learning may disrupt the ability to induce LTP in peri-lesion cortex. After reach training with one forelimb in intact animals, structural and functional changes have mostly been identified in the motor cortex contralateral to the trained limb. However, the “non-trained” motor cortex also tends to show some changes with training (Greenough et al., 1985), including subtle potentiation (Monfils & Teskey, 2004). It could be that these changes are exaggerated with intact forelimb training, leading to a potentiation of synapses in peri-lesion cortex either devoted to, or as a side product of, the newly learned skill in the intact forelimb. In either event, the intact forelimb might thereby confiscate circuits that would otherwise be changed to subserve skill re-learning with the impaired forelimb.

The onset of intact forelimb training or control procedures for rats in the delayed groups began 20 days after the lesions. Although maximum dendritic growth in the
contralateral cortex was previously found at 18 days post lesion (Jones & Schallert, 1992), it may be that there is still a time period for facilitation of skill acquisition. Hsu and Jones (2005) found that rats which were trained with their intact forelimb beginning 25 days after lesions, though not significantly different, did not perform at the same level as rats with intact training beginning 4 days after lesions. A similar tendency was found in this study. Thus it appears that unilateral lesions still prime skill acquisition with the intact forelimb, though this effect diminishes as the time period increases before rehabilitation is implemented. In the present study, if intact training was initiated at an even later time point (e.g., 45 days after lesions), its impact on impaired forelimb recovery might be weakened if not absent. However, at least within the time span examined in this study, delayed intact training still impeded recovery of the impaired forelimb, suggesting that peri-lesion cortex is vulnerable to experience with the intact body side for at least the first several weeks after the lesion.

It is very interesting that animals that served as controls for early intact forelimb training (being placed in the reaching chamber and fed pellets) had a higher spontaneous recovery rate than did rats with no behavioral manipulations (delayed groups). This possibly suggests a role of contextual cues. Cont rats, paired with the early intact animals, were exposed to the reaching chamber for two weeks, during which time they had no reaching experience and only ate pellets off the chamber floor. However, this occurred in the same environment in which they attained and practiced reaching skill prior to the SMC lesions. These cues alone may have been sufficient to facilitate spontaneous recovery effects. However, animals in delayed groups were not handled on a regular basis.
between 6-18 days post-lesion, and it is not possible to rule out that handling associated with early intact training and control procedures could also facilitate recovery. The role of contextual cues and handling in mediating recovery from brain damage should be considered in future investigations.

Data from this study provide additional support for the importance of post-stroke behavioral management. The infarcted cortex becomes vulnerable to experiences with the intact forelimb that are strong enough to override spontaneous recovery effects. In the present study, the maladaptive effect of intact forelimb training was not blocked by delaying post-lesion experience with this limb. These data provide some insight into the possible mechanisms by which this experience affects the ability of peri-lesion sensorimotor cortex to drive functional recovery of the impaired forelimb. Intact forelimb training caused a significant loss in peri-lesion motor cortex devoted to use of the impaired forelimb.
6.1. Abstract

Following stroke-like injury to the sensorimotor cortex in rats, experience with the ipsi-to-lesion (“intact”) forelimb worsens deficits in the contra-to-lesion (“impaired”) forelimb. We tested whether the maladaptive effects of intact forelimb experience are mediated through callosal connections and the contralesional sensorimotor cortex. Adult male rats that were proficient on a skilled reaching task with their dominant (for reaching) forelimb, received ischemic bilateral sensorimotor cortex lesions, or unilateral lesions with and without callosal transections. After assessing dominant forelimb function (the impaired forelimb after unilateral lesions), animals were trained with their non-dominant/intact forelimb or underwent control procedures for 15 days. Animals were then tested with their dominant/impaired forelimb. In animals with unilateral lesions only, intact forelimb training worsened subsequent performance with the impaired forelimb, as found previously. This effect was blocked by callosal transections. Rats with bilateral lesions that were trained first with the non-dominant limb had no decrement in function when switched to the dominant limb compared with controls. Thus, the maladaptive effects of training the intact limb on impaired forelimb recovery depend upon the contralesional cortex and transcallosal projections.
6.2. Introduction

Stroke affects approximately 795,000 Americans annually and accounts for 1 of every 18 deaths in the US (Lloyd-Jones et al., 2009). Many strokes are survived, but long term disabilities remain a prevalent problem. A now well established model for treatment of upper arm extremity impairments is constraint induced movement therapy (CIMT; (Mark, Taub, & Morris, 2006; Taub et al., 2006), where use of the impaired arm is encouraged through restraint of the intact or “less-affected” arm for most waking hours. This is intended to counteract the effects of learned disuse of the impaired arm, an effect thought to occur as a result of repeated incompetent use of this arm (Taub et al., 2003.) Data from clinical trials indicate that this therapy can significantly improve upper arm deficits (Wolf et al., 2006; Park et al., 2008). Frequently, however, stroke survivors use their intact body side in order to carry out daily tasks (e.g., Dobkin, 2006). Though use of this body side may convey immediate functional benefits, the long-term neural and behavioral consequences of compensatory behavior with the intact body side are not well understood.

Unilateral sensorimotor cortex (SMC) damage in the caudal forelimb representation region in rats results in major impairments in the contralesional (impaired) forelimb and a compensatory reliance on the ipsilesional (intact) forelimb, as well as an increased capacity to learn a skilled motor task with the intact forelimb (e.g., (Allred & Jones, 2004; Bury & Jones, 2002; Hsu & Jones, 2005; Luke et al., 2004). Recently we found that rats trained with their intact forelimb early after unilateral SMC damage have worsened motor function, a decreased responsiveness to rehabilitative training of the
impaired forelimb and a reduced peri-lesion neuronal activation of FosB/ΔFosB compared to rats without intact forelimb training (Allred & Jones, 2008; Allred et al., 2005). This may point to possible plasticity-inhibiting effects of the intact forelimb on peri-infarct neural plasticity. However, the mechanisms underlying this effect are entirely unknown.

Several lines of evidence suggest that following unilateral brain injury there are abnormalities in interhemispheric activity that are associated with reduced functional outcome. For example after visual cortex lesions in cats there is an increase in neural activity in contralesional structures. Visual neglect to stimuli presented in the contralesional field can be reduced with transient lesions of the contralateral cortex (Rushmore et al., 2006; Ward & Cohen, 2004). Interhemispheric inhibition (as measured using a paired-pulse transcranial magnetic stimulation protocol) from the intact to the lesion hemisphere is increased following stroke in human patients (Duque et al., 2005; Murase et al., 2004; see also Perez & Cohen, 2009), and this finding was correlated with deficits in motor performance (Murase et al., 2004). Functional magnetic resonance imaging (fMRI) studies indicate that better functional outcome tends to correlate with more normal lateralized cortical activity during paretic hand movements (reviewed in Cramer, 2008). It seems logical to think that experience with the intact body side may also further disrupt interhemispheric activity and contribute to worsened recovery.

The present studies were designed to test whether the maladaptive effects of intact forelimb experience are mediated by the contralesional SMC and intercortical connections. If so, then loss of these intercortical connections, which can be induced with
partial transections of the corpus callosum (Bury et al., 2000), would result in a blocking of the maladaptive effect of intact forelimb experience on functional recovery of the impaired forelimb. Furthermore, the effect should not be found in animals with bilateral SMC lesions.

6.3. Materials and Methods

6.3.1. Animals

Forty-four, 6 to 7 month old adult male Long-Evans rats were housed in pairs on a 12:12 light/dark cycle in standard laboratory cages. Animals were provided with standardized housing supplementation (a 10.5 cm diameter PVC pipe, small wooden objects, and cardboard paper rolls). At the beginning of each experiment, animals were fed a restricted diet of 16-19g/day/rat standard rat chow given once per day so that they were motivated to perform the skilled reaching task. Animal protocols were approved by the University of Texas at Austin Animal Care and Use Committee.

Experiment 1. This study was designed to determine if the maladaptive effects of intact forelimb training on impaired forelimb recovery are mediated through callosal projections of the SMC. It was hypothesized that if contralesional callosal fibers were denervated through transection of the corpus callosum, the disruptive influence of intact forelimb training on impaired forelimb function would be absent. Rats were divided into 4 groups based on post-operative impaired forelimb reaching deficits: two groups
received control procedures (Cont, n = 7; CCX_Cont, n = 10) and two groups received intact forelimb training (IntactT, n = 7; CCX_IntactT, n = 9).

**Experiment 2.** This study was designed to test whether bilateral sensorimotor cortex (SMC) damage would also block the maladaptive effects of sequential forelimb training after the lesions. It was hypothesized that animals with bilateral SMC damage trained with their non-dominant (for the task) forelimb (Bilat_ND, n = 6) would perform at a similar level with their dominant (for the task) forelimb compared to animals receiving control procedures (Bilat_Cont, n = 5). Figure 6.1 outlines the experimental designs.

**6.3.2. Ischemic Sensorimotor Cortex lesions**

All animals were given unilateral SMC lesions opposite their dominant-reaching forelimb (Experiment 1) or in both hemispheres (Experiment 2) using the endothelin-1 method (Adkins et al., 2004; Allred & Jones, 2004). Animals were anesthetized with i.p. injections of ketamine (10mg/kg) and xylazine (120mg/kg) and maintained under a surgical plane of anesthesia throughout the procedure, with ketamine boosters when necessary. A craniectomy was made by connecting four drill holes (A/P: -1.0, +2.0, M/L: 2.0, 4.5) and dura was removed just prior to topical application of 3.0 µl (Experiment 1) or 4.0 µl (Experiment 2) of endothelin-1 (ET-1; 80 pmol, American Peptide, Inc.). (Animals in Experiment 2 received a larger amount of ET-1 to ensure that the lesions were large enough to adequately determine the contribution of the non-dominant homotopic cortex on dominant forelimb recovery.) Animals were then left
undisturbed for 10 minutes before suturing. Buprenorphine (10mg/kg), an analgesic, was administered subcutaneously post-surgery when the animal began to arise from anesthesia. Reaching behavioral assessment began 5 days after surgeries. For animals with corpus callosum transections (see below), ET-1 was applied to the cortical surface directly following the completion of the transection. Animals in Experiment 2 received bilateral craniectomies, and ET-1 was applied to one cortex and then immediately to the other cortex with the first side chosen based on flipping a coin.

6.3.3. Corpus Callosum Transections

Transections were made using methods described previously which focus callosal lesions in the region of the interhemispheric projections of the SMC (Bury et al., 2000; Bury & Jones, 2004). Midline skull between -2.0 and +1.5 mm was thinned and removed in all animals. For those animals receiving a transection (CCX_Cont, CCX_IntactT), the dura and sagittal sinus were pushed to the side and a size 00 ethyl cyanocrylate coated insect pin with an exposed tip was lowered 4.7 mm into the brain at -1.5 mm posterior to Bregma. The side of approach was opposite the SMC lesion. After lowering, 0.7mV of anodal current was passed through the electrode while it was moved rostrally to Bregma over 9 seconds. The electrode was then raised 0.7 mm and current was again passed while the electrode was moved rostrally 1 mm anterior to Bregma over 6 seconds.
6.3.4. Behavioral Measures

Single Pellet Retrieval Task

Reach training was carried out in a Plexiglas reaching chamber as previously described (Allred & Jones, 2008), adapted from Whishaw and others (Jones et al., 2008; Miklyaeva & Whishaw, 1996; Whishaw, 1992; Whishaw, Pellis, & Gorny, 1992). For shaping, briefly, animals were placed in the reaching chamber with their cage mate for 10 minutes. Forty-five mg banana flavored pellets (Bioserve, Inc.) were dropped into the chamber and placed on a 3 cm high shelf located outside of the reaching chamber (see Figure 6.1). On each subsequent day animals were placed into the reaching chamber alone for 10 minutes and permitted to reach for pellets on the shelf. Once a limb of preference (the dominant forelimb) was established (greater than 50% reaching with the same forelimb), the next day, the task was configured so that they could only successfully reach pellets with the dominant forelimb. A Plexiglas wall was placed ipsilateral to the reaching limb and pellets were placed in a shallow well 1 cm from the center window as described previously (Allred & Jones, 2008; Hsu & Jones, 2005; Maldonado et al., 2008) for 30 trials or 10 minutes, whichever came first. To discourage tongue use, a small 2 mm diameter drill bit was adhered to the platform where it made contact with the reaching chamber. Pre-operatively animals were trained to a proficient level (≥ 50% success/reach attempt).

On each trial animals were given up to 5 reach attempts to obtain a single pellet. Trials concluded when the pellet was knocked from its well or greater than 5 reach attempts took place (failures), the pellet was retrieved but dropped inside the chamber.
before consumption (drop), or the pellet was retrieved and taken directly to the mouth (success). Post-operative non-dominant (intact in Exp. 1) reach training was for 60 trials/day for 15 days. Post-operative performance was calculated based on % successful retrievals (success + drops/total reach attempts).

Reach training focused on the impaired/dominant forelimb was used to assay the initial effects of the lesions and the effects of experience with the other limb (see Figure 6.1).
Figure 6.1. Experimental Design.

A. Schematic of the reaching chamber. The inner chamber wall and pellet placement are adjusted to train the left versus right limb. This chamber is configured reaching with the right forelimb. B. A rat aiming and reaching
for a banana flavored food pellet. C. Unilateral SMC lesion and corresponding intact and impaired forelimbs (transections of the corpus callosum are not depicted). D. Bilateral SMC lesions and corresponding non-dominant and dominant (for reaching) forelimbs. E. Time line of experimental procedures. Transections, in Experiment 1, were given at the same time as ischemic lesions.

Pre-operatively and during the post-operative impaired/dominant forelimb assessment and training periods, animals received up to 30 trials per day for either 9 days (Experiment 1) or 13 days (Experiment 2).

Reach training focused on the intact/non-dominant forelimb was used as an experimental manipulation. Post-operative non-dominant (intact in Experiment 1) reach training was for 60 trials/day for 15 days. (The larger number of trials in this phase was intended to ensure its robustness as an experimental manipulation, which may vary with training intensity (Allred et al., 2008)). Animals in control conditions were placed in a reaching chamber, without an inner wall, and given banana flavored food pellets on the cage floor at approximately the same rate as trained animals.

**Schallert Cylinder Test**

To test forelimb use asymmetries, the Schallert cylinder test (Schallert, Kozlowski, Humm, & Cocke, 1997) was used pre-operatively and post-operatively. Animals were placed into a 19 cm diameter Plexiglas cylinder for approximately 2 minutes to
encourage upright postural support behaviors. Use of the forelimbs (ipsilateral, contralateral, or bilateral) on the cylinder walls was recorded from slow motion playbacks of videotape. Percent use of the non-dominant forelimb was calculated based on: # of non-dominant touches/sum of all touches multiplied by 100.

6.3.5. Histology and Lesion Evaluation

At the conclusion of each experiment, animals were overdosed with sodium pentobarbital and perfused trans-cardially with .1M phosphate buffer and 4% paraformaldehyde in the same buffer. Brains were removed and sliced coronally on a vibratome at 50µm thick sections collected in 6 alternating sets. Sliced brains were stored in cryoprotectant at 4 degrees Celsius. One set of sections was immediately mounted onto subbed slides and stained with toluidine blue, a Nissl stain.

The volume of remaining sensorimotor cortex was measured by tracing seven 50µm (between 2.2 mm anterior to and 0.80 mm posterior to Bregma) coronal Nissl stained sections using Neurolucida (Microbrightfield Inc.) perimeter tracing software at 17X magnification. Moving caudally, the first section containing the head of the caudate was chosen and subsequent sections were 600 µm apart. Volume was obtained by applying the formula: \( \Sigma A \times \text{section thickness} \) (Gundersen et al., 1988) where \( \Sigma A \) is the total area summed across all sections.
6.3.6. Statistical Analyses

All statistical analyses were carried out using SPSS statistical software (SPSS, Inc) with a priori planned comparisons. For dominant/impaired forelimb training, the following comparisons were made: 1) Cont vs. IntactT, 2) CCX_Cont vs. CCX_IntactT, 3) Bilat_Cont vs. Bilat_ND. For intact forelimb training InatctT and CCX_IntactT were compared. Behavioral analyses were performed with repeated-measures ANOVAs and student’s t tests. Volume analyses were performed with one-way ANOVAs. All data are expressed as mean ± SEM. Effects were considered significant at p < .05. Three rats in the CCX_Cont group (Experiment 1) had particularly large damage resulting from the callosal transection procedure. Excluding these animals did not change statistical outcome on behavioral measures and therefore they remained in the study, however they are considered separately, as described below.

6.4. Results

6.4.1. Corpus callosum transections block the negative impact of intact forelimb training.

As shown in Figure 6.2A, when rats with unilateral SMC lesions in Experiment 1 were trained with their intact forelimb, there was no difference in acquisition of the skilled reaching task in rats with or without callosal transections (F(1, 15) = 0.97, p > .05, Fig. 6.2A). Consistent with previous findings, this intact forelimb training led rats with SMC lesions to perform significantly worse than Cont rats when later trained with their impaired forelimb (F(1,12) = 5.82, p < .05, Figure 6.2B). However,
transections of the corpus callosum blocked this detrimental effect of the intact forelimb training on impaired forelimb function (F(1,17) = 1.44, p > .05); CCX_Cont vs. CCX_IntactT, Figure 6.2C). These results cannot be explained by differences in activity in the use of the impaired forelimb. There was no significant group or group by day interaction for impaired reach attempts during the impaired limb reaching period.

Transections did not result in significant deficits in reaching behavior in the impaired forelimb. As measured on day 5 post-lesion, rats without transections had a 57.1 ± 7.83% reduction from pre-operative performance levels whereas rats with both unilateral lesions and callosal transections had a drop of 53.3 ± 7.21% drop from baseline (Figures 6.2B-C).
Figure 2. Intact forelimb training worsens performance of the impaired forelimb in rats without corpus callosum transections.

Experiment 1 A. There was no significant difference in acquisition of the skilled reaching task with the intact forelimb between rats with and without prior transections of the corpus callosum. The first day of intact limb training was 6 days after lesions. B. After unilateral lesions and intact limb reach training, rats had major deficits in the contralesional, impaired forelimb compared to control animals. C. Rats with corpus callosum transections with and without intact forelimb experience performed at similar levels with their impaired forelimb. The first impaired limb training day was 22 days after lesions. Data in panels B and C were calculated as %[(pre-operative postoperative)/preoperative] successful retrievals per reach attempt.

6.4.2. No detrimental effects of non-dominant forelimb training on the other limb were found in rats with bilateral SMC lesions.

Non-dominant forelimb training after bilateral lesions did not worsen subsequent performance with the dominant forelimb compared to control rats (F(1,9) = 0.29, p > .05, Figure 6.3). Animals with bilateral lesions also did not differ on the number of reach attempts made with the dominant limb during this training period (Bilat_ND = 37.27 ± 0.85; Bilat_Cont = 35.67 ± 1.36, means ± SEM).
In addition to the lesion-induced deficits in the dominant limb, rats with bilateral lesions tended to perform poorly during the non-dominant forelimb training period (Fig. 6.3A) compared to animals in Experiment 1, as expected because unlike Experiment 1 this limb was contralateral to a SMC lesion.
Figure 6.3 Non-dominant forelimb training in rats with bilateral SMC lesions does not worsen performance of the dominant forelimb.

Experiment 2. A. Non-dominant forelimb learning curve of rats with bilateral lesions. Rats that had learned the task with the dominant limb were learning it for the first time with the non-dominant limb after the lesion. The first training day was 6 days after the lesions. B. Rats with bilateral lesions had a similar rate of re-acquisition of the skilled reaching task with their dominant forelimb regardless of whether they received earlier post-lesion training with the non-dominant forelimb (Bilat_ND) or earlier control procedures (Bilat_Cont). Panel B shows %[(pre-operative-postoperative)/preoperative] successful retrievals per reach attempt. The first dominant limb training day was 22 days after lesions. Note the differences in scales in comparison to Figure 6.2.
6.4.3. Training the non-dominant/intact forelimb leads to its perseverative use after switching to the other limb

Consistent with findings from chapter 5, intact forelimb training also increased attempts to use this limb during the impaired limb training period (even though the apparatus was configured to only permit successful retrievals with the impaired limb). However, perseverative reaching with the intact forelimb cannot explain the worsening of function of the impaired forelimb in IntactT compared to controls, because this perseverance effect was also found in rats with callosal transections and bilateral SMC lesions.

In Experiment 1, all animals with intact forelimb training made futile reaches with this forelimb (mean = 20.10 ± 2.87 reaches) on the first day of the impaired forelimb training period. This compares with only 1 reach attempt made by only 1 animal in the Cont group. This effect did not vary significantly as a result of corpus callosum transections. All animals in the CCX_IntactT made reach attempts with the intact limb on the first day of the switch in sides (23.67 ± 4.65 reaches). In contrast, 4 of 10 rats in the CCX_Cont group made intact side reaches (1.9 ± 0.95 averaged over all animals in this group). The number of reaches with the intact forelimb in these groups significantly declined over days of impaired forelimb training and, by day 4, the IntactT groups were no longer significantly different from Cont in this measure.

Consistent with data from Experiment 1, animals trained with their non-dominant forelimb after bilateral lesions in Experiment 2 also made significantly more reaches with this limb (mean = 22.0 ± 4.51 reaches) on the first day of dominant limb training
compared to Bilat_Cont animals (mean = 6.6 ± 1.78 reaches, p < 0.05). Reach attempt number with this limb significantly declined over days of training, though in contrast to Experiment 1, animals in both groups (n = 6, Bilat_Intact; n = 3, Bilat_Cont) were still making reach attempts with this limb on day 13 of dominant limb training (Bilat_Intact = 7.2 ± 2.03; Bilat_Cont = 2 ± 0.95).

This perseverance effect was not linked with performance on the skilled reaching task during the earlier intact/non-dominant forelimb training period. There were no significant correlations in either experiment between intact/non-dominant forelimb success levels and the number of reach attempts made with this forelimb during the subsequent impaired/dominant training period (r’s < .5, p’s > .05). Furthermore, there was no relationship between the severity of the perseverance and impaired/dominant forelimb performance. However unlike chapter 5, there was no significant correlation between perseverative intact/non-dominant reach attempts and impaired/dominant successful retrievals during the impaired/dominant training period in IntactT, CCX_IntactT, or Bilat_ND (r’s < .6, p’s > .05). This failure to replicate a previous finding may be related to the greater intensity of intact forelimb training (1500 trials) in chapter 5 animals compared to intact forelimb trials (900) in this present study.

6.4.4. Unilateral, but not bilateral, lesions resulted in asymmetrical forelimb use for postural support behavior

Consistent with previous findings (Allred & Jones, 2004; Allred et al., 2008), unilateral, but not bilateral, SMC lesions increased reliance on one forelimb (i.e., the
intact forelimb in Experiment 1) as measured on the Schallert cylinder test. Before the unilateral lesions, rats used the intact forelimb solely for 36.47 ± 2.12% of wall touches and after the lesions they used it for 57.92 ± 2.68%. There were no significant differences in the initial post-lesion effects in rats with transections versus no transections (57.54 ± 3.81% and 58.44 ± 3.94%, respectively). Also consistent with previous findings (Allred et al., 2005), training the intact forelimb tended to increase reliance on this forelimb compared with controls (61.43 ± 3.16 vs. 55.12 ± 3.67%), however, this failed to reach significance (F(df,df) = 3.61, p = .11). This same tendency was not found in animals with transections (CCX_IntactT = 53.73 ± 5.69%; CCX_Cont = 54.23 ± 6.39%) and it was also not found in animals with bilateral lesions (Bilat_ND = 41.38 ± 5.03%; Bilat_Cont = 40.0 ± 2.36%).

6.4.5. Differences in injuries do not explain differential effects of intact/non-dominant limb training

Sensorimotor Cortex Lesions. SMC Lesions produced damage to the forelimb representation area in the region between approximately 2.7 mm rostral and 0.8 mm caudal to bregma (Figure 6.4). Lesions also frequently resulted in some superficial white matter damage directly below the lesion (72% of animals in Exp. 1; 55% of animals in Exp. 2). The striatum was considered damaged if the lesions penetrated the white matter under the lesions. With this criterion, striatal damage was incurred in approximately one third of the rats (38% of animals in Exp. 1; 36% of animals in Exp. 2). However, more than superficial striatal damage was not found in any animal.
Placement and extent of SMC lesions were similar between groups within experiments. There was no difference between groups in either experiment in volume of remaining SMC of the dominant hemisphere (Table 1) though animals with bilateral lesions in Experiment 2 tended to have larger lesions than animals with the unilateral lesions in Experiment 1, likely due to the larger amount of endothelin-1 used in Experiment 2.

<table>
<thead>
<tr>
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<th>Impaired/Dominant Hemisphere Volume (mm³)</th>
<th>Intact/Non-Dominant Hemisphere Volume (mm³)</th>
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<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>84.51(2.68)</td>
<td>95.43(1.61)</td>
</tr>
<tr>
<td>IntactT</td>
<td>82.11(3.28)</td>
<td>95.61(1.11)</td>
</tr>
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<td>CCX_Cont</td>
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<td>88.63(1.79)</td>
</tr>
<tr>
<td>CCX_IntactT</td>
<td>83.52(2.07)</td>
<td>94.38(1.36)</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilat_Cont</td>
<td>80.85(2.63)</td>
<td>81.96(5.25)</td>
</tr>
<tr>
<td>Bilat_ND</td>
<td>76.61(1.29)</td>
<td>81.30(5.94)</td>
</tr>
</tbody>
</table>

Note. Data are means with SE in ()..

**Callosal Transections.** All transections resulted in some damage to the corpus callosum between +1.2 and -0.3 relative to Bregma. Most transections also produced damage to the septal nucleus (n = 8, CCX_IntactT; n = 9, CCX_Cont; See Fig. 6.4). In
four brains, complete dorsal to ventral transections were not found in any single coronal plane, but major superficial damage was evident in several coronal planes between 0.7 and -0.3 mm relative to bregma. These variations in callosal injury characteristics were not clearly linked to differences in reaching performance with either limb.

Callosal transections were made using a side of approach opposite the SMC lesions and, as intended, there was no damage from the electrode track evident in the cortex of the dominant hemisphere (the side of the SMC lesions) in any animal. However, despite being matched for initial impairment levels and despite similarity in white matter damage, in histological analysis, the CCX_Cont group was found to have significantly more cortical tissue loss in the side of the transection procedure compared to the CCX_Intact group (F(1,18) = 6.31, p < .05). This is unlikely to contribute to the results because the matched groups had similar deficits in the impaired forelimb as measured 5 days post-operatively (CCX_IntactT = 54.26 ± 9.54% drop from pre-operative levels; CCX_Cont = 52.20 ± 10.79% drop from pre-operative levels) as a result of matching groups for initial impairment levels. Furthermore, the transected groups were not different on the first day of impaired forelimb training (see Figure 6.2C). Finally, in secondary analyses of the behavioral results, excluding animals with larger cortical tissue loss on the side of the transection approach did not change statistical outcome on behavioral measures. For example, when 3 animals with the largest cortical damage in the CCX_Cont group were excluded such that, in the remaining animals, the cortical volume (91.78 ± X 1.15 mm³) was similar to that of CCX_Intact (Table 1), this resulted in little effect on mean values of reaching success during the impaired limb training. In
another example, the subgroup of CCX_Cont (excluding the three animals with the largest cortical damage) performed at $17.43 \pm 6.38\%$ of preoperative levels on impaired limb training days 7-9, which was similar to the inclusive group and to CCX_Intact (Figure 2C). The subgroup of CCX_Cont continued to be nonsignificant compared with CCX_Intact ($F(1,14) = .04, p = .85$). Furthermore, there was no correlation between transection-associated cortical injury and impaired limb performance in either group ($r’s < .5, p’s > .05$). Thus, there is no clear relationship between this secondary cortical damage and the attenuation of the intact limb training effects by CCX.

**Figure 6.4 Representative lesions and callosal transections.**

A. Experiment 1, representative lesion and corpus callosum transection.

B. Representative lesion from Experiment 2. Large scale bars in panels A and B are 1 mm. Scale bar in inset (panel C) is 250µm. * indicates damage.
6.5. Discussion

Consistent with previous findings (Allred et al., 2005; Allred & Jones, 2008), training the intact forelimb after unilateral lesions resulted in a worsening of function of the impaired forelimb compared to rats without intact forelimb experience. This maladaptive effect was blocked in animals with unilateral lesions that also had a transection of the corpus callosum. Furthermore, the effect was not reproduced in rats with bilateral lesions of the sensorimotor cortex that underwent equivalent sequential training of the two limbs. These data suggest an involvement of both the SMC of the contralesional hemisphere and of transcallosal projections in the maladaptive effects of intact forelimb training on impaired forelimb recovery.

The present findings also add more support for the finding that learning a skilled motor task with the intact forelimb worsens performance and re-learning with the impaired forelimb (Allred et al., 2005; Allred & Jones, 2008). This may be a contributor to the more general phenomenon known as “learned disuse” (Mark et al., 2006; Taub et al., 2003) where negative experiences with the impaired body side leads to pronounced neglect of its use. This is an effect frequently seen in stroke survivors (e.g. Taub et al., 2003) and following stroke-like damage in rats (see Alaverdshvili et al., 2008). The task learned with the intact forelimb does not have to be one that was originally performed with the impaired side. When rats are naïve to the reaching task prior to the lesions, post-lesion training of the intact forelimb results in a pronounced deficit in learning this task later with the impaired forelimb compared with controls trained only on the impaired limb (Allred et al., 2005). This may indicate that establishment of pre-injury dominance
for the task is not a critical factor in this effect. The detrimental effects of intact forelimb experience could result from an exaggeration of disrupted interhemispheric interactions. Although transcallosal cortical projections are primarily excitatory in adults, they terminate on both excitatory and inhibitory neurons (e.g., Innocenti et al., 1986; Kawaguchi, 1992; Karayannis et al., 2007; Carr & Sesack, 1998; Cisse et al., 2003) and there is bilateral and intercortical activity involved in unilateral somatic sensory stimulation (Liepert et al., 2006) and movement (Cisek et al., 2003; Brus-Ramer et al., 2009). Unilateral lesions or transient inactivation of cortex are known to alter activity in the other hemisphere (Li, Rema & Ebner, 2005; Clarey, Tweedale & Calford, 1996). In humans, an abnormal inhibitory drive from M1 of the intact hemisphere to M1 of the damaged hemisphere, as measured using a TMS paired-pulse protocol, was found in stroke patients preceding voluntary paretic hand movement, an effect which was associated with increased reaction time with this hand (Murase et al., 2004).

Following unilateral lesions there is an increase in excitability in the homotopic contralateral cortex. (e.g., Que et al.1999; Witte et al., 2000; Witte & Stoll, 1997). It seems possible that intact forelimb experience can induce an even greater disruption of interhemispheric activity, changes which seem likely to have been blocked by transecting callosal fibers and creating bilateral lesions in the present studies.

Though the present results indicate that the contralateral SMC and callosal projections are necessary for the effects, the time window for their involvement remains unknown. It could be that the contralateral SMC instigates a long-lasting disruption of the impaired limb during the intact limb training period but is not necessary for the
endurance of this effect. However, there might also be an ongoing disruption from the intact to the lesion hemisphere during impaired forelimb training. Bilateral cortical activity occurs during unimanual task performance (e.g., Cisek et al., 2003; Sehm et al., 2009).

Reorganization in peri-lesion cortex is important for recovery after unilateral damage (e.g., Kleim et al., 1998). Training the intact forelimb reduces at least some aspects of peri-lesion plasticity which is evident in a worsening of impaired forelimb function right after this experience (see Allred & Jones, 2008). Previously it was found that intact forelimb training disrupts the peri-lesion neuronal expression of FosB/ΔFosB resulting from impaired limb training (Allred & Jones, 2008). Thus, it is possible that activity-dependent plasticity in peri-lesion cortex is suppressed by experience with the intact forelimb and that this can be attenuated when transcallosal connections are severed. It is possible that this disruptive influence arising from the intact cortex onto the lesion cortex carries into the impaired forelimb training period. There could be an ongoing abnormal intercortical activity that has nothing or little to do with what the intact forelimb is doing while the impaired forelimb is being trained. Alternatively, or in addition to, activity in the intact cortex driven by intact forelimb experience may instigate changes that result in a lasting disruption of peri-lesion plasticity during intact forelimb training. However, the timing of this interference is at present unknown.

One possibility is that intact limb training confiscates neurons in perilesion cortex via intercortical connections to participate in the skill learning of the intact forelimb, disrupting their ability to contribute to improvements in the impaired limb.
Alternatively, the intact limb might constrain peri-infarct experience-dependent plasticity of the impaired limb independent of any such confiscation. Unilateral deprivation of sensory input enhances somatosensory and motor abilities (O’Bryant et al., 2007; Floel et al., 2004) and experience-dependent plasticity (Glazewski et al., 2007) involving the non-deprived side, suggesting that ipsilateral activity is normally constraining of lateralized experience-dependent plasticity. But the mechanisms of this constraint are not well understood.

Intact training induced a perseverative use in reach attempts with this forelimb during impaired forelimb training. However, the present results also suggest that this effect cannot be responsible for exacerbating deficits in the impaired limb as it was seen in all animals with intact/non-dominant training, including those with corpus callosum transections and bilateral lesions. Furthermore in correlation analyses, it was not associated with deficits on impaired forelimb skilled reaching performance. There was no correlation between the number of intact/non-dominant reach attempts and performance with the impaired/dominant forelimb. This suggests that ongoing experience with the intact forelimb while the impaired forelimb is being used is not necessarily maladaptive. This adds to earlier findings that rats trained at the same time to reach with both forelimbs in alternating trials after unilateral lesions did not show a corresponding worsening of impaired forelimb function (Allred & Jones, 2008). Thus the maladaptive effects of intact forelimb training require focused training of this limb.

While IntactT rats do show some motor recovery from initial deficits with training of the impaired forelimb, their rate of relearning is slowed greatly compared to control
rats. It is possible that longer training of the impaired forelimb could overcome the maladaptive effects of prior intact forelimb experience and learned disuse of the impaired forelimb. Iny and colleagues (2006) found that, following monocular deprivation in rats, there was a decrease in visual acuity of the deprived eye and a compensatory enhanced visual acuity of the non-deprived eye. This effect recovered to near control levels after visual experience was restored with the deprived eye, although maximum acuity in this eye took greater than 70 days to manifest.

Also consistent with previous findings (Allred et al., 2005), intact forelimb training tended to increase reliance on this forelimb compared to control rats in upright exploratory movements, an effect which was not seen in transected animals or in animals with bilateral lesions. Motor skill training with the intact limb in animals with unilateral lesions may therefore induce a greater use of this limb, at a cost of disuse of the impaired forelimb. However, in the present study, this effect failed to reach significance in contrast with previous findings. A more sensitive measurement of forelimb use for postural support may reveal greater experience-dependent effects in asymmetrical forelimb use.

Learning a motor skill with the intact body side appears to restrict the ability of the impaired body side to recovery. Following unilateral SMC lesions, even in the absence of any training, animals begin to rely more on their intact forelimb. Compensatory reliance on this side for postural support is evident in home cage observations (Jones & Schallert, 1992) and it may be that these self-taught behaviors are also limiting recovery of the impaired forelimb. By training them in a skilled motor task that is not performed in the home cage environment, we may be exaggerating these effects. Furthermore, in the
present model system of training on one type of motor task (skilled reaching), we are likely capturing only a small aspect of the compensation with the intact side that human stroke survivors develop in the performance of daily activities. Rats in standard laboratory cages lead more sedentary lives with all basic requirements of living provided. This may not challenge the use of the intact body side as much as would be found in a more demanding environment.

Though unilateral lesions to the SMC result in a robust plastic response in the contralateral homotopic cortex including increases in dendritic surface density and synaptogenesis (Bury & Jones, 2002; Jones, 1999; Jones et al., 1999; Luke et al., 2004) and an increased capacity to learn a motor skills task with the ipsilesional/non-dominant forelimb (Allred & Jones, 2004; Bury & Jones, 2002; Hsu & Jones, 2006) taking advantage of that situation to learn new skills may not be beneficial to recovery of function with the dominant forelimb (Allred & Jones, 2008; Allred et al., 2005), at least not when the learning requires focused lateralized use of the intact limb. This seems likely to exacerbate learned disuse or learned bad use (Taub et al., 2003; Alaverdashvili et al., 2008). The exact mechanism(s) mediating this effect are still unknown; however, data from this study point to interhemispheric involvement and disruptive behavioral experience on recovery.

The maladaptive effects of experience with the intact body side may need to be overcome with treatment approaches. One promising avenue has been to use peri-lesion cortical stimulation, including stimulation coupled with training focused on the impaired forelimb, to improve function (Adkins-Muir & Jones, 2003). However, the optimal
application of these strategies might be improved with a better understanding of the exact neural mechanisms of the present phenomenon, including the time periods in which intercortical interference is high. Though the present results indicate that the effect is mediated by intercortical connections, further investigation is needed to isolate the time period of their involvement as well as to understand exactly how they are disrupting the function of the impaired forelimb. A better understanding of the phenomenon seems likely to illuminate processes involved in neglect and learned nonuse.
Chapter 7

General Discussion

7.1. Summary

These dissertation studies provide substantial support for how experience with the intact body side following unilateral stroke-like damage can maladaptively influence behavioral recovery of the impaired forelimb and disrupt peri-lesion neural plasticity. Intact forelimb training reduces FosB/ΔFosB neural expression and caudal forelimb motor representations in peri-lesion cortex. Intact forelimb experience was also shown to result in a worsening of performance with the impaired forelimb even when initiated at a later post-lesion time point. However, if intact training is carried out in conjunction with training of the impaired forelimb (Experiment 2, Chapter 4), it is not detrimental to impaired forelimb recovery, indicating that it is focused use of this forelimb which results in a worsening of impaired forelimb performance.

IntactT animals appear to develop an exaggerated compensatory reliance on the intact forelimb following this training period. For example, there is a tendency to increase reliance on this limb in a measure of postural support movements (chapters 3, 6). Anesthetization of the intact forelimb led to an unmasking of impaired forelimb deficits on a coordinated forelimb placement test and these deficits that emerged were greater in rats with intact forelimb training (Experiment 2, Chapter 4). The maladaptive effect of intact forelimb experience appears to come at a greater cost when learning a motor skill task with the impaired forelimb for the first time after unilateral SMC damage (chapter 3). In this experiment, animals with intact forelimb experience showed little improvement
on the task over 10 days of training with their impaired forelimb, and these animals additionally had an enduring reliance on the intact forelimb in postural support behaviors. Because “recovery” on the cylinder task (Humm et al., 1998) and on skilled reaching (Whishaw et al., 1992) is thought to be due in large part to the development of compensatory movements with the impaired forelimb it may be that intact forelimb experience is particularly detrimental to the development of functionally beneficial behavioral compensation with the impaired limb. The damaging effect of intact forelimb experience is blocked in the presence of corpus callosum transections and is not evident after bilateral SMC lesions, strongly suggesting that transcallosal projections play a role in mediating this effect and that, there is a disruptive influence originating in the contralesional hemisphere projecting onto the lesion hemisphere.

7.2. Effects of intensity of intact forelimb experience on impaired forelimb recovery

It may be the case that the greater the intact forelimb experience (i.e., more reaching experience), the greater the detriment to impaired limb functional recovery. A direct statistical comparison across studies is not appropriate, however, animals with the most intact forelimb training (1500 trials over days of impaired forelimb training, chapter 5) had the worst recovery with their impaired forelimb compared to animals which received less intense intact forelimb training (chapters 3, 4, 6). These animals also had greater perseveration of intact forelimb reach attempts throughout impaired forelimb training, while animals with less intense intact forelimb experience showed a significant decline in intact reach attempts over days of impaired forelimb training (chapter 6). This
suggests that intensity of post-lesion experience with the intact forelimb may matter for recovery of impaired forelimb function.

7.3. Potential exaggeration of learned disuse of the impaired forelimb

Following unilateral SMC lesions even animals without intact forelimb experience show an increased reliance on the intact forelimb on other sensorimotor tasks (chapters 3, 4, 6) and also in home cage experiences (Humm et al., 1998). This effect is amplified following focused experience with the intact forelimb (chapters 3, 4, 6) and may lead to an enhanced disuse of the impaired forelimb in home cage activities. Disuse of the impaired forelimb is unrelated to activity with this limb during impaired forelimb training as IntactT animals did not reach less with this forelimb compared to control animals. Learning a skilled reaching task with the intact forelimb leads to a perseveration of reaching with the intact forelimb during impaired forelimb training, though this is unlikely to lead to learned disuse of the impaired forelimb, as animals with transections of the corpus callosum or bilateral SMC lesions (chapter 6) also showed this perseveration effect, but without increased deficits in the impaired (dominant) limb.

7.4. Possible mechanisms mediating maladaptive intact training phenomenon

7.4.1. Disruption of synaptic plasticity in peri-lesion cortex

Motor skills training induces synaptic plasticity (Kleim et al., 2002) and an expansion of motor maps in the motor cortex opposite the trained forelimb (Nudo, 2003; Kleim et al., 1998; Monfils et al., 2005). The ability for motor maps to reorganize is
related to strengthening of synaptic connections, which is experience dependent (e.g., Kleim et al., 2002). The reorganization of peri-lesion cortex after stroke, including reorganization of motor maps, has been linked with functional recovery (e.g., Nudo, 2003). Conversely, increased activity in intact cortex, evident following unilateral brain damage (e.g., Que et al., 1999; Reinecke et al., 1999; Murase et al., 2004), is related to greater inhibition and disruption of peri-lesion cortex and poorer recovery (Murase et al., 2004). In these dissertation studies it was shown that intact forelimb training disrupts the organization of peri-lesion forelimb motor maps. IntactT rats had significantly increased deficits in reaching with the impaired forelimb. In the presence of callosal transections, this effect was blocked.

The finding that FosB/ΔFosB expression is reduced in peri-lesion cortex following intact forelimb experience (chapters 3 and 4) may indicate a reduced capacity for protein synthesis. ΔFosB has been shown to both down regulate (in an acute stage) and up regulate (at a more chronic time-point) various genes, including GluR2 (an AMPA receptor subtype) (Mclung & Nestler, 2003). It is unknown if expression of FosB/ΔFosB is the first in a cascade of events occurring in peri-lesion cortex in response to intact forelimb training. It is clear that intact forelimb training disrupts peri-lesion plasticity as revealed with smaller caudal forelimb motor maps (chapters 4 and 5) and that the contralesional (intact) cortex is involved in mediating this effect (chapter 6). Intact forelimb training maladaptively influences peri-lesion plasticity via disruptive transcallosal inputs. These inputs may be acting in a confiscating manner, inducing a depotentiation of the peri-lesion cortex during intact forelimb training and therefore
having a possible saturation effect, making it more difficult to drive synaptic
strengthening through behavioral LTP-like mechanisms during impaired forelimb
training. Thus it is possible that the intact cortex (during intact forelimb training)
activates peri-lesion cortex (chapter 7) in a maladaptive way that causes later resistance
to reorganization (chapter 5). This may be indicated by the reduction in FosB/\Delta FosB
expression in peri-lesion cortex in IntactT rats after the period of impaired forelimb
training, which presumably reflects reduced neuronal activity in this region and, if so,
may indicate reduced activity-dependent plasticity resulting from experience with the
impaired forelimb (chapter 4). FosB/\Delta FosB can regulate gene transcription and a
reduction in the FosB/\Delta FosB gene product could suppress LTP-like mechanisms in peri-
lesion cortex, which depend on glutamate activity. If the mechanisms mediating learning
and memory (e.g., synaptic strengthening) are blocked through maladaptive peri-lesion
activity, then improvements in impaired forelimb recovery would be minimal and
difficult to unmask.

7.4.2. Experience-dependent changes in inhibitory activity in peri-lesion cortex

Another possibility is that lesion animals with intact forelimb experience have an
increase in inhibitory activity in peri-lesion cortex. It has previously been suggested that
intracortical inhibition is due primarily to activity of GABAergic interneurons that
receive transcallosal excitatory input (e.g., Liepert et al., 2000; Lee et al., 2007; Perez &
Cohen, 2009). A reduction in peri-lesion GABA activity may be a possible mediator of
recovery of function in the impaired forelimb. Patients with less pronounced initial
deficits have a more pronounced disinhibition of peri-lesion cortex, and this has been proposed to be linked with reduced GABAergic activity (Liepert et al., 2000). Following neocortical damage in rats, motor recovery was blocked in animals receiving diazepam (Schallert, Hernandez, & Barth, 1986). Moreover, GABA agonists have been shown to increase intracortical inhibition (Ziemann, Lönnecker, Steinhoff, Paulus, 1996), while local administration of GABA antagonists uncover motor cortical representations (Jacobs & Donoghue, 1991). In the presence of GABA antagonists, motor map representations are changed because the boundaries between movement areas become less distinct (Jacobs & Donoghue, 1991; Hickmott & Merzenich, 1998), suggesting a role of GABA in mediating the boundaries and segregation of movement representations. GABA activity in peri-lesion cortex of intact trained animals could conceivably be responsible, at least in part, for the reduction in forelimb motor map size. It is not clear exactly what role transcallosal inhibitory effects may have over the constraint of motor maps, but there is evidence for transcallosal control over somatosensory representations in barrel cortex (Glazewski et al., 2007). Thus it seems reasonable to suspect that these transcallosal connections could be involved in the maladaptive effect of intact forelimb training.

7.4.3. Role of the ipsilesional corticospinal tract

Most of the motor cortex contribution to the corticospinal tract (CST) is crossed in the rat, such that the contralateral cortex is responsible for moving the limb. However, there is some ipsilateral contribution (about 5-10%, Brosamle & Schwab, 1997). A question is whether the 10% of this pathway arising from the contralesional cortex
mediates recovery of the impaired forelimb. If so, learning with the intact forelimb may also interfere with motor relearning of the skilled task with the impaired forelimb because it interferes with the take over of the impaired limb by the ipsilateral CST. The likelihood of this effect, however, seems low. In animals with transections of the corpus callosum the maladaptive effect of intact forelimb training on impaired recovery was absent. If this pathway was involved in mediating recovery of the impaired forelimb, the maladaptive effect should have still been present in transected animals. Additionally, after lesion animals had recovered on a skilled reaching task, a second lesion in the contralateral (intact) hemisphere did not reinstate impaired forelimb deficits greater than deficits seen in animals with a unilateral lesion, suggesting that it is not normally responsible for its recovery, at least after these focal SMC lesions (Maldonado, Allred, Hsu, & Jones, 2006).

The contribution of the ipsilateral, uncrossed pathway has previously been shown not to contribute noticeably to skilled reaching behavior and the uncrossed pathway also does not contribute to motor recovery when the crossed pathway is severed (Whishaw & Metz, 2002). However, the ipsilateral components of the CST may at least sometimes play a larger role in mediating recovery following some types of brain damage, perhaps particularly after very large lesions, when there is little of the peri-lesion cortex remaining.

7.4.4. Possible mechanisms conclusions

These potential mechanisms mediating the maladaptive effect of intact forelimb training, including a disruption of peri-lesion synaptic plasticity, the role of GABAergic
activity in peri-lesion cortex, and the role of the ipsilateral CST, are not mutually exclusive, and it is possible that they are interacting together. These proposed mechanisms are also not entirely inclusive. There are many other considerations to take into account, including the role of other neurotransmitter systems, trophic factor expression, and other brain regions. Basal forebrain cholinergic lesions block proficient learning of a skilled reaching task (Conner, Chiba, & Tuszyinsky, 2005), and it is likely that acetylcholine and other neurotransmitters contribute to this effect. There are additional brain regions that are also likely to be of importance in recovery of motor function. For example, the cerebellum plays a role in motor movement timing and fine motor control (Glickstein & Doron, 2008) and is likely involved in motor recovery after brain damage (e.g., Fregni & Pascual-Leone, 2006). In the present studies, examination of FosB/ΔFosB expression in the nucleus accumbens, dorsolateral striatum, and the basolateral amygdala did not reveal any definitive role of these brain regions in this particular phenomenon. However, this is a simple marker of neuronal activity and does not rule out those structures’ involvement. Brain structures that were not examined in these studies are likely to contribute to impaired limb functional recovery.

7.5. Generalizability and limitations of the model system

These studies use an animal model of sensorimotor cortical stroke, which can be loosely translated into potential post-stroke therapeutic strategies in human populations. Rats make good models of stroke-induced behavioral deficits that mimic, in some fashion, deficits seen after cortical strokes in humans (Whishaw et al., 1992; Sacrey et al., 140
2009). A major concern following stroke is the loss of functionality. Frequently this loss is compensated for by overuse of the less-affected body side. While this may be advantageous especially during the acute post-stroke phase, these dissertation studies provide substantial evidence that this experience is detrimental to impaired forelimb recovery.

A major limitation of these studies is the inability to directly apply this knowledge to human stroke populations, which are inherently diverse. Stroke incidence increases with age. These dissertation studies used young adult rats (approximately 5-6 months of age at stroke onset). In general, in clinical populations there are a variety of strokes, both subcortical and cortical, and recovery is dependent upon a multitude of factors, including size and location of the infarct (Kleim, 2006). Animal studies are very well controlled, and the lesions produced in these dissertation studies were focal and highly conserved across animals and across studies. This control restricts data variability, making it feasible to ask these questions in an experimental model, but it also makes it more difficult to generalize to clinical populations.

It is not known whether this effect generalizes to other forelimb activities, though unpublished data suggest that learning the single pellet retrieval task can lead to an increase in dexterous forepaw use on a separate task, the vermicelli handling test (Allred et al., 2008). Rats trained pre-operatively on the single pellet retrieval task that reached a proficient level (> 50% success/reach attempt) quickly (2 days or less) made significantly more adjustments with their dominant forepaw on the pasta handling task (see Allred et al., 2008) than rats that took more than 2 days to reach proficiency. However, this effect
was not found to be bidirectional: experience with pasta handling prior to any skilled reaching experience did not facilitate single pellet performance (unpublished data).

We found that bilateral training did not have maladaptive effects and it could be that even more lateralized training would have more detrimental effects. The single pellet reaching task is not a truly lateralized task because the non-reaching forelimb is used to help aid in reaching behaviors. It is possible that even greater impaired forelimb deficits would be revealed after training on a more unilateral task like the Montoya staircase task (Montoya, Campbell-Hope, Pemberton, & Dunnett, 1991). This task requires animals to reach for pellets placed on descending stairs in an apparatus configured to allow reaches with only one forelimb (the side ipsilateral to placed pellets).

It is also not known what this effect would be if animals were made to reach pre-operatively with their non-dominant forelimb and then given lesions opposite this forelimb. The effect, however, seems likely to be the same, based on supportive findings. Previously, rats given lesions opposite their non-dominant forelimb and then trained with their dominant forelimb still had a facilitation of learning with this limb compared to sham animals (Bury and Jones, 2002). Additionally, rats pre-operatively trained with their non-dominant (for the skilled reaching task) forelimb took on average twice as long to reach proficiency with this forelimb (4 days) than rats trained with their dominant forelimb (unpublished data). In animals without pre-operative training, learning the skilled reaching task for the first time with their intact forelimb lead to a blunted acquisition of the task with their impaired forelimb, suggesting that pre-operative forelimb dominance may not matter in this effect. Taken together these data suggest that
the maladaptive effect of intact limb training would still be present even if the non-dominant forelimb was the impaired forelimb, though the magnitude of the effect may not be as great.

7.6 Future directions and overall conclusions

There remain many unanswered questions and possibilities for future studies. One question is, if there is a time period of quiescence following intact forelimb training (e.g., two weeks), would there still be a disruption of impaired forelimb recovery? In animals with delayed training of the intact forelimb (chapter 5), there was a tendency for spontaneous recovery tended to be facilitated by contextual cues of the training environment and disrupted by intact forelimb experience. It may be possible to regain this spontaneous recovery effect after intact forelimb training if there is a period of no training or a period of control procedures.

Would intact forelimb training have a maladaptive effect on impaired forelimb recovery if the intact cortex was inhibited during training of this limb? This could be accomplished through muscimol infusion into the homotopic, contralesional SMC. Muscimol, a GABA agonist, is known to enhance GABAergic activity (e.g., Wahab, Heinemann, & Albus, 2009). The maladaptive effect of intact forelimb training is mediated (at least in part) by the corpus callosum. If transmission from intact SMC causes the peri-lesion cortex to react in a disadvantageous way, then blocking activity in this cortex during intact forelimb training would not lead to a further decrement in impaired forelimb function. Consistent with this idea, peripheral anesthetization of the
intact arm transiently improves function in the paretic arm of human stroke patients (Werhan & Cohen) and the impaired forelimb in rats (O’Bryant et al., 2007).

Taken all together, these data provide substantial evidence that peri-lesion cortex is vulnerable to post-lesion experience with the intact forelimb, and experience with this limb, even if it occurs following a longer recovery period, is detrimental to impaired forelimb recovery. There are many obstacles to recovery of lost function following damage, including overcoming self-taught behaviors (e.g., compensatory movements performed with the intact body side), which may lead to disuse of the impaired body side. While constraint induced movement therapy (CIMT) is an effective therapy for overcoming learned disuse of the affected arm after stroke in humans (Taub et al., 2006), the best solution may be to avoid over reliance on the intact body in the first place. However, it may be unsafe to do this by restricting the intact arm early after injury, because this is related to exaggeration of injury extent due to overuse of the impaired limb (Humm et al., 1998). The most efficacious management of upper extremity post-stroke behavior might include a training paradigm using both arms. There may be a way to permit the associated plasticity in the contralesional cortex without limiting peri-lesion plasticity and impaired forelimb function. This requires a better understanding of the neural mechanisms involved in the phenomenon.
## Appendix A

Table A1. FosB/ΔFosB expression in nucleus accumbens.

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain Region</th>
<th>FosB/ΔFosB pos Nv (10^4)</th>
<th>FosB/ΔFosB +/- ratio</th>
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</thead>
<tbody>
<tr>
<td>Cont (n = 8)</td>
<td></td>
<td>8.30(0.61)</td>
<td>7.08(0.86)</td>
</tr>
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<td>IntactT (n = 7)</td>
<td>Nucleus Accumbens (peri-lesion)</td>
<td>9.38(0.72)</td>
<td>6.60(0.58)</td>
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<td>Sham_C (n = 7)</td>
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<td>8.69(0.49)</td>
<td>6.88(0.66)</td>
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<tr>
<td>Sham_ND (n = 9)</td>
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<td>9.40(0.59)</td>
<td>5.24(0.99)</td>
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</tbody>
</table>

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<tr>
<th>Group</th>
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<tr>
<td>Cont (n = 8)</td>
<td></td>
<td>8.31(0.98)</td>
<td>8.83(1.77)</td>
</tr>
<tr>
<td>IntactT (n = 7)</td>
<td>Nucleus Accumbens (contralesion)</td>
<td>9.32(0.71)</td>
<td>6.88(1.14)</td>
</tr>
<tr>
<td>Sham_C (n = 7)</td>
<td></td>
<td>8.64(0.55)</td>
<td>7.17(1.31)</td>
</tr>
<tr>
<td>Sham_ND (n = 9)</td>
<td></td>
<td>8.83(0.50)</td>
<td>4.20(0.41)</td>
</tr>
</tbody>
</table>
Table A2. FosB/ΔFosB expression in dorsolateral striatum.

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain Region</th>
<th>FosB/ΔFosB pos Nv (10^4)</th>
<th>FosB/ΔFosB +/- ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont (n = 9)</td>
<td></td>
<td>5.91(0.27)</td>
<td>9.55(1.05)</td>
</tr>
<tr>
<td>IntactT (n = 9)</td>
<td>Dorsolateral Striatum (peri-lesion)</td>
<td>6.00(0.34)</td>
<td>13.36(2.44)</td>
</tr>
<tr>
<td>BiT (n = 9)</td>
<td></td>
<td>5.69(0.30)</td>
<td>11.67(3.27)</td>
</tr>
</tbody>
</table>


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Rachel Allred was born in Galveston, TX. Her family moved to Flagstaff, Arizona and she was raised there attending high school at Flagstaff High School, and later, college at Northern Arizona University (NAU) where she received two bachelors in psychology and religious studies in 2000. Finding her passion in neuroscience, she continued to attend school at NAU as a non-declared graduate student, gaining knowledge in more basic science. In September of 2002 she entered graduate school in the department of psychology at the University of Texas, at Austin.

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