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The Development of Complex Movement Motor Maps Using Long Duration Intracortical Microstimulation (LD-ICMS) in Rats

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UNIVERSITY OF CALGARY

The Development of Complex Movement Motor Maps Using Long Duration Intracortical
Microstimulation (LD-ICMS) in Rats

by

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A THESIS

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Abstract

The adult motor cortex is topographically organized into representations (maps) of different body parts (Fritsch & Hitzig, 1870; Ferrier, 1873). Recent evidence, however suggests a movement-based, rather than, muscle-based cortical motor organization (Graziano et al., 2002; Brown & Teskey, 2014). In the adult rat, four specialized multi-joint forelimb movements have been consistently evoked using long-duration intracortical microstimulation (LD-ICMS; limb elevation, advancement, grasping, and retraction) that are thought to recapitulate components of the basic walking and reaching movements in rats (Karl & Whishaw, 2013; Brown and Teskey, 2014). The present experiment characterized the behavioural and cortical development of these four movement categories in Long-Evans hooded rats and how skilled reach training could affect forelimb development. This study was the first to find that LD-ICMS evoked single-joint movements developed before multi-joint movements within the neocortex. In addition, the forelimb behavioural capacity of the rat predated the emergence of LD-ICMS-evoked forelimb responses indicating that subcortical structures might be mediating behaviour. Moreover, this study revealed for the first time that grasp movements were not restricted to the “grasp region” at early stages of development, suggesting that the motor cortex underwent large cortical changes as the rat developed, leaving a refined grasp region in adulthood. Finally, after skilled reach training, the LD-ICMS motor map of the hemisphere contralateral to the skilled reach trained forelimb contained more grasp movements than the ipsilateral hemisphere, which is not observed in adult rats (Ramanathan et al., 2006; Brown & Teskey, 2014). This novel finding revealed that rat motor maps might be more plastic throughout development than in

adulthood. It is important to understand how forelimb movements develop within the cortex in order to begin finding solutions for when these areas become damaged, such as during a stroke.

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List of Symbols, Abbreviations and Nomenclature

<u>Symbol</u>	<u>Definition</u>
ANOVA	Analysis of variance
CFA	Caudal forelimb area
CST	Corticospinal tract
E	Embryonic day
GABA	γ -Aminobutyric acid
GPI	Globus pallidus interna
ICMS	Intracortical microstimulation
LD-ICMS	Long-duration intracortical microstimulation
M	Mean
PFA	Posterior forelimb area
PND	Postnatal day
RFA	Rostral forelimb area
SD	Standard deviation
SD-ICMS	Short-duration intracortical microstimulation
SEM	Standard error of the mean
vDF	Ventral aspect of the dorsal funiculus

Chapter One: **GENERAL INTRODUCTION**

1.1 Introduction

The adult motor cortex is topographically organized into representations (maps) of different body parts (Fritsch & Hitzig, 1870; Ferrier, 1873). Since the earliest days of motor mapping there has been a long-standing debate as to whether the motor cortex encodes activity separately for individual muscle groups, or integrates collective activity among many muscle synergies to encode whole, multi-joint movements (Graziano et al., 2002). Short-trains (40ms) of intracortical microstimulation (ICMS) reveal short, single-joint (simple) twitch-like muscle contractions (Asanuma & Sakata, 1967; Asanuma & Ward, 1971; Asanuma & Rosen, 1972; Young et al., 2011b). On the other hand, long-trains (500ms) of ICMS, which are approximately the same duration as a motor neuron firing during a reaching and grasping movement (Graziano et al., 2002), reveal several topographically organized areas of multi-joint (complex) movements in rats (Ramanathan et al., 2006; Bonazzi et al., 2013; Brown & Teskey, 2014). Recently, four specialized areas of the motor cortex in the adult rat evoked cohesive multi-joint forelimb movements (limb elevation, advancement, grasping, and retraction) that recapitulate components of the basic walking and reaching movements in rats (Karl & Whishaw, 2013; Brown and Teskey, 2014). Moreover, temporarily and reversibly inactivating the grasp area specifically impairs grasping, suggesting that the motor cortex is indeed topographically organized to make complex movements (Brown & Teskey, 2014).

This thesis investigated the development of rat forelimb motor behaviour from post-natal day (PND) 13 to 60 and the development of rat forelimb motor maps from

PND 13 to 60 using the novel technique of long duration intracortical microstimulation (LD-ICMS). Specifically, this thesis investigated when LD-ICMS forelimb motor maps emerged during development in relation to the use of the forelimb in freely behaving pups. In addition, the effect of skilled reach training on the development of complex movement representations derived using LD-ICMS was investigated. The present chapter will present an overview of the background information on neural networks, motor mapping and development as a precursor to the empirical investigation found in Chapter 2.

1.2 Development of the cortical laminar structure

The development of the laminar structure of the rat neocortex begins prenatally with four layers (the ventricular zone, the intermediate zone, the cortical plate, and the marginal zone) and gradually progresses into the adult-like, six-layer formation. There are four types of cells that are found within these four layers, Cajal-Retzius cells, subplate cells, interneurons and projection neurons. Pyramidal neurons are a specified type of projection neuron, which are the most abundant neuron in the cerebral cortex and use the neurotransmitter glutamate. On the other hand, interneurons are predominantly inhibitory neurons that use the neurotransmitter GABA. A cell is considered to be within a certain laminar layer based on the position of the cell body; however the cell's axons may extend to other layers (Sanes, Reh & Harris, 2006).

From approximately embryonic day (E) 11 to 14, progenitor cells in the germinative area, or ventricular zone, of the telencephalon are thought to develop into the first cortical neurons (Molnár et al., 2014; Sanes et al., 2006). These neurons migrate

radially from the ventricular zone to form a specified layer below the pial surface, called the preplate. The preplate is divided into two layers: the superficial marginal zone (known as Layer I in the adult rat; Rice et al., 1985; Shipp, 2007), which consists of Cajal-Retzius cells, and a deeper layer, which consists of subplate cells (Sanes et al., 2006). It was previously believed that subplate cells also migrated radially from the ventricular zone, however more recently the cells have been found to migrate tangentially from an extracellular area of the telencephalon called the *cortical hem*. The preplate covers the entire exterior of the cerebral cortex within 24 hours of proliferation (Molnár et al., 2014).

Next, an abundance of mature, or post mitotic, cells accumulate within the preplate to form the cortical plate; located below the marginal zone but above the intermediate zone, which consists mainly of subplate cells and incoming axons (Sanes et al., 2006). At this point, there are three distinguishable immature layers: 1) the marginal zone, or Layer I, *the molecular layer* (Kandel et al., 2013), which is just below the pial surface and consists mainly of axons and dendrites of cells from other layers, as well as minimal of GABAergic cell bodies (Douglas & Martin, 2004), 2) the cortical plate, which is an undifferentiated grouping of cells that will become layers II to V, and 3) Layer VI or *the multiform layer*, which consists of eclectic cell types and axons that project to and from the cortex. The differentiation of the cortical plate occurs at approximately PND 0 (Rice et al., 1985) with the help of radial glia, which are a set of cells that function as scaffolding from the ventricular zone to the pial surface. Neurons migrate up the radial glia and begin to accumulate in the cortical plate, greatly increasing cortical thickness.

These new neurons rest in progressively more peripheral zones of the cortical plate, creating the inside-out development of the cortical layers (Sanes et al., 2006).

The first layer to differentiate within the cortical plate is Layer V (Rice et al., 1985), or the *internal pyramidal cell layer*. This layer is densely populated with large pyramidal cells and is the main output to subcortical targets, such as the spinal motor neurons (Donoghue & Wise, 1982; Sanes et al., 2006; Shipp, 2007). This differentiation process is marked by a rapid decrease in the number of cells within the deeper half of the cortical plate beginning on PND 0 and ending on approximately PND 2 with the complete separation of layer V from the cortical plate. At this point, layers V and VI have matured to adult-like thickness, whereas layers II, III and IV remain undifferentiated within the cortical plate and are half of the thickness of adult cortical layers (Rice et al., 1985).

The cortical plate again differentiates into a trilaminar appearance on approximately PND 3 (Rice et al., 1985); revealing layers II, III and IV and results in the adult six-layer laminar formation (Kandel et al., 2013). Layer II, or the *external granule cell layer*, consists of many granule cells, which project to neighbouring layers. Layer III, *the external pyramidal cell layer*, contains larger pyramidal neurons than layer II, however they have a similar function of mediating intracortical communication within each layer and other layers. Layer IV, *the internal granular cell layer*, contains small, round neurons that are a main output to the thalamus (Kandel et al., 2013). This layer also gives rise to GABAergic inhibitory neurons (Sanes et al., 2006). Areas of the cortex that have a large layer IV, such as the sensorimotor cortex, are deemed *granular cortex*,

whereas areas of the cortex that have a thin layer IV grouped as the *agranular* frontal cortex (Kandel et al., 2013).

1.3 Development of the corticospinal tract (CST) and projections to muscles

The corticospinal tract (CST) is the main descending pathway to initiate voluntary control of muscles in humans (Martin, 2005) and other species (Terashima, 1995; Canty & Murphy, 2008). In the rat, the development of this tract begins between E15 and E17 (Terashima, 1995), when pyramidal neurons proliferate in the subventricular zone of the telencephalon and migrate to layer V within the cortical plate. These pyramidal neurons differentiate from the cortical plate into a distinct layer V within the first 24 hours after birth and are the main output to the spinal cord (Canty & Murphy, 2008).

The leading or pioneer axons of these neurons begin to grow and elongate, beginning the descent through subcortical structures and the CST to reach target cells. This descent begins with the internal capsule, where CST axons separate from corticothalamic projections to enter the cerebral peduncles. By approximately E17, the axons reach the upper levels of the brainstem and by approximately E19, axons pass through the midbrain and ventral pons. Between E21 and P0, the CST decussates in the caudal medulla and descends to the ventral aspect of the dorsal funiculus (vDF) in rats or the dorsolateral white matter in primates (Terashima, 1995; Canty & Murphy, 2008). Approximately 5-15% of pyramidal axons do not cross the caudal medulla and project instead to the ipsilateral ventral CST (Terashima, 1995; Martin, 2005; Canty & Murphy, 2008).

The projections to the spinal cord develop in a rostro-caudal fashion meaning that the cervical spinal cord, which projects to the forelimb, develops before the lumbar spinal cord, which projects to the hindlimb. The corticospinal fibers initially reach the cervical enlargement of the spinal cord between PND 0 (Terashima, 1995) and PND 1 but do not reach the lumbar region until PND 6 (Schreyer and Jones, 1982; Canty & Murphy, 2008). For the three to four days following the arrival of the initial pioneer axons to the cervical spinal cord, additional axons make their descent. In the thoracic and lumbar region, the addition of axons can last up to a week. In addition, the myelination of the spinal cord begins on PND 10 in a rostro-caudal direction. Therefore the cervical projections, which control the forelimb, are myelinated, and mature, before the lumbar projections, which control the hindlimbs (Canty & Murphy, 2008). This directional myelination of the CST is also seen in humans within the first two years of life, however myelination can continue for several years (Martin, 2005).

In the primate CST, motor neurons are grouped functionally into columns within the neural tube and throughout development. For example, the medial motor column projects to postural muscles in the dorsal body region, whereas the lateral motor column projects exclusively to limb muscles (Kanning et al., 2010). The corticospinal axons in rats, however, do not have obvious functional groupings during their descent through the brain and brainstem. Once they reach the spinal cord, however, axons are separated into a topographic organization. The axons significantly increase in number and, after a waiting period of a few days (Donatelle, 1977; Schreyer and Jones, 1982), form collateral branches. These branches then leave the vDF to locate their target interneurons within the topographical organization of the grey matter of the dorsal horn. These connections form

within the first week of life, however the ventrolateral connections are eliminated by PND 14 (Kamiyama et al., 2006). Similarly, transcranial magnetic stimulation studies show that humans develop bilateral CST connections and then become more specific to the contralateral CST over time (Martin, 2005). The maturation of this topographic organization does not occur until at least PND 21 in rats and can occur as late as PND 28, after the completion of dendritic arborization, axonal rerouting and axonal pruning, which results in a 50% decrease in the number of axons from PND 14 (Chung and Coggeshall, 1987). By the completion of myelination at PND 28, the CST cross sectional area has experienced a significant expansion, and axonal growth and reorganization are thought to be unlikely (Canty & Murphy, 2008).

1.4 CST innervation of muscles

Once a signal from the layer V pyramidal neurons in the rat descends the CST to reach the ventral horn, they synapse onto lower motor neurons indirectly through interneurons (Isa et al., 2007; Lemon, 2008). There are two types of lower motor neurons in the ventral horn. First, the alpha motor neurons, which innervate extrafusal muscle fibers, which causes the muscle to contract and 2) the gamma motor neurons, which innervate intrafusal muscle fibers and control the stretch of the muscle as well as proprioception (Kanning et al., 2010). Using the ICMS technique with an adult rat, layer V pyramidal cells are electrically stimulated and depolarize, sending a signal through the CST and interneurons to lower motor neurons. The signal causes lower motor neurons to release acetylcholine onto the muscle fibre at the neuromuscular junction, which activates the nicotinic acetylcholine receptors. This activation causes a depolarization of the

endplate (i.e. endplate potential) at the muscle fibre, which causes the muscle to contract (Purves et al., 2001). Complete lesions to the medulla pyramids of the rat CST have been found to prevent muscle movements from being evoked during the ICMS procedure (Piecharka et al., 2005).

1.5 Development of motor behaviour

The development of motor function varies significantly depending on species. Precocial species, such as horses, have highly developed motor abilities from birth, which allow them to complete complicated activities, such as walking, within minutes of birth. These species are thought to have well developed motor systems, with pathways linking supraspinal motor centers and the spinal cord. On the other hand, altricial species, such as rats and cats, are born with only limited motor abilities, such as breathing, which allow them to sustain life. The altricial animals have a full brainstem motor system, but do not have a fully functioning corticospinal system. In addition, axon projection patterns to the ventral spinal cord between species can develop at significantly different rates depending on the postnatal skills that are required. For example rhesus monkeys are thought to have developed forelimb topography prenatally, giving them the ability to cling tightly to their mother during transport, whereas neonatal rats and cats are unable to crawl due to forelimb weakness and incomplete topographic projections. The development of the forelimb topography can also vary within a species (Martin, 2005), where some animals develop skills faster than others depending on environmental factors such as enrichment (Coq & Xerri, 1998; Simonetti et al., 2009; Young et al., 2012) or skilled forelimb training (Young et al., 2012).

The development of locomotor skills of the rat pup begins prenatally and progresses rapidly after birth. Small kicks, jerks and twitches occur *in utero* as early as E16, however they lack coordination or organization (Hamburger, 1973). These first movements are thought to be spontaneous motor neuron activity (Sanes et al., 2006) and coincide with the arrival of axons to immature muscles (Westerga & Gramsbergen, 1993). Unlike the adult movement repertoire, the rhythmicity of these prenatal movements does not require communication between the right and left dorsal horn of the spinal cord. In the adult rat, a negative feedback loop controls motor output, beginning with a signal from motor axons collaterals to the interneurons of the ventral cord called R-interneurons. This signal is attenuated by GABAergic signals to the flexor motor neurons (Wenner & O'Donovan, 2001). In the prenatal rat, however, GABA is excitatory; therefore the depolarization causes mass excitatory signalling to all surrounding neurons. This period of excitation gradually diminishes, resulting in a period of inexcitability, which indicates that the rhythmicity is likely an intrinsic property of the developing network (Sanes et al., 2006).

By E18, the rat can produce slight movements of the forelimb, head and trunk, which are more organized than E16 (Smotherman & Robinson, 1986). Prenatal movements are thought to be extremely important in the proper development of postnatal movements. For example, it is thought that certain small movements may be involved in the development of breathing movements after birth, whereas paralyzing an animal embryo can cause negative effects postnally, such as joint deformations and underdeveloped lungs (Westerga & Gramsbergen, 1993). These twitches produced by rats *in utero* are therefore thought to help with the development of motor behaviours

postnatally (Smotherman & Robinson, 1986).

Directly after birth on postnatal day zero (PND 0), rat pup movements mainly consist of curling and extending the body (Whishaw, 2004). Next, the beginning of locomotion can be seen when the head of the rat moves from side to side. The hindlimbs of the rat however are mostly immobile (Westerga & Gramsbergen, 1993). At approximately PND 4 or 5, the rat uses the contralateral forelimb to turn the trunk and in a desired direction. At this age, the hindlimbs of pups still do not function well, partially because the posterior (hindlimb) portion of the motor cortex develops later than anterior (forelimb) portion, which modulates locomotion (Whishaw, 2004; Canty & Murphy, 2008) and partially due to a lack of myelination to the spinal cord, which intrinsically mediates locomotion (Joosten et al., 1992). Rat pups are therefore unable to walk but can easily move in circles (Altman & Sudarshan, 1975).

From PND 4 to PND 10 the forelimb strength and locomotor skills of rat pups improves, producing the new ability of maintaining a quadruped stance (Whishaw, 2004). From PND 9 to 13, the hindlimbs are outwardly rotated and dragged behind the body in a swimming-like motion (Westerga & Gramsbergen, 1993). The rats are therefore able to walk approximately 25cm in three minutes, however the movement is clumsy and the hindlimbs often slip (Whishaw, 2004).

By two weeks of age, rats demonstrate a large improvement in hindlimb function during locomotion. This improvement begins with an exaggerated lifting of the hips and forelimb while the limb is swinging forward, similar to the exaggerated step sequence seen in human infants. Within a few days, the rats gain an adult-like hindlimb posture and stepping repertoire, which drastically improves locomotion (Westerga & Gramsbergen,

1993). This two-week mark also corresponds to the maturation of the myelination of the corticospinal tract (Joosten et al., 1992), indicating that the integration of supraspinal system to the spinal circuit may be essential to locomotor improvement (Westerga & Gramsbergen, 1993). By three weeks of age, there is still evidence that locomotion has not fully matured as rats travel a significantly shorter distance on smooth surfaces compared to a rough surfaces; a task that adult rats can easily complete (Altman & Sudarshan, 1975).

The arm of humans and the forelimb of rats, cats and primates have been found to develop in a proximodistal fashion, where proximal shoulder actions develop significantly earlier than distal digit actions (Armand & Kably, 1993; Armand et al., 1994; Berthier et al., 1999; Martin, 2005). Early in rat and human development, reaching and grasping are not performed or are functionally different from the mature behaviours (Porter & Lemon, 1993; Berthier et al., 1999). For example, soon after birth children are able to move their hands toward objects, at four months old they are able to grasp objects with their palms and between 12 and 18 months old they develop independent use of their fingers (Berthier et al., 1999). These findings are thought to be a result of axon specification to the topographic organization within the ventral horn of the CST that occurs during development (Armand et al., 1994; Berthier et al., 1999; Martin et al., 2004). Monkeys (Armand et al., 1994), cats (Martin, 2005), rats (Terashima, 1995) and humans (Berthier et al., 1999) are unable to complete a grasp movement or independent use of the digits before the maturation of this topographic organization therefore indicating that the maturation of the CST is a prerequisite for independent digit use.

Preventing forelimb use by restraint or neuromuscular blockade during the period of axon specification can cause reductions in axonal branching to proper topographic terminals in the ventral horn of the CST. This malformed topography has been found to cause a permanent disruption of grasping and supination behaviours during food manipulation later in development (Martin et al., 2004; Martin, 2005). Similarly, ischemia or trauma to the developing human CST can cause misconnections or reduced connections of CST axons to the topographic terminals resulting in cerebral palsy-like, spastic movements. These results suggest that proper functional development of the forelimb is necessary for the growth and maintenance of CST axon connections (Martin et al., 2004). On the other hand, performing skilled training during CST axonal specification can cause significant improvement in behaviour due to morphological and physiological changes of the CST terminals that lead to more effective synaptic activation of spinal motor neurons (Martin, 2005).

Recently, it has been suggested that environmental factors may also play a role in reaching and grasping abilities. For example, rat reaching abilities are thought to have developed from the advancement of the forelimb during the step sequence of walking, whereas grasping, which involves fine motor control, is thought to derive from food handling behaviours (Karl & Whishaw, 2013). The grasping action is typically not seen until PND 21 (Altman & Sudarshan, 1975; Donatelle, 1977), which corresponds to the typical weaning of pups from their mother; requiring pups to manually manipulate food pellets in order to eat (Karl & Whishaw, 2013). Similarly, cats show a dramatic increase in grasping abilities after weaning in order to catch prey (Martin et al., 2004). This

finding suggests that reaching and grasping movements may be functionally distinct (Karl & Whishaw, 2013).

Within the adult rat cortex, the reach-to-grasp action has been found in the posterior portion of the motor cortex or the CFA of a motor map, whereas grasp has strictly been found in the anterior portion of the motor cortex, or the RFA of a motor map (Brown and Teskey, 2014). This cortical and functional distinction between movement areas within the motor cortex supports the observed difference in behavioural development between reaching and grasping.

1.6 Behavioural assessments of forelimb movements

Manual dexterity of rodents has been found to mimic that of humans and primates (Iwaniuk & Whishaw, 2000; Cenci et al., 2002). Two common methods of measuring manual dexterity in the rat are the sunflower seed task (Whishaw et al., 1998) and the vermicelli noodle task (Allred et al., 2008). In the sunflower seed task, rat must balance on the hindlimbs and hold a seed with both forelimbs. The rat adjusts and rotates the seed to a preferred position and then bites the large end of the shell. The top portion of the shell is removed longitudinally and discarded in order to expose the seed within. Finally, the rat adjusts the manual position on the seed and consumes it (Whishaw et al., 1998). The vermicelli task measures bimanual dexterity of handling an uncooked piece of pasta. The number of manual adjustments, or grasp and release movements, of the digits are counted in order to reveal any functional asymmetries. Normally developing rats should demonstrate a similar number of manual adjustments between forelimbs (Allred et al., 2008). Previous reports indicate that rats do not gain manual dexterity to manipulate food

until the weaning age of PND 21 (Karl & Whishaw, 2013), thus I hypothesized that rats will not be able to successfully hold and manipulate the vermicelli noodles, sunflower seeds and other food objects until this time. The first experiment assessed the abilities of handling vermicelli noodles, sunflower seeds and a variety of food pellets at PND 13, 15, 20, 25 and 30.

The motion of reaching-to-grasp an object has been found to be remarkably similar in rats, monkeys and humans (Karl & Whishaw, 2013). In these species, the reaching motion can be broken down into 10 distinct movements (Whishaw et al., 2003). The number of these movements that can successfully be performed has been found to increase over time (Altman & Sudarshan, 1975; Donatelle, 1977; Armand et al., 1994; Terashima, 1995; Berthier et al., 1999; Martin, 2005). For example, when a child is born, they are first able to point the hand in the direction of an object, using mainly the torso and the shoulder; however they do not have the capabilities to grasp the object. As the child's arm develops in a proximodistal fashion, the ability to reach and grasp objects significantly improves (Berthier et al., 1999). This result corresponds with an increase in cortical synaptogenesis in the rat (Kleim et al., 1998) and reorganization of axons within the ventral horn of the spinal cord of the cat (Martin et al., 2004).

Single pellet skilled reach training is a process by which a rat reaches through a slit in the front of a Plexiglas box to obtain a sugar pellet reward. A successful reach attempt is defined as reaching through the opening to grasp the pellet, pulling the pellet toward the body and placing the pellet in the mouth (Whishaw et al., 2003). At the beginning of training, success on this task is low, however rats drastically improve

overtime to achieve a 50 to 90% success rate within the final few days of training (Whishaw, 1992; Monfils & Teskey, 2004; Young et al., 2012).

Unskilled reach training is a method used to teach rats how to reach, however the sugar pellet reward is removed before the rat is able to successfully grasp it. Rats are therefore unable to judge the distance of the pellet reward from the body and do not practice the grasping technique (Kleim, Barbay & Nudo, 1998; Monfils & Teskey, 2004; Young et al., 2012). On a final testing day, rats are allowed to obtain the sugar pellet on all trials, however the resulting number of successful reach attempts is typically extremely low, similar to the performance of a skilled rat on the first day of training (Kleim, Barbay & Nudo, 1998; Young et al., 2012). By comparing the differences within the 10 small movements of reaching between and skilled and unskilled training groups, the differences in reaching techniques that are causing a disparity in reach success can be elucidated.

1.7 History of Motor Mapping

For centuries, scientists have been trying to solve the enigma of how the motor cortex functions. In the mid 1700s to mid 1800s, the prevailing view was that the cortex was inexcitable and did not control motor functions (Gross, 1997). This idea changed after the observation that seizures were occurring in a sequential order in the body, for example beginning in the fingers and moving up to the shoulder. Neurologist John Hughlings Jackson observed this series of movements, later termed “the Jacksonian March”, as the movements appeared to be marching up the arm. Based on this observation, Jackson suggested that these areas of the body were perhaps located in adjacent brain regions (Jackson, 1870).

Fritsch and Hitzig (1870) confirmed the idea that the motor cortex was excitable when they stimulated the surface of a dog's cortex with a short duration direct current battery, which elicited twitch-like movements in the animal's contralateral forelimb. They described a "map" of the motor cortex, which involved a number of cortical islands that would elicit movements, each surrounded by areas of inactivity. These regions of excitation were thought to be undifferentiated organizations of muscles. Ferrier (1873) expanded on this finding by conducting stimulation experiments in a variety of other species including cats, rabbits and monkeys. Ferrier, however, used a biphasic stimulation current that lasted up to several seconds, which elicited coordinated, multi-joint movements in the contralateral forelimb of the animals rather than the simple muscle twitches elicited by Fritsch and Hitzig (1870).

Fourteen years later, Beevor and Horsley (1887) conducted a number of experiments using both short and long duration stimulation. They noticed that the short duration stimulation produced a map of the muscle twitches that followed the organization of the body. The long duration stimulation, however, revealed highly coordinated, multi-joint movements such as reaching and grasping. Beevor and Horsley (1890) later performed an experiment where they removed the cortex of a monkey and stimulated the fibers of the pyramidal tract, which produced a similar map of twitch-like muscle movements as the short duration cortical surface stimulation. They concluded that the cortex was actually not involved in complex movements rather the cortex was simply a starting point to the activation of the pyramidal tract, which controlled muscles.

Grunbaum and Sherrington (1901) discounted Beevor and Horsley's (1890) results when they discovered that the cortex was highly plastic and varying the

stimulation parameters could change an efferent movement. Specifically, Grunbaum and Sherrington (1901) discovered that the stimulation of a certain cortical point could alter the movement evoked from the next stimulation point, indicating that the way in which the cortex was mapped was important in how the cortex activated the pyramidal tract. This finding indicated that although the surface stimulation could evoke a muscle twitch, the cortex was also extremely important in the organization of, and the evoking of, complex movements. In addition, this finding indicated that the borders of the motor cortex were not strict and could easily carry over to what is thought to be the sensory cortex, revealing what is now labeled the sensorimotor cortex.

Almost 50 years later, Penfield and Rasmussen (1950) extended this cortical mapping procedure to humans. They used short duration surface stimulation to map the precentral (motor) and postcentral (sensory) gyrus in awake epilepsy patients and observed the movements that were elicited or the sensations that were reported by patients. From this information, the experimenters formed what are now referred to as the motor and sensory homunculi. The motor homunculus is a graphical representation of the movement that is elicited when a specific portion of the motor cortex is stimulated. Areas that control fine motor movements, such as the fingers, cover more area of the cortical surface than areas that control gross motor movements, such as the torso (see Figure 1.1). Although the image is thought to be a simplified version of the true cortical somatotopy (Graziano, 2009), which in reality has a lot of overlap between body parts (Penfield & Rasmussen, 1950), it is still an iconic representation of the motor and sensory cortices.

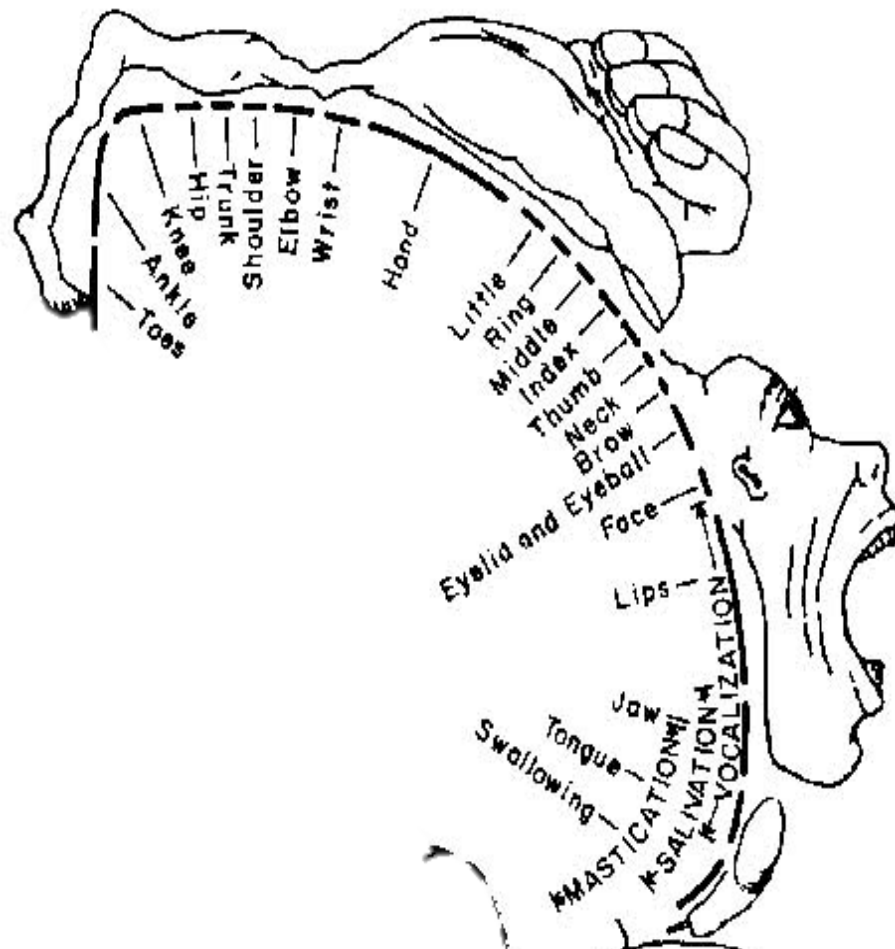


Figure 1.1. A cortical slice, which demonstrates Penfield and Rasmussen's (1950) motor homunculus.

1.8 Short-Duration-Intracortical Microstimulation

The main issue with the method of surface stimulation was the large electrical current that was required to evoke a movement, which resulted in widespread neural activation. Asanuma and Sakata (1967) believed that these stimulation parameters were causing the overlap in the cortical representations of body parts within the homunculus, and created the technique of short duration (less than 50ms) intracortical microstimulation (SD-ICMS), which used a microelectrode to stimulate layer V pyramidal cells in the motor cortex of cats (see Figure 1.5). Similar to surface stimulation, SD-ICMS evoked single-joint (simple) movements, such as an elbow flexion or a wrist extension (Asanuma & Sakata, 1967; Castro-Alamancos & Borrell, 1995; Young et al., 2012). This technique required 1/100th of the electrical current and train duration compared to surface stimulation (Asanuma & Sakata, 1967). SD-ICMS therefore allowed for closer mapping of cortical points, producing a higher resolution motor map than was ever achieved using surface stimulation. The least amount of stimulation required to produce a movement, or the threshold for each movement, was noted and the movement was coded onto the corresponding location of a brain schema, resulting in a motor map. The thresholds, however, have been found to change following treatments, such as seizures and neurotransmitter depletions (Teskey et al., 2002; Scullion et al., 2013).

This SD-ICMS technique has since been used to derive motor maps in rats (Castro-Alamancos Borrell, 1995; Donoghue & Wise, 1982; Kleim et al., 1998; Gioanni & Lamarche, 2002; Nudo et al., 1990), monkeys (Asanuma & Rosen, 1972; Nudo et al., 1996; Rosen & Asanuma, 1972) and mice (Tennant et al., 2011; Young et al., 2011b;

Young et al., 2012). Maps of the motor cortex in rats have been found to contain two consistent areas: the rostral forelimb area (RFA) and the caudal forelimb area (CFA; Neafsey et al., 1986; Neafsey & Sievert, 1982). A third area, the posterior forelimb area (PFA) was discovered later due to an increase in cortical excitation following repeated seizures (Henderson et al., 2011). The CFA is typically within 1mm anterior and 2mm lateral of bregma (Hall and Lindholm, 1974), whereas the RFA is typically located at 3mm anterior and 2mm lateral of bregma and is separated from CFA by neck, whisker and jaw movements (Neafsey and Sievert, 1982). The CFA is typically larger than the RFA and is thought to be analogous to the primary motor cortex in primates (Rouiller et al., 1998). The RFA and the CFA are predictably found under normal mapping parameters, whereas the PFA is typically located after cortical inhibition is removed (Henderson et al., 2011). The three forelimb regions, however, can be thought of as “islands” on top of a continuous underlying forelimb network that can be completely revealed when cortical inhibition is removed (Young et al., 2012).

As the rat (Young et al., 2012) and the cat motor cortices develop (Martin, 2005), the SD-ICMS threshold required to elicit a forelimb movement decreases. It has been found that cats can behaviourally produce forelimb movements before forelimb movements can be elicited using SD-ICMS. For example, from the birth of a cat to two months of age, behavioural forelimb movements are present but SD-ICMS motor maps are not. As the cat gets older and the forelimb movements develop, SD-CMS movements are gradually elicited in a proximodistal fashion. Motor maps first contain responsive shoulder movements and gradually consist of more elbow movements, then wrist movements, and finally grasp movements once the cat approaches adulthood (Martin,

2005). In the rat, a similar pattern is found where movements cannot be elicited prior to PND 35 at typical SD-ICMS intensities and without pharmacological reductions in cortical inhibition. The motor maps then gradually increase in number of responsive forelimb points and evolve in a proximodistal fashion beginning with shoulder movements and lastly producing digit movements (Young et al., 2012). It was previously believed that the discrepancy between the behavioural forelimb development and SD-ICMS elicited movements was due to the slow development of connections between the motor cortex neurons and spinal motor circuits (Martin, 2005). More recently, however, it has been found that SD-ICMS evoked rat forelimb movements can be elicited as early as PND 13 by injecting bicuculline methiodide into layer V of the neocortex. Bicuculline is used to reduce cortical inhibition, therefore lowering movement thresholds and allowing SD-ICMS movements to be elicited with typical current intensities (Young et al., 2012). This result therefore demonstrates that motor neuron and spinal motor circuit connections likely exist, however the high degree of cortical inhibition early in development prevents motor map from being revealed. The use of bicuculline in chapter 2 was essential to uncovering the development of LD-ICMS motor maps.

1.9 Long-Duration Intracortical Microstimulation

Graziano and colleagues (2002) have combined the long duration stimulation used by past scientists (Beever and Horsley, 1887) and the ICMS technique created by Asanuma and Sakata (1967) to form long-duration (500ms) intracortical microstimulation (LD-ICMS). LD-ICMS has been found to evoke single-joint (simple) forelimb movements, such as an elbow flexion, as well as multi-joint (complex) forelimb

movements, such as reaching-to-grasp. The longer duration of stimulation is thought to mimic the approximate amount of time it takes for an animal to behaviourally produce a reaching movement (Graziano et al., 2002) as well as the approximate amount of time that it takes for a motor cortex neuron to fire during reaching (Georgopoulos et al., 1982). In addition, LD-ICMS forelimb movements have been found to travel toward a specific end posture, regardless of starting position, similar to natural movements in monkeys (Graziano et al., 2002, Graziano et al., 2005; Stepniewska et al., 2009), rats (Ramanathan et al., 2006; Brown & Teskey, 2011, Bonazzi et al., 2013) and mice (Harrison et al., 2012).

LD-ICMS results in a greater number of movement categories than SD-ICMS and therefore more elaborate maps. Rather than forelimb movements being limited to the categories of elbow flexion, wrist extension, digit flexion and shoulder, LD-ICMS motor maps contain the additional categories of grasp, advance (i.e. the anterior displacement of the elbow with a simultaneous wrist extension), elevate (i.e. elbow flexion with a simultaneous wrist extension), retract (i.e. the posterior displacement of the shoulder and a wrist flexion), digit extension and supination. Moreover, motor maps derived using SD-ICMS have revealed a similar mosaic spread of movement categories over the RFA and the CFA in mice and rats (Young et al., 2009; Brown & Teskey, 2014), which are not predictable in their location. LD-ICMS, on the other hand, has revealed highly reliable clusters of movements (Brown & Teskey, 2014) therefore providing a more specific topographic organization of forelimb movements than can be elicited using SD-ICMS.

This greater degree of specificity may be key to elucidating why one can lose function of a very specific movement, such as supination, but maintain a different

movement, such as grasping, depending on the size and location of a cortical lesion (Whishaw et al., 1991; Brown & Teskey, 2014). In addition, rather than observing a significant increase in distal forelimb movements after reach-training using SD-ICMS (Tennant et al., 2012; Young et al., 2012), the LD-ICMS technique could allow the discovery of the specific distal movement that is affected by training, such as grasp. Understanding how these specific forelimb movement representations develop and change with experience within the motor map will be essential in furthering the understanding of forelimb motor control and function. In addition, greater knowledge of the timing and cortical location of the development of these movements could help in the understanding of deficits that develop from cortical injuries. Therefore, although SD-ICMS is an invaluable tool to measure cortical excitability, LD-ICMS is the ideal technique for studying the development of simple and complex forelimb movements in the rat, as well as the cortical plasticity involved in behavioural training such as skilled reach training.

1.10 Cortical development of GABA

γ -Aminobutyric acid (GABA) is the main inhibitory neurotransmitter within the neocortex and is present in the neural tissue throughout development (Yu et al., 2006). Surprisingly, however, GABA in the rat neocortex is an excitatory neurotransmitter early in development (Miles, 1999; Rivera et al., 1999) but, after the transition from NKCC1 to KCC2 at approximately PND 13, GABA becomes inhibitory (Miles, 1999; Rivera et al., 1999; Dzhalala et al., 2005). The GABA_A receptor expression, which is a ligand-gated chloride ion channel, is then upregulated until it peaks at PND 30 and then gradually

declines until the cortical maturity of the rat at approximately PND 60. If GABA_A declines too much, it can cause severe behavioural issues such as an inability to properly orient or direct attention to visual cues (Leventhal et al., 2003) or a decrease in cortical functioning in late stages of aging (Hua et al., 2006). However, housing rats in an enriched environment has been found to decrease GABAergic inhibition in the visual cortex, which has had positive effects such as restoring visual acuity after amblyopia (Sale et al., 2007).

GABA is present in the neocortex from birth and is an important neurotransmitter for inhibitory interneurons and the proper production of forelimb movements (Sanes et al., 2006). Varying the level of GABA in the neocortex can dramatically alter forelimb motor maps. For example, raising rats in an enriched environment has been found to accelerate GABA development in the cortex (Micheva & Beaulieu, 1997) and significantly increase motor map size (Young et al., 2012). Furthermore, bicuculline, a GABA_A antagonist, is a drug used to prevent the inhibitory effect of GABA and allow neural firing to occur more easily (Curtis et al., 1970). Bicuculline has been found to decrease movement thresholds and increase SD-ICMS motor map size in pups (Young et al., 2012) and in adults, using both SD-ICMS and LD-ICMS (Brown & Teskey, 2014). Infusing a GABA agonist into the cat motor cortex however has been found to permanently impair map expression later in life (Chakrabarty & Martin, 2005). SD-ICMS forelimb motor maps are unable to be revealed without the use of bicuculline prior to PND 35 (Young et al., 2012) therefore, in chapter 2, bicuculline was applied to the surface of the cortex during LD-ICMS at PND 13 and throughout development until adulthood.

1.11 Plasticity of motor maps

Plasticity or reorganization of the motor cortex is essential for learning (Monfils & Teskey, 2004) and recovery after injury (Nudo et al., 1996; Kleim et al., 2002; Adkins et al., 2006). SD-ICMS and LD-ICMS motor maps of the rat forelimb are highly plastic and can be altered by a variety of techniques (Graziano, 2002; Teskey et al., 2002; Kleim et al., 2004; Ramathan et al., 2006; Brown and Teskey, 2009; Scullion et al., 2012). For example, pups raised in an enriched environment were found to have significantly larger SD-ICMS motor maps at PND 45 with significantly lower movement thresholds compared to pups that were raised in standard housing conditions (Young et al., 2012). High frequency stimulation, which leads to long-term potentiation, has also been found to significantly increase map size (Henderson et al., 2012) whereas low frequency stimulation, which leads to long-term depression, has been found to significantly decrease map size (Monfils & Teskey, 2004). Repeated seizures have been found to significantly increase and reorganize map expression (Teskey et al., 2002), whereas serotonin and dopamine depletion can significantly decrease map size (Brown et al., 2009; Scullion et al., 2012).

The cortical changes in the rat forelimb motor map are consistent and predictable, making it an ideal location to study the effects of behavioural training on cortical plasticity. Skilled motor learning is a common technique used to demonstrate motor plasticity (Whishaw & Pellis, 1990; Whishaw, Pellis & Gorny, 1992). Results from humans (Tyc et al., 2005), non-human primates (Nudo et al., 1996) and rats (Kleim et al., 2004) have revealed an increase in the cortical area associated with the trained motor behaviour. For example, under SD-ICMS, young rats (PND 45; Young et al., 2012) and

adult rats (PND 60; Kleim et al., 1998) that have undergone single pellet reach training have been found to have a significantly larger proportion of distal forelimb movements within the motor map contralateral to the trained forelimb compared to controls. However motor maps of adult rats derived using LD-ICMS have revealed that the cortical regions associated with complex forelimb movements involved in reaching, such as grasp and advance, do not change in size (Ramanathan, 2006), however there is significant cortical overlap between forelimb and non-forelimb movements (Brown & Teskey, 2014). These changes in the cortical representations have been found to be associated with changes in dendritic length and spine density (Monfils and Teskey, 2004) as well as changes in protein synthesis (Kleim et al., 2003), and synaptogenesis (Kleim et al., 1998; Kleim et al., 2004; Kleim et al., 2002). Since rats have been found to demonstrate dexterous forelimb reaching abilities, which are incredibly similar to non-human primates (Iwaniuk & Wishaw, 2000; Cenci et al., 2002) and humans (Whishaw et al., 2002), rats are an ideal model for studying reach-training.

1.12 Hypotheses

Although LD-ICMS evoked forelimb movements of elevate, advance, grasp and retract have been documented in adult rats (Brown & Teskey, 2014), when these movements develop within the motor map has not yet been investigated. In the present study, I first characterized the behavioural development of limb elevation, advancement, grasp and retraction in Long-Evans hooded rats at PND 13, 15, 20, 25 and 30. I then characterized the development of motor maps evoked by LD-ICMS in Long-Evans hooded rats at PND 13, 15, 20, 25, 30, 35, 45 and 60. First, motor maps were derived

with a cortical surface application of saline. Next, bicuculline was applied to the surface of the cortex from PND 13 to PND 60 to assure that GABA mediated inhibition was reduced, allowing for the expression of the whole motor map. I hypothesized that LD-ICMS evoked complex movements, such as elevate, advance, grasp, and retract, would emerge in the motor maps after the rat pups are behaviourally able to perform those complex movements. Finally, I hypothesized that hemisphere contralateral to the skilled reach trained forelimb would have more grasp movements than the ipsilateral hemisphere.

Chapter Two:

The Development of Complex Movement Motor Maps Using Long Duration Intracortical Microstimulation (LD-ICMS) in Rats

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Anna Singleton performed craniotomies, anaesthetics and LD-ICMS procedures, reach trained rats, video recorded all rats, data analyses, created motor maps, statistical analyses, contributed to the experimental design and wrote the manuscript.

Sarah Park reach trained rats and created motor maps.

Andrew Brown trained A.C.S. on LD-ICMS and edited the manuscript.

Cam Teskey conceived and funded the project, created the experimental design and edited the manuscript.

2.1 Introduction

The adult motor cortex is topographically organized into representations (maps) of different body parts (Fritsch & Hitzig, 1870; Ferrier, 1873). Since the earliest days of motor mapping there has been a long-standing debate as to whether the motor cortex encodes activity separately for individual muscle groups, or integrates collective activity among many muscle synergies to encode whole, multi-joint movements (Graziano et al., 2002). Short-trains (40ms) of intracortical microstimulation (ICMS) reveal short, single-joint (simple) twitch-like muscle contractions (Asanuma & Sakata, 1967; Asanuma & Ward, 1971; Asanuma & Rosen, 1972; Young et al., 2011b). On the other hand, long-trains (500ms) of ICMS, which are approximately the same duration as a motor neuron firing during a reaching and grasping movement (Graziano et al., 2002), reveal several topographically organized areas of multi-joint (complex) movements in rats (Ramanathan et al., 2006; Bonazzi et al., 2013; Brown & Teskey, 2014).

SD-ICMS forelimb motor maps are limited to flexions or extensions of the elbow, wrist, and digits, as well as abductions of the shoulder, and have revealed a similar mosaic spread of movement categories over the rostral forelimb area (RFA) and the caudal forelimb area (CFA) in mice and rats (Young et al., 2009; Brown & Teskey, 2014). The particular movement that is elicited within the motor map at each electrode placement cannot be accurately predicted throughout development or in adulthood (Young et al., 2012). In addition, SD-ICMS is typically used to measure the sensitivity of cortical movement representations via assessment of movement thresholds (i.e. the least amount of stimulation required to produce a movement) and how these movement thresholds change with experience-dependent learning (Kleim et al., 2004), throughout development

(Young et al., 2012) or in response to disease (Boychuk et al., 2011; Brown et al., 2011; Young et al., 2011a). LD-ICMS motor maps, however, have been found to elicit eight movement categories in adult rats, including four specialized areas of the motor cortex that evoke cohesive forelimb movements (limb elevation, advancement, grasping, and retraction) that recapitulate components of the basic walking and reaching movements in rats (Karl & Whishaw, 2013; Brown and Teskey, 2014). This added specificity of movements within the LD-ICMS motor maps is important for elucidating how specific areas of the cortex relate to behavioural movements. For example, reversibly inactivating the RFA or the “grasp area” within a LD-ICMS motor map during single-pellet reach training has been found to negatively affect grasping behaviour specifically. This result suggests that the motor cortex is indeed topographically organized to make complex movements (Brown & Teskey, 2014). Therefore, although SD-ICMS is an essential tool for understanding cortical excitability, LD-ICMS is the ideal technique for studying the development of simple and complex forelimb movements in the rat, as well as the cortical plasticity of specific movements involved in behavioural training, such as single-pellet skilled reach training.

Although the LD-ICMS forelimb movements of elevate, advance, grasp and retract have been documented in adult rats (Ramanathan et al., 2006; Bonazzi et al., 2013; Brown & Teskey, 2014), when these movements develop and are expressed within the motor map has not yet been investigated. In the present study, I first characterized the behavioural development of limb elevation, advancement, grasp and retraction in Long-Evans hooded rats at PND 13, 15, 20, 25 and 30. I then characterized the development of motor maps evoked by LD-ICMS in Long-Evans hooded rats at PND 13, 15, 20, 25, 30,

35, 45 and 60. First, motor maps were derived with a cortical surface application of saline. Next, bicuculline was applied to the surface of the cortex on rats mapped between PND 13 to PND 60 to assure that GABA mediated inhibition was reduced, allowing for the expression of the whole motor map. I hypothesized that LD-ICMS evoked movements of elevate, advance, grasp, and retract, would emerge in the motor maps after the rat pups were behaviourally able to perform those complex movements. Finally, I hypothesized that the hemisphere contralateral to the skilled reach trained forelimb would have more grasp movements in the overall map expression than the ipsilateral hemisphere.

2.2 Methods and Procedure

2.2.1 Subjects

Seventy-five male Long-Evans Hooded rats were obtained from Charles River Laboratory (St. Constant, Quebec). Six rats were used in for the behavioural observations. Next, fifteen rats were used as the PND 35, 45 and 60 groups in the LD-ICMS development study. In addition, thirteen pregnant female Long-Evans Hooded rats were obtained from Charles River Laboratory and resulting litters were culled to six males. If the litter did not contain six male offspring, female rats were used to achieve six offspring in an attempt to maintain consistency of maternal care. The thirty-one male offspring were used in the PND 13, 15, 20, 25 and 30 LD-ICMS development groups. In the reach training experiment, 25 rats total were used in the skilled and unskilled reach training conditions from PND 30 to PND 45. All rats were housed in pairs in clear holding containers (23cmx43cmx20cm) and were given *ad libitum* access to food and

water. Rats raised at the university of Calgary were housed with the mother rat until weaning age at PND 21, at which point rats were housed in pairs. Reach trained animals were placed on a restricted diet for the duration of training in order to achieve 90% of a normal free-feeding body weight and in order to maintain motivation. The housing facility remained on a 12-hour light/dark cycle (on at 07:00) and all testing was performed during the light phase. Rats were food deprived for the 24-hour period before surgery. All rats were cared for, and handled, according to the Canadian Council for Animal Care guidelines and the institutional Health Sciences Animal Care Committee approved the experimentation.

2.2.2 Behavioural observations.

Six rats were placed one at a time in a 39cm x 18cm glass chamber for 15 minutes per day at PND 13, 15, 20, 25 and 30. A mirror at 45° was placed under the glass bottom of the chamber to allow for viewing the side and bottom angles of the movements. Rats were video-recorded using a Panasonic HD 1080p video recorder on a tripod with 60 frames per second.

2.2.3 Reach Training.

All rats ($N=25$) were placed in a Plexiglas reaching chamber (13.5cm wide, 40cm tall and 40cm long). Along the center of the front of the reaching chamber, there was a 1.5 cm opening through which a rat could place its forelimb. On the outside of the opening was a platform (7.5 cm wide, 3.5 cm long, and 5cm above the ground) with two small circular grooves that were 2cm from the edges of the opening. During two days of pretraining (PND 28 and PND 29), dustless precision banana-flavoured pellets (45mg, #F0059, Bio Serv) were placed in both grooves to encourage the rat to reach through the

slit to grasp the pellets. The preferred reaching forelimb was determined when a rat made five consecutive reach attempts with the same forelimb. Rats were then trained in either a skilled or unskilled reach training condition from PND 30 to PND 45.

In the skilled reach training group ($n=14$), the 15 days of training consisted of placing the banana pellets, one at a time, in the groove contralateral to the preferred reaching forelimb. A successful reach attempt was defined as reaching through the opening to grasp the pellet, pull the pellet toward the body and place the pellet in the mouth. If the attempt was unsuccessful, the pellet was immediately removed from the platform to avoid multiple unsuccessful reach attempts. On training days 1-3, successful and unsuccessful reach attempts were rewarded with a banana pellet placed at the back of the reaching chamber to ensure that the rat reset its initial reaching posture. On training days 4-15, rats were only rewarded with a banana pellet at the back of the cage on successful attempts. Between trials, all rats were required to move to the back of the cage to ensure a reset of the proper reaching posture, regardless of the result of the reach attempt. Incorrect initial reaching posture can result in improper reaching technique and therefore more unsuccessful reach attempts (Young et al., 2012). The percent of successful reach attempts was calculated each day (number of successful reach attempts divided by the total number of reach attempts $\times 100$).

In the unskilled reach training group ($n=11$), rats were trained to reach for the banana pellet in the groove contralateral to the forelimb, however the pellet was removed from the platform with tweezers before the rat could touch it. On training days 1-3, rats were rewarded with one or two banana pellets at the back of the reaching chamber after every reach attempt to mimic the reward pattern of the skilled reach-trained group. On

training days 4-14, rats were given an equivalent amount of pellets as the reach-trained group, alternating between zero and two banana pellets to mimic the reward pattern of the skilled reach-trained group. On the final day of training, rats were given the opportunity to reach and grasp the banana pellets and the percent of successful reach attempts was calculated.

Rats in both groups were video-recorded on the final day of reach training using a Panasonic HD 1080p video recorder on a tripod with 60 frames per second.

2.2.4 Long-duration intracortical microstimulation (LD-ICMS).

LD-ICMS methodology was used according to Brown and Teskey (2014) at PND 13 (n=6), PND 15 (n=5), PND 20 (n=6), PND 25 (n=5), PND 30 (n=5), PND 35 (n=5), PND 45 (n=5), PND 60 (n=5) and reach trained animals. The age of rats in the 60-day group consisted of rats ranging from PND 57 to PND 63 and one rat in the PND 30 group was PND 29. All rats were weighed and rats weighing over 70g were given an initial intraperitoneal injection of ketamine (100 mg/kg, i.p) and xylazine (5 mg/kg, i.p.). For rats under 70g, ketamine and xylazine were diluted 10 times and rats were given half of the amount stated above to avoid complications due to respiratory failure resulting in 5mg/kg of ketamine and 0.25 mg/kg of xylazine. The ratio of xylazine to ketamine, however, was consistent across all animals. Throughout the LD-ICMS procedure, additional injections of ketamine or cocktail (one part xylazine and two parts ketamine, in the concentrations stated above) were administered in alternating order as needed. Injection need was determined by a behavioural reaction to a gentle foot pinch, changes in breathing rate and vibrissae whisking. Anaesthetics can alter movement expression

(Tandon et al., 2008; Young et al., 2012) and were therefore be closely monitored throughout the study.

After the initial injection, the rat was placed on a platform and in a stereotaxic apparatus, which oriented the rat to prone position. Typically rats under 70g would need a couple of supplementary injections before they were anesthetised enough to be put in the stereotaxic ear bars. The forelimbs hung slightly in front of the platform to allow freedom of movement. A craniotomy was performed on the left hemisphere unless a preferential reaching limb was determined; in which case, the craniotomy was performed on the hemisphere contralateral to the preferred forelimb. The dura was carefully removed to expose the cortex and the cisterna magna was punctured with a 1.2mm diameter needle in order to reduce pressure due to edema. A 32x photo was taken of the cortex using a Canon digital camera and was altered using Canvas 11 software. A grid containing 500 μ m squares was overlaid on the picture and bregma and the sagittal suture were identified with an overlaid white line. The intersections of the grid, as well as the center of the squares, were used as stimulation points unless obstructed by a blood vessel. Obstructed points were either skipped, mapped at the nearest unobstructed area of the cortex, or mapped last.

Bicuculline (30 μ L of 50 μ M) was applied to the surface of the cortex 30 minutes before beginning the LD-ICMS procedure and was applied every 30 minutes thereafter (Stojic et al., 2000) unless the contralateral forelimb produced tonic-clonic seizure movements (Velíšková et al., 1990) that resembled elbow flexions or retractions; at which point, bicuculline was applied at the next 30-minute interval. Between applications, when the amount of bicuculline did not fill the craniotomy window, saline

was applied to maintain a consistent moisture level on the cortex.

A platinum (80%), iridium (20%) micro-electrode (FHC, Inc., Bowdoin, ME, USA) was used throughout LD-ICMS. The electrode had a shank diameter of 125 μ m, a standard blunt tip taper angle with a standard profile and an exposed tip with glass insulation. In addition, the electrode had impedance between 0.3 and 0.5 M Ω . The microelectrode was lowered to a depth of 1500 to 1550 μ m using a microdriver (Narishige, Tokyo) in order to stimulate the layer V pyramidal cells (Young et al., 2011b). The stimulation consisted of 100 μ A intensity with a biphasic pulse to avoid tissue damage (Tehovnik, 1996; Graziano et al., 2002). Five hundred millisecond trains of 200 μ s pulses were delivered at a frequency of 333Hz. A single point was stimulated a maximum of six times to avoid the spread of neuronal activation and to maintain the integrity of the map borders (Nudo et al. 1990; Brown and Teskey, 2014). A point was deemed non-responsive if it did not evoke a movement at 100 μ A (Graziano et al., 2002). If the forelimb did not reset to its original position after stimulation, the experimenter lightly adjusted the limb to its baseline position. The movements were then colour coded according to the coding scheme below and placed on the associated map point on the Canvas image.

The map began at 1mm anterior and 2mm lateral of bregma. After achieving a responsive point, the next point was chosen 0.5mm posterior to the responsive point. This procedure continued until the border was reached, which was defined as any non-forelimb movement, such as vibrissae, hindlimb, tail, neck, jaw, or a non-responsive point. Mapping then continued 0.25mm lateral and 0.25mm anterior to that point and proceed anteriorly with the same method until the border was reached again. This process

was repeated until the bottom half of the map is complete, after which the top half of the map was performed. This entire map section was the caudal forelimb area (CFA; Neafsey et al., 1986; Neafsey & Seivert, 1982). After all points in the CFA were mapped and bordered, the point 3mm anterior and 2mm lateral of bregma was mapped in order to locate the rostral forelimb area (RFA; Neafsey and Sievert, 1982). The same mapping procedure was performed for the RFA and PFA as for CFA until non-responsive or non-forelimb points bordered all responsive forelimb points. Since it was unknown where responsive points would be located in rats under PND 35, a 50-point grid was mapped to locate all possible responsive points. Anaesthetic levels were monitored throughout mapping by returning to a responsive point to check for alterations in movements. Forelimb movements were video-recorded during LD-ICMS and reviewed to ensure the accuracy of movement coding. All behavioural surveillance was performed using a Panasonic (HD) 1080p video recorder on a tripod in 60 frames per second with a 1/1000 second shutter speed. After mapping, rats were humanely euthanized using 1ml of Euthanyl (pentobarbital sodium USP, 240mg/ml).

2.2.4.1 Coding scheme. Eight movement categories were defined and colour coded: 1) elbow flexion (navy blue), 2) elbow extension (dark blue) 3) wrist extension (light green), 4) supination (forest green), which involved a clockwise rotation of the right forelimb until the palm faces upward or the counter clockwise rotation of the left forelimb, 5) Retraction (light blue), which was a posterior displacement of the elbow with or without a wrist flexion. 6) Elevate (orange), which was defined as a simultaneous elbow flexion and wrist extension. 7) advance (yellow), which involved an anterior displacement of the forelimb in combination with the flexion of the wrist and 8) grasp

(red), which was a wrist extension followed by a wrist flexion, while simultaneously the digits open then close. The additional category of digit extension (dark purple) was also included.

2.2.5 *Statistics.*

The mean number of responsive bicuculline forelimb points was analyzed across age groups (PND 13, 15, 20, 25, 30, 35, 45, 60) in an analysis of covariance (ANCOVA) with the amount of ketamine (mg/kg/min) administered to rats during LD-ICMS as the covariate. In addition, the mean number of responsive forelimb points with a cortical application of saline were analyzed across age groups (PND 13, 15, 20, 25, 30, 35, 45, 60) in a one way between subjects analysis of variance (ANOVA). The mean number of responsive saline and bicuculline forelimb points were analyzed across age groups (PND 13, 15, 20, 25, 30, 35, 45, 60) in a multivariate analysis of covariance (MANCOVA) with the amount of xylazine (mg/kg/min) administered to rats during LD-ICMS as the covariate.

In the behavioural analysis of reach training, the ten components of the reaching motion (digits to midline, digits semi-flexed, elbow to midline, advance, digit extension, arpeggio, grasp, supination1, supination2 and release) were scored on a 0 to 1 scale; 0 indicating that the movement was present with no impairment, 0.5 indicating ambiguity of the movement and impairment and 1 indicating that the movement was either not present or impaired (Whishaw & Metz, 2002). The reach training video from three rats in the skilled and three rats in the unskilled condition were lost and not available for analysis; therefore the training videos from six skilled and eight unskilled reach trained

rats were analyzed. The first five successful reaches from each rat were scored and all movements were analyzed in a Mann-Whitney U non-parametric test with the reach training group (skilled versus unskilled) as the independent variable and the movement scores as the dependent variable.

Two rats in the skilled reach training condition died during the LD-ICMS procedure and three rats were eliminated from the analysis for achieving a mean success rate below 30% on the final three days of testing and never achieving above 40% success.

If multiple paired or independent samples t-tests were performed, the Tukey correction was used. Mauchly's test of sphericity and/or Levene's test of homogeneity of variance were calculated for all analyses.

2.3 Results

2.3.1 Behavioural observations

Six rats were observed for 15 minutes per day at PND 13, 15, 20, 25 and 30. All rats had access to vermicelli noodles, sunflower seeds, large and small banana pellets and large and small rat chow pellets. The following are the behavioural observations at each age. It should be noted that once a particular behaviour was observed, it was consistently present at all proceeding ages.

PND13. The eyes of the rats were closed and rats performed a quadruped stance. Rats were able to pivot in circles by lifting and placing the forelimbs on the ground using elbow flexions. The forelimb locomotor pattern included elevate, advance, placing the extended digits on the ground and retracting the forelimb. The rats performed the elliptical grooming stroke where the digits and elbows were flexed and stroked away from the face and then forelimbs retracted.

PND15. The eyes of the six rats were open. Rats performed small unilateral grooming strokes, which involved flexing the elbow and the digits of one forelimb at a time while stroking the nose and retracting the forelimb.

PND20. For the first time, rats handled and adjusted digits on the large banana pellets using the power grip or a grasp with all of the digits simultaneously. One rat handled and adjusted digits around a vermicelli noodle, holding the noodle between the third and fourth digit. Two rats manipulated sunflower seeds however only one rat opened the shell and consumed the seed inside. In addition, rats simultaneously licked both paws during grooming using a retraction movement.

PND25. For the first time, rats picked up the large rat chow pellet and held it in a power grip (Whishaw and Coles, 1996) or like a child would hold a beach ball.

PND30. Rats grasped large banana pellets and rat chow pellets with the power grip but once the large banana pellet became smaller during consumption, the rats used the precision grip, which involved holding an object between the first two digits and the thumb-like stump (Whishaw and Coles, 1996). The rats were proficient at manipulating and consuming sunflower seeds. Typically, once the rats removed the shell, the precision grip was used to hold and then consume the seed.

2.3.2 Observation of food manipulation

The mean time spent manipulating food was analyzed across age groups (PND 13, 15, 20, 25, 30) in a one-way within subjects analysis of variance (ANOVA) and revealed a significant difference, $F(4,20)=26.74, p<0.001$, see figure 2.1. Rats at PND 13 and 15 did not manipulate food whereas PND 30 rats spent significantly more time manipulating food than all other age groups, (see table 2.1) suggesting an improvement in

manual dexterity over development.

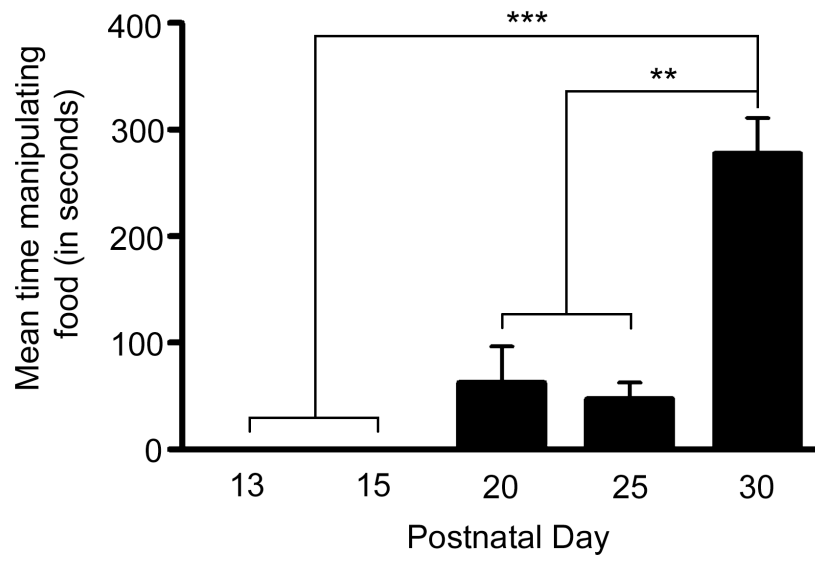


Figure 2.1. The mean time spent manipulating food in seconds as a function of age (PND 13, 15, 20, 25 and 30). Error bars represent the standard error of the mean. (** $p < 0.01$, *** $p < 0.001$).

Table 2.1 The mean time spent manipulating food (in seconds) was analyzed across age groups (PND 13, 15, 20, 25, 30).

Postnatal Day	Mean	Standard deviation	Significance
13	0.00	0.00	
15	0.00	0.00	
20	62.50	83.26	
25	47.00	38.40	>PND 13, $t(5)=3.00$, $p=0.030$ >PND 15, $t(5)=3.00$, $p=0.030$
30	277.50	82.05	>PND 13, $F(1,5)=68.63$, $p<0.001$ >PND 15, $F(1,5)=68.63$, $p<0.001$ >PND 20, $F(1,5)=19.76$, $p=0.007$ >PND 25, $F(1,5)=33.60$, $p=0.002$

The mean amount of time spent manipulating the five food options (sunflower seed, large banana pellet, small banana pellet, large rat chow pellet and small rat chow pellet) during a 15 minute observation period was analyzed across three age groups (PND 20, 25 and 30) in a two-way within subjects ANOVA. Results revealed a significant time by food type interaction, $F(8,40)=6.61, p<0.001$, indicating that the time spent manipulating food types varied as rats got older. At PND 20, there was a significant difference in the amount of time spent manipulating the food types, $F(4,20)=3.091, p=0.039$, however due to the large variability between rats, the posthoc paired t-tests between food types did not reveal a significant difference. For example, one rat did not manipulate any food pellets whereas two others spent 11% and 22% of the 15-minute observation period manipulating a variety of food pellets. At PND 25, rats spent significantly more time manipulating the small banana pellet than the small rat chow pellet, $F(1,5)=10.36, p=0.023$, and a trend toward a preference for the large banana pellets over the sunflower seed and the large and small rat chow pellets, $F(1,5)=4.26, p=0.094$. At PND 30, rats spent more time manipulating sunflower seeds and large banana pellets than small banana pellets and large and small rat chow pellets (see figure 2.2 and table 2.2).

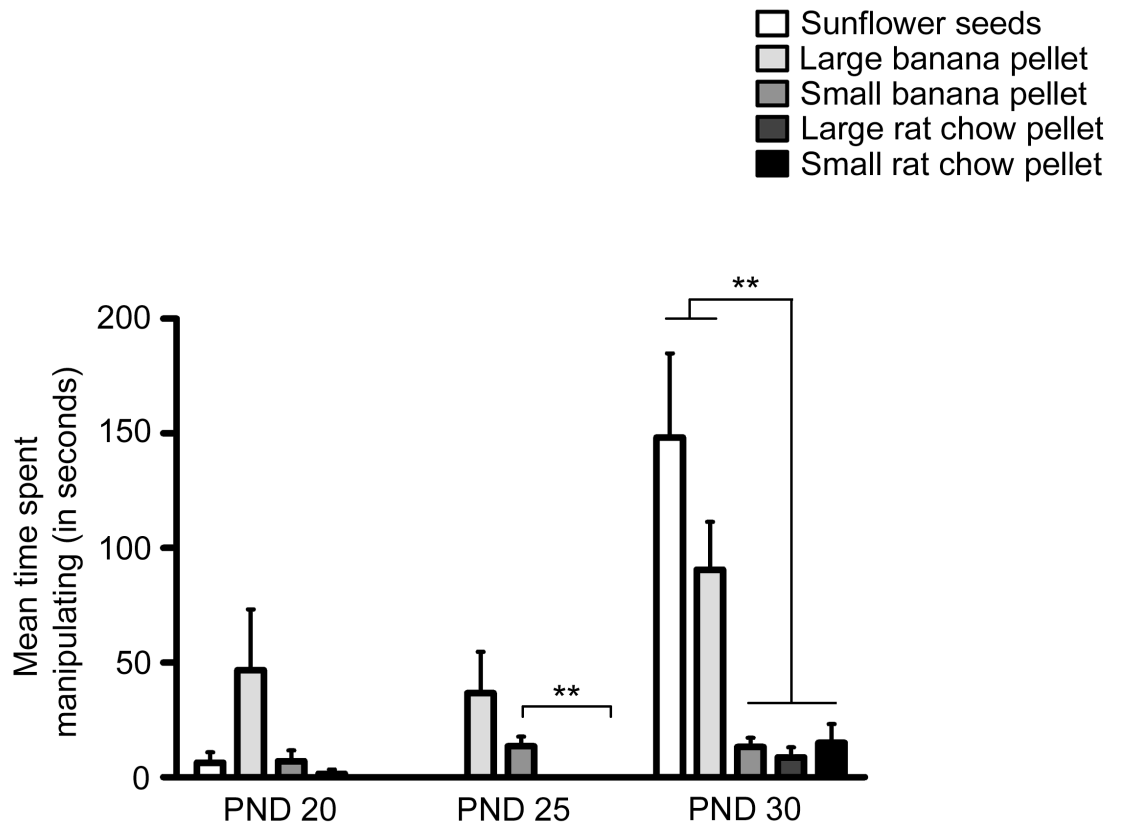


Figure 2.2. The mean time spent manipulating each type of food (sunflower seed, large banana pellet, small banana pellet, large rat chow pellet, small rat chow pellet) as a function of age (Postnatal Day (PND) 20, 25, 30). Error bars represent the standard error of the mean. (** $p < 0.025$).

Table 2.2. *The mean (M) amount of time spent manipulating the five food options (Sunflower Seed (SS), Large Banana Pellet (LBP), Small Banana Pellet (SBP), Large Rat Chow Pellet (LRC) and Small Rat Chow Pellet (SRC)) across three age groups (Postnatal Day 20, 25 and 30). Standard deviations (SD) are also noted.*

Postnatal Day	Food type	Mean, Standard deviation	Significance
20	Sunflower seed (SS)	$M=6.33, SD=11.34$	
	Large banana pellet (LBP)	$M=46.67, SD=65.01$	
	Small banana pellet (SBP)	$M=7.17, SD=11.21$	
	Large rat chow pellet (LRC)	$M=1.67, SD=4.08$	
	Small rat chow pellet (SRC)	$M=0.00, SD=0.00$	
25	Sunflower seed (SS)	$M=0.00, SD=0.00$	SBP>SRC, $F(1,5)=10.36, p=0.023$
	Large banana pellet (LBP)	$M=36.83, SD=43.71$	
	Small banana pellet (SBP)	$M=13.50, SD=10.27$	
	Large rat chow pellet (LRC)	$M=0.00, SD=0.00$	
	Small rat chow pellet (SRC)	$M=0.00, SD=0.00$	
30	Sunflower seed (SS)	$M=148.17, SD=90.10$	SS>SBP: $t(5)=3.61, p=0.015$ SS>LRC: $t(5)=3.99, p=0.010$ SS>SRC: $t(5)=3.41, p=0.019$ LBP>SBP: $t(5)=3.47, p=0.018$ LBP>LRC: $t(5)=3.78, p=0.013$ LBP>SRC: $t(5)=3.92, p=0.011$
	Large banana pellet (LBP)	$M=90.67, SD=50.86$	
	Small banana pellet (SBP)	$M=13.17, SD=10.14$	
	Large rat chow pellet (LRC)	$M=8.83, SD=10.50$	
	Small rat chow pellet (SRC)	$M=15.00, SD=20.39$	

The number of manual adjustments for the right and left forelimb was counted for each food type; sunflower seeds, large and small banana pellets, and large and small rat chow pellets. As rats rarely manipulated the small rat chow pellet, the results will not be discussed. In addition, even at PND 30, rats did not lift the large rat chow pellets off of the ground; therefore they will not be discussed.

The mean number of manual adjustments for the sunflower seeds, large banana pellets and small banana pellets were analyzed in a two-way within subjects ANOVA with right and left forelimb as one within subjects factor and age (PND 20, 25 and 30) as another within subjects factor. Results revealed a significant two-way interaction for sunflower seeds, large and small banana pellets, $F(1,5)=23.04$, $p=0.005$, $F(2,10)=37.48$, $p<0.001$ and $F(12,24)=3.47$, $p<0.05$, respectively. However, there was no significant difference between right and left manual adjustments at any age, except for the large banana pellet, where rats had a higher number of right forelimb adjustments at PND 20, $t(5)=8.34$, $p<0.001$, and a higher number of left forelimb adjustments at PND 25, $t(5)=6.49$, $p=0.001$, see figure 2.3. In summary, rats did not demonstrate an asymmetry between forelimb adjustments during food manipulation, indicating normal functioning of both forelimbs.

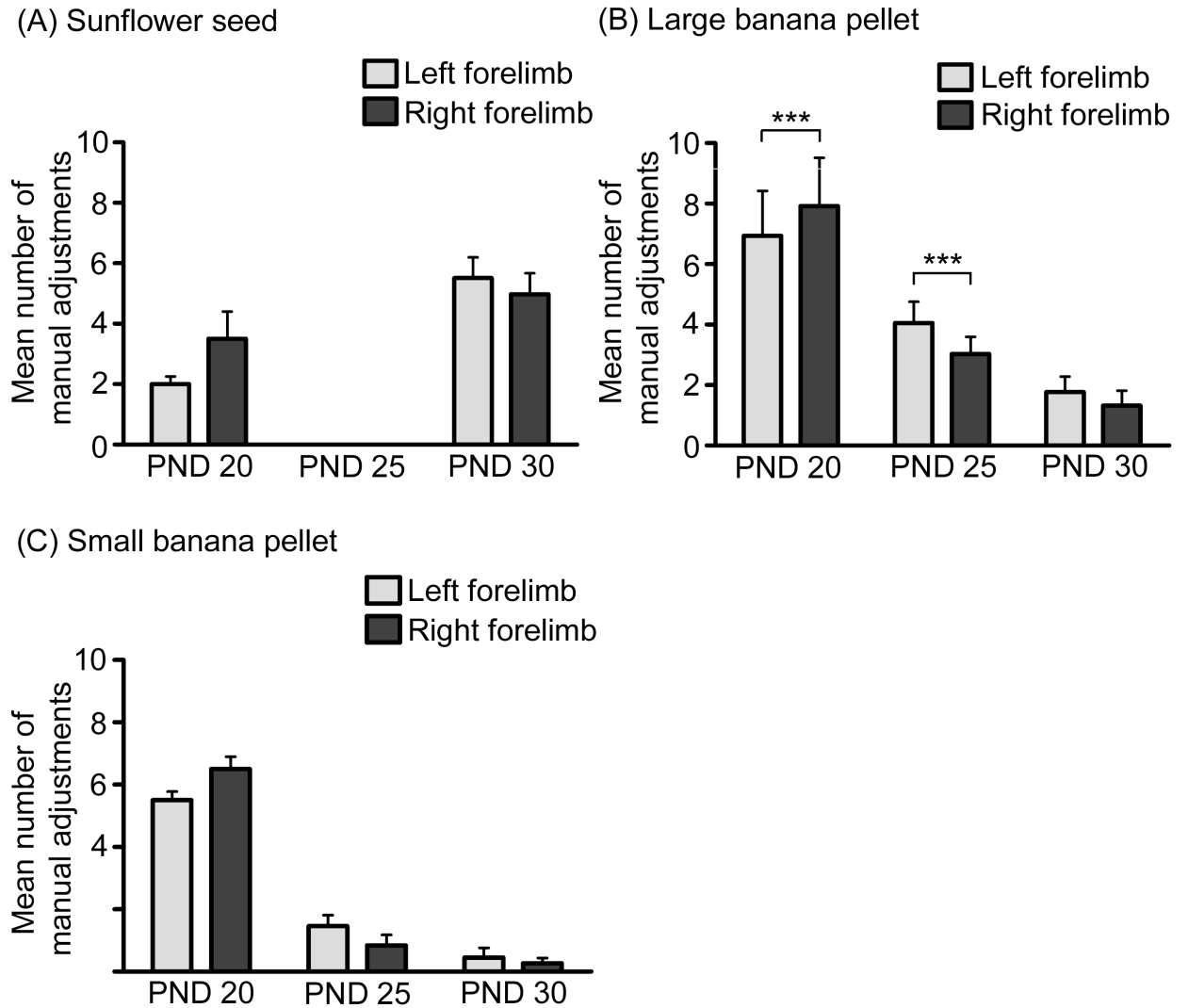


Figure 2.3. The mean number of manual adjustments of the right and left forelimb on (A) sunflower seeds (B) large banana pellets and (C) small banana pellets at each observation age (PND 20, 25 and 30, $N=6$). Error bars represent the standard error of the mean. (***) $p \leq 0.001$).

2.3.3 LD-ICMS Development

2.3.3.1 Anesthetics. The amount of ketamine (mg/kg/min) and xylazine (mg/kg/min) are shown in table 2.3. In order to reveal the relationship between the amount of ketamine administered during LD-ICMS and the number of responsive forelimb points with a cortical application of saline or bicuculline, two Pearson correlations were performed. The results indicated that there was a non-significant correlation between the amount of ketamine administered and the number of saline points, $r=0.230$, $p=0.143$, however there was a significant correlation between the amount of ketamine administered and the number of bicuculline points, $r=0.354$, $p=0.021$.

The mean number of responsive bicuculline forelimb points was analyzed across age groups (PND 13, 15, 20, 25, 30, 35, 45, 60) in an analysis of covariance (ANCOVA) with the amount of ketamine (mg/kg/min) administered to rats during LD-ICMS as the covariate. An ANCOVA was deemed the appropriate analysis for this data set because the interaction between the amount of ketamine administered and the age groups was non-significant for responsive bicuculline points, $F(7,26)=2.08$, $p=0.082$, indicating homogeneity of regression across groups. When we accounted for the amount of ketamine administered, the between-subjects test revealed a significant difference in the number of responsive bicuculline points between age groups, $F(7,26)=5.67$, $p<0.001$. In addition, the amount of ketamine administered accounts for a significant amount of variability in the number of bicuculline responsive points, $F(1,26)=4.27$, $p=0.049$ and results revealed a linear trend between the number of responsive bicuculline points across age, $F(1,34)=3.94$, $p=0.004$. In summary, after accounting the amount of ketamine

administered during the LD-ICMS procedure, the number of bicuculline points increased across age groups (see table 2.4).

The mean number of responsive forelimb points with a cortical application of saline were analyzed across age groups (PND 13, 15, 20, 25, 30, 35, 45, 60) in a one way between subjects analysis of variance (ANOVA) and was significant, $F(1,34)=45.71$, $p<0.001$. Results also revealed a significant linear trend, $F(1,34)=303.45$, $p<0.001$, indicating that the number of responsive saline forelimb points increased across age groups (table 2.4).

The mean number of responsive saline and bicuculline forelimb points were analyzed across age groups (PND 13, 15, 20, 25, 30, 35, 45, 60) in a multivariate analysis of covariance (MANCOVA) with the amount of xylazine (mg/kg/min) administered to rats during LD-ICMS as the covariate. The results indicated that there was a significant correlation between the amount of xylazine administered and the number of saline points, $r=0.354$, $p=0.021$, and bicuculline points, $r=0.456$, $p=0.002$.

A MANCOVA was deemed the appropriate analysis for this data set because the interaction between the mean amount of xylazine administered across the age groups was non-significant for saline, $F(7,26)=1.46$, $p=0.223$, and bicuculline, $F(7,26)=0.76$, $p=0.625$, indicating homogeneity of regression across groups. When we statistically controlled for the amount of xylazine administered during the LD-ICMS procedure, the between-subjects test revealed a significant difference in the number of responsive saline and bicuculline points between age groups, $F(7,26)=4.77$, $p=0.001$ and $F(7,26)=4.13$, $p=0.004$, respectively. In addition, the amount of xylazine administered accounts for a significant amount of variability in the number of saline responsive points, $F(1,26)=4.56$,

$p=0.042$, but not the bicuculline responsive points, $F(1,26)=0.11$, $p=0.741$. In summary, when taking pretreatment weight into account, the number of saline points and the number of bicuculline points increased across age groups (see table 2.4).

Table 2.3. *The mean amount of ketamine (mg/kg/minute) and xylazine (mg/kg/minute) administered across age (PND 13, 15, 20, 25, 30, 35, 45, 60). Standard deviations (SD) are also noted.*

Postnatal Day	Ketamine (Mean, SD)	Xylazine (Mean, SD)
13	$M=0.56, SD=0.10$	$M=0.022, SD=0.004$
15	$M=0.68, SD=0.15$	$M=0.020, SD=0.006$
20	$M=0.70, SD=0.20$	$M=0.024, SD=0.006$
25	$M=1.26, SD=0.22$	$M=0.044, SD=0.014$
30	$M=1.36, SD=0.43$	$M=0.047, SD=0.014$
35	$M=1.08, SD=0.05$	$M=0.040, SD=0.003$
45	$M=1.00, SD=0.24$	$M=0.038, SD=0.010$
60	$M=0.97, SD=0.34$	$M=0.040, SD=0.008$

Table 2.4. *The mean (M) number of responsive forelimb points across age group (Postnatal Day 13, 15, 20, 25, 30, 35, 45, 60 and 90) and Drug condition (Saline, bicuculline). Standard deviations (SD) are also included.*

Postnatal Day	Saline (Mean, SD)	Bicuculline (Mean, SD)
13	$M=0.00, SD=0.00$	$M=0.00, SD=0.00$
15	$M=0.00, SD=0.00$	$M=3.00, SD=2.83$
20	$M=0.00, SD=0.00$	$M=11.33, SD=10.15$
25	$M=2.00, SD=3.46$	$M=21.6, SD=6.95$
30	$M=18.60, SD=15.27$	$M=39.00, SD=25.26$
35	$M=36.40, SD=13.44$	$M=64.40, SD=21.82$
45	$M=44.80, SD=11.43$	$M=100.60, SD=14.44$
60	$M=72.80, SD=12.41$	$M=100.20, SD=13.16$
90	$M=42.20, SD=8.94$	$M=80.00, SD=12.86$

2.3.3.2 Bicuculline. This portion of the experiment was performed to assess if the application of a drug (within-subjects independent variable) to the cortex would alter the first appearance of motor maps, as well as the size of the motor maps across age (between subjects independent variable). Map size (dependent variable) was defined as the number of responsive forelimb points in a motor map. The data from 45 rats was analyzed in a 2 (Saline, Bicuculline) x 8 (PND 13, 15, 20, 25, 30, 35, 45, 60) split-plot ANOVA.

Results indicated the first responsive points during LD-ICMS with a cortical application of bicuculline first occurred at PND 15 (see figure 2.4 and 2.5), whereas responsive points during LD-ICMS with a cortical application of saline occurred at PND 25 (see figure 2.5). Within the saline maps, the first single-joint movement was elicited at PND 25 and the first multi-joint movement was elicited PND 30 (see figure 2.4 and 2.5). However, after reducing cortical inhibition, the first movement that became responsive was the single-joint movement of elbow flexion at PND 15 and the first multi-joint movement was not present within the motor map until PND 25 (figure 2.4 and 2.5). Results also revealed a significant two-way interaction between drug and age, $F(7,37)=9.20, p<0.001$, indicating that the effect of the saline and bicuculline varied across age groups. Results revealed that the bicuculline maps were significantly larger than the saline maps at all ages except for PND 13, which is not represented, as there were no responsive forelimb points in either drug condition (see table 2.5 and figure 2.6).

Data from a naïve (saline) and bicuculline PND 90 group were received from Andrew Brown and therefore were analyzed separately in an age-matched paired samples t-test. Results indicated, again, that bicuculline maps were significantly larger than the

saline maps ($t(1)=-5.04$, $p=0.007$, see figure 2.4 and table 2.5).

The number of responsive saline and bicuculline forelimb points of the nine movement categories (wrist extension, elbow flexion, elbow extension, digit extension supination, elevate, advance, grasp, retract) were analyzed in a between subjects MANOVA with age (PND 13, 15, 20, 25, 30, 35, 45, 60) as the between subjects factor. Results from both drug conditions (saline and bicuculline) revealed a significant linear trend in the number of responsive forelimb points for each movement type except for saline elbow extension and digit extension, indicating that the number of responsive points of a particular movement category increased with age (see figure 2.4 and 2.5, and table 2.6 and 2.7).

In table 2.8, the first appearance of elevate, advance, grasp and retract during behavioural observations are noted according to age (PND 13, 15, 20, 25 or 30) and compared to the day that those movements were first evoked using LD-ICMS (PND 13, 15, 20, 25, 30, 35, 45 or 60).

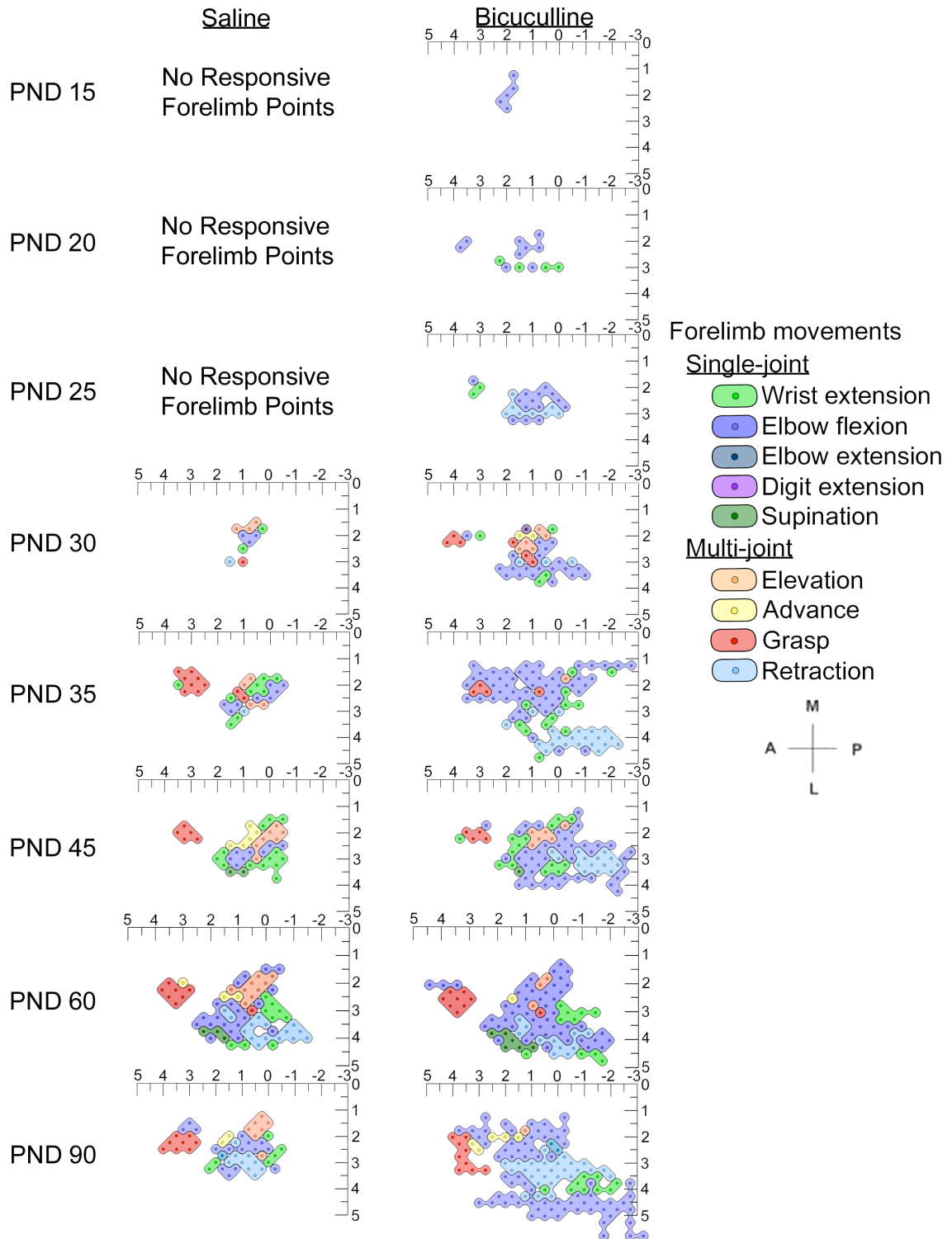
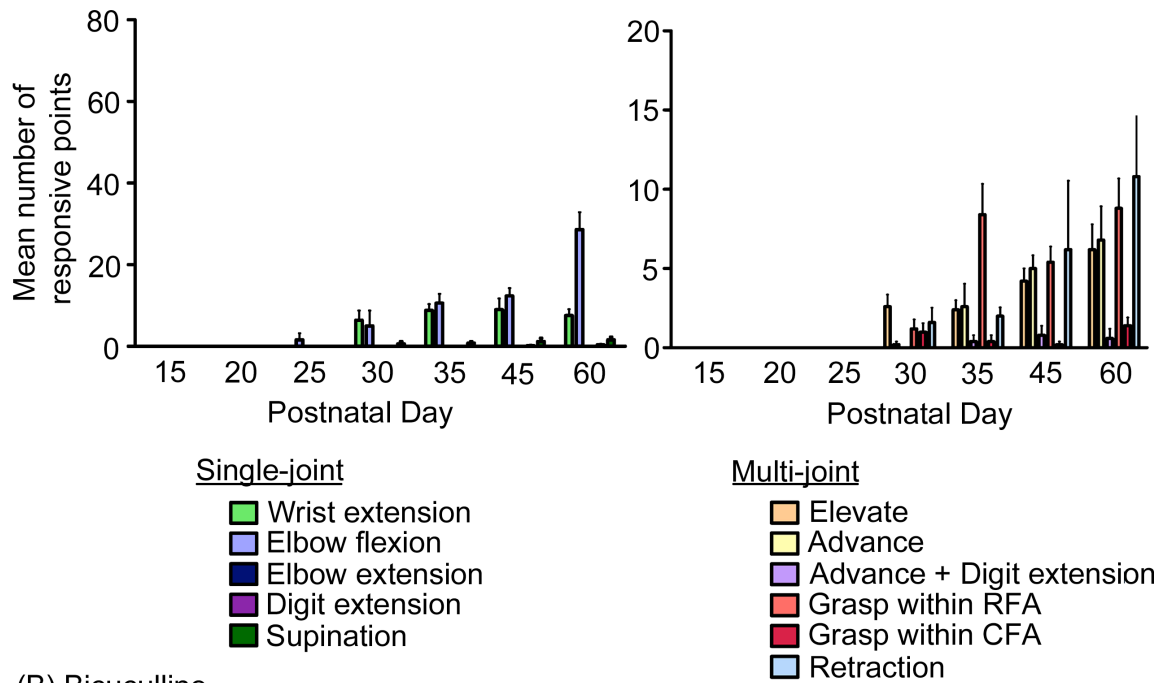


Figure 2.4. Representative LD-ICMS motor maps from one rat at each age group (PND 15, 20, 25, 30, 35, 45 and 60) with a cortical application of saline and then remapped with a cortical application of bicuculline. PND 90 maps are representative from Andrew Brown of one rat within a saline mapped group and one rat within a bicuculline mapped group.

(A) Saline



(B) Bicuculline

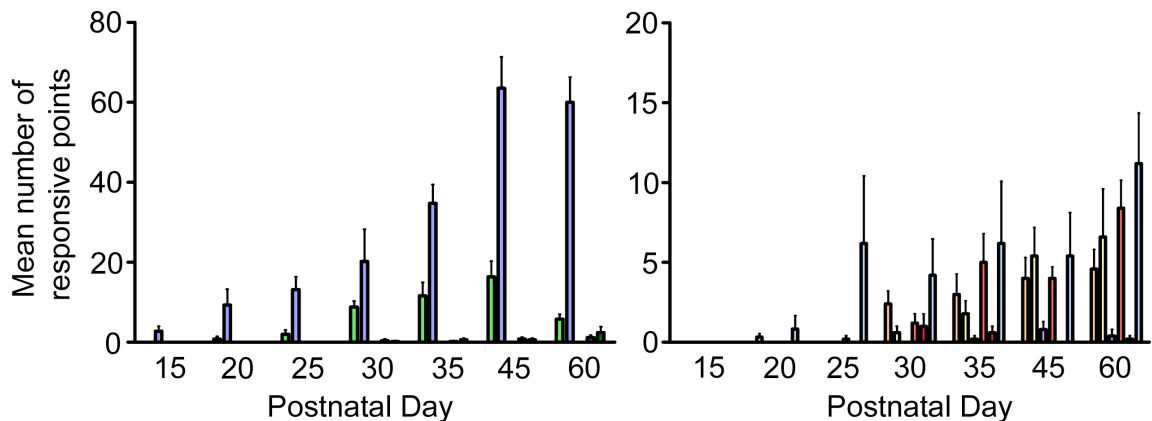


Figure 2.5. The mean number of responsive single-joint and multi-joint forelimb points during LD-ICMS with a cortical application of (A) saline and (B) bicuculline across age (PND 15, 20, 25, 30, 35, 45, 60). Error bars represent the standard error of the mean. PND 13 was not included as there were no responsive forelimb points in either drug condition condition.

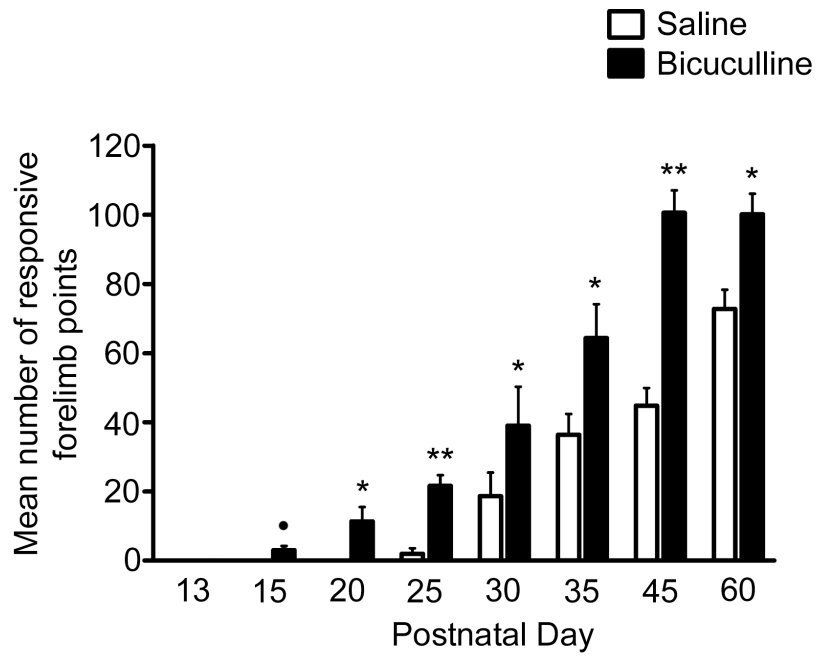


Figure 2.6. A comparison between the mean number of responsive forelimb points of bicuculline maps and saline maps across age groups (PND 13, 15, 20, 25, 30, 35, 45, 60; $N=42$). Error bars represent the standard error of the mean. (• $p<0.08$, * $p<0.05$, ** $p<0.01$).

Table 2.5. *The mean number of responsive forelimb points across age group (PND 13, 15, 20, 25, 30, 35, 45, 60 and 90) and drug condition (Saline, bicuculline).*

Postnatal Day	Saline (Mean, SD)	Bicuculline (Mean, SD)	Significance
13	$M=0.00, SD=0.00$	$M=0.00, SD=0.00$	<i>n.s.</i>
15	$M=0.00, SD=0.00$	$M=3.00, SD=2.83$	$S<B, F(1,4)=2.37, p=0.077$
20	$M=0.00, SD=0.00$	$M=11.33, SD=10.15$	$S<B, F(1,5)=2.73, p=0.041$
25	$M=2.00, SD=3.46$	$M=21.6, SD=6.95$	$S<B, F(1,4)=5.60, p=0.005$
30	$M=18.60, SD=15.27$	$M=39.00, SD=25.26$	$S<B, F(1,4)=3.15, p=0.035$
35	$M=36.40, SD=13.44$	$M=64.40, SD=21.82$	$S<B, F(1,4)=3.25, p=0.031$
45	$M=44.80, SD=11.43$	$M=100.60, SD=14.44$	$S<B, F(1,4)=6.37, p=0.003$
60	$M=72.80, SD=12.41$	$M=100.20, SD=13.16$	$S<B, F(1,4)=3.38, p=0.028$
90	$M=42.20, SD=8.94$	$M=80.00, SD=12.86$	$S<B, t(1)=-5.04, p=0.007$

Table 2.6. The mean (*M*) number of responsive saline forelimb movements across age groups (Postnatal Day 13, 15, 20, 25, 30, 35, 45, 60). Standard deviations (*SD*) are provided.

Movement	PND 15	PND 20	PND 25	PND 30	PND 35	PND 45	PND 60	ANOVA	Linear Trend
Wrist extension	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=6.40 SD=5.32	M=8.80 SD=3.56	M=9.00 SD=6.08	M=7.60 SD=3.44	$F(6,31)=8.92$, $p<0.001$	$F(1,31)=34.52$, $p<0.001$
Elbow flexion	M=0.00 SD=0.00	M=0.00 SD=0.00	M=1.60 SD=3.58	M=5.00 SD=8.48	M=10.60 SD=5.13	M=12.40 SD=4.16	M=28.60 SD=9.56	$F(6,31)=19.58$, $p<0.001$	$F(1,31)=110.22$, $p<0.001$
Elbow extension	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	n/a	n/a
Digit extension	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.20 SD=0.44	M=0.40 SD=0.54	$F(6,31)=1.97$, $p=0.100$	n/a
Supination	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.60 SD=1.34	M=0.80 SD=0.84	M=1.20 SD=1.79	M=1.60 SD=1.52	$F(6,31)=2.16$, $p=0.074$	$F(1,31)=12.22$, $p=0.001$
Elevate	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=2.60 SD=1.67	M=2.40 SD=1.34	M=4.20 SD=1.79	M=6.20 SD=3.56	$F(6,31)=11.78$, $p<0.001$	$F(1,31)=66.55$, $p<0.001$
Advance	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.20 SD=0.45	M=2.60 SD=3.21	M=5.00 SD=1.87	M=6.80 SD=4.76	$F(6,31)=8.80$, $p<0.001$	$F(1,31)=48.00$, $p<0.001$
Grasp	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=1.20 SD=1.30	M=8.40 SD=4.34	M=5.40 SD=2.19	M=8.80 SD=4.21	$F(6,31)=15.63$, $p<0.001$	$F(1,31)=69.15$, $p<0.001$

								01	01
Retract	M=0.0 0 SD=0. 00	M=0.0 0 SD=0. 00	M=0.0 0 SD=0. 00	M=1.6 0 SD=2. 07	M=2.0 0 SD=1. 22	M=6.2 0 SD=9. 70	M=10. 80 SD=9. 09	$F(6,31)$)= 3.74, $p=0.0$ 06	$F(1,31)$)= 20.74, $p<0.0$ 01

Table 2.7. The mean (M) number of responsive bicuculline forelimb movements across age groups (Postnatal Day 13, 15, 20, 25, 30, 35, 45, 60). Standard deviations (SD) are provided.

Move ment	PND 15	PND 20	PND 25	PND 30	PND 35	PND 45	PND 60	ANO VA	Linea r Trend
Wrist extensi n	M=0. 00 SD=1 .28	M=0. 833 SD=1. 60	M=2. 00 SD=2. 45	M=8.8 0 SD=3. 27	M=11. 60 SD=7. 53	M=16. 40 SD=8. 68	M=5.8 0 SD=2. 59	$F(6,31)=10.00$, $p<0.001$	$F(1,31)=22.86$, $p<0.001$

Elbow flexion	M=2.00 SD=2.52	M=9.33 SD=9.60	M=13.20 SD=7.08	M=20.20 SD=18.02	M=34.80 SD=10.38	M=63.60 SD=17.43	M=60.00 SD=14.00	$F(6,31)=23.19$, $p<0.001$	$F(1,31)=124.92$, $p<0.001$
Elbow extension	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	n/a	n/a
Digit extension	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.40 SD=0.89	M=0.20 SD=0.44	M=0.80 SD=1.10	M=1.20 SD=1.30	$F(6,31)=2.30$, $p=0.059$	$F(1,31)=12.59$, $p=0.001$
Supination	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.20 SD=0.45	M=0.60 SD=0.89	M=0.60 SD=0.89	M=2.40 SD=3.29	$F(6,31)=2.37$, $p=0.053$	$F(1,31)=11.76$, $p=0.002$
Elevate	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.00 SD=0.00	M=2.40 SD=1.81	M=3.00 SD=2.83	M=4.00 SD=2.92	M=4.60 SD=2.70	$F(6,31)=6.23$, $p<0.001$	$F(1,31)=32.75$, $p<0.001$
Advance	M=0.00 SD=0.00	M=0.33 SD=0.52	M=0.00 SD=0.00	M=0.60 SD=0.89	M=1.80 SD=1.79	M=5.40 SD=3.97	M=6.60 SD=6.73	$F(6,31)=4.70$, $p=0.002$	$F(1,31)=25.28$, $p<0.001$
Grasp	M=0.00 SD=0.00	M=0.00 SD=0.00	M=0.20 SD=0.45	M=1.20 SD=1.30	M=5.00 SD=4.00	M=4.00 SD=1.58	M=8.40 SD=3.91	$F(6,31)=12.04$, $p<0.001$	$F(1,31)=64.01$, $p<0.001$
Retract	M=0.00 SD=0.00	M=0.83 SD=2.04	M=6.20 SD=9.44	M=4.20 SD=5.07	M=6.20 SD=8.67	M=5.40 SD=6.07	M=11.20 SD=7.05	$F(6,31)=2.20$, $p=0.053$	$F(1,31)=10.77$, $p=0.003$

Table 2.8. *The number of rats (#) eliciting elevate, advance, grasp and retract within the behavioural observations and within the saline and bicuculline LD-ICMS motor map according to age (measured in days) and group size (n).*

Movement	Age in days	Number of rats performing the movement during behavioural observations (#/n)	Number of rats eliciting the movement during LD-ICMS + saline motor map (#/n)	Number of rats eliciting the movement during LD-ICMS + bicuculline motor map (#/n)	Difference between first appearance of a movement during the behavioural observation and in the motor map (in days)
Elevate	13	6/6	0/6	0/6	17
	15	6/6	0/5	0/5	
	20	6/6	0/6	0/6	
	25	6/6	0/5	0/5	
	30	6/6	4/5	4/5	
	35	n/a	4/5	4/5	
	45	n/a	5/5	5/5	
	60	n/a	5/5	5/5	
Advance	13	6/6	0/6	0/6	7
	15	6/6	0/5	0/5	
	20	6/6	0/6	2/6	
	25	6/6	0/5	0/5	
	30	6/6	1/5	2/5	
	35	n/a	4/5	3/5	
	45	n/a	5/5	4/5	
	60	n/a	5/5	5/5	
Grasp	13	0/6	0/6	0/6	5
	15	0/6	0/5	0/5	
	20	2/6	0/6	0/6	
	25	6/6	0/5	1/5	
	30	6/6	4/5	4/5	
	35	n/a	5/5	4/5	
	45	n/a	5/5	5/5	
	60	n/a	5/5	5/5	
Retract	13	6/6	0/6	0/6	7
	15	6/6	0/5	0/5	
	20	6/6	0/6	1/6	
	25	6/6	0/5	3/5	
	30	6/6	3/5	4/5	

	35	n/a	5/5	5/5	
	45	n/a	3/5	4/5	
	60	n/a	5/5	5/5	

2.3.3.3 Map size. The rats weights were strongly correlated with the number of responsive points in the saline, $r=0.927$, $p<0.001$, and bicuculline motor maps, $r=0.902$, $p<0.001$, indicating that as the weights of the rats increased, the number of responsive forelimb points also increased, see figure 2.7.

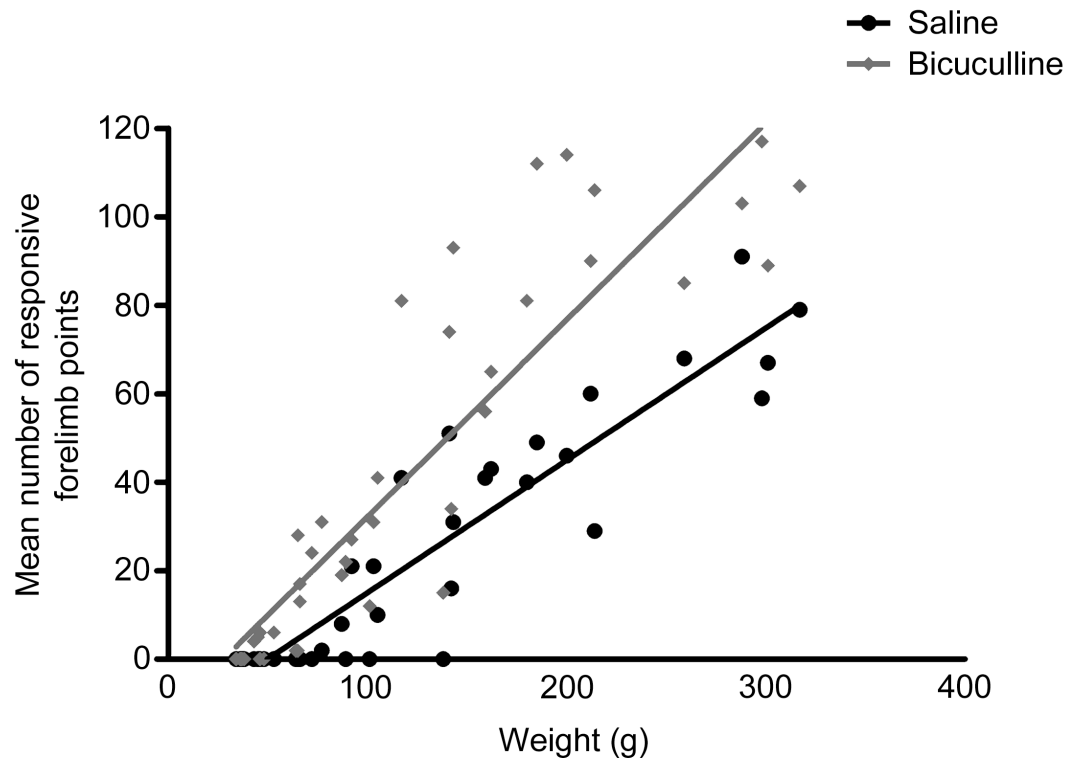


Figure 2.7. The correlation between the number of responsive forelimb points in a saline (black circles) and bicuculline (grey diamonds) maps relative to the weights of the rats.

2.3.4 Reach training qualitative measures

Results of the Mann-Whitney U test revealed that the mean rank score of supination 2 in the skilled reach training group was significantly lower than the mean rank score of the unskilled reach training group ($U=405.00$, $Z=-1.87$, $p=0.003$) indicating more impairment in the unskilled condition (see figure 2.8). Supination 1 was trending on significance ($U=480.00$, $Z=1.87$, $p=0.062$). During reach training, however, it was obvious that the main cause of unsuccessful reach attempts was the inability of rats to advance the forelimb far enough forward to contact the pellet. Since skilled reach training has been known to produce proficient reachers and poor reachers (Henderson, Pittman, & Teskey, 2012), the data was reanalyzed after separating these groups, using a cut off of 55 percent success. Results revealed a significantly higher mean rank score of the advance and grasp movements for poor reachers compared to the proficient reachers ($U=22.50$, $Z=-4.32$, $p<0.001$ and $U=37.50$, $Z=-3.67$, $p<0.001$, respectively) indicating more impairment of the movement (see figure 2.8).

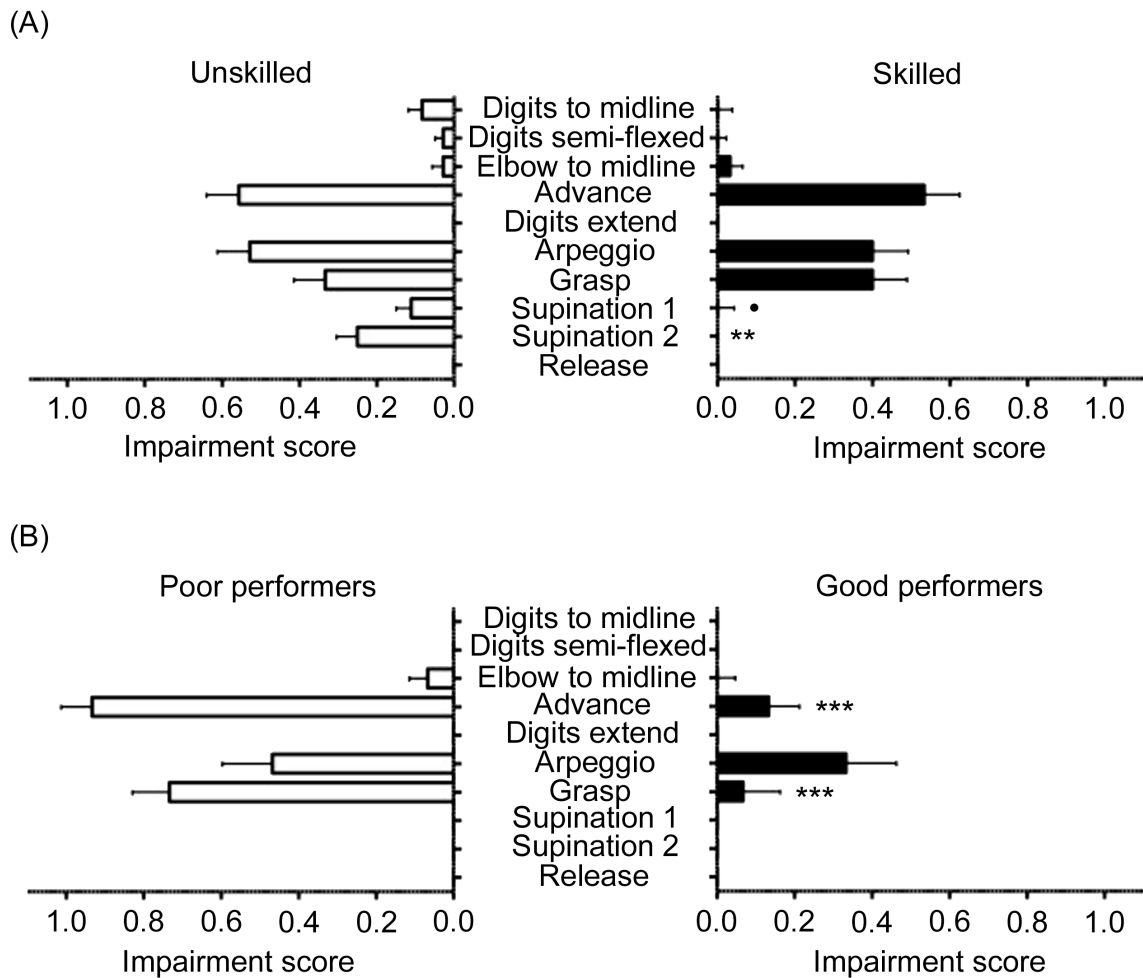


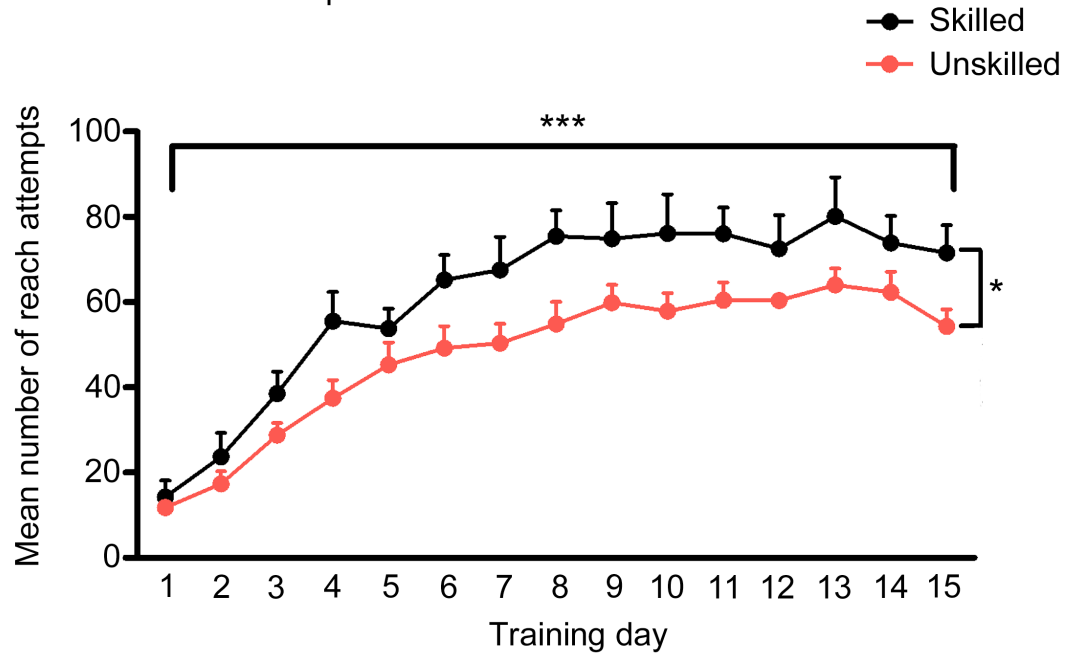
Figure 2.8. The forelimb movements involved in reaching on a scale from 0 to 1, zero indicating that the movement was present and not impaired, and 1 indicating that the movement was not present or impaired. The difference in performance on single pellet reaching between (A) skilled ($n=6$) and unskilled reach trained rats ($n=8$) and (B) poor ($n=3$) and good skilled reach trained rats ($n=3$). ($\bullet p<0.07$, $**p<0.01$, $***p<0.001$).

2.3.5 Reach training quantitative measures

The number of total reach attempts and the number of successful reach attempts were each analyzed in a one-way repeated measures ANOVA. Results revealed a significant difference in reach attempts across training days in the skilled, $F(14,112)=24.01, p<0.001$ and unskilled reach training conditions $F(14,140)=25.82, p<0.001$. Specifically, a significant increase in reach attempts from training day 1 and day 15 occurred in the skilled, $t(8)=10.77, p<0.001$, and unskilled reach training conditions $F(1,10)=98.12, p<0.001$, indicating that the rats learned the task, see figure 2.9. Furthermore, the skilled reach training group had significantly more reach attempts on training day 15 compared to the unskilled reach training group, $t(18)=2.36, p=0.030$. This result cannot be explained by a difference in the number of food pellets given throughout reach training, as there was no difference between the number of pellets given to each training group (skilled vs. unskilled), $F(1,17)=1.49, p=0.239$, see figure 2.10.

The percent of successful reach attempts varied from training day 1 to 15 in the skilled reach training group, $F(14,112)=8.79, p<0.001$. Specifically there was a significant increase in the percent of successful reach attempts between training day 1 and 15, $t(8)=6.27, p<0.001$, indicating that the rats became proficient at the task. In addition, on training day 15, all rats underwent a testing day and the percent of successful reach attempts was collected and analyzed. Results revealed that on training day 15, rats in the skilled condition had a significantly higher percent of successful reach attempts than rats in the unskilled condition, $t(18)=2.73, p=0.014$, see figure 2.9.

(A) Number of reach attempts



(B) Percent of successful reach attempts

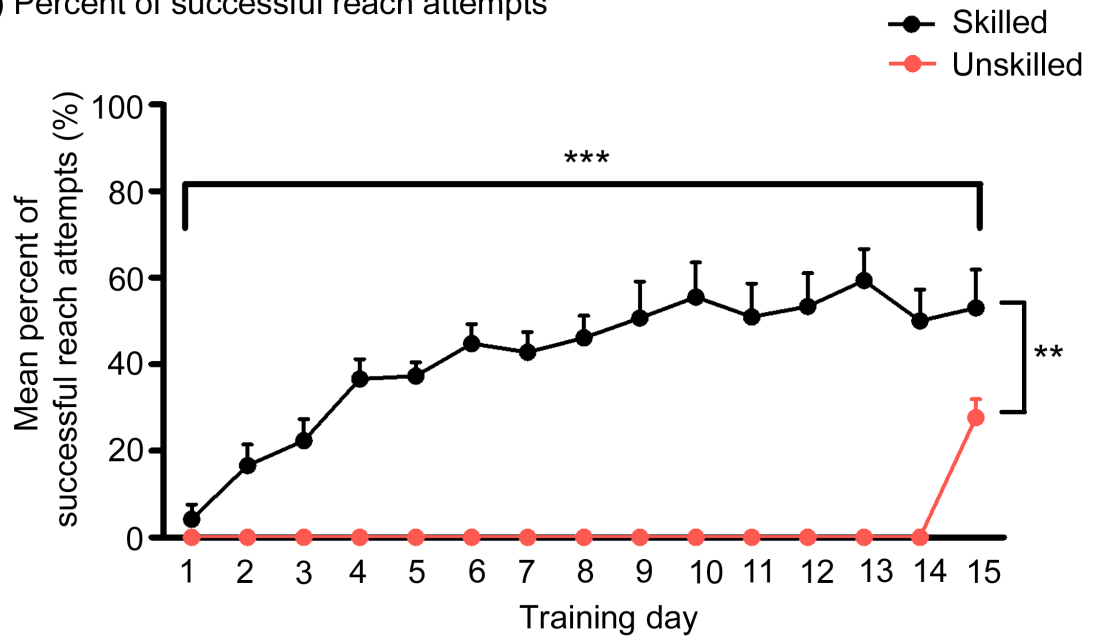


Figure 2.9. The mean number of (A) reach attempts and (B) the percent of successful reach attempts across skilled (black) and unskilled (red) reach training groups and training day (1-15). Error bars represent the standard error of the mean. (* $p < 0.05$, ** $p < 0.025$, *** $p < 0.001$).

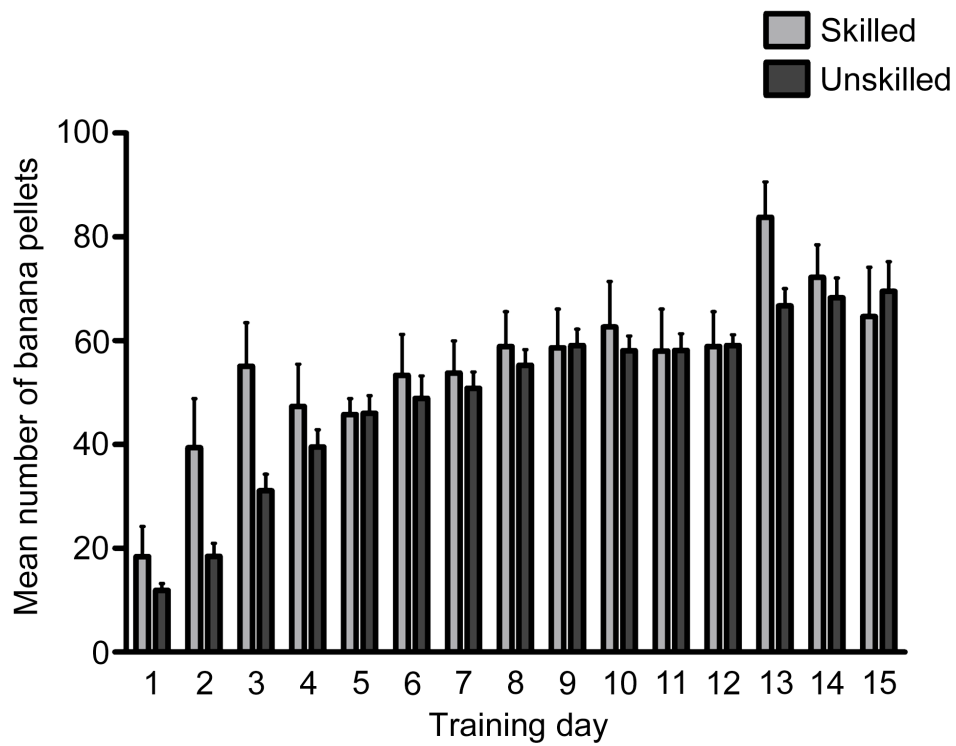


Figure 2.10. The mean number of banana pellets administered during reach training across reach training days (1-15) and between training groups (skilled vs. unskilled). Error bars represent the standard error of the mean.

2.3.5.1 LD-ICMS anesthetics for reach trained rats. The amount of ketamine (mg/kg/minute) and xylazine (mg/kg/minute) were each analyzed in a one-way between-subjects ANOVA with mapping condition (Skilled reach training with a cortical application of saline, Skilled reach training with a cortical application of bicuculline, Unskilled reach training with a cortical application of saline and unskilled reach training with a cortical application of bicuculline) as the between-subjects factor. Results revealed a non-significant difference in ketamine and xylazine administration across mapping conditions, $F(3,16)=1.335, p=0.298$ and $F(3,16)=1.20, p=0.341$, respectively (see figure 2.11).

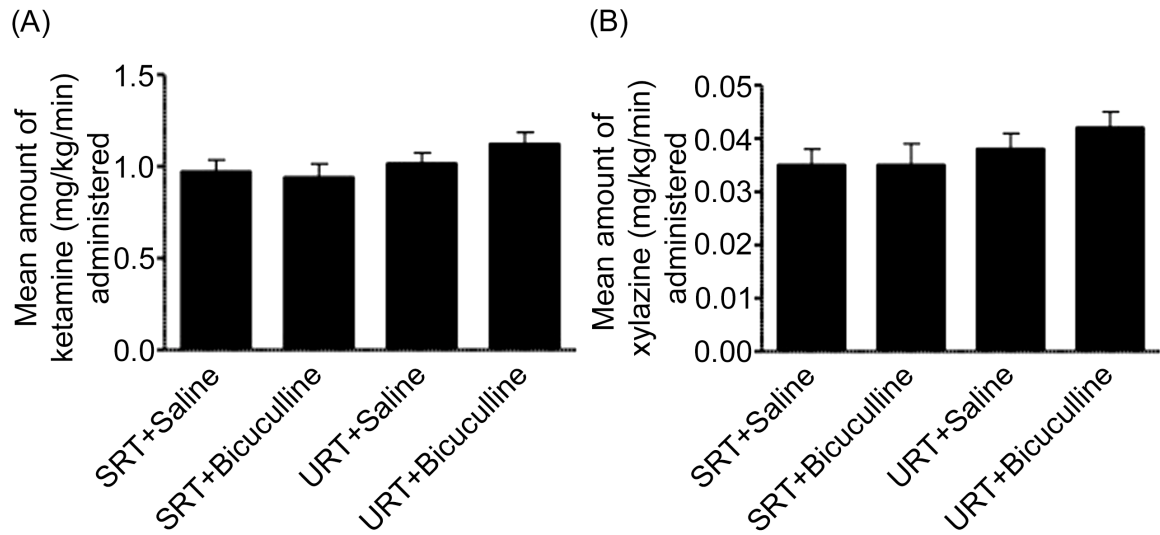


Figure 2.11. The mean amount of (A) ketamine (mg/kg/minute) and (B) xylazine (mg/kg/minute) administered across rats in the skilled reach training condition (SRT) or unskilled reach training condition (URT) and who received a cortical application of saline or bicuculline. Error bars represent the standard error of the mean.

2.3.5.2 Cortical application of saline. Five rats in the skilled and six rats in the unskilled reach training groups underwent LD-ICMS on the contralateral and ipsilateral hemispheres to the reach-trained forelimb with a cortical application of saline. It was hypothesized that the hemisphere contralateral to the reach-trained forelimb would contain more grasp movements than the ipsilateral hemisphere in the skilled but not unskilled reach training group. There was no significant difference in map size between hemispheres in the skilled ($t(4)=0.29, p=0.781$) or unskilled groups, $t(5)=1.67, p=0.158$. When comparing the number of grasp movements between hemispheres, results revealed that there were significantly more grasp points in the contralateral hemisphere than in the ipsilateral hemisphere in the skilled, $t(4)=2.75, p=0.051$ and unskilled training groups, $t(5)=2.44, p=0.058$, see figure 2.12, 2.13 and 2.14.

In order to reveal what other movements may have changed to compensate for the larger number of grasp points, planned paired sample t-tests were performed between the contralateral and ipsilateral hemisphere of the reach trained forelimb with all of the movement categories: wrist extension, elbow flexion, grasp points in the RFA, grasp points in the CFA, advance, retraction, digit extension, elevate, supination, elbow flexion, elbow/wrist/digit extension. In the skilled reach training group, results revealed that retraction was the only movement that had a significantly larger number of responsive points in the contralateral compared to the ipsilateral hemispheres, $t(4)=4.36, p=0.012$, see figure 2.13). In the unskilled reach training group, results revealed that there were significantly more wrist movements in the contralateral compared to ipsilateral

hemisphere, $t(5)=2.77$, $p=0.039$, and significantly less and elbow movements in the contralateral compared to ipsilateral hemisphere, $t(5)=3.21$, $p=0.024$, see figure 2.14).

(A) Skilled reach training

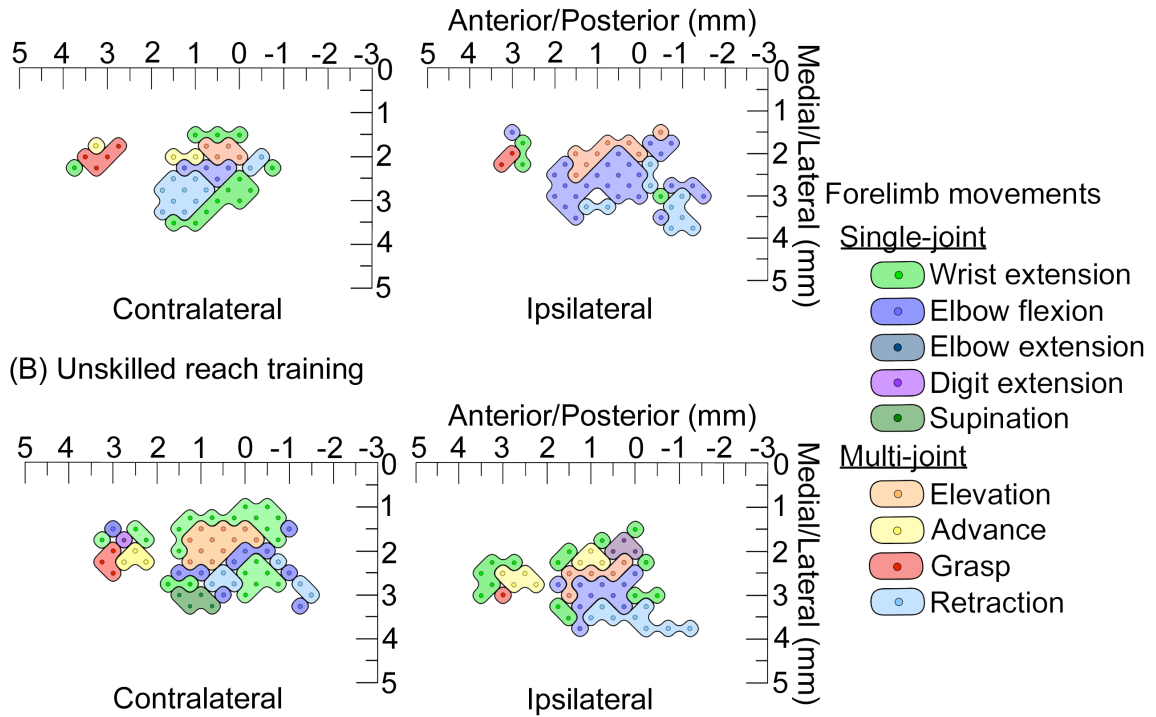


Figure 2.12. Representative saline LD-ICMS motor maps of the contralateral and ipsilateral hemispheres of one rat in each the (A) skilled and (B) unskilled reach trained conditions.

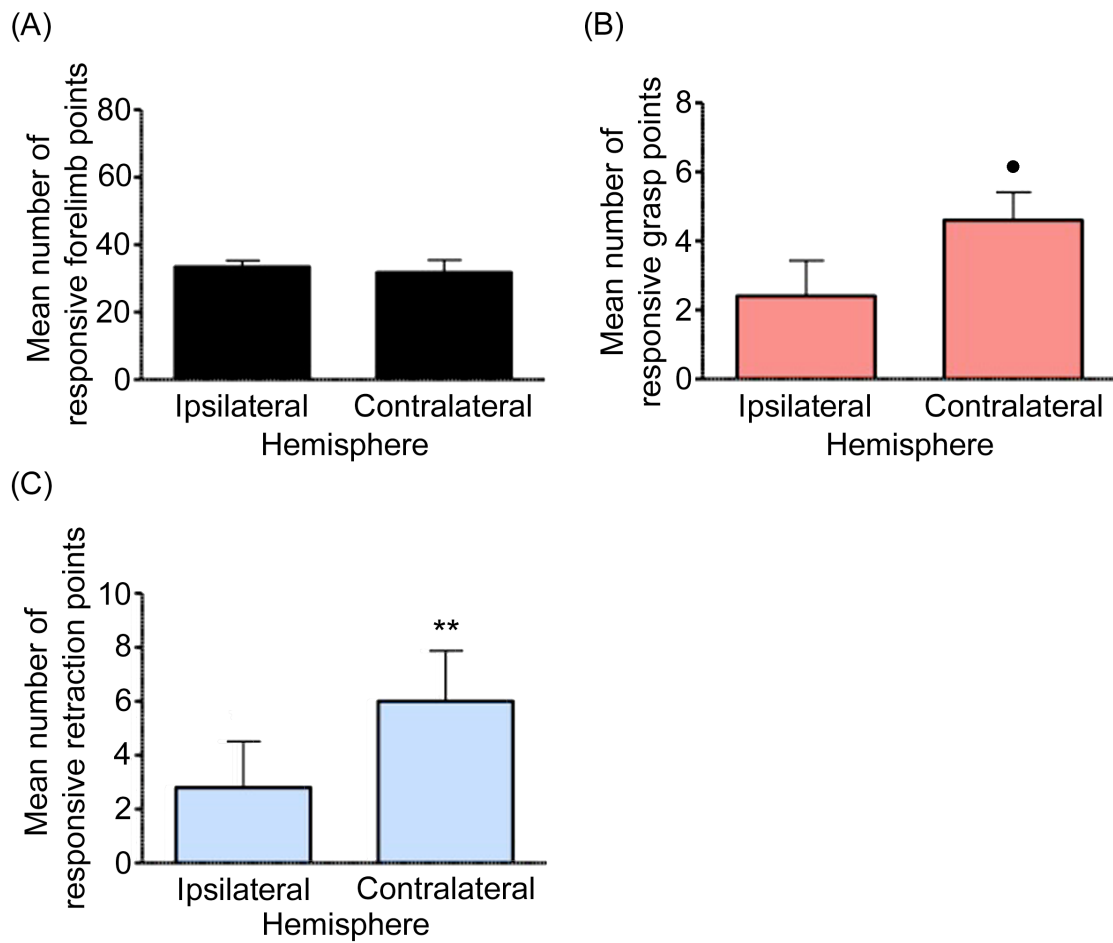


Figure 2.13. The mean number of responsive (A) total forelimb, (B) grasp, and (C) retraction points as a function of hemisphere (contralateral vs. ipsilateral) of rats that completed skilled reach training and underwent a cortical application of saline during LD-ICMS. Error bars represent the standard error of the mean. (• $p < 0.06$, ** $p < 0.025$).

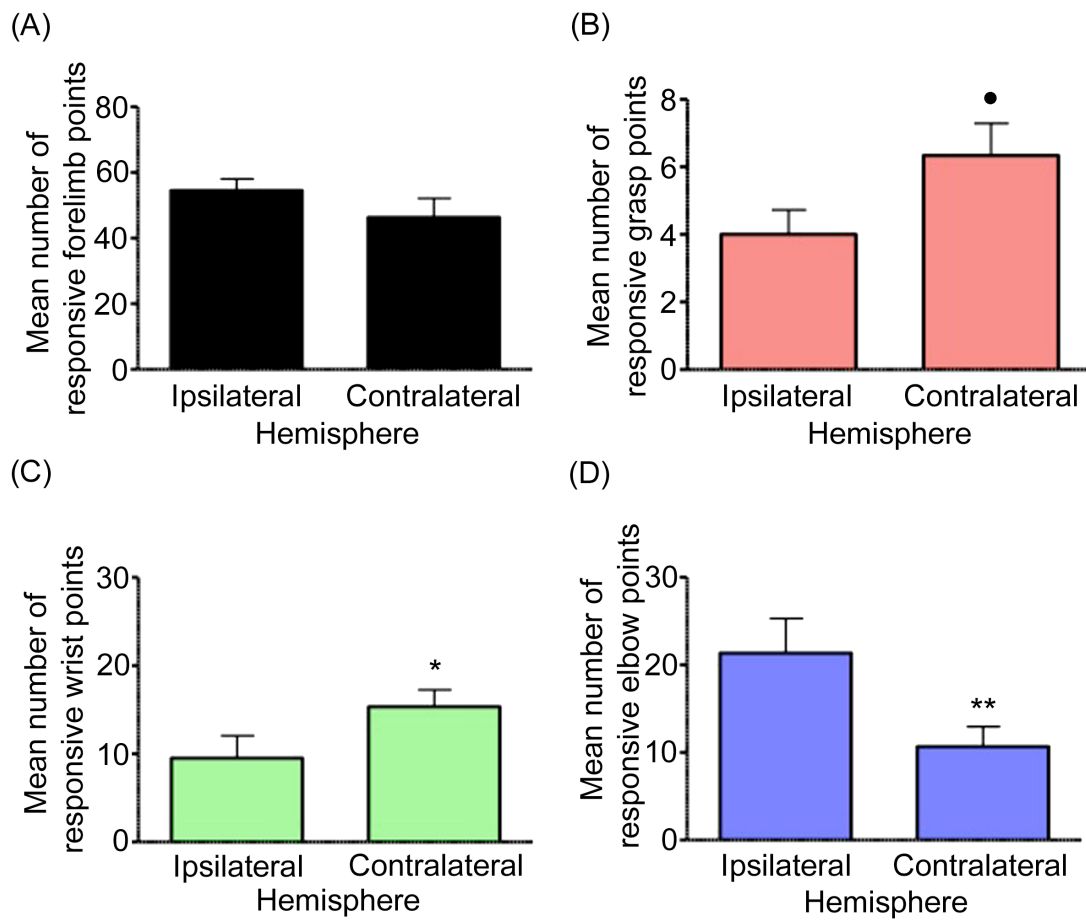


Figure 2.14. The mean number of responsive (A) total forelimb, (B) grasp, (C) wrist, and (D) elbow points as a function of hemisphere (contralateral vs. ipsilateral) of rats that completed unskilled reach training and had a cortical application of saline during LD-ICMS. Error bars represent the standard error of the mean. (• $p < 0.06$, * $p < 0.05$, ** $p < 0.025$).

2.3.5.3 Cortical application of bicuculline. Four rats in the skilled and five rats in the unskilled reach training group underwent ICMS on the contralateral and ipsilateral hemispheres to the trained forelimb with a cortical application of bicuculline. The total map size of the contralateral and ipsilateral hemisphere of the skilled and unskilled reaching condition were not significant different ($t(3)=-1.97, p=0.143$ and $t(4)=0.20, p=0.851$, respectively; see figure 2.17 and 2.18). There was also no significant difference between the grasp regions of the contralateral and ipsilateral hemispheres in the skilled and unskilled reach training groups, $t(3)=0.378, p=0.731$ and $t(4)=1.30, p=0.263$, respectively see figure 2.15, 2.16 and 2.17).

In order to identify if other movements may have differed between hemispheres, planned paired sample t-tests were performed between the contralateral and ipsilateral hemisphere of the reach trained forelimb with all of the movement categories: wrist extension, elbow flexion, grasp points in the RFA, grasp points in the CFA, advance, retraction, digit extension, elevate, supination, elbow flexion, elbow/wrist/digit extension. Results revealed that there were significantly more retraction movements in the ipsilateral compared to contralateral hemisphere in the skilled reach training group, $t(5)=5.55, p=0.012$, see figure 2.16) and no differences in movements between hemispheres in the unskilled reach training group.

(A) Skilled reach training

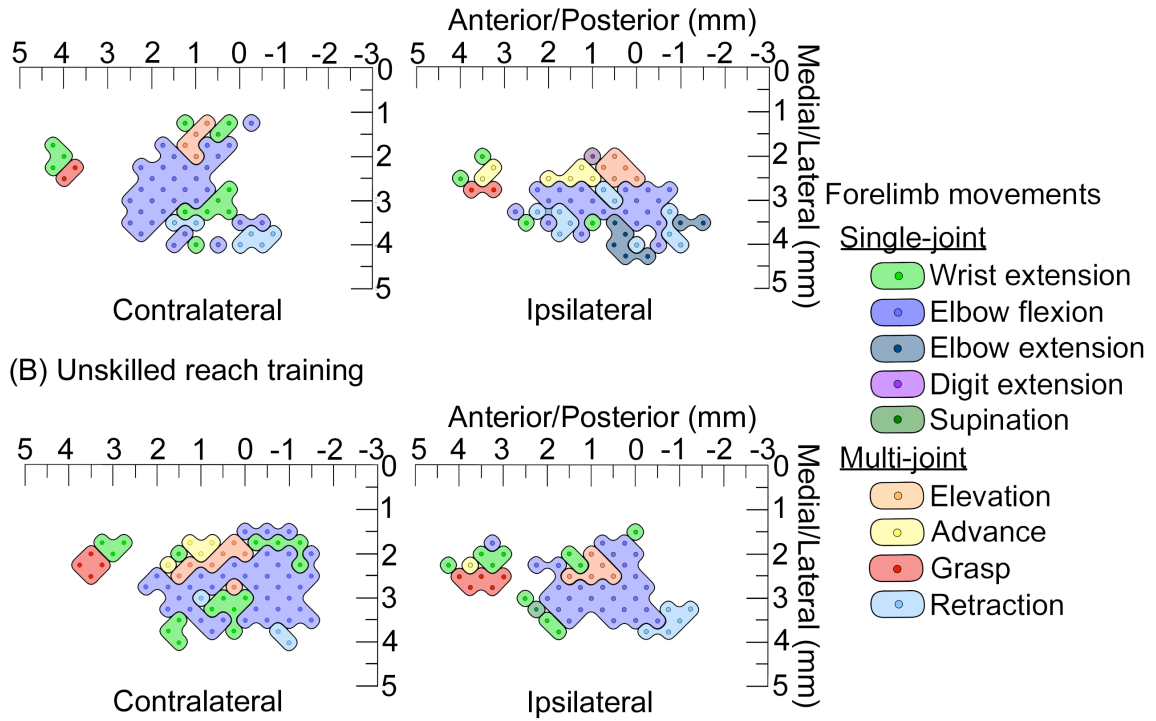


Figure 2.15. Representative bicuculline LD-ICMS motor maps of the contralateral and ipsilateral hemispheres of one rat in each the (A) skilled and (B) unskilled reach trained conditions.

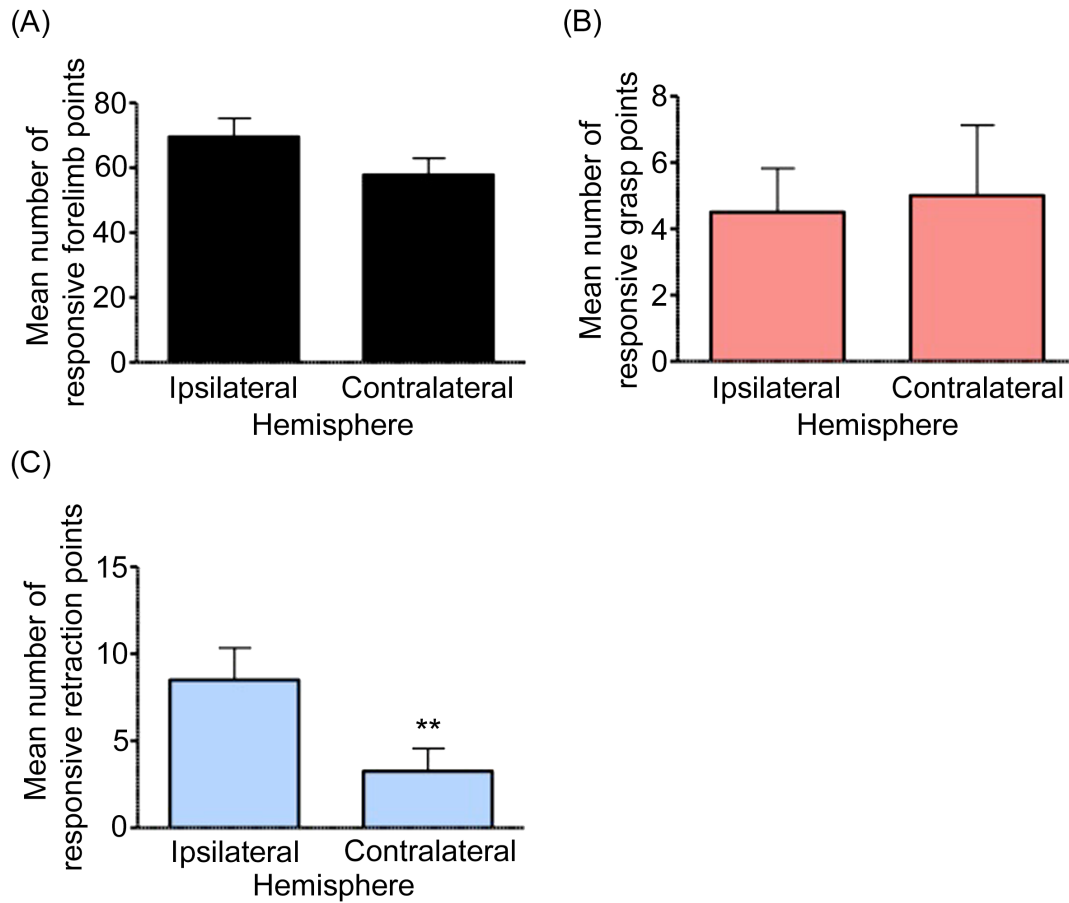


Figure 2.16. The mean number of responsive (A) total forelimb, (B) grasp, and (C) retraction points as a function of hemisphere (contralateral vs. ipsilateral) of rats that completed skilled reach training and underwent a cortical application of bicuculline during LD-ICMS. Error bars represent the standard error of the mean. (** $p < 0.025$).

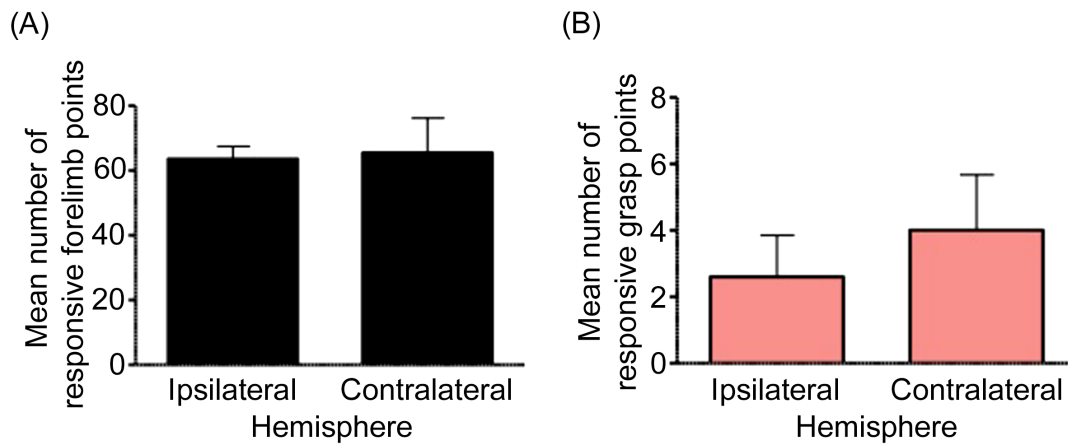


Figure 2.17. The mean number of responsive (A) total forelimb, and (B) grasp points as a function of hemisphere (contralateral vs. ipsilateral) of rats that completed unskilled reach training and underwent a cortical application of bicuculline during LD-ICMS. Error bars represent the standard error of the mean.

2.4 Discussion

The present study was the first to demonstrate the development of LD-ICMS evoked forelimb motor maps in rats. First, I found that LD-ICMS evoked multi-joint movements, such as elevate, advance, grasp and retract, develop later within the neocortex than single-joint movements, indicating that the cortical connections associated with multi-joint movements may need more time to develop. I also found that grasp movements are not restricted to the “grasp region” throughout development, revealing for the first time that the topographic representations of multi-joint movements undergo a large change as rats develop, leaving a refined grasp region in adulthood. Second, the present study found that rats were behaviourally able to perform elevate, advance, grasp and retract before these multi-joint movements were elicited with LD-ICMS. This result indicated that subcortical structures were likely mediating behaviours before the maps appeared, whereas the neocortex may be functioning to refine movements and cortical connections in response to environmental changes. Finally, the present study was the first to demonstrate that unlike in adulthood (Ramanathan et al., 2006; Brown & Teskey, 2014), single-pellet skilled reach training can alter the motor maps of complex movements in developing rats. Specifically, skilled reach training resulted in an increase in the number of grasp points within the motor map contralateral to the reach-trained forelimb. This novel finding indicates that motor maps may be more plastic throughout development than in adulthood.

2.4.1 Behavioural development, LD-ICMS Development and Bicuculline

Throughout development, rats first manipulated food at PND 20. By PND 30 rats

were spending almost three times longer manipulating food than at PND 20 or 25, indicating a significant improvement in manual dexterity. At PND 20, rats used the power grip, to hold any food that was manipulated, which requires gripping food with all digits simultaneously. As the rats got older, the precision grip was used, which involved holding an object between the first two digits and the thumb-like stump (Whishaw and Coles, 1996). This progression from general grasping to precise use of the digits is similar to the development of manual dexterity in children. When children first learn how to grasp objects, they consistently use the power grip. As the child gains skill and practice with the hands and digits, the child develops individual use of the digits and is able to produce a precision grasp with the index finger and the thumb (Berthier et al, 1999). This development of skilled digit use is thought to be a result of axon terminal specification to the topographic organization within the ventral horn of the CST in both humans (Berthier et al, 1999) and rats (Terashima, 1995). In rats, the maturation of this axonal specification occurs between PND 21 and PND 28 (Chung & Coggeshall, 1987), which is only slightly earlier than the LD-ICMS elicited behaviours were observed at PND 30.

This study was the first to demonstrate the development of LD-ICMS forelimb motor maps with a cortical application of saline or bicuculline from PND13 to PND 90. In both conditions, the total number of responsive forelimb points increased with age, even after accounting for the effects of ketamine and xylazine administration during the LD-ICMS procedure. Similar to the findings of Young and colleagues (2012), the motor map size of rats increased as the weight of the rats increased. In addition, the number of responsive forelimb points for all movement categories (wrist extension, elbow flexion, supination, digit extension, elevate, advance, grasp, retract) except for saline digit

extension and saline and bicuculline elbow extension increased with age, indicating that movements were occupying more area within the motor cortex as the rats developed. This is not surprising due to the increase in cortical area that occurs in rats throughout development, which corresponds to an increase in the number of neurons within the cortex and an increase in dendritic and axonal density (Eayrs & Goodhead, 1959). LD-ICMS stimulates groups of pyramidal neurons within layer V of the motor cortex (Graziano et al., 2002; Bonazzi et al., 2013; Ramanathan et al., 2006; Brown & Teskey, in revision), and since the number of neurons and cortical area increases, the area from which LD-ICMS can evoke movements also increases.

After reducing cortical inhibition with a cortical application of bicuculline, LD-ICMS motor maps contained significantly more responsive forelimb points than saline maps across all age groups except for PND 13, which had no responsive points in both drug conditions. LD-ICMS with a cortical application of bicuculline also resulted in responsive forelimb points at PND 15, which is approximately when the CST becomes myelinated (Canty & Murphy, 2008). Under standard mapping conditions with a cortical application of saline however, responsive points could not be evoked until PND 30. These converging results are important as they further support the finding that bicuculline, which decreases the GABAergic inhibition, (Velíšková et al., 1990) decreases movement thresholds and allows responsive forelimb movements to be revealed (Young et al., 2012; Brown & Teskey, in prep).

At PND 13, movements were not evoked using LD-ICMS, despite the rats being behaviourally capable of complex movements such as advance, elevate and retract. Although reducing cortical inhibition is an important factor in evoking movements during

LD-ICMS, eliciting movements also relies on the level of synaptic connectivity within layer V of the neocortex (Monfils et al., 2005) and the development and myelination of the CST (Piecharka et al., 2005). Low levels of synaptic connectivity have been found within layer V of the rat cortex at PND 12, where there is very little overlap between the dendritic fields of nearby neurons compared to adult neurons (Eayrs & Goodhead, 1959). A signal from the layer V pyramidal neurons needs to be strong enough to descend the CST to reach the ventral horn, then indirectly synapse onto lower motor through interneurons in order to create a muscle movement (Isa et al., 2007; Lemon, 2008). Although myelination increases the speed of the signal through an axon and promotes neuron-to-neuron communication (Sherman & Brophy, 2005), myelination develops over time. A transcranial magnetic stimulation (TMS) study measuring the conductance of corticospinal axons in humans revealed that the latency to produce bicep and digit movements was significantly longer in neonates than in adults. The movement latency, however, dropped significantly between 6 and 18 months of age, reaching adult-like speeds by 24 months (Eyre et al., 2000). A similar axonal conductance speed has been found in the corticospinal axons of newborn macaque monkeys (Olivier et al., 1997) indicating that human and monkey corticospinal axons are likely poorly myelinated at an early age. In addition, the TMS threshold required to elicit a skilled and distal digit movement was significantly higher than the threshold to elicit a proximal forelimb movement in infants (Muller, Homberg, Lenard, 1990), indicating that the CST may need more time to develop and myelinate before grasp movements can be elicited. Myelination of the forelimb projection of the rat CST begins at PND 10, however this process is not completed until PND 28 (Canty & Murphy, 2008). If the descending signal is not strong

enough due to lack of myelination, small axon diameter, or an interruption of the signal to the lower motor neurons due to a lesion, the muscle movements cannot be evoked using the ICMS procedure (Piecharka et al., 2005). Taken together, it is possible that these weak intracortical signals combined with incomplete myelination of the CST are contributing factors to the inability to produce a complex movement under the current LD-ICMS parameters at PND 13, despite the rat being behaviourally able to do so.

Alternatively, although the motor cortex is important for the output of voluntary motor behaviour, it is not the sole executer; there are other structures that likely mediate the motor behaviours before movements are expressed within the LD-ICMS motor maps. For example, five days after injecting a retrograde transporter into the forelimb region of M1 in a monkey, many second-order neurons, which project from the spinal cord and thalamus, were labeled within the output nuclei of the basal ganglia and the cerebellum (Hoover & Strick, 1993, Zemanick et al., 1991). The basal ganglia are thought to send signals to the primary motor, premotor and prefrontal cortices, which are essential for planning movements (Alexander, DeLong & Strick, 1986). The basal ganglia and the motor cortex are both important structures within the thalamocortical circuit, which gathers signals from the frontal cortex and sends them through the putamen to the globus pallidus then thalamus and back to the cortex to produce a movement (Johnston & Hoon, 2000). For example, if the GPi is overactive in children (Johnston & Hoon, 2000) or adults (Hoover & Strick, 1993), the thalamocortical projections are silenced, resulting in the inability to initiate movements. Although LD-ICMS-evoked movements mimic natural forelimb movements in a rat by simulating layer V pyramidal cells (Ramanathan et al., 2006, Bonazzi et al., 2013, Brown & Teskey, 2014), the lack of input from the

other motor-loop structures may be why we do not see movements within the LD-ICMS motor map at the same time that the rat is behaviourally able to perform it.

The observation of rats being able to behaviourally complete movements before those movements were present within the LD-ICMS motor map occurred throughout development. Retraction movements emerged within the bicuculline motor maps (PND 25) twelve days after the rat pups were behaviourally able to perform those multi-joint movements, whereas advance and elevate were present within the bicuculline maps at PND 20 and 30, respectively, which is approximately 7 and 17 days after the behaviour was performed. Furthermore, rats were capable of grasping vermicelli noodles and a variety of food pellets at PND 20, which is consistent with the approximate weaning period where rats are required to manipulate their food (Karl & Whishaw, 2013). The first grasp point, however, was not seen within a motor map until five days later at PND 25 under bicuculline. This delay of LD-ICMS evoked movements within the motor map after acquiring a new ability is consistent with results found after skilled reach training. Typically, two weeks of single-pellet skilled reach training causes a reorganization of movements within a motor map. However, this reorganization does not occur if training is stopped before the acquisition phase, where rats experience a significant increase in success with the task and the movement has been consolidated (Kleim et al., 2004). A similar occurrence can be found in humans, where after five days of learning digit techniques on the piano, the representation of the digits expands within the contralateral hemisphere. This change, however, was not seen in the untrained hand (Pascual-Leone et al., 1995). It appears the neocortex and corresponding motor maps may be refining the function of subcortical structures by responding to changes within the environment

(Teskey & Valentine, 1998). The strengthening or pruning of neural connections that occurs after environmental changes, or learning, refines the abilities of the rats, resulting in better behavioural performance. Movements are therefore refined and revealed within the LD-ICMS motor maps after the rat is behaviourally able to perform them.

The rat cortex is thought to be extremely plastic throughout development until it reaches its adult form (Young et al., 2012). LD-ICMS evoked movements have been found to be very consistent in terms of orientation within motor maps of adult rats. The present study found similar results at PND 60, with the RFA or the “grasp region” located consistently around 3mm anterior and 2mm lateral from bregma (Brown and Teskey, 2014). A consistent clustering of grasping movements, however, was not found throughout development. Specifically, there were many grasp points located in the CFA at PND 30, 35 and 45 whereas grasp movements were more consistently located within the grasp region at PND 60 and PND 90. This finding has also been identified in the auditory cortex, where the topography of low to high characteristic frequency tones (tonotopy) has been found to reorganize throughout development (Kandler, Clause & Noh, 2009). High frequency tones are heard easily during childhood, however these tones become pruned and inaudible by adulthood (Sanes, Merickel, & Rubel, 1989). A similar trend of tonal pruning and specification occurs in cats (Kandler, Clause & Noh, 2009), gerbils (Sanes, Merickel & Rubel, 1989), rats (Chang & Merzenich, 2003) birds and reptiles (Mann & Kelley, 2011). This pruning and cortical plasticity is experience-dependent, therefore tonal critical periods can be extended or shortened based on early life experiences (Sanes et al., 1989; Chang & Merzenich, 2003). Based on this information, it is possible that this study has found a similar result of cortical

reorganization and pruning within the sensorimotor cortex. In the future it would be essential to alter the environment of developing rats to identify if corresponding motor map changes occur. For example, enriched environments, which are conducive to complex movements such as climbing and manual manipulations, have been found to significantly increase map size in rats that underwent SD-ICMS (Young et al., 2012). Since LD-ICMS contains many more movement categories than SD-ICMS, it would be interesting to see what movements are contained within these larger maps after this complex forelimb training.

2.4.2 Skilled Reach Training

In the present study, rats were trained on a single-pellet reaching task. Rats in the skilled reach training condition successfully learned and became proficient at the task, evident by their high reach attempt and percent of successful reach attempt rates. The unskilled reach condition required that the rats reach for a banana pellet, however the pellet was removed before the rat was able to touch, grasp or consume the pellet.

Although the skilled reach training group achieved a significantly higher percentage of successful reach attempts than the unskilled reach training group on the final test day, the skilled group also had a significantly greater number of reach attempts than the unskilled group. This result was not due to a difference in banana pellet rewards administered during training as this was balanced between groups. Similar findings have occurred when using unskilled reach training, where the unskilled reach training group made significantly less reach attempts than the skilled reach training group until the 14th and 15th day of training (Monfils & Teskey, 2004). It is unclear why the present experiment

did not see this matching of reach attempts toward the end of training, however the significant difference between the percent of successful reach attempts between groups is consistent with prior studies (Monfils & Teskey, 2004; Young et al., 2012).

Typically, reach training causes a distal expansion or reorganization when mapping with SD-ICMS (Young et al., 2011b). Since LD-ICMS motor maps have many more movement categories than SD-ICMS maps, it was hypothesized that the number of grasp movements would increase in the contralateral hemisphere to the reach trained forelimb compared to the ipsilateral hemisphere. After statistically controlling for the amount of ketamine and xylazine administered across groups, results revealed that this hypothesis was not supported for rats that were mapped with a cortical application of bicuculline. Bicuculline has been found to significantly increase the size of motor maps by lowering movement thresholds (Young et al., 2012; Brown & Teskey, in prep), whereas reach training has mainly been found to cause a higher percentage of distal than proximal forelimb movements within the motor map (Young et al., 2012). It is therefore possible that the large effect of bicuculline overshadowed the reorganization that typically occurs after single pellet skilled reach training.

In addition, the variability of the number of grasp movements within the bicuculline maps was larger than with the saline maps, making an overall group difference difficult to find. Although bicuculline can reveal responsive grasp points that were previously unresponsive by lowering movement thresholds, it can also alter responsive movements, therefore eliminating grasp points that were previously present. This result was obvious during the developmental LD-ICMS study, whereby a movement elicited at a certain cortical location mapped under saline would change after being

mapped with bicuculline. For example, after completing a LD-ICMS motor map with saline at PND 60 (Rat 100), there were 13 movements that had changed from their original movement to a different movement during the bicuculline remap. The main issue is that bicuculline can at times lower cortical inhibition too much, which causes tonic-clonic seizures of the forelimb (Velíšková et al., 1990). The movement therefore becomes masked by the seizure during the LD-ICMS. Maintaining a constant level of bicuculline within a cortical application was difficult and was a constant struggle throughout the experiment. In the future, it would be interesting to use a different method of lowering cortical inhibition if possible in order to avoid these difficulties.

A significant increase in grasp points was found when LD-ICMS was performed with a cortical application of saline in skilled reach trained rats. Surprisingly this result was also found in the unskilled reach training condition with a cortical application of saline. After analyzing the reach training videos, it was obvious that rats in the unskilled condition were still achieving the grasp motion. The main issue with the unskilled reaching technique was that the advance movement was not extending far enough forward, causing rats to grasp the pellet with the ends of the digits. Results did not, however, reveal a significant difference in the number of advance movements between hemispheres. After reviewing the behavioural reaching videos, there were some rats in the skilled reach training group that were exhibiting similar deficits in the advance action. It has previously been found that there are good performers and poor performers within a skilled reach training group (Henderson, Pittman, & Teskey, 2012). Once these groups were distinguished, the poor performers used a poor advancing technique more often by than the good performers. Due to this high variability between movement proficiency

within the skilled reaching group, it may have been difficult to reveal a difference in the number of advance movements within the motor map. In the future, it would be essential to have a higher percent success inclusion threshold for skilled reach training in order to be confident in the mapping differences.

Alternatively, the advance movement involves the anterior displacement of the shoulder and the extension of the wrist. The anterior displacement of the shoulder is a proximal movement, which typically does not expand after skilled reach training under SD-ICMS; in fact, the number of proximal movements decreased to account for the expansion of the distal movements (Young et al., 2012). Since there are minimal advance movements typically found in the LD-ICMS maps, this difference would be difficult to uncover. Although there are distal movements within the reaching motion, it appears that the specificity required to grasp the pellet by closing the digits may cause more of a plastic change than the proximal movement of the shoulder. In the future, an untrained reach training group should be added to the analysis. This group would be placed in the reach training box with banana pellets for 15 minutes per day for 15 days but would not reach. This group would be a good comparison to identify if it is the successful grasp that is necessary to cause a plastic change in motor maps.

In conclusion, the present study found that LD-ICMS movements could not be evoked at PND 13, despite the rats being behaviourally capable of quite complex movements. This result may be due to a combination of heightened cortical inhibition (Dzhala et al., 2005; Miles, 1999; Rivera et al., 1999), a low level of synaptic connectivity within layer V of the neocortex (Eayrs & Goodhead, 1959) and an underdeveloped CST (Piecharka et al., 2005). This study also supported previous findings

that bicuculline lowered cortical inhibition (Young et al., 2012) and allowed forelimb movements to be evoked using LD-ICMS much earlier in development (PND 15) than is possible mapping with a cortical application of saline (PND 30). Early in development, movements within the map were simple, however as the rat got older and heavier, complex movements began to appear. Furthermore, there were significantly more grasp movements within the contralateral map compared to the ipsilateral saline map, however the advance region remained the same, which demonstrated a reorganization of distal rather than proximal forelimb points. Finally, grasp movements were not restricted to the grasp region throughout development as they are in adulthood indicating that there may be cortical reorganization and pruning that occurs within the sensorimotor cortex that is similar to the tonotopic reorganization that occurs within the auditory cortex (Kandler, Clause & Noh, 2009; (Sanes, Merickel, & Rubel, 1989). In the future, it would be interesting to see how enriched environments alter the developing motor maps and it would be ideal to have an unskilled reach training group to compare the skilled and unskilled mapping results.

Chapter Three: **General Discussion**

My goal during this thesis was to investigate how changes in the behavioural capacity and proficiency of the rat forelimb related to changes in the functional organization of the motor cortex during development. In addition, I sought to investigate how motor cortical organization during development would be altered during skilled motor learning. Towards these aims, I first monitored six rats for 15 minutes per day at PND 13, 15, 20, 25 and 30 and found that as rats aged, more time was spent manipulating food pellets and manual dexterity improved. I then performed LD-ICMS on rats at PND 13, 15, 20, 25, 30, 35, 45 and 60 and found that multi-joint movements, such as elevate, advance, grasp and retract, developed later than single-joint movements, indicating that time is needed for these movements to develop cortical connections (Eayrs & Goodhead, 1959). Moreover, the present study found that rats were behaviourally able to perform elevate, advance, grasp and retract movements before these multi-joint movements were elicited with LD-ICMS. These results indicated that subcortical structures might be mediating such behaviours and have a function in shaping cortical networks (Alexander, DeLong & Strick, 1986; Zemanick et al., 1991; Hoover & Strick, 1993; Teskey & Valentine, 1998). The neocortex, however, is functioning to refine movements and cortical connections in response to environmental changes (Teskey & Valentine, 1998). Finally, I found that unlike in adulthood, grasp movements were not restricted to the grasp region during development, indicating that cortical reorganization within the sensorimotor cortex may be occurring.

During my thesis research, I found that behavioural movements preceded LD-ICMS evoked movements in rats. There are a few reasons why this result may have

occurred. First, cortical connections are weaker in early development compared to adulthood and myelination of the CST is not complete. Evidence from TMS studies on monkeys (Olivier et al., 1997) and children (Eyre et al., 2000) have revealed that the speed of CST conduction significantly increased across age. In addition, Young and colleagues (2012) found that rat forelimb movements could not be evoked with SD-ICMS until PND 13, even after decreasing cortical inhibition with bicuculline, indicating that cortical excitability may not be strong enough to evoke movements before this age, despite the rat being behaviourally able to perform movements. Second, the motor cortex is one of many cortical structures that aid in the production of voluntary movements. For example, the primary motor, premotor and prefrontal cortices are essential for planning movements (Alexander, DeLong & Strick, 1986). In addition, silencing thalamocortical projections from the output nuclei of the basal ganglia has been found to produce hypokinesia in both children (Johnston & Hoon, 2000) and adults (Hoover & Strick, 1993). In turn, hypokinesia has been associated with decreased SD-ICMS motor map size, indicating a decrease in cortical excitability (Brown et al., 2011). It is therefore possible that subcortical structures are modulating behaviours before movements can be evoked with LD-ICMS, whereas the neocortex may be functioning to refine movements and cortical connections in response to environmental changes or changes in subcortical structure functioning.

In order to develop potential therapeutic solutions for impaired forelimb movements, it is important to understand how the forelimb develops behaviourally and cortically. For example, an ischemic stroke of the middle cerebral artery typically results in hemiparesis in neonates, children and adults; often leaving patients unable to reach for,

and grasp, objects. During post-stroke recovery, it has been widely observed that neonates and children are more likely to recover to normal (or pre-stroke) neurological and behavioural functioning (De Vries & Levene, 2001; Nelson, & Lynch, 2004) when compared to adults (Hendricks et al., 2002). This difference in post-stroke recovery outcome between children and adults has been attributed to a greater degree of cortical plasticity throughout childhood development than in adulthood. The underlying mechanism of this age-dependent cortical plasticity, however, is not understood (Lee et al., 2005; Bernard et al., 2008). Within the auditory cortex, tonotopic frequencies are pruned and reorganized depending on experience (Sanes et al., 1989; Chang & Merzenich, 2003; Kandler, Clause & Noh, 2009; Mann & Kelley, 2011). In my thesis research, I found that grasp movements were not restricted to the grasp region during early development, indicating that experience-dependent reorganization and pruning may also be occurring within the sensorimotor cortex. In addition, it was found that skilled reach training significantly increased the number of responsive grasp points within the grasp region in the contralateral but not ipsilateral hemisphere to the reach trained forelimb of developing rats, but not adults (Ramanathan et al., 2006; Brown & Teskey, 2014). In humans, performing skilled training during CST axonal specification during development can cause significant improvement in reaching behaviour due to morphological and physiological changes of the CST terminals that lead to more effective synaptic activation of spinal motor neurons (Martin, 2005). Together, these findings suggested that the topographic organization of forelimb movements in the rat cortex is more plastic throughout development than in adulthood.

In adult rats, LD-ICMS evoked grasp movements have been found to be restricted to the grasp region. Reversible inactivation of the grasp region has been shown to cause grasp specific behavioural deficits. Impairments in grasping, however, did not occur after inactivating the CFA, which has been found to consist of elevate, advance and retract movements (Brown & Teskey, 2014). An interesting future direction for this study would therefore be to perform cortical cooling in the grasp region and CFA at PND 13, 15, 20, 25, 30, 35, 45 and 60 to identify if a similar impairment in grasping would occur when cooling the grasp region during development as in adulthood. Since the present LD-ICMS development experiment found that the grasp region and the CFA both elicited grasp movements during development, it is possible that inactivating the grasp region earlier in development may spare impairment to the grasping motion. This result would further support the theory of greater plasticity in the neonatal and childhood motor cortices compared to adulthood, and help to explain why neonates and children recover forelimb movements significantly better than adults after a stroke (Lee et al., 2005; Bernard et al., 2008).

After unskilled reach training, the number of LD-ICMS evoked grasp points significantly increased in the hemisphere contralateral, but not ipsilateral, to the reach trained forelimb, indicating use-dependent plasticity. In addition, there was an increase in wrist movements and a decrease in elbow movements in the contralateral hemisphere compared to the ipsilateral hemisphere. This result is consistent with previous results of SD-ICMS motor maps after skilled reach training, where the contralateral hemisphere motor map experienced an increase in the percentage of distal forelimb movements and a decrease in proximal forelimb movements (Kleim et al., 1998; Young et al., 2012).

Although the skilled reach training condition experienced an increase in the grasp region in the contralateral hemisphere, there was no change in the wrist or elbow movements. One limitation to this study was the large degree of variability within the skilled reaching task. In adult rats, it has been found that there are proficient and poor performers within the skilled reach training group (Henderson, Pittman, & Teskey, 2012); therefore it is not surprising that a similar finding would arise in younger rats. This issue, however, should have been addressed before rats underwent LD-ICMS. If I could redo this project, I would assure that only proficient skilled reach trained rats undergo LD-ICMS and are included in the final analysis by implementing a precise cut off of successful reach attempts before training began.

In summary, the present study found rats were behaviourally able to perform elevate, advance, grasp and retract movements before these multi-joint movements were elicited with LD-ICMS, indicating that subcortical structures may be mediating forelimb behaviours. In addition, I found that grasp movements were not restricted to the grasp region during early development, indicating that experience-dependent reorganization and pruning may also be occurring within the sensorimotor cortex. Finally, cortical plasticity was seen in LD-ICMS evoked grasp movements throughout development and after skilled reach training, which does not occur in adult rats. These results may compliment the theory that the cortex is more plastic during development than in adulthood. More research is needed to connect the cortical plasticity found in this study with stroke recovery research.

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