MODELING MIDDLE CEREBRAL ARTERY STROKE IN RATS: AN EXAMINATION OF THE SKILLED REACHING IMPAIRMENTS

OMAR A. GHARBAWIE
Bachelor of Science, University of Toronto, 1999
Masters of Science, University of Lethbridge, 2002

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Abstract

Middle cerebral artery (MCA) stroke can produce chronic incapacitating motor impairments. Understanding the neural basis of the motor syndromes is complicated by the diversity of neural structures damaged but the problem can be addressed in laboratory rats by inducing selective infarcts. Nevertheless, the motor syndromes that ensue from stroke in rats remain poorly understood and undermine its potential as a model for clinical stroke. The objective of the present thesis was to document the skilled reaching impairments from neocortical and subcortical MCA infarcts in rats. In addition, the integrity of the motor system components spared by the infarct was assessed neurophysiologically and neuroanatomically. Characteristic reaching impairments emerged from each infarct but there were also some overlapping features that might be explained by neural dysfunction extending beyond the boundaries of the infarct. The present studies showed that the laboratory rat is an ideal animal model for studying stroke, which should be of interest to both clinical and research scientists studying stroke.

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Chapter 1

General Introduction

Introduction

In 2002, an estimated 300,000 Canadians were living with the effects of stroke according to the Heart and Stroke Foundation of Canada. In each year since, an additional 40,000-50,000 Canadians suffered a stroke. Of every 100 people who suffer a stroke only 10 recover completely. The remainder either: die (15), recover with minor disability (25), are left with moderate to severe impairment (40), or are severely disabled to a point that they require long-term care (10). There is no sign that these figures are on a downward trend. Thus, a conservative estimate from these statistics would predict that each year 4,000 Canadians will suffer a lethal stroke and an additional 20,000 will suffer a stroke yielding a severe disability that will require daily assistance with their needs. The ramifications of the ensuing disability are not limited to the sufferer's reduced quality of life because an economic burden in lost jobs and health care is absorbed by all segments of society. To put the magnitude of the problem in perspective, more Canadians are disabled by stroke each year than the entire Canadian population of patients with Parkinson's disease, Huntington's disease, and spinal cord injury combined. It is not surprising that vast experimental resources are devoted to the study of stroke. At least two publicly funded federal granting agencies (The Canadian Stroke Network, The Canadian Institute of Health Research), one privately funded granting agency (The Heart and Stroke Foundation), and provincial initiatives, sponsor clinical and experimental stroke research in Canada.

The range of disabilities produced by stroke broadly varies from cognitive, sensory, or motoric in nature. To sufficiently cover both in a single thesis would be a formidable venture, and so this thesis is directed toward the study of motoric

impairments. Specifically, the objective of the present thesis is to explore a rat model of the motor impairments that ensue from middle cerebral artery (MCA) stroke. The following introduction will consider:

(1) The features of human stroke

(2) The necessary features of a successful rat model of stroke. Humans and rats will be compared on:

(A) anatomy of the motor system.

(B) vasculature

(C) induction of stroke

(D) motor analysis

The introduction will aim to convince the reader that the motor impairments that ensue from stroke could be faithfully modeled in laboratory rats.

Features of stroke

What is stroke?

Stroke is the sudden loss of brain function due to an interruption of blood flow to the brain. Approximately 20% of strokes are caused by a blood vessel rupturing (hemorrhagic stroke). Neurons in the vicinity of the leaky vessel are flooded by blood and killed. Rupturing of a blood vessel is related to structural problems within the vessel. A weakened area of the blood vessel wall could yield to pressure, bulge, and fill with blood creating an aneurysm. High blood pressure or trauma tear the bulge and bleeding ensues. Congenital malformations in artery structure can also cause the vessel to weaken, making it susceptible to bursting. A hemorrhagic stroke can occur in the subarachnoid

space (the space separating the skull from brain) on the surface of the brain. Alternatively an intracerebral hemorrhage can occur deep within the brain.

The remaining 80% of strokes are caused by a blood clot interrupting blood flow in an artery (ischemic stroke). The blood clot can form within the artery it is blocking (thrombus) or the clot may develop in a separate vessel and travel until it is lodged in a narrower vessel (embolism). Narrowing of the blood vessels (arteriosclerosis) caused by deposits of fatty material, calcium, and scar tissue inside the vessels increases the chances of a circulating blood clot being sufficiently stuck to interrupt blood flow. With time (3-6 hrs), the neural tissue irrigated by the blocked vessel will have been sufficiently deprived of blood that delivers glucose and oxygen necessary for cell metabolism, and will have accumulated ample lactic acid for cells to enter a death cycle. Complete maturation of an infarct (ischemic lesion) progresses over subsequent days or weeks.

Stroke is typically associated with some early warning signs including: sudden loss of muscle strength and/or numbness in the face or limbs on one side of the body, inability to articulate or comprehend speech, blindness in a visual field, and severe headache and dizziness. The signs need not be chronic because a transient encounter could reflect a stroke. Nevertheless, many strokes are not detected by the individual or people around them. This is in part related to an underestimation of the significance of the warning signs, especially when they are short lived. But it is also related to the possibility that the warning signs in a transient ischemic attack may not be sufficiently alarming and pass undetected.

Treatment of stroke

Stroke treatment is most effective if administered within the immediate post stroke period. Estimates of this time window vary, but three hours is an approximate average. The nature of the stroke (hemorrhagic versus ischemic) has to be first identified, however. The strategies for treating the two classes of stroke are essentially polar opposite. In treating a hemorrhage, the objective is to reduce blood from the ruptured artery, whereas in an ischemic stroke treatments are directed at promoting blood flow through the blocked artery. Treating stroke by promoting blood flow in the case of hemorrhage or slowing down blood flow in the case of ischemia would certainly worsen outcome over receiving no treatment. Diagnosis of the kind of stroke cannot be easily made from the immediate symptoms, however, as they usually indicate little more than the hemisphere experiencing the stroke. Imaging the brain makes a definitive diagnosis. A CAT (computerized axial tomography) scan, which uses x-rays to image cross sections of the brain, is the most efficient procedure in emergency to classify the stroke and identify its location.

At the present time the only intervention available for hemorrhagic stroke involves surgery. The objective is to repair the ruptured blood vessel to prevent further bleeding and to remove as much of the pooled blood from the brain as possible. Apparent aneurysms that are still intact can be treated at this time by applying a clip to the neck of the aneurysm, which isolated it from the rest of the artery and prevents blood from flowing into it. A less invasive procedure for treating aneurysms introduces a coil into the aneurysm, which fills the bulge preventing blood from flowing into it.

Treatment options are more diverse in ischemic stroke. The first line of defense is a pharmacological intervention that involves the intravenous administration of

thrombolitic drugs, which break up clots and so improve blood flow. Tissue plasminogen activator, more commonly known as t-PA, is delivered intravenously to break up the clot. The efficiency of the drug declines substantially three hours after the onset of ischemia. But even if delivered within the three-hour time window, t-PA is not always effective in restoring blood flow. Surgical intervention to remove the clot, clear plaque deposits, or even widen the artery might be necessary.

Outcome from stroke

Although most stroke patients are eventually admitted to hospital, only a subgroup arrives at the emergency wing within a few hours from the onset of stroke when treatment options are most effective. An even smaller subgroup, 25% of the total population with ischemic stroke, receives t-PA within the three-hour critical time window. It is therefore not surprising that a permanent infarct forms in the neural territory of the blocked artery in the majority of stroke survivors.

The nature and the severity of the resulting disability depend primarily on the location and the size of the ischemic infarct. Because a stroke can occur in any cerebral blood vessel, all brain regions are subject to ischemic infarct and the range of potential disabilities is vast. Motor movements are particularly vulnerable to stroke because the motor system is distributed throughout the entire brain. In the most debilitating cases, a patient may be incapable of executing the hand movements necessary for basic self care such as feeding, dressing, grooming, etc. Quality of life deteriorates and dependency on others becomes inconvenient and expensive. If the outcome is more favorable, the patient may overcome the impairments using compensatory strategies. Those typically involve relying on a set of movements that survived the stroke but would not have been used to

accomplish the same objectives before the stroke because they lacked efficiency. Nevertheless, complete recovery whereby pre-stroke motor function is restored is unlikely.

From the statistics provided earlier, it is apparent that far more people are disabled by stroke than those that recover. Efforts in raising awareness about stroke and promoting preventative measures can reduce the number of stroke accidents. Nevertheless, at least for the foreseeable future, stroke will afflict unsuspecting individuals and will not be treated in time to alleviate a permanent infarct.

Modeling stroke in the laboratory

That stroke continues to pose a threat to quality of life is sufficient reason to invest research efforts into it. Some progress in stroke treatment has been advanced in the clinic. Often this work is an application of a discovery made in the laboratory on an animal model of stroke. It is safe to assume that laboratory research will continue to contribute to stroke research and that the clinical work will adapt to the laboratory discoveries and apply them to the stroke population.

The reasons for this division of labor between preclinical and clinical research are at least two fold. First, ethical concerns are by comparison less in the laboratory than they are in the clinic. Investigating innovative stroke treatments that might be too risky to experiment with stroke patients is more acceptable in the laboratory. Second, treatments can be objectively measured on animals with homogenous ischemic infarcts and similar life histories, which cannot be expected from a clinical population.

To study stroke in the laboratory, the ischemic attack is modeled either *in vitro* on brain slices taken from a laboratory animal or *in vivo* in a laboratory animal. Effective

modeling of ischemic stroke requires: (1) the use of an animal with a neural organization that parallels that of humans. (2) Sudden interruption of blood flow to induce ischemia in a prescribed region of the brain. (3) A task for assessing behavioural function that is sensitive to the ensuing impairments and that is ethologically relevant to both the laboratory animal and humans (applicable to *in vivo* work). (4) Confirmation of infarct location and size with post mortem histological analysis or in vivo imaging.

Stroke is modeled in a host of mammals including: rats, mice, gerbils, cats, dogs, and monkeys. Because humans and non-human primates belong to the same order, the biological and ecological parallels between them makes non-human primates ideal for modeling stroke. Ethical concerns and cost effectiveness related to their use are prohibitive, however. The Glires clade, which consists of Rodentia and Lagomorpha, is a sister clade to the Euarchonta clade that includes the primate order. Rodents or Lagomorphs can therefore serve as an alternative to non-human primates for modeling human stroke. In terms of number of species, Rodentia comprises approximately half the number of mammalian species. So it is not surprising that rodents such as mice, rats, and gerbils (family Muridae) are the most common laboratory models for human stroke.

The laboratory rat (*Rattus norvegicus*) has emerged as a clear favorite for investigating stroke because it meets the above criteria for effective modeling of stroke. That rats are the animal of choice for stroke research is most evident in light of the large number of ischemia procedures designed specifically for rats as well as the large number of behavioural tests designed to assess neurological function in rats. The rat is suitable for modeling both the cognitive and the motor dysfunctions that ensue from stroke. It is also suitable for modeling stroke both *in vitro* and *in vivo*. Devotion to rats in stroke research

is reflected in a disproportionate number of publications on experimental stroke in rats than any other rodent. A PubMed search in October 2006 on the following terms: (1) ischemia or stroke and (2) rats, yielded 38,919 citations, whereas the same search with mice only yielded 7,654 citations and 2,249 for gerbils.

The focus of this thesis will be on modeling one of the motor syndromes of middle cerebral artery (MCA) stroke, that is the skilled reaching impairments in rats. This specific area of study was selected because: (1) the MCA is the most commonly occluded artery in clinical stroke, (2) a large portion of the motor system resides in the forebrain, which is primarily irrigated by the MCA, (3) the largest part of the motor forebrain is involved in skilled limb use. Furthermore, components of the motor system distant from the forebrain are connected to the forebrain structures of the motor system by fibers that course through territory irrigated by the MCA. Thus, motor syndromes from MCA stroke are almost inevitable.

Comparing the human and the rat motor systems.

The motor system includes all the central neurons and peripheral joints, tendons, and muscles necessary for producing movement. The present description will focus on the neural components of the motor system that are: (1) involved in controlling the proximal and distal musculature of the forelimbs, (2) in the territory of the MCA. The motor system of humans will be compared to that of rats. The objective is to demonstrate that despite differences, the motor systems of both animals are quite similar, which makes the rat motor system suitable for modeling human stroke.

Human motor system

The overall organization of the motor system is summarized in Figure 1.1. The system is responsible for generating voluntary movements. This is achieved by the brain relaying commands to the spinal cord and from there, motoneurons excite or inhibit combinations of muscles. This moves the appropriate body part while stabilizing others thus effectuating the brain commands into movements. Components of the motor system are distributed throughout the brain. Several subdivisions of the frontal cortex and nuclei in the midbrain and brain stem send projections to the motoneurons of the spinal cord. The activity of the cortical areas is modified through feedback from subcortical structures including the basal ganglia and the cerebellum.

The corticospinal tract is the main communicating pathway from the frontal neocortex to the spinal cord, and is thus the main pathway for motor action. Corticospinal projections originate from several cytoarchitectonically distinct regions of the frontal cortex (Fig 1.1 A). The precentral gyrus (Brodamann's area 4) is referred to as the primary motor cortex (M1) because it is the neocortical region that projects most densely to the spinal cord. Also, the most direct projections to the alpha motoneurons of the ventral horn of the spinal cord arise from M1. For both reasons electrical stimulation of M1 requires less current than any other neocortical area to generate muscle contractions. The premotor cortex (Brodmann's area 6) lies rostral to M1. Both its ventral and dorsal components project to M1 and send corticospinal projections to the motoneurons of the spinal cord. The supplementary motor cortex (Brodmann's area 8) lies medial to the dorsal premotor cortex and is also connected to M1 and the spinal cord. Corticospinal projections from the premotor cortex and the supplementary motor cortex account for the second major set of neocortical axonal projections to the spinal cord (Dum and Strick

1991). The remainder of the corticospinal fibers arises from the somatosensory cortex and from M1 in the opposite hemisphere.

Despite differential corticospinal fiber density contribution from the various neocortical regions, their pattern of projections is similar. Pyramidal neurons in layer 5 of the neocortex send their axons into the corona radiata. The fibers fuse immediately to form the posterior limb of the internal capsule, which separates the striatum into a caudate nucleus and a putamen in the forebrain. En route to the spinal cord, these fibers course through the base of the midbrain, then through the motor nuclei of the pons, to form the pyramidal shaped tract of the medulla. The fibers cross to the other side of the brain at the junction of the medulla and spinal cord (pyramidal decussation). The fibers then descend in the lateral portion of the spinal cord and enter the gray matter in the cervical sections of the spinal cord. There they either synapse directly onto motoneurons of lamina IX or indirectly by first making synaptic contact with interneurons in the intermediate zone, which in turn synapse onto motoneurons.

The motor cortex forms multiple closed loops with the basal ganglia and the cerebellum. Motor cortex projections enter the striatum, which is the input zone of the basal ganglia. From there two pathways originate and eventually terminate in the neocortex to regulate its activity. A direct pathway that involves the internal pallidum provides excitatory feedback to the cortex via the anterior thalamic nuclei (VA), whereas, an inhibitory pathway involving the subthalamic nucleus and internal pallidum suppresses VA activity and consequently neocortical activity as well.

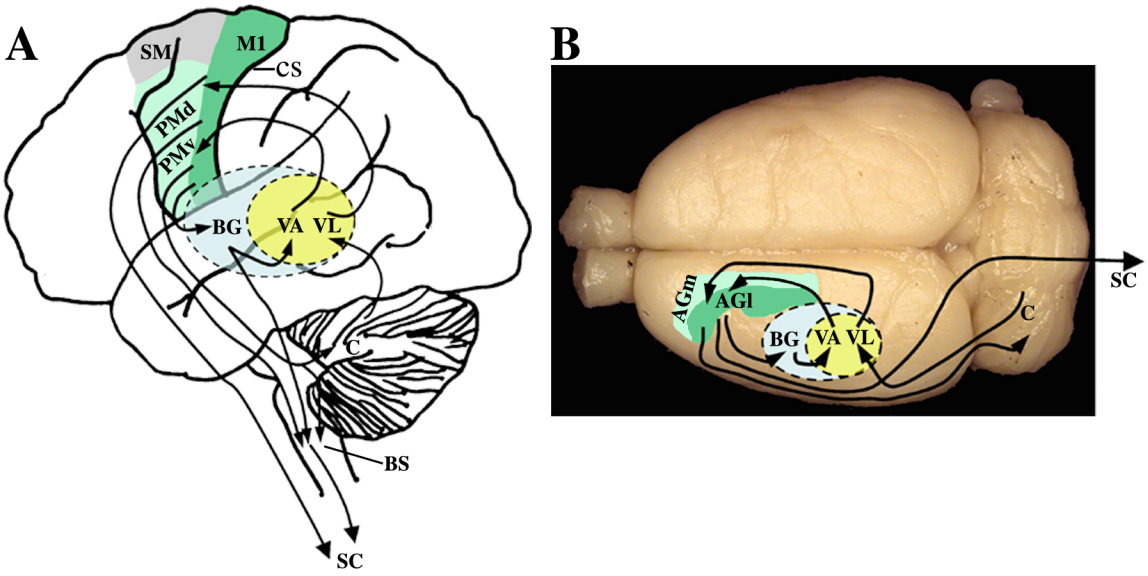


Figure 1.1. Schematic diagram of (A) human and (B) rat motor systems. The cortical areas of the motor system are anterior to the central sulcus (cs) and are shaded in different colours to distinguish their cytoarchitectonics (M1: primary motor cortex, SM: supplementary motor cortex, PMd: dorsal premotor cortex, PMv: ventral premotor cortex). The bulk of the cortical projections to the spinal cord (sc) and brain stem (BS) arise in M1. The basal ganglia (BG) and the thalamic nuclei (VA, VL) are hidden beneath the cortex but their approximate location is indicated to complete the motor circuitry diagram. The motor cortical areas form two closed with the basal ganglia and the cerebellum. The cortical regions of the rat motor system are the agranular medial cortex (AGm) and the agranular lateral cortex (AGl).

Cortical projections reach the cerebellum indirectly via pontine nuclei. Cerebellar nuclei also receive proprioceptive feedback from the spinal cord. A comparison of the motor efference copy and actual position of the limb is therefore thought to take place in the cerebellum and its allied structures. Corrective feedback from the cerebellum reaches the motor cortex via the lateral thalamic nuclei (VL) to close the loop. The projections to and from the cerebellum course through the forebrain before reaching their destination. Projections from other neocortical areas to M1 are typically direct. Primary somatosensory areas of the postcentral gyrus project to M1 and the rostral portions of the

posterior parietal cortex project to M1 as well as to the dorsal and ventral premotor cortex.

The red nucleus, a prominent nuclei of the midbrain, is a target of motor cortex and is also thought to be involved in motor control of the upper limb. Magnocellular neurons of the red nucleus give rise to the rubrospinal tract. Almost all of the fibers decussate to the contralateral side in the midbrain and run parallel to the corticospinal tract in the pons and the medulla. In the spinal cord the rubrospinal tract descends ventral to the corticospinal tract and forms synaptic contact with the intermediate zone or directly with motoneurons.

Rat motor system

The overall organization of the rat motor system (Fig 1.1 B) is similar to the human motor system (Fig 1.1 A). Nevertheless, anatomical and nomenclature differences in the rat motor system warrant clarification. First, the rat brain is lisencephalic (devoid of sulci and gyri) and therefore neocortical subdivisions are not defined in relation to sulci. Instead, references are made to cytoarchitecture, connections to thalamic nuclei and neocortical areas, and neurophysiological properties. The anatomical and neurophysiological properties of the primate M1 are inherent to the lateral agranular (AGl) necortex of the rat frontal lobe (Wise and Donoghue 1986). The AGl is known as such because it is devoid of layer 4 granular cells and contains a disproportionately larger layer of pyramidal cells (layer 5), which is the origin of the corticofugal projections. Anatomical and electrophysiological data have suggested that the agranular medial (AGm) neocortex, which is medial and rostral to the AGl may be the equivalent of the premotor and supplementary motor areas in primates (Neafsey et al. 1986; Sievert and

Neafsey 1986). Lateral to AGl is the dysgranular zone where layers 4 and 5 are comparable in size. Because the anatomical makeup of this region supports both sensory and motor functions, it is commonly referred to as sensorimotor cortex. The granular zone is lateral to the dysgranular zone and extends as far as the insular cortex. An elaborate layer 4 in the granular zone makes it the centre of sensory input from the ventral posterior nucleus of the thalamus (VPL). Although layer 5 is significantly diminished here, sparse corticospinal projections arise from this neocortical region (Wise and Donoghue 1986).

The course of the corticospinal fibers is somewhat different between rats and primates. In rats, some corticospinal fibers exit the cortex and immediately enter the internal capsule. The remainder of the fibers project through the striatum before fusing with the internal capsule more caudally. The path of the fibers through the brain stem and the decussation at the junction of the medulla and spinal cord is similar in both species. In the rat spinal cord, corticospinal fibers descend in the base of the dorsal horn, however. In the cervical sections, most fibers enter the spinal grey and synapse in the intermediate zone. It remains unclear whether there is direct contact between corticospinal fibers and motoneurons or if all contact is mediated through the interneurons. Nevertheless, any direct contact between corticospinal projections and motoneurons in rats is likely insignificant in number as compared to primates.

The rat M1 also forms closed loops in which M1 sends projections to the basal ganglia and the cerebellum that return to it via thalamic nuclei. The associated fibers course through the forebrain. Perhaps the most salient anatomical feature of the rat basal ganglia is that the boundaries of the caudate and putamen are blurred. They are

collectively recognized as the dorsally located dorsal striatum and the ventrally located ventral striatum. Although the subdivisions of the globus pallidus are comparable between rats and primates, the external globus pallidus of the primate is known as the entopeduncular nucleus in the rat.

The rubrospinal tract of the rat decussates in the mesencephalon as does the human tract. Also, it descends in the dorsolateral section of the spinal cord and enters the spinal gray at the intermediate and ventral zones to synapse on interneurons and motoneurons respectively.

Thus, as stated earlier the neural building blocks of the motor system and its organization are similar in primates and rats.

The human and rat cerebral vasculature systems are similar

Blood arrives at the brain through four main arteries: (1) two common carotid arteries (CCA) and (2) two vertebral arteries. The two vertebral arteries join at the base of the medulla and form the basilar artery, which irrigates the brain stem. This system also gives rise to the posterior cerebral artery (PCA), which irrigates the temporal and occipital lobes as well as the cerebellum. The CCA bifurcates approximately at the level of the thyroid gland into an external carotid artery (ECA) and an internal carotid artery (ICA). The ECA does not enter the cranium but irrigates mastication muscles and surrounding tissue. The ICA branches into the pytergopaladine artery, which irrigates extracranical zones. Downstream the ICA enters the cranium through the carotid canal. At the base of the brain it branches into a middle cerebral artery (MCA) and an anterior cerebral artery (ACA).

The origins of the ACA and MCA are continuous at the base of the brain. This in addition to an anterior communicating artery that connects the ACAs of the opposite hemispheres and a posterior communicating artery that connects the PCA to the MCA and ACA, makes for a closed circle that connects the major blood vessels of the brain. The circle is commonly known as the circle of Willis named after Sir Thomas Willis who first described it. The circle of Willis can be envisioned as a watershed in which the main arteries irrigating the forebrain originate and are connected. The arteries are also connected end-to-end at the their terminals. For example, the ACA and MCA are connected end-to-end by anastomosis within the motor cortex, whereas the MCA and PCA are connected at the junction of the temporal and parietal lobes. The frequency and size of anastomosis is highest between the ACA and MCA, followed by those between the MCA and PCA. Additional anastomosis exist within each artery via the pial network of vessels on the surface of the brain.

The forebrain is primarily irrigated by both the ACA and the MCA. The ACA ascend through the interhemispheric fissure (Fig 1.2 A). The anterior communicating artery arises from its proximal segment to connect it to the contralateral ACA. Distal branches of the ACA irrigate the medial walls of the cortex throughout the entire brain (e.g. cingulate cortex, supplementary motor cortex). The MCA ascends on the lateral surface of the brain and ramifies into several branches that irrigate an extensive neocortical region from the frontal pole to the occipital cortex. Near their proximal segments, both the ACA and MCA give off smaller arterial branches (lenticulostriate arteries) that penetrate deeper into the brain to irrigate the white matter and subcortical

structures. Lenticulostriate and are not connected by anastomosis at the terminal ends (Fig 1.2 A,B).

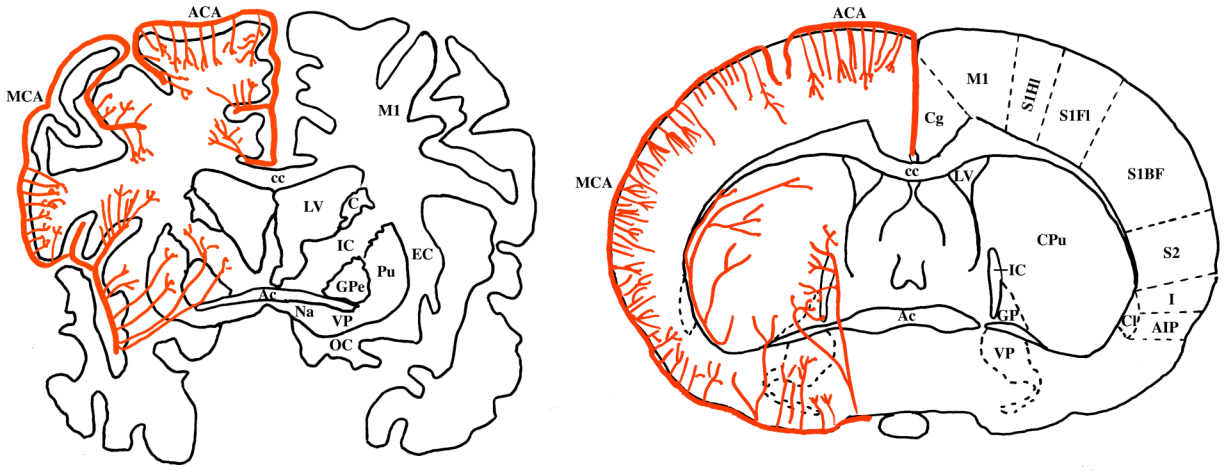


Figure 1.2. Distribution of the middle cerebral artery (MCA) and anterior cerebral artery (ACA) as seen in coronal schematics from a (A) human and a (B) rat brain. The arteries irrigate comparable structure in both species. Both arteries give off smaller arteries that irrigate the grey matter. Lenticulostriate arteries arise close to the proximal trunk of the MCA and penetrate deep into the brain to irrigate both the white matter as well as subcortical structures. Abbreviations: anterior commisure (ac), primary motor cortex (M1), orbital cortex (OC), internal capsule (IC), external capsule (EC), corpus callosum (cc), lateral ventricle (LCV), GPe (external globus pallidus), ventral pallidum (VP), putamen (Pu), nucleus accumbens (Na), striatum (CPu), cingulate cortex (Cg), forelimb sensorimotor cortex (S1FL), hindlimb sensorimotor cortex (S1HL), secondary sensory cortex (S2), insular cortex (I, AIP), claustrum (Cl).

The organization of the cerebral vasculature is similar in rats (Scremin 2004), but with differences (Fig 1.2 B). The internal carotid artery is integrated into the arterial circle at the base of the brain before bifurcating into an ACA and an MCA (Fig 1.3 A). At the frontal pole, the ACAs on either hemisphere fuse via an azygos communicating artery (Fig 1.3 A). The MCA branches from the ICA approximately 2 mm behind Bregma and ascend on the lateral surface of the brain towards the midline (Fig 1.3 B). End-to-end anastomosis between the ACA and MCA are 4-5 times more abundant in rats than in humans (Lee 1995).

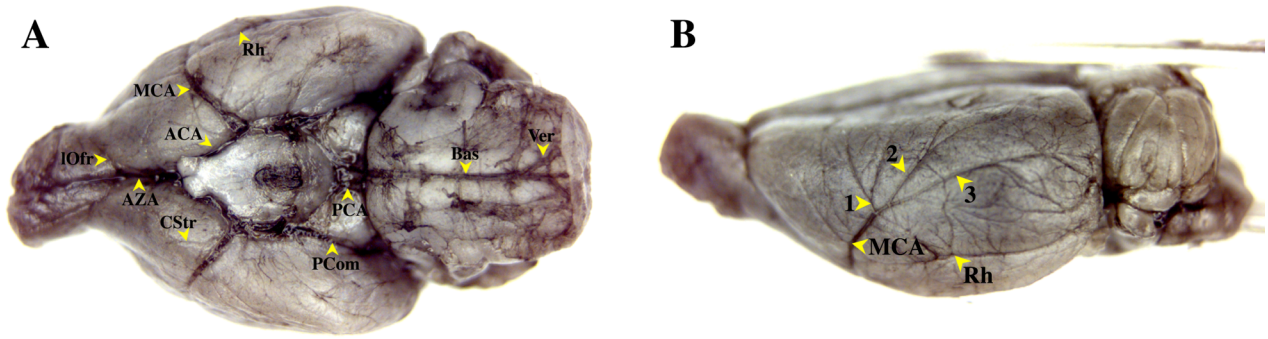


Figure 1.3. Brain extracted from a rat perfused with black ink (procedure conducted by Claudia L. R. Gonzalez & Omar A. Gharbawie). The arterial circle is evident from the (A) ventral perspective. The middle cerebral artery (MCA) ascends the cortex as a single artery and ramifies into multiple branches distal to the rhinal fissure (Rh). (B) The main branches of the MCA (1,2,3) irrigate most of the lateral and dorsolateral aspects of the neocortex. Abbreviations: Basilar artery (Bas), verterbral artery (ver), posterior cerebral artery (PCA), posterior communicating artery (PCom), corticostriate artery (CStr), azygos of the anterior cerebral artery (AZA).

Stroke procedures.

A blood clot could travel freely throughout the cerebral vasculature, but once it is lodged into a blood vessel, flow through that vessel is rapidly interrupted, which is the onset of ischemia. Narrow vessels and lenticulostriate arteries that lack end-to-end anastomosis are particularly susceptible to blockade. Thus, to faithfully model ischemic stroke in an animal, blood flow has to be abruptly interrupted.

Rats rarely have spontaneous strokes, however. This may in part be related to a healthy laboratory diet that minimizes the possibility of mineral deposits that contribute to arteriosclerosis. In addition, the well-developed arterial circle and end-to-end anastomosis between and within the main arteries of the brain, provide collateral blood flow such that if a vessel is occluded its terminal branches may fill with flood in the retrograde direction (Lee 1995; Scremin 2004). The efficiency of this system in compensating for ischemia is dependent on the duration of the occlusion as well as the

arterial blood pressure in the unoccluded vessels. Thus, not only is the circulatory system of the rat brain protective against ischemia, it complicates the experimental induction of ischemic stroke.

A pinpoint occlusion of a vessel in experimental ischemia as occurs in clinical blood clots, is ineffective in interrupting blood flow to ischemic levels (Ginsberg and Busto 1989). Ischemic procedures designed for rats overcome this problem in one of three ways: (1) by occluding the vessel of interest over its entire length to ensure that blood from collateral supplies does not enter into any of the occluded segments; (2) by occluding vessels within a prescribed region to ensure that blood flow has been interrupted in the vessel of interest as well as its collaterals; and, (3) by occluding the vessel of interest under systemic hypotension (reduced blood pressure), which reduces blood flow in general indirectly limiting collateral blood flow to the target vessel.

Most of the experimental stroke procedures induce ischemia in the MCA. There are two overarching features of stroke procedures. The first pertains to the localization of the infarct. In global ischemia, blood flow is interrupted in a wide area of the brain, often bilaterally, whereas in focal ischemia blood flow is restricted to a circumscribed brain region. The second feature is related to the restoration of blood flow. In permanent ischemia, specific blood vessels are irreversibly closed or removed altogether, whereas in transient procedures, vessels are occluded for a controlled duration and then blood flow is restored. Any given stroke procedure can be concurrently classified according to both features (e.g. transient global ischemia, permanent focal ischemia, etc.). A few standard stroke procedures and their advantages and limitations, will be described. The procedures described will be grouped according to blood flow characteristics. Because the focus in

this thesis is on MCA stroke, only focal ischemic procedures that can target the MCA will be described.

Permanent ischemia

Pial strip. The objective of pial stripping is to devascularize a target neocortical area (Kolb et al. 1997; Sofroniew et al. 1983). The skull overlaying the area of interest is trephinated and the underlying dura is cut and deflected. The exposed blood vessels are wiped away with a saline-soaked cotton swab. The tissue turning from a healthy pink colour to white confirms the ischemia. The procedure is valuable for modeling the motor syndromes of stroke because a focal infarct can be produced in the motor cortex or part of it (e.g. rostral or caudal).

Advantages. The parameters of the infarct including size and location are guided by the size and location of the trephination, which is controlled by the experimenter allowing reliable reproducibility from one rat to the next. Also, infarcts could be stereotaxically placed in neocortical areas of interest.

Limitations. In wiping away the vasculature, both arteries and veins are physically damaged in a way that does not resemble clinical stroke. To produce ischemia in motor cortex for example, branches of the ACA, MCA, and the veins in between, have to be wiped away. Also, because the target vessels have to be exposed through the skull, subcortical ischemia is not possible with this procedure.

Electrocoagulation. The objective is to permanently occlude the target blood vessels by heating them (Tamura et al. 1981). The skull overlaying the blood vessels of interest is trephinated and dura is cut and deflected. Target blood vessels are approached with the tip of a pair of forceps that is connected to a bipolar electrocoagulator. Low

current intensity is passed through the forceps, which generates heat at the tip of the forceps that occludes the underlying blood vessel. This is repeated for the length of the blood vessel of interest or for the blood vessels irrigating a specific neocortical area until they are permanently closed and the surrounding tissue discolours to white. The procedure is valuable for motor cortex stroke because ischemia can be directly induced in it. The procedure can also be applied to occlude distal or proximal branches of the MCA. Ischemia in distal MCA branches produces a neocortical infarct lateral to M1, whereas delivered to proximal MCA branches (lateral to the rhinal fissure) the ensuing infarct includes dorsolateral striatum as well as the neocortex lateral to M1.

Advantages. The fine-tip forceps used for vessel electrocoagulation allow the experimenter to occlude a single vessel. This makes the procedure reminiscent of clinical stroke as well as adds versatility in controlling infarct location. For example, electrocoagulating the MCA medial to the rhinal fissure limits the infarct to the neocortex, where occlusion lateral to the rhinal fissure produces an infarct in both the striatum and the neocortex. Alternatively, the location of the infarct can be guided by stereotaxically exposing the vasculature of the neocortical area of interest, which are then electrocoagulated. The number of vessels electrocoagulated within the trephinated area dictates the size of the infarct. Thus, both the size and the location of the infarct can be tightly controlled for inducing infarcts in specific neocortical regions.

Limitations. Although infarct size and location is in the main reproducible, variability can arise from collateral blood flow. Also, inducing subcortical infarcts is limited to electrocoagulating lenticulostriate vessels before they penetrate the neocortex

to reach subcortical structures. Thus, the parameters for subcortical ischemia are less controlled than the neocortical counterpart.

Transient Ischemia

Vasconstricting agent. The objective is to transiently interrupt blood flow by infusing an agent that temporarily constricts the vessel (Macrae et al. 1993; Sharkey et al. 1993). The substance is microinfused via a steel cannula or a Hamilton syringe and flow rate is controlled with an infusion pump. For neocortical infusions, the skull above the region of interest is either removed and the dura is cut and deflected, or holes are drilled into it for cannula access. Subcortical infusions are delivered through holes in the skull. Stereotaxic positioning of the infusion cannula allows the delivery of vasocontricting agents such endothelin (ET-1) to a specific blood vessel or a region of interest. The infusion creates a brief vasodilation that is followed by a longer and transient vasconstriction due to contraction of smooth muscles that line the walls of the blood vessel. Neocortical ischemia delivered through a window in the skull is readily confirmed by tissue discoloration to white. Blood flow is restored after ET-1 has been metabolized. The procedure is useful for producing focal neocortical or subcortical infarcts of the motor system. It can also be directed at occluding distal or proximal branches of the MCA.

Advantages. That ischemia can be directed at a single blood vessel with this procedure makes it more reminiscent of clinical stroke. Delivery of the vasoconstricting agent through a cannula offers versatility for producing stereotaxic infarcts in neocortical or subcortical regions alike.

Limitations. The effectiveness of vasoconstriction is dependent on the concentration of ET-1 as well the position of the infusing cannula in relation to the target blood vessel. Because the response of smooth muscle that line the vessel walls can vary from one vessel to the next and from one rat to the next, variability is expected in the effectiveness of vasoconstriction and consequently infarct size. Also, because blood vessel architecture varies from rat-to-rat, stereotaxic coordinates standardized for occluding a specific blood vessel is bound to occlude slightly different combinations of vessels in each subject, contributing to infarct variability. Visual confirmation of ischemia is only possible in neocortical ischemia and only in procedures when a window of skull is trephinated as opposed holes.

Intraluminal suture. The objective is to transiently interrupt blood flow in the MCA by occluding its origin with a suture (Longa et al. 1989). A monofilament is advanced through the internal carotid artery and into the arterial circle to block the origin of the MCA. Occlusion duration and blood pressure during ischemia are the primary parameters that guide infarct size. Ischemic severity increases with longer durations and with lower blood pressures. Occlusion durations of 30-90 min are typical for this procedure. Hypotension in the range of 60-80 mm Hg is also typical for this procedure and can be regulated with anhilatory anesthesia. Removal of the suture slowly restores blood flow. Smaller infarcts are expected to include the striatum, globus pallidus, and fibers of passage, whereas larger infarcts include the same structures but may extend laterally into the adjacent neocortex and medially into the hypothalamus and internal capsule.

Advantage. The procedure is reminiscent of clinical stroke in that the ischemia is localized to a single major artery, is transient, and the skull is undisturbed. That ischemic parameters such as hypotension and occlusion duration can be controlled by the experimenter allows versatility in inducing subcortical infarcts or larger infarcts that include both subcortical and neocortical regions.

Limitations. The procedure is technical and demands lots of practice. To adequately monitor blood pressure, rats are intubated for artificial ventilation and their tail artery is cannulated and connected to a blood pressure monitor. Although regulating blood pressure during ischemia adds considerable experimental control over infarct variability, it further complicates an already technical procedure. But even with tight control over ischemic parameters, variability in infarct size and mortality are high in comparison to other procedures. This is likely understated in most studies that use the intraluminal suture as rats are typically perfused within 48 hrs of ischemia before their health completely deteriorates from aphagia and they eventually die. We have been successful however in sustaining rats that did reject food with tube feeding several times a day until voluntary feeding is restored. The chances of survival at that point are promising.

Photothrombosis. The objective is to cause blood platelets to aggregate temporarily and form a clot (Watson et al. 1985). A light sensitive dye such as rose Bengal is infused into the circulatory system through the tail or femoral arteries. The skull over the target area is shaved with a dental drill to reduce its thickness and then a cold light is shined over the prepared area for a prescribed duration. The light reaction causes the dye, which should have circulated throughout the brain at that point, to

aggregate blood platelets. The resulting clot formation is sufficient for inducing ischemia. Blood flow is restored when the clots are displaced or metabolized. The procedure is effective in inducing motor system stroke because it can be directed at the motor cortex.

Advantage. The invasiveness of the procedure is minimal because the skull is only shaved and not trephinated. Shaving the skull according to stereotaxic coordinates allows experimental control over infarct location.

Limitations. Because the light beam is mostly absorbed by the neocortex, subcortical infarcts are not possible. In addition, ischemia cannot be directed at a specific blood vessel because the light sensitive dye is ubiquitously circulated in the vasculature.

Selecting a stroke procedure

The selection of an experimental stroke procedure is the subject of an ongoing debate. That is not surprising given the vast number of procedures to choose from (the ones listed above only represent a sample). It is probably safe to say that the criteria for selecting an appropriate stroke procedure differs somewhat between clinical scientists and behavioural neuroscientists. Clinicians are likely to advocate procedures that faithfully mimic ischemic events of stroke. Thus, they favor procedures of focal transient ischemia because of the potential for blood flow restoration. Infarct location and the ensuing impairments are a secondary priority from this perspective. Behavioural neuroscientists modeling stroke in rats might be less concerned with the restoration of blood flow as long as the infarct is positioned in a specific neural circuitry with little variability. This allows basic scientists to characterize the behavioural impairment in groups of rats with homogenous infarcts, an approach that is consistent with the classical

behavioural neuroscience framework, where function is linked to structure by identifying the specifics of the behavioural disorganization that ensue from a circumscribed lesion.

Behavioural testing

Behavioural testing serves as an assessment of motor capacity after stroke. Selecting an appropriate task can be complicated given the abundance of behavioural tests developed for rats. Adhering to some general guidelines narrows the possibilities, however. An appropriate laboratory test has to capitalize on behavioural features that are ethologically relevant to the rat. The task has to assess a motor function that is expected to be compromised by the infarct. The task should be sensitive enough to distinguish spontaneous recovery from compensatory movements. Additionally, the task has to be readily related to human stroke if the results are to make a contribution to clinical work. Assessing rats with stroke on reflexive tests that are idiosyncratic to the rat may reveal if an animal is impaired but it may be impossible to translate the results to the human stroke population.

The laboratory test that satisfy these criteria generally involve limb placing or reaching for food. Several limb placing tasks have been developed for rats, but the essence of the assessment is similar for all tests in that the actions of the forelimb and/or hindlimb are examined as the limbs contact a surface. For example, in vibrissae placing, the animal is held by the torso and moved toward the edge of a table. Vibrissae contact with the table triggers a forelimb placing onto the table. In rung walking, the rat traverses a horizontal ladder to reach a goal box (Metz and Whishaw 2002). To avoid forelimb or hindlimb slipping between rungs, the digits and the toes grip the rungs in each step. In the cylinder test, rats are placed inside a small cylinder for few minutes to allow spontaneous

exploration (Schallert et al. 1997). They rear and place their paws on the walls of the cylinder to support their weight. The primary measure of performance in these placing tests is an asymmetry score in which one side of the body is compared to the other. Because MCA stroke is typically administered unilaterally in rats, the contralateral-to-lesion limbs score poorly as compared to the ipsilateral-to-lesion limbs, giving an asymmetry score that is significantly different from control rats. This scoring system presents a critical confound for studying recovery of function from stroke because bilateral lesions impair both paws and produce a low asymmetry score that is comparable to control rats. The reliability of a scoring system that does not differentiate a rat with bilateral lesions from a control rat is problematic, even if the scoring system reliably identifies unilateral lesions. Furthermore, it is not clear what the incentive is for the rats to perform any of the placing tasks because not performing, or performing poorly, is of no major consequence. Interpreting limb disuse after stroke is therefore complicated as it might be related to a motor incapacity or a lack of motivation.

Reaching for food tasks satisfy the criteria of a behavioural measure of stroke deficits without the confounds of the limb placing tasks. Evidence supporting the use of skilled reaching for assessing rat models of stroke will be provided in the sections to follow. First, the similarities between the human hand and rat paw will be illustrated. Second, the sophistication of the behavioural analysis possible in skilled reaching tasks will be demonstrated and its value in this line of research explained.

Comparing the human hand to the rat paw

Significant components of the motor system are dedicated to controlling the hand of humans and the paw of rats. Perhaps this is best exemplified by the disproportionately

large hand representation in M1 of humans and paw representation in M1 of rats. Also, given that rodents and primates are orders of sister clades, it is not surprising that the paw of the rat (Fig 1.4 A) is also similar to the hand of the human (Fig 1.4 B). The architecture of the hand and paw will be briefly discussed in the next section. Emphasis will be placed on features of the hand/paw that make it adaptable to the skilled movements of reaching and grasping.

The human hand is specialized for manipulating small objects and grasping. All fingers, except the thumb, are composed of three phalanges that are connected by a synovial joint allowing them to flex. The tendons that move the fingers are attached to forearm muscles that are attached to the elbow (Napier and Tuttle 1993). The tendons pass through a narrow tunnel of the carpal bones and attach to the fingers. The flexor tendons also adduct the fingers toward the midline, whereas the extensor tendons abduct the fingers. Flexion creases on the phalanges of the digits and the palm reflect the vast number of postures that the digits and palm can form. Perhaps the most revered is the opposition between the thumb and the fingers, particularly the index finger. This is the posture of the fingers that allows fine manipulation of small objects and generally thought to be exclusive to primates. The most significant of the different finger postures is the pincer grasp in which the tip of the index finger opposes that of the thumb. Size ratios of certain phalanges also seem to correlate with dexterity. For example, dexterous animals such as humans have a middle finger that is approximately half the length of the full hand. They also have a thumb that approximates the length of the index finger.

The rat paw is strikingly similar in structure and function to the human hand although this has not been adequately acknowledged. Some primate researchers argued

that clawed animals, such as rodents are incapable of skillfully grasping or manipulating small objects. To quote Napier and Tuttle (Napier and Tuttle 1993)

"Claws are not compatible with prehensility because of the mechanical obstruction of claw overgrowing the fingertips; occasionally a marmoset will grip its food between the tips of the claws and the proximal part of the palm but the action lacks any element of precision. Many clawed mammals, especially the smaller ones like squirrels and marmots, hold their food between their two paws as one would hold a beach-ball; in this way they get around their single-handed prehensility" (Napier, Hands, p.40).

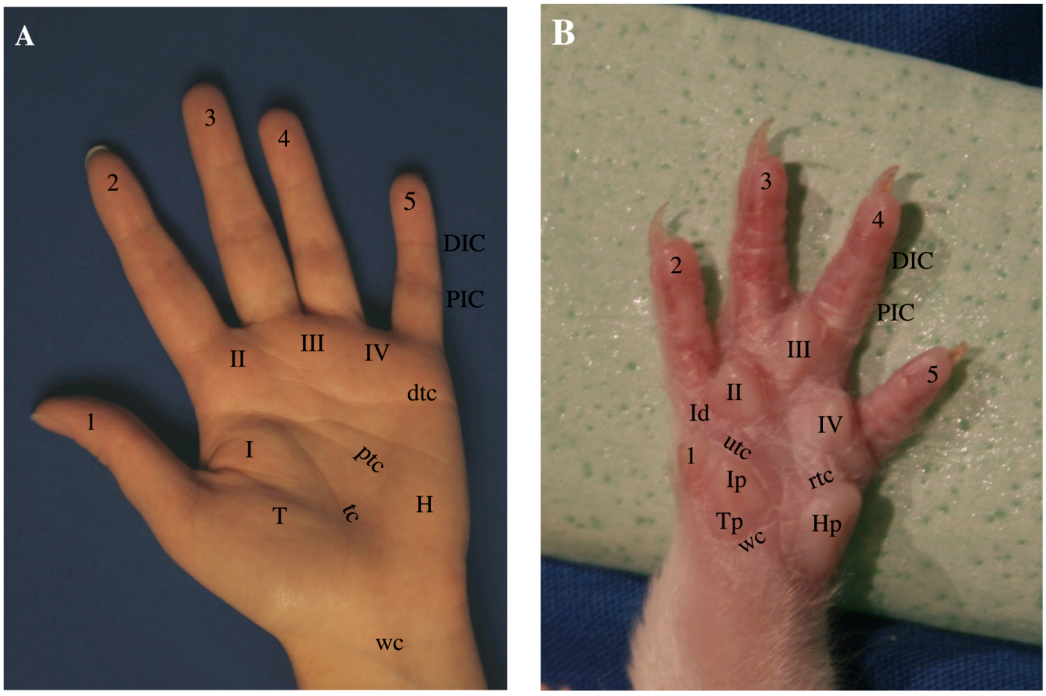


Figure 1.4. Similarities between a (A) human left hand and (B) rat left paw. The fingers/digits are labeled 1-5. The thumb is 1 and the index finger/digit is 2. Interdigital pads are labeled I-IV. The thenar (T) and hypothenar (H) pads in the rat are divided into proximal (Ip) and distal (Id) in the rat. Creases are evident at borders where flexion is possible: wrist crease (wc), thenar crease (tc), proximal thenar crease (ptc), distal thenar crease (dtc), distal interdigital crease (dic), proximal interdigital crease (pic), ulnar thenar crease (utc), radial thenar crease (rtc). Labeling is according to (Kimura et al. 2002).

Rats do indeed have claws on their digits, but they also have a nail on their thumb. Furthermore, the pahalangeal composition of their digits is similar to humans. Flexion creases on the digit phalanges show that the each digit is capable of flexing at the joints (Fig 1.4 B). Although the ratio of the length of the thumb to the index is skewed in relation to humans, a pincer grasp has been shown in rats in which the thumb is in opposition with digit 2 to grasp slim items such as a strand of uncooked pasta. Opposition between digits 4 and 5, akin to the human scissor grasp, also supports prehensile grasping of small objects.

Comparing reaching target identification in humans and rats

Visuomotor transformations in humans

Human reaching is in the main guided by vision. Reaching to grasp tasks designed for humans involve subjects reaching for different objects including blocks of different sizes and shapes. Alternatively, they can reach for standard household tools as though preparing to use them. In fewer tests, subjects reach for a small piece of food, which they grasp, withdraw, and consume. Target versatility is possible because subjects are verbally instructed and rewards are not necessary for compliance. Reaching in non-human primates is motivated by food or drink rewards. For example, monkeys can reach for food targets or can be trained to reach for and grasp a switch that dispenses food to them.

Accurate reaching necessitates visual information about the target such as location, size, and shape. This information is then transformed into appropriate reaching action. The neural circuitry implicated in visuomotor transformation originates in primary visual cortex and ends in M1, as exemplified in anatomical studies in the Rhesus monkey. Visual information is sent to association areas of parietal cortex, specifically zones in the

medial intraparietal sulcus (MIP) and the anterior intraparietal sulcus (AIP). Both areas, collectively known as the parietal reach area, then project to the ventral premotor cortex (PMv or F5) thought to encode the "lexicon of actions" (Rizzolatti et al. 1988). For reaching, the lexicon includes various digit postures for grasping (precision grasp Fig 1.5 A,C vs. whole hand grasp). Premotor cortex then projects to M1, which presumably encodes a "lexicon of movements" (Rizzolatti et al. 1988), or the muscle synergies for executing a specific grasp. Thus, the dorsal stream linking the parietal cortex with the premotor cortex is responsible for the visual transformation that guides limb trajectory and shaping of the digits for grasping.

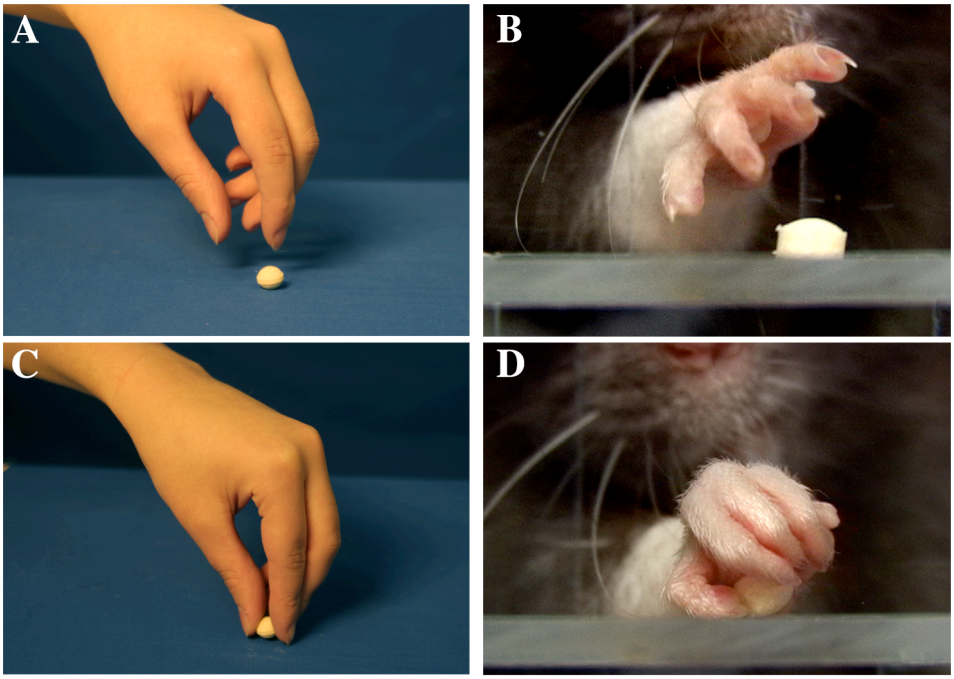


Figure 1.5. The posture of the fingers/digits during reaching for a small food pellet in a human (A) and a rat (B). The pellet is grasped between the thumb and index finger in the human (C), whereas the pellet is grasped between digit 4 and 5 in the rat (D). The two food pellets are identical in shape and texture but the one for the human hand is larger and heavier (300 mg) than the one for the rat paw (45 mg).

Olfacto-motor transformations in rats

The stimuli that could serve as reaching targets for rats are more limited than the ones possible for primates. Although rats can be trained to reach for and press a lever to receive a food or a drink reward, reaching-to-grasp tasks are generally limited to food targets. Following Petersen's description of rat reaching, a number of tasks have been developed in which rats reach through bars, tubes, or slots, to retrieve food from wells, troughs, and rotating platforms. But in principle the tasks are similar in that an animal reaches for and grasps a small piece of food, withdraws its paw with the food, and consumes it immediately. Despite limiting the reaching target to food, design manipulations involving target accessibility and target size are possible to modify the difficulty of the task.

For most reaching tasks the target is a flavoured, sugar pellet. The pellets are palatable to the rats and they will readily consume them over the standard laboratory diet if given the choice. Rats will consume the sugar pellets even when they are not hungry. This should not be surprising to anyone who could not resist consuming a cookie or a sweet treat in the absence of any desire for eating. Nevertheless, rats are typically maintained on a feeding schedule limited to once a day feeding of laboratory food to ensure that they are motivated to reach even after stroke when they are most likely to be unmotivated to participate in behavioural tasks.

Visual information does not play a significant role in guiding skilled reaching in rats. Rats can accurately reach for food pellets in complete darkness and with their eyes' patched. In addition, the position of the food pellet in most reaching apparati may be hard to see from eyes on the side of the rat's head. Also, the close proximity of the food is well

within the rat's focal point. Furthermore, that large parietal cortex lesions do not significantly disrupt the paw movements for opening puzzle latches in rats (Kolb and Walkey 1987), undermines the significance of the dorsal stream in guiding the rat's limb movements. Reaching in rats is guided by olfaction. Rats sniff to determine the presence and location of a food target before transporting their limb towards it and they reach as if "blind" when olfaction is blocked (Whishaw and Tomie 1989). The extent of the limb advance is typically to the tip of the snout, which is fixated to the food target. Thus, it is conceivable that the rat limb is guided by an olfactomotor-transformation instead of the visuomotor counterpart in primates. Although such a circuit has not been investigated, direct connections between pyriform cortex (olfactory cortex) and motor cortex, might be at the core of such a circuit. Nevertheless, the shaping of the digits during reaching and grasping is similar between rats (Fig 1.5 B, D) and humans (Fig 1.5 A, C).

Reaching tasks

The assessment of reaching performance evolved over several decades. In the first reaching task designed for rats, Petersen was merely interested in handedness (Peterson and Gucker 1959). It later became clear that transfer of handedness as a sole measure of reaching performance was insufficient for identifying impairments that could ensue from a lesion without transfer of handedness. Quantitative measures of performance such as success scores or reaching speed were therefore implemented. But even these fell short of fully capturing reaching impairments. Perhaps this is most evident at later stages of recovery when pre-operative success scores are spontaneously restored yet the actions of reaching are different from the normal form. The issue is addressed in reaching tasks that

allow a qualitative analysis of the submovements that comprise a reach in addition to quantitative measures.

The reaching tasks present today for rats can be broadly classified according to the level of sophistication of the analysis the task permits, as well as the involvement of the experimenter in the administration of the task. In automated reaching tasks, experimenter involvement is minimal because food targets are mechanically dispensed for the rat. Performance is quantified by a computer in terms of success scores. On the opposite end of the spectrum are reaching tasks in which an experimenter administers every reach trial. Numerous quantitative and qualitative measures of performance can be derived in such tasks. A separate group of reaching tasks falls in between the automated and manually administered tasks. These tasks are not automated, but the experimenter involvement is limited to the start and the end of the testing session. Some quantitative and qualitative measures are feasible in these tests.

The advantages and limitations of the different reaching task categories will be briefly discussed. The draw to automated tasks is that they are not labour intensive. That the assessment is limited to success scores and the prohibitive cost of assembling the apparatus has precluded the use of automated tests in stroke research. Reaching tasks that are only partly labour intensive are widely adopted in stroke research. The staircase test and the tray test are among the most popular.

In the staircase test (Montoya et al. 1991), rats straddle a platform and reach onto steps of a staircase on either side bated with food pellets. The food targets present varying degrees of difficulty because they are located on stairs at progressively longer distances from the animal's reach. The reaching abilities of each limb are assessed independently

by counting the number of pellets remaining on the staircase after the animal has been removed from the apparatus. The task is attractive to experimenters because reaching can be simultaneously assessed in large groups of rats. In addition, test sessions are brief, lasting only a few minutes, and provide a score for both paws. Nevertheless, the seemingly straightforward quantitative analysis is of little value without video records. For one, food pellets on the two top steps can be lapped with the tongue. This may be mistaken for a successful reach unless monitored by video. Also, a food pellet displaced from a step due to an inaccurate reach can fall onto any number of steps below it. It would not be possible to document the step at which the reaching error was committed in the absence of a video record. Of equal concern is the scenario in which the animal knocks the pellet off one step and is able to retrieve it from the step it landed on. In this case the reaching error escapes undetected all together. Although these limitations cannot be alleviated without major modifications to the apparatus, they can at least be documented from video records. This jeopardizes the low labour intensity advantage of the task. Last, the apparatus imposes an awkward reaching posture on the animal and only permits filming from a side view. Kinematic analysis of reaching movements are therefore complicated in this task (Whishaw et al. 1997). Furthermore, that each food pellet is located on a different step means that the limb movements differ from one reach to the next making it impossible to generate a profile of limb kinematics based on multiple trials. The nature of the reaching impairment in a stroke rat is not likely to be detected.

In the tray task (Whishaw et al. 1986), rats reach through bars into a tray filled with chick feed or sugar food pellets. Performance is assessed in terms of success, which

is determined from the ratio of limb advances through the bars and the number of successful reaches in which food was grasped and released into the mouth. Other than ensuring that the tray is filled with the food target during the test session, the administration of the task does not involve additional work on part of the experimenter. Thus, multiple rats can be tested simultaneously but performance of individual rats has to be filmed to calculate success scores. Another advantage of this reaching setup is that the floor holding the rat is constructed of a wire grid. Food grasped from the tray has to be released into the mouth or is otherwise lost through the floor beyond reach. This feature is particularly useful after stroke when rats are most likely to "cheat" by dragging food from the tray instead of grasping it. Perhaps, the most important drawback of the test is that rats reach into a tray that holds an abundance of food. Rats can displace large amounts of food by reaching inaccurately without major consequences because plenty of food will still be available in the tray. Thus, rats may develop "sloppy" reaching habits and there is little incentive for them to improve. Another limitation of the task is that performance assessment is limited to quantitative measures because the front wall of the apparatus is constructed from metal bars that obstruct the primary perspective necessary for analysis of limb kinematics.

The single pellet reaching task is an example of a labour intensive task (Whishaw and Pellis 1990). The experimenter administers the food targets one at a time, each as a single trial, for 20-30 trials/test session. The experimenter withholds pellet presentation in between trials so that animals walk to the back of the reaching box (Fig 1.6 A,B) and then again to the front. This allows the animal to reposition its body at the front of the box in

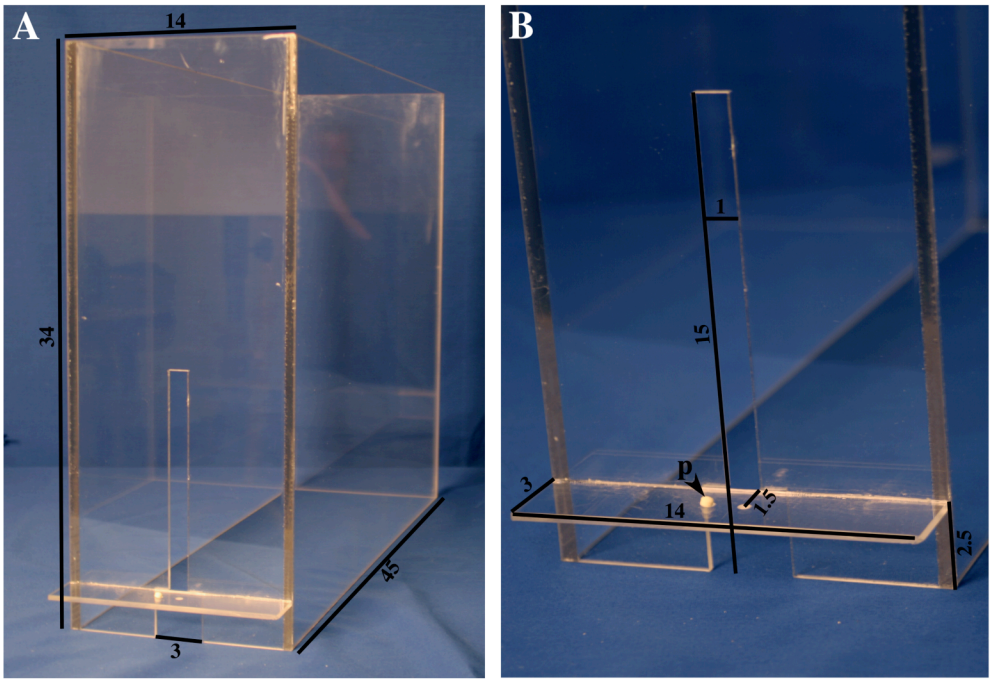


Figure 1.6. (A) Full perspective of the single pellet reaching box. (B) Close up view of the slot, shelf, and 45 mg pellet food pellet (P), which is the reaching target. All measurements are in centimeters.

preparation for the next trial. It also makes for a distinct break that differentiates one trial from the next. A successful reach is one in which the rat reaches through the slot with a single paw, grasps the food pellet, withdraws the paw with the pellet, and presents it to the mouth. Performance is measured quantitatively in terms of: success percentage, single reach success percentage, reaches/pellet retrieved (Whishaw et al. 1991). The multiple quantitative measures are useful for identifying rats that seem recovered in terms of success scores but demonstrate impairments in terms of multiple reach attempts to retrieve a food pellet. Perhaps the most valuable utility of the single pellet task is the possibility of analyzing limb kinematics and body posture during a reach (Whishaw et al. 1993). The construction of the reaching box allows cameras to be positioned in various perspectives to capture unobstructed views of any part of the body during a reach. A reach is comprised of 10 submovements, which are rated for quality from video records.

Because the performance of stroke rats is compared to control rats and to their own performance before injury, true recovery may be differentiated from compensation with this rating scale. Also, because the position of the pellet in relation to the box is the same for each trial, the reaching objective is consistent across trials. This allows the experimenter to generate a profile of the submovements of reaching that is based on multiple trials for each rat. Perhaps the only reason the single pellet reaching is still rivaled by other reaching tasks is that it is labor intensive. Rats are individually trained, tested, and their performance video recorded. Not to mention that hours are spent on offline analysis for adequate profiling of limb movements.

The importance of qualitative analysis of reaching performance

The importance of analyzing reaching performance beyond quantitative scores is evident considering the potential erroneous conclusions drawn in their absence. First, reaching is impaired by stroke for numerous reasons. Perhaps during the early post stroke period, success scores may be sufficient for capturing failed reaching attempts or even lack of reach attempts if animals are too impaired to even engage in the task. Nevertheless, preoperative success levels are usually restored within a few weeks of stroke (Whishaw 2000). End point measures of success would suggest complete recovery implying that the area damaged by the stroke is either only remotely important for skilled reaching, or that its functions are readily adopted by another brain region spared by the injury. But frame-by-frame video analysis demonstrates that many of the movements involved in reaching such as aiming and advancing of the limb, pronation and supination of the paw, are lost after stroke and not restored even when success scores recover. That the rats adopt compensatory strategies to overcome the lost submovements suggests that

the movements lost were unique to the brain area damaged. Conclusions about complete recovery are therefore not supported. The second pitfall of collecting reaching data without qualitative analysis of movement is the assumption that different strokes impair reaching in similar ways. Most forebrain strokes produce reaching impairments followed by spontaneous recovery that is reflected by a steady improvement in success scores over days. But the recovery curves for different stroke groups may be similar in shape. A quantitative analysis alone would assume that the stroke groups have similar impairments that abate at a comparable rate. While that may be true in some strokes, it is unlikely in others, especially when stroke infarcts are in disparate brain regions. For example, an impairment in limb aim may have a similar impact on success scores as an impairment in limb advance, or an impairment in paw supination, yet the nature of these impairments is obviously different and will escape unnoticed without qualitative measures.

Given the advantages of the single pellet reaching task and the importance of qualitative analysis of skilled reaching for stroke research, the laboriousness of administering the single pellet task will be overlooked and it will be used as the standard behavioural measure throughout the thesis.

The value of the laboratory rat as an animal model for stroke

The evidence provided above demonstrates that rats have comparable motor and vasculature systems to humans. Also, rats have paws that are similar in structure and grasping function as human hands. Finally, numerous procedures have been developed for inducing transient and permanent ischemia that parallel clinical stroke. Thus, experimenters have adopted procedures for inducing stroke and assessing skilled reaching

impairments and applied them to an animal that shares a similar anatomy to humans, making the rat an attractive animal model of clinical stroke.

But the rat represents more than a feasible alternative to studying stroke in the clinic. The rat offers a means for investigating aspects of stroke that are not be feasible in humans. For example, anatomical studies in clinical stroke are limited to magnetic resonance imaging (MRI). At best the procedure identifies the location and size of the infarct. With consent from the patient or family, the brain may be subjected to histological analysis postmortem. The same imaging technique is possible in rats. Moreover post mortem histological analysis is standard practice at the termination of stroke studies. This makes it possible to accurately reconstruct the infarct and to identify morphological changes at the cellular and protein levels that are only possible in freshly harvested tissue, which is rarely a viable option in clinical stroke. The advantages of studying stroke in rats are also evident in the sophistication of neurophysiological studies. For example, the function of ion channels is investigated *in vitro* in brain slices extracted from ischemic rats. In addition, the topography of motor maps (Kleim et al. 2003; Nudo and Milliken 1996; Teskey et al. 2003) is generated with intracortical microstimulation (ICMS) (Stoney et al. 1968) that is superior in resolution over the less invasive transcranial magnetic stimulation (TMS) counterpart applied in humans (Barker et al. 1985).

Despite the vast resources allocated to studying stroke, surprisingly little progress has been made in understanding the neural correlates of the wide range of motor symptoms caused by the injury. Even less has been gained in terms of advancing therapies. It is not clear whether the shortcomings lie in the experimental, clinical, or

translational (from experimental to clinical) sides of the research. It is clear however, that advances in the experimental aspects of stroke research may offer the most return on the investment because: (1) tight experimental control allows precise manipulation of independent variables, which facilitates the interpretation of the results and makes for sound science in general, (2) the experimental phase of the research can be the bottle neck for advancing both the translational and clinical aspect of the research. One approach to improving the experimental aspect of stroke research is to ensure that the behavioural impairments are clear and readily translated to clinical research.

Objective of the present thesis

The present thesis attempted to resolve some of the potential confounds of experimental stroke research by providing an in depth description of the long-term skilled reaching impairments that ensue from three different MCA strokes. The experiments focused on MCA ischemia because: (1) the majority of clinical stroke accidents occur within the MCA or one of its branches; (2) major portions of the forebrain motor system are irrigated by MCA branches in both humans and rats, so the probability that MCA stroke will produce an infarct that causes motor impairment in humans and rats is significant; and, (3) several procedures have been developed for inducing MCA stroke in rats.

Motor cortex stroke

The first three studies investigated motor cortex stroke. The motor cortex is in part irrigated by the MCA and also irrigated by the ACA. To effectively damage motor cortex, branches of both arteries have to be occluded within the circumscribed region. Thus, procedures such as endothelin-1 and photothrombosis are suitable for temporary

occlusion, whereas pial strip and electrocoagulation are suitable for permanent occlusion. Given that the motor cortex preparation was intended to serve as the standard against which strokes in later experiments were to be compared, the pial strip procedure was used to ensure consistent infarcts were produced.

In the first study, the effects of motor cortex stroke on skilled reaching and the neural basis of recovery were investigated in a series of experiments. As a prelude to this study, the location of the forelimb movement representations within motor cortex was identified in a group of rats with intracortical microstimulation (ICMS). Some of these rats received an ischemic stroke in the caudal forelimb area and others received a stroke in the rostral forelimb area. Skilled reaching was assessed using the single pellet task until recovery was complete. The brains were harvested, sectioned, and the location of the infarct reconstructed in relation to Bregma. Thus, the first experiment examined the effects of rostral, caudal, and complete motor cortex stroke on skilled reaching in three different groups of rats. The expectation was that the three groups would be differentially impaired, but that significant recovery would take place within two weeks of the stroke.

The second and third experiments explored the neural basis of this recovery. It was hypothesized that recovery would be mediated both within remaining motor cortex tissue as well as in other neocortical regions. In the second experiment, rats that recovered from caudal or rostral motor cortex strokes received an additional stroke within the same hemisphere such that after this stroke they had a complete motor cortex injury. The animals were tested on the single pellet task after the second stroke and until recovery was complete. The additional stroke was expected to impair all rats to the same extent as a complete motor cortex stroke. The third experiment examined how neural

tissue outside motor cortex supports recovery. Although the majority of the corticospinal fibers that control the paw contralateral to the injury would have been lost after motor cortex stroke, the subset of corticospinal fibers that originate: from the cortex lateral to the damaged motor cortex, from the cingulate cortex medial to the damaged motor cortex, and from the motor cortex in the opposite hemisphere, should have been preserved. The present experiment investigated whether two of these areas support recovery after motor cortex stroke. After rats recovered from a complete motor cortex stroke, they received an additional stroke either in the cortex lateral to the damaged motor cortex, or in the opposite motor cortex. Skilled reaching was assessed until performance reached asymptote. Additional injury lateral to the damaged motor cortex was expected to devastate reaching.

In the second study, the exploratory behaviour of rats in small enclosures was investigated. Most laboratory tasks designed for rats, including all reaching for food tasks, involve testing in a small enclosure. Nevertheless, there has been little attempt to document how rats explore their testing environment, which is critical when testing stroke rats in a reaching box because they are likely to interact differently with their environment than control rats. Thus, the exploratory behaviour of rats was documented as they investigated a small cylinder.

In the third study, the stages of recovery from motor cortex stroke was investigated. Several reports indicate that rats are severely impaired on skilled reaching after motor cortex injury but that preoperative success scores are restored within two weeks of injury. It is not clear, however, if this recovery is punctuated by specific milestones or if it represents a gradual improvement in reach accuracy. To investigate

this, the behaviour of rats with motor cortex stroke (experimental group) and rats with parietal cortex stroke (control group) was documented while in the reaching box in the immediate postoperative period and for the following two weeks. Reaching behaviour was decomposed into three phases: (1) orientation to the food pellet, (2) limb transport to the food pellet, (3) limb withdrawal with the food pellet and release into the mouth. The primary measures in this study were the impairment and recovery of these three phases of reaching.

Distal MCA stroke

The focus of the following three studies was distal MCA stroke. The injury was induced with electrocoagulation of MCA branches that are distal to the rhinal fissure. The infarct was expected to damage the neocortex lateral to motor cortex and spare motor cortex and subcortical structures.

In the fourth study, the effects of distal MCA stroke on skilled reaching with the contralateral-to-lesion paw was examined. A group of rats received a distal MCA stroke with electrocoagulation and was compared to a motor cortex group as well as a sham group. Because the neocortical area damaged by distal MCA stroke gave rise to corticospinal fibers, it was expected that the MCA rats would demonstrate a skilled reaching impairment. Quantitatively, this impairment may not have been readily distinguished from the motor cortex counterpart, but the deficits were expected to be qualitatively different because of minimal overlap between the lesions in the two strokes.

In the fifth study, the effects of distal MCA stroke on motor cortex function was explored. Because the neocortical area damaged by distal MCA stroke sends corticospinal projections and is intricately connected to the primary motor cortex, there

was reason to suspect that the motor cortex, although seemingly spared from distal MCA stroke, would be dysfunctional. To explore this possibility, the integrity of the motor cortex adjacent to the infarct was explored with ICMS several weeks after skilled reaching had recovered. A subset of rats with distal MCA stroke from the second study was used for this one.

In the sixth study, the effects of distal MCA stroke and motor cortex stroke on the ipsilateral-to-lesion paw was explored. Although the neural control of each paw is primarily in the contralateral hemisphere, each paw is to a lesser extent also controlled by the ipsilateral hemisphere because a small percentage of the corticospinal fibers that control a paw arise in the ipsilateral frontal cortex. Also, the striatum receives bilateral neocortical projections. Thus, it was expected that distal MCA stroke or motor cortex stroke would produce skilled reaching impairments in the ipsilateral-to-lesion paw, albeit to a lesser degree than contralateral stroke. Because lesions have been reported to induce morphological changes in the homotopic neocortex, neurophysiological adaptations may ensue there as well. To explore this possibility, the topography of the forelimb movement representations was explored in the contralateral-to-lesion motor cortex with ICMS several weeks after skilled reaching had recovered. Reorganization of forelimb movement representations was expected to reflect the morphological adaptations.

Proximal MCA stroke

The next two studies investigated proximal MCA stroke. Ischemia was induced transiently with an intraluminal suture. A range of infarcts was expected, on the smaller end, the infarct would be limited to subcortical structures (striatum, globus pallidus, associated fibers). At the larger end, the infarct was expected to damage the same

subcortical structure but more severely as well as neocortical zones typically damaged in distal MCA stroke.

In the seventh study, the effects of proximal MCA stroke on skilled reaching was examined in both paws. Rats with subcortical infarcts were expected to be comparatively less impaired with the contralateral-to-lesion paw than other stroke groups are with the same paw. Rats with conjoint subcortical and neocortical damage were expected to be more impaired than most groups, but perhaps not as impaired as rats with motor cortex stroke.

In the eighth study, the functional integrity of motor cortex was explored after transient proximal MCA ischemia. Although infarcts from this stroke were expected to spare motor cortex, they were also expected to damage its output fibers that course through the striatum en route to fuse with the internal capsule, which may compromise the neurophysiological function of the motor cortex. To explore this, the functional integrity of the motor cortex ipsilateral-to-infarct was investigated with ICMS several weeks after recovery. Motor cortex dysfunction was expected to be reflected in smaller forelimb motor maps.

Chapter 2

Recovery of skilled reaching following motor cortex stroke: Do residual corticofugal fibers mediate compensatory recovery?

Abstract

Motor cortex injury impairs skilled reaching in rats but success scores are eventually restored to approximate preoperative levels. The improvement is attributed to compensatory strategies such as substituting trunk rotations for the chronically lost rotatory movement of the limb made during transport and withdrawal. The present study examined the contributions of the rostral motor cortex (RMC) and the caudal motor cortex (CMC) to skilled reaching performance. The study also examined the role of the ipsilateral and the contralateral hemispheres in supporting the recovery in success scores characteristic of motor cortex injury. Rats were trained to reach for single food pellets and their recovery was documented with quantitative and qualitative measures in three experiments: (1) devascularization of the caudal motor cortex (CMC), or the rostral motor cortex (RMC), or both (MC), in the hemisphere contralateral to the reaching paw, (2) additional lesions to the CMC and RMC injuries such that the conjoint damage amounted to a MC lesion, and (3) MC lesion followed by damage in the neocortex lateral to the injury or in the opposite MC. The results showed that the CMC made the main contribution to skilled reaching performance followed by the RMC. MC damage was exacerbated by additional damage to the ipsilateral neocortex as compared to the contralateral neocortex. The results are discussed in relation to the idea that the proportional involvement of the neocortical areas to skilled reaching performance and its recovery may be related to the density of corticofugal fiber projections.

1. Introduction

Rats are adept at reaching for, grasping, and bringing food to the mouth with a single paw, a behavior termed skilled reaching (Whishaw and Pellis, 1990; Whishaw, 2005). The motor cortex and its fiber projections contribute to the skill, and are especially involved in the rotatory movements of the forelimb. The demand that skilled reaching places on the motor cortex, is reflected in motor map reorganization. Intracortical microstimulation (ICMS) shows an expansion of distal movement forelimb representations (wrist and digits) at the expense of proximal forelimb movement representations (shoulder and elbow) within motor cortex in response to reach training but not strength training or repetitive bar pressing (Nudo et al., 1996b; Remple et al., 2001; Kleim et al., 2004). The reorganization is supported by enhancement in synaptic efficacy (Rioult-Pedotti et al., 1998; Monfils and Teskey, 2004), synaptogenesis (Kleim et al., 2002; Kleim et al., 2004), and protein synthesis (Kleim et al., 2003; Luft et al., 2004). The importance of motor cortex to skilled reaching is also reflected in the reaching impairments ensuing from motor cortex injury (Castro, 1972b; Whishaw et al., 1986; Whishaw et al., 1991; Miklyaeva et al., 1993; Gonzalez and Kolb, 2003; Emerick and Kartje, 2004; Metz et al., 2005) or pyramidal tract section (Castro, 1972a; Whishaw et al., 1993; Z'Graggen et al., 1998; Piecharka et al., 2005). Nevertheless, rats eventually recover the ability to reach for and grasp food after injury, as evident by near complete restoration of success scores within 2-3 weeks of injury (Whishaw, 2000). The reaching movement is not normal, however, because trunk rotation compensates for rotatory movements once performed by the forelimb (Whishaw et al., 1991; Whishaw, 2000; Metz et al., 2005).

There are several views concerning the neural basis of the recovery from motor deficits produced by motor cortex injuries. One view is that the recovery is mediated by peri-infarct tissue within the same hemisphere including the motor and/or premotor cortex (Glees and Cole, 1950; Nudo et al., 1996a; Liu and Rouiller, 1999; Frost et al., 2003; Fridman et al., 2004; Jaillard et al., 2005). A second view is that neocortical areas in the opposite hemisphere mediate recovery (Cramer et al., 1997; Marshall et al., 2000; Carey et al., 2002; Schallert et al., 2003; Werhahn et al., 2003). Yet a different view suggests bilateral hemispheric involvement in recovery with the size of the injury guiding the extent of involvement of each hemisphere (Ward et al., 2004; Biernaskie et al., 2005; Gerloff et al., 2006; Nair et al., 2007). Comparing the competing views is complicated because they are based on studies with lesions in different locations. Also the capacity of the intact tissue to mediate recovery was explored with methods that varied between studies. Despite the inherent challenge of evaluating contrasting views derived with different methodologies, all of the studies share a common ground in attributing recovery to neocortical areas that have descending corticofugal projections, especially projections forming the corticospinal tract. Accordingly, it may be hypothesized that the contribution of neocortical regions to skilled reaching is proportional to the density of descending fibers involved in the control of the reaching forelimb. Because the densest contribution of fiber projections arises in the forelimb motor cortex contralateral to the reaching paw, and within this region of motor cortex, the caudal motor cortex (CMC) sends more descending fiber projections than the rostral motor cortex (RMC) (Wise and Donoghue, 1986; Nudo and Masterton, 1990a, b), damage to these regions should have the greatest effect on limb performance. Because neocortical areas in cingulate cortex, lateral frontal

cortex, and parietal cortex, make the second largest descending fiber contribution, their role in skilled reaching should be smaller but still greater than the contribution from the motor cortex of the opposite hemisphere, which contributes less than 5% of its corticospinal projection to its ipsilateral forelimb.

The hypothesis was tested in three experiments. The first experiment examined the role of the CMC and RMC subdivisions of motor cortex in skilled reaching. Rats were trained on a single pellet reaching task (Whishaw and Pellis, 1990) using a single paw and then received a lesion in the CMC, a lesion in the RMC, or a combined motor cortex lesion (MC), in the hemisphere contralateral to the reaching paw. Postoperative testing on the single pellet reaching task lasted two weeks. Then rats received additional rehabilitative training for one week on an easier tray reaching task (Whishaw et al., 1986) to ensure that recovery was complete before retesting on the single pellet reaching task. The second experiment examined the role of the remaining MC in the CMC and RMC groups in sustaining skilled reaching. Rats with CMC and RMC lesions, received an RMC and CMC lesion respectively to produce complete MC lesions. This lesion was followed by the same reach testing and rehabilitation paradigms in experiment 1. The third experiment examined the role of the hemisphere ipsilateral to MC lesion and the hemisphere contralateral to MC lesion in sustaining skilled reaching after MC damage. Rats with MC damage received an additional lesion in the neocortex lateral to the MC injury or in the opposite MC followed by testing for two weeks.

2. Materials and Methods

Animals

Fifty-six female Long-Evans hooded rats, 150-200 days old, and weighing 290 - 320 g at the start of the experiments from the University of Lethbridge vivarium were used. The experiments were conducted in compliance with the guidelines of the University of Lethbridge animal care committee and the Canadian Council for Animal Care. Rats were housed in Plexiglas cages (36 cm long, 20 cm wide, and 21 cm deep) with sawdust bedding, in groups of three, in a colony room maintained on a 12/12 h light/dark cycle (08:00-20:00 h).

Feeding

For initial and subsequent training, the rats were gradually food deprived to 90-95% of their body weight by once a day feeding of a measured quantity of Purina rat chow. For the week prior to reach training, each rat received twenty 45 mg dustless precision banana-flavoured pellets (product #F0021, Bioserve Inc., Frenchtown, NJ, USA) one hour prior to the daily chow ration. The objective was to introduce rats to the pellets, which would later serve as reaching targets, in a familiar environment. Once reach training began and until the end of the study, only rat chow was served in the home cage once a day in sufficient amounts to maintain all rats at 90-95% of their original body weight. The number of banana-flavoured pellets a rat received on a given training day depended on the rat's reaching accuracy, but it did not exceed 25 pellets (1.125 g).

Surgery

Intracortical Microstimulation

Motor mapping was conducted with the objective of investigating the topography of the caudal forelimb area (CFA), the rostral forelimb (RFA), and their borders. Rats were anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) and xylazine (5 mg/kg,

i.p.). A craniotomy was performed, the dura was retracted, and the pia was covered with inert silicon oil (37 ^{o}C). To relieve edema, a puncture to the cisterna magna drained cerebrospinal fluid. A grid (500 mm^2) was superimposed onto a digital image of the cortex to guide microelectrode penetrations (Remple et al., 2001).

A tungsten filament was inserted into a glass microelectrode (15-30 μm tip diameter) filled with concentrated saline (3.5 M). The tip of the electrode was lowered into the approximate location of layer V pyramidal neurons at 1550 μm below the surface of the cortex with a hydraulic microdrive. Electrical stimulation was delivered as trains of thirteen, 200 μs, 350 Hz cathodal pulses, from a stimulation isolation unit. At each site, current intensity was increased from 0 μA to 60 μA. An experimenter supported the rat's forelimb from underneath the elbow and recorded forelimb movements as proximal (elbow/shoulder) or distal (wrist/digit). In the case of two simultaneous movements, the movement obtained at the lowest threshold was recorded along with the current intensity. Movements of hind limb, vibrissae, jaw, head/neck were also recorded. Area representations were analyzed using the software Canvas (version 3.5, ACD Systems, http://www.deneba.com)

Pilot lesion

After the CFA and RFA were defined in a subject with ICMS, neocortical injury was induced while the cortex was still exposed. The blood vessels irrigating the CFA or the RFA were permanently occluded with microforceps connected to a bipolar electrocoagulator as they penetrated the cortical surface (Nudo et al., 1996a). The incision was closed and the animal's condition was monitored in a recovery room for 12 hours, at which time it was returned to the colony room.

First lesion

The experimental groups received either a partial motor cortex lesion in the first surgery: caudal motor cortex (CMC), or rostral motor cortex (RMC), or received a complete motor cortex (MC) lesion. For surgery, rats were injected with atropine nitrate (0.1 mg/kg i.p.; Sigma-Aldrich, St. Loius, MO) to facilitate respiration and then anesthetized with sodium pentobarbital (65 mg/kg, i.p.; Sigma-Aldrich, St. Loius, MO). Isoflourane (2%) was delivered via oxygen inhalation when necessary to maintain sedation. Four holes were drilled using a fine dental burr in the skull overlaying the target area at the following stereotaxic coordinates measured from Bregma [anterior (A) and lateral (L)]:

CMC: A = -0.5 mm, L = +1.0 mm; A= +2.0 mm, L= +1.0 mm; A= -0.5 mm, L= +4.0 mm; A= +2.0 mm, L= +4.0 mm

RMC: A = +2.0 mm, L = +1.0 mm; A= +4.5 mm, L= +1.0 mm; A= +2.0 mm, L= +4.0 mm; A= +4.5 mm, L= +4.0 mm

MC: A = -1.0 mm, L = +1.0 mm; A= +4.0 mm, L= +1.0 mm; A= -1.0 mm, L= +4.0 mm; A= +4.0 mm, L= +4.0 mm

The rectangular area enclosed by the four points was then trephinated and the dura was removed. The underlying tissue was devascularized by gently wiping away the pia mater and blood vessels with a saline-soaked cotton swab (Sofroniew et al., 1983). The incision was closed and the animal's condition was monitored in a recovery room for 12 hours, at which time it was returned to the colony room. For sham operation, the rats were

anesthetized and the superficial layers of the skull were drilled within the parameters of the trephination for partial motor cortex lesion.

Second lesion

In the second experiment, rats with partial motor cortex lesions received an additional lesion such that the conjoint damage amounted to a complete motor cortex lesion. Thus, the experimental groups were: CMC+RMC and RMC+CMC. In the third experiment, a group of MC rats received an additional lesion either in the lateral frontal cortex (LC) ipsilateral to the MC injury or in the opposite motor cortex (MC+MC group). For surgery, rats were anesthetized following the same procedures of the first lesion. Identifying Bregma was difficult because the first surgery shifted the skull plates. Thus, the coordinates for the second lesion were measured from the borders of first trephination. For the CMC+RMC group, the rostral edge of the CMC trephination served as the posterior edge of the additional RMC trephination. The RMC trephination measured 2 mm in length and was aligned to the same width as the CMC trephination. For the RMC+CMC group, the caudal edge of the RMC trephination served as the anterior border of the additional CMC trephination. The CMC opening measured 2 mm in length and was aligned to the same width as the RMC trephination. For the MC+LC group, the lateral edge of the MC trephination served as the medial edge of the LC trephination. The trephination was the same length as the MC opening and also measured 3 mm in width. Sham operation involved superficial skull drilling. For the MC+MC group, the rostral and caudal edges of the MC trephination were guided by the MC trephination in the opposite hemisphere. The lateral coordinates were measured from the skull midline to encompass an area that is 3 mm in width.

Skilled reaching

Reaching boxes

Single pellet reaching boxes were made of clear Plexiglas, with the dimensions 45 x 14 x 35 cm (Whishaw and Pellis, 1990). In the center of each front wall was a vertical slit 1 cm-wide, which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-deep shelf. Two indentations on the surface of the shelf were located 2 cm from the inside of the wall to hold the food targets. The indentations were aligned with the edges of the slit where rats could reach.

The tray boxes were 26 cm high, 28 cm deep, and 19 cm wide (Whishaw et al., 1986). The front of the boxes was constructed of 2mm bars separated from each other by a 9 mm gap. Clear Plexiglass tops allowed access to the inside of the box. A 4 cm wide and 0.5 cm deep tray containing food was mounted in front of the bars. Drinking water was accessible at the rear of the box.

Video recording

Reaching performance was video recorded using a Sony 3CCD camcorder (1000th of a second shutter speed) and a cold light source. A Sony videocassette recorder DSR-11 was used for subsequent frame-by-frame analysis. Representative still frames were captured from digital video recordings with Final Cut Pro HD (V.4.5 www.apple.com).

Bracelets for limb restraint

A bracelet made of Elastoplast fabric adhesive tape (Smith & Nephew Inc., Lachine, Quebec) was wrapped around the wrist of a particular paw to enlarge it such that

it could not be inserted through the slot of the reaching box (Whishaw et al., 1986). The treatment limited access to the food pellet to the non-bandaged paw. The bracelet did not cover the digits of the paw or otherwise impede the rat's movement during stepping, grooming, or supporting the other paw during reaching. The bracelet could be easily slipped off by the experimenter without discomfort to the rat's forelimb or denuding its fur.

Reach training

Single Pellet. The first week of training consisted of daily, 10 min sessions, for each rat. In the second and third weeks, rats also received daily training sessions but a session now consisted of 25 pellets, which may be considered 25 trials per session. The first five trials were considered practice trials but the succeeding 20 trials counted towards the score for the session. A session began when a rat was individually placed in a reaching box and ended when the rat was removed from the box. Training involved the achievement of three stages, in which the rat learned to orient to the food pellet through the slot, transport its limb through the slot to grasp the food pellet, retract its paw through the slot to release the food into its mouth.

For a naive rat, a number of food pellets were placed on the shelf and directly in front of the slot. The objective was to make the pellets accessible to the tongue or paw through the slot. In addition, crushed food pellets were positioned on the shelf as well to intensify the scent of the pellets to prompt the rat's interest. Once a rat was successfully taking food from the shelf, pellets were moved further from the slot to encourage use of a paw. Once a rat demonstrated a preference for one paw by making more reaching attempts with it, individual pellets were placed into the indentation contralateral to that

paw. Pellets so placed are accessible to that paw and not the other paw because the reaching paw pronates medially to grasp. A delay preceded the presentation of successive pellets. During the delay, the rats were shaped to leave the slot, go to the back of the box, and then return to the slot. Once this aspect of training was completed, food was placed on the shelf on a random schedule and the rats were shaped to return to the back of the box if they could not confirm by sniffing that food was located on the shelf. At preoperative asymptote, rats retrieved pellets successfully with a single paw on more than 50% of trials.

Tray reach rehabilitation. For reach rehabilitation, animals were allowed to reach in the tray task through the bars, grasp the food and retract it. Animals were given access to the reaching boxes for two 1-hr sessions per day, for 7 days.

Quantitative scoring of single pellet reaching

(1) *Trials attempted.* Each pellet presentation constituted a trial. An attempted trial is one in which the rat transported its paw through the slot at least once to retrieve the pellet. The percentage of trials attempted was calculated using the following formula:

Percent of trials attempted = (number of trials attempted/20) x 100

(2) *Total success.* A successful reach is one in which the food was grasped and transferred into the mouth using the designated paw. Success was measured using the following formula:

Percent of total success = (number of pellets retrieved/20) x 100.

(3) *Single reach success.* A single reach success is one in which the designated paw grasped the pellet with a single limb transport through the slot and was then withdrawn to

transfer food to the mouth. Single reach success was calculated using the following formula.

Percent of single reach success = (number of successful single reaches/20) x 100

(4) *Reaches/success.* This measure calculates the number of limb transports through the slot for successfully grasped pellets. Reaches/success were calculated using the following formula:

Reaches/success = total number of reaches on successful trials/number of successful trials

Qualitative scoring of single pellet reaching

For a qualitative analysis of single pellet reaching, a reach was subdivided into 10 components (Whishaw et al., 1993). (1) *Digits to midline*: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body. (2) *Aim*: using the upper arm, the elbow is adducted so that the forearm is aligned along the midline of the body, with the paw located just under the mouth. This movement involves fixation of the distal portion of the limb, so that digits remain aligned with the midline of the body. This is likely produced by a movement around the elbow that reverses the direction of movement of the paw to compensate for the adduction of the elbow. (3) *Digits semiflexed*: as the limb is lifted, the digits are semiflexed and the paw is supinated so that the palm faces the midline of the body. (4) *Digits open*: as the limb is advanced the digits are extended. (5*) Advance*: the head is slightly lifted to allow the limb to be advanced directly forward above and beyond the food pellet. (6) *Pronate*: using a movement of the upper arm, the elbow is abducted, pronating the paw over the food while the digits open. A movement of the paw around the wrist aids pronation of the paw onto the food. (7) *Grasp*: as the pads of the palm or the digits touch the food, the food is grasped by closure

of the digits. This can occur as an independent movement, or the grasp can occur as the paw is withdrawn. (8) *Supination I*: as the limb is withdrawn, the paw is dorsiflexed and is supinated 90^0 by a movement around the wrist and by adduction of the elbow. These movements can occur as soon as the food is grasped or can occur as the limb is withdrawn. (9) *Supination II*: as the rat sits back with food held in the paw, the paw is further supinated by 90^0 and ventroflexed to present the food to the mouth. (10) *Release*: the digits are opened and the food transferred to the mouth.

The first three successful reaches were analyzed frame-by-frame on the videotapes by two experimenters blind to the animals' group membership. Each component was rated on a three-point scale. If the movement appeared normal, it was given a score of "0". If it appeared slightly abnormal but recognizable it was given a score of "0.5"; for example if the wrist was only supinated (supination 1) 45° instead of a complete 90°. A score of "1" was assigned if the movement was absent or completely unrecognizable; for example if the forelimb was dragged across the shelf and retracted into the slot without any supination (supination 1) such that the dorsal surface of the forepaw faced upwards.

Statistical analysis

All quantitative scores were assessed with repeated measures ANOVA and Fishers's LSD test was used as a post hoc test for the main effects. Qualitative scores were assessed with the Kruskal-Wallis for non-parametric statistics. $P < 0.05$ was deemed statistically significant for all of the above. All post hoc multiple comparisons were carried out using the Bonferonni correction procedure to control family wise error inflation ($\alpha = 0.05$).

Histology

Rats were euthanized with an overdose of sodium pentobarbital and intracardially perfused with saline (0.9%) followed by 4% paraformaldehyde. The brains were removed, post-fixed and cryoprotected in 30% sucrose and 4% paraformaldehyde at 4°C for coronal sectioning (40 μm) and cresyl violet staining. Digital images from sections spanning the length of the lesion were captured for subsequent analysis.

3. Procedures

Experiment 1. The skilled reaching impairments from caudal and rostral motor cortex lesions

In a pilot study, rats were trained on the single pellet reaching task for three weeks. Then the forelimb movement representations were mapped with intracortical microstimulation (ICMS, n=6). At the end of the mapping session, the vessels irrigating the caudal forelimb area (CFA) were occluded with electrocoagulation (n=3) and in separate rats (n=3) the vessels irrigating the rostral forelimb area (RFA) were occluded. The rats were tested on the single pellet task every three days for three weeks and then sacrificed. The brains were extracted, sectioned, stained, and the lesions were digitally reconstructed in relation to Bregma. The stereotaxic coordinates corresponding to the two lesions were determined from the Rat Brain Atlas (Paxinos and Watson, 1997).

The same stereotaxic coordinates were used to damage the caudal and rostral motor cortex in larger groups of rats. The intention was to approximate the damage produced in the CFA and the RFA but without ICMS mapping, which may have been impractical in the large number of surgeries planned. The present approach also reduced the amount of time each rat received general anesthesia. The coordinates for the caudal

motor cortex lesion (CMC, n=8) were intended to damage the CFA, whereas the coordinates for the rostral motor cortex lesion (RMC, n=8) were intended to damage the RFA. Because the RFA is smaller in size than the CFA and because it was critical for the RMC and CMC lesions to be comparable in size with little or no overlap, the RMC coordinates were extended in the rostral direction to match the target area of the CMC. The third set of coordinates was intended to damage areas of the motor cortex (MC, n=7) to include both the CFA and the RFA. Sham surgery involved surface skull drilling within the MC coordinates (sham, n=7).

For the experiment, rats were trained on the single pellet reaching task for three weeks. They were then randomly assigned to a group and received a stereotaxic devascularization of the CMC, or RMC, or MC, or sham operation in the hemisphere contralateral to the reaching paw. Reaching performance was tested and video recorded on postoperative days 1, 3, 5, 7, 10, 12, and 14. To ensure that recovery was complete, rats received additional rehabilitative reach training in the tray reaching task (Vergara-Aragon et al., 2003). The tray task is less demanding than the single pellet reaching task because rats reach into a tray that holds an abundance of the food target. Rehabilitation consisted of access to the tray reaching apparatus for one hour in the morning (9:00 a.m.) and one hour in the afternoon (4:00 p.m.). Most rats reached throughout the duration of each rehabilitation session, which ensured repetition of the reaching, grasping, and withdrawing movements with the contralateral-to-lesion paw. After seven days of rehabilitation, rats were retested on the single pellet reaching task on three consecutive days and their behaviour was video recorded on the third day. Four rats (CMC, n=2;

RMC, n=2) were euthanized at the end of the experiment and the brains were extracted for histological analysis.

Experiment 2. The role of motor cortex remnants in sustaining skilled reaching after partial motor cortex damage

All of the rats from experiment 1 except four were used in the present experiment. The objective was to induce additional lesions within the damaged hemisphere of the two groups with partial motor cortex damage. The conjoint damage was intended to produce a lesion similar to that of the MC group in the first experiment. Thus, the CMC group received RMC lesions (CMC+RMC, n=6), whereas the RMC group received CMC lesions (RMC+CMC, n=6). The other two groups received sham surgery (MC+sham, n=7) and (Sham+Sham, n=7). Performance in the single pellet reaching task was assessed and video recorded on post operative days 1, 3, 5, 7, 10, 12, and 14. Then the same tray reach rehabilitation regiment was administered to ensure that recovery was complete. After seven days of rehabilitation, rats were retested on the single pellet reaching task for three consecutive days and their behaviour was video recorded on the third day. All rats were euthanized at the end of the experiment and the brains were extracted for histological analysis.

Experiment 3. The role of the ipsilateral and the contralateral hemispheres in sustaining skilled reaching after complete motor cortex damage

A naive group of rats was trained on the single pellet reaching task for three weeks then received a motor cortex devascularization in the hemisphere contralateral to the reaching paw (MC, n=20). Postoperative reach testing and rehabilitation was administered using the same protocol in the previous experiments. MC rats were then

randomly assigned to one of three groups for additional surgery: one group received a lesion in the neocortex lateral to the damaged motor cortex (MC+LC, n=6); a second group received a motor cortex lesion in the opposite hemisphere (MC+MC, n=6); a third group received sham surgery (MC+Sham, n=8). Performance on the single pellet reaching task was assessed and video recorded on postoperative days 1, 3, 5, 7, 10, 12, and 14. All rats were euthanized at the end of the experiment and the brains were extracted for histological analysis.

4. Results

Histology

Experiment 1. The skilled reaching impairments from caudal and rostral motor cortex lesions & Experiment 2. The role of motor cortex remnants in sustaining skilled reaching after partial motor cortex damage

The devascularization lesions of the CMC and RMC matched the corresponding electrocoagulation lesions from the ICMS pilot study, for size and location. But the present description will only focus on the rats that received a devascularization injury. Damage was localized to dorsal aspects of the neocortex in all lesion groups. It was evident from the coronal sections that damage was confined to rostral aspects of the motor cortex in the RMC group (Fig 2.1, left), caudal aspects of the motor cortex in the CMC group (Fig 2.1, centre), but included both rostral and caudal aspects of motor cortex in the MC group (Fig 2.1, right). Minor overlap between the caudal aspects of the RMC lesion and the rostral aspects of the CMC lesions was evident in otherwise exclusive injuries (Fig 2.1 E, left and centre). Corpus callosum fibers degenerated directly beneath all lesions. Disfiguration in dorsal aspects of the striatum was evident in both the CMC

and the MC groups and was likely due to swelling and ventricular enlargement.

Otherwise, subcortical structures were intact in all groups.

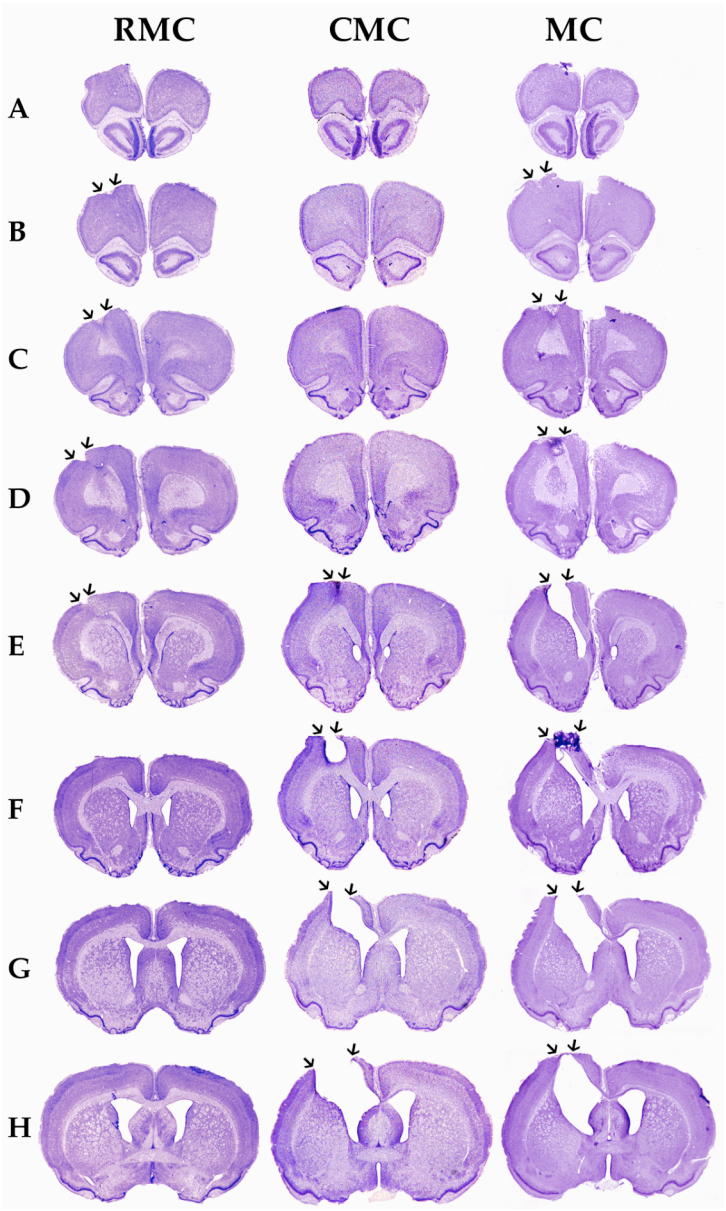


Figure 2.1. Representative coronal sections stained with cresyl violet of brains extracted from rats that received: rostral motor cortex lesion (RMC), caudal motor cortex lesion (CMC), or motor cortex lesion (MC) that included both RMC and CMC. The injury extended into all layers of the neocortex within the infarct boundaries, which is marked with arrows. There may have been minimal overlap between the caudal aspects of the RMC lesion and the rostral aspect of the CMC lesion (section E) but otherwise the lesions were disparate between the groups. The MC lesion encompassed the same sections as the conjoint RMC and CMC damage.

Most rats with partial motor cortex lesions received an additional devascularization injury to intact remnants of the motor cortex. The objective was to make their final lesion comparable in size and location to that of the MC+Sham (Fig 2.2, right). The RMC+CMC (Fig 2.2, left) and CMC+RMC (Fig 2.2, centre) lesions extended into all layers of the neocortex. Deformation of the striatum was evident in both groups and it was likely due to swelling and ventricular expansion. Subocortical structures were otherwise intact.

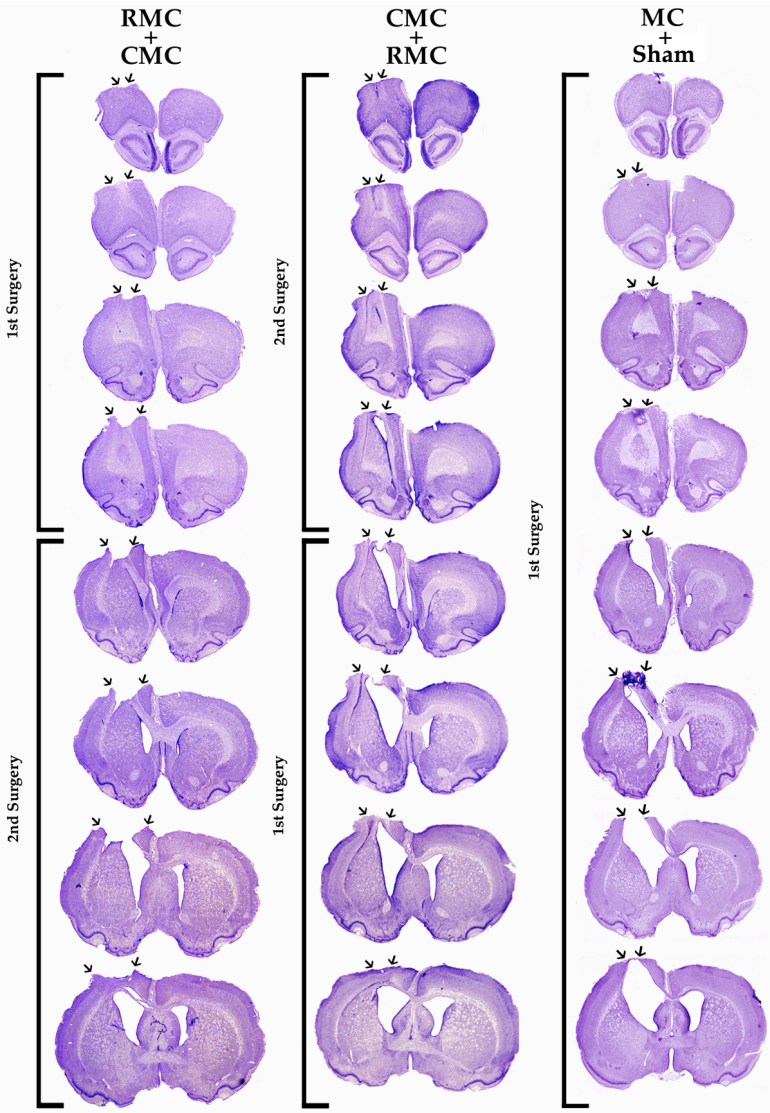


Figure 2.2. Representative coronal sections stained with cresyl violet of brains extracted from rats that received motor cortex lesions in one or two surgeries. The RMC+CMC group received a RMC lesion in the first surgery and a CMC lesion in the second surgery. The CMC+RMC group received a CMC lesion in the first surgery and a RMC lesion in the second surgery (CMC+RMC). The MC+Sham group received a motor cortex lesion encompassing the RMC and CMC in the first surgery and sham operation in the second surgery. The RMC+CMC and CMC+RMC produced comparable lesions that were also similar to the MC lesion. Lesion boundaries are marked with arrows.

Experiment 3. The role of the ipsilateral and the contralateral hemispheres in sustaining skilled reaching after complete motor cortex damage

A naive group of rats that received MC devascularization sustained a similar neocortical injury to the experimental groups above (Fig 2.3 left hemisphere in all panels). The MC+Sham group was sham operated in the second surgery and thus did not sustain any additional damage (Fig 2.3, left). The MC+MC group received a motor cortex lesion in the opposite hemisphere that was slightly smaller in the rostral aspect but otherwise comparable in size and location to the first lesion (Fig 2.3, centre). The MC+LC group received its second lesion in the neocortex lateral to the motor cortex lesion and the two injuries formed a contiguous lesion (Fig 2.3, right). This LC injury damaged neocortical sensory areas of the forelimb, hindlimb, and face.

Skilled reaching

Experiment 1. The skilled reaching impairments from caudal and rostral motor cortex lesions

The experiment investigated the contribution of caudal and rostral MC subdivisions to skilled reaching. According to quantitative and qualitative measures of skilled reaching, the effects of RMC damage were mild in comparison to the more

severely impaired CMC group. The MC group was the most impaired group on both measures of skilled reaching.

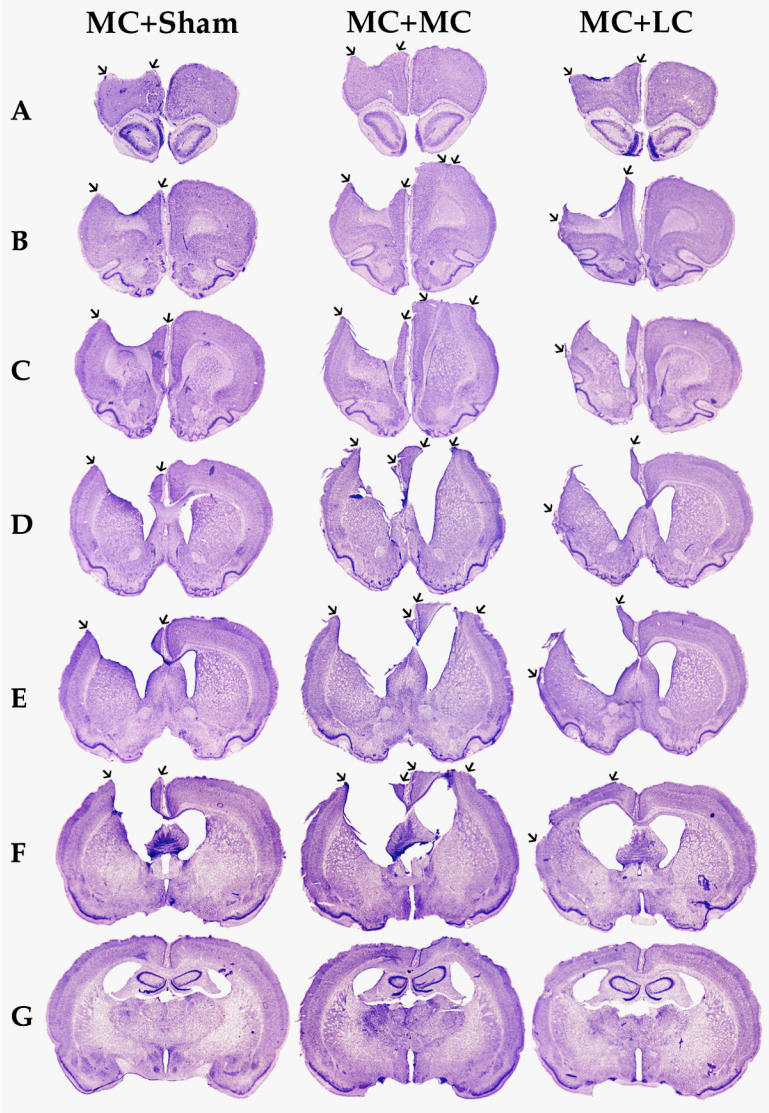


Figure 2.3. Representative coronal sections stained with cresyl violet of brains extracted from rats that first received motor cortex lesions in the left hemisphere. In the second surgery, the MC+Sham group was sham operated, the MC+MC group received a motor cortex lesion in the opposite hemisphere, and the MC+LC group received a lesion in the neocortex lateral to the already damaged motor cortex to create a contiguous lesion (MC+LC). Lesion boundaries are marked with arrows.

Quantitative scoring of single pellet reaching

(1) Trials attempted. After seven days of preoperative training, all rats attempted 100% of the trials in a reaching session. The percentage of trials attempted declined significantly after injury, however [$F(3,26) = 7.92$, $p<0.01$]. Follow up Fisher's LSD indicated that the MC group attempted fewer trials than the other three groups. A significant day effect [$F(10,260) = 12.00$, $p<0.01$], a day X group interaction [$F(30,260) = 6.24$, $p<0.01$], and follow up Fisher's LSD, suggested that the decline in reach attempts for the MC group was most severe in the first three postoperative days.

(2) Total success. The groups had comparable success scores before surgery but differed significantly from each other after injury [$F(3,26) = 15.01$, $p<0.01$]. Follow up Fisher's LSD indicated that success scores were hierarchically distributed: MC<CMC<RMC=Sham (Fig 2.4 A). Success scores improved across test days [$F(10,260) = 20.01$, $p<0.01$]. Most notably are the significantly improved success scores after tray reach rehabilitation as compared to all other test days including postoperative day 14. The rate of improvement was also different between groups as evident by a day X group interaction [$F(30,260) = 2.07$, $p<0.01$]. After tray reach rehabilitation, when improvement appeared to have reached asymptote, the success scores of the MC group were still significantly poor in comparison to the sham and RMC groups but not different from the CMC group.

(3) Single reach success. Performance was comparable between groups before surgery but differed significantly after injury [$F(3,26) = 19.92$, $p<0.01$]. Follow up Fisher's LSD indicated that single reach success scores were hierarchically distributed: MC<RFA<Sham and CMC<Sham (Fig 2.4 B). Performance improved across test days [F

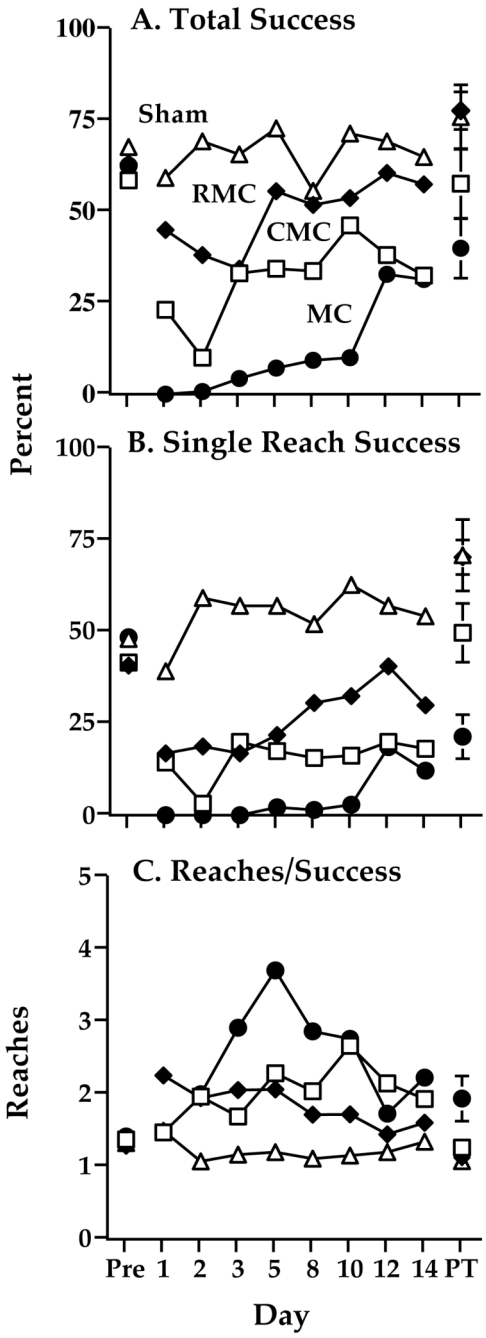


Figure 2.4. Performance was assessed on three quantitative measures of skilled reaching. Animals were preoperatively trained for three weeks. Performance during the last week of training is averaged to one time point (Pre). Postoperative testing was conducted on days 1, 2, 3, 5, 8, 10, 12, and 14 after surgery. Performance after tray reach rehabilitation is summarized in one time point (PT). The experimental groups: rostral motor cortex lesion (RMC), caudal motor cortex lesion (CMC), and motor cortex lesion (MC) were differentially impaired when compared to one another and to Sham on measures of (A) total success, (B) single reach success, and (C) reaches/success. Recovery rate also varied between groups.

(8,208) = 20.51, p<0.01] and success scores were significantly better after tray reach rehabilitation than on the next best performance (postoperative day 14). The rate of improvement was group dependent as evident by a day X group interaction [F (24,208) = 2.31, p<0.01]. Nevertheless, the MC group was still significantly impaired in relation to the other groups even after tray reach rehabilitation.

(4) Reaches/success. Groups had similar reaches/success scores before surgery but their scores differed significantly after injury [F (3,26) = 7.04, p<0.01]. Follow up Fisher's LSD indicated that the three lesion groups had more reaches/success than the sham group (Fig 2.4C), as did the MC group in relation to the RMC group. The number of reaches/success declined across recovery days [F (10,260) = 9.33, p<0.01] and approached its lowest score after tray reach rehabilitation. Improvement on this measure varied between groups across recovery days as confirmed by a day x group interaction [F (30,260) = 5.66, p<0.01]. The MC group was impaired in relation to the Sham and RMC groups even after tray reach rehabilitation.

Qualitative scoring of single pellet reaching

Postoperative day 1. Significant impairments were evident on: sniffing to locate the food pellet [Sniff; H=10.89, p<0.01], pronating the paw onto the food pellet [Pronation; H=6.84, p<0.05], supinating the paw after the pellet was grasped [Supination 1; H=9.93, p<0.01], supinating the paw to present food to the mouth [Supination 2; H=13.08, p<0.01], extending the digits to release the food pellet into the mouth [Release; H=9.30, p<0.01]. Post hoc tests showed that the group differences arose from significantly high scores by the CMC and RMC groups in relation to the Sham group (Fig 2.5 A, Fig 2.6, and Fig 2.7). The CMC and RMC groups were not different from one

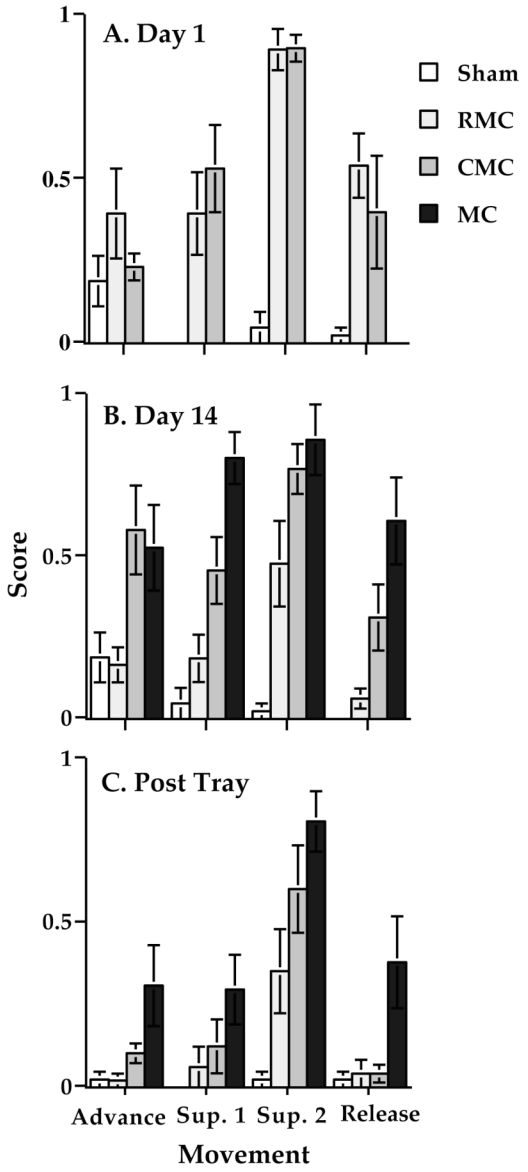


Figure 2.5. Qualitative scores on four of the elements that comprise a reach: limb advance during transport (Advance), paw supination after grasping (Supination 1), paw supination to present the food pellet to the mouth (Supination 2), digit extension to release the food (Release). The experimental groups: rostral motor cortex lesion (RMC), caudal motor cortex lesion (CMC), and motor cortex lesion (MC) were compared to each other and to a sham operated group. (A) Postoperative day 1. The RMC and CMC groups were equally impaired on the four elements shown. The MC group could not be assessed because it did not successfully grasp any pellets. (B) Postoperative day 14. The RMC scores improved almost to sham levels except on supination 2. The CMC and MC groups were impaired on the four elements, with the MC group being more severe. (B) Post tray. Tray reach rehabilitation improved all the elements comprising a reach in all experimental groups. Nevertheless, the MC group was impaired in relation to the other groups and chronic impairments in supination 2 were evident in all experimental groups.

another however. Scoring the MC group was not possible because rats in this group did not successfully grasp pellets during this session.

Postoperative day 14. Significant impairments were evident on: advancing the limb through the slot to reach the pellet [Advance; $H=8.22$, $p<0.05$; post hoc CMC>RMC], Supination 1 [$H=16.86$, $p<0.01$; post hoc MC>CMC>Sham and MC>RMC], Supination 2 [$H=16.12$, $p<0.01$; post hoc MC=CMC=RMC>Sham], and Release [$H=15.40$, $p<0.01$; post hoc MC>RMC=Sham and CMC>Sham] (Fig 2.5 B, Fig 2.6, and Fig 2.7).

Post tray reach rehabilitation. Chronic impairments were evident on: Advance [$H=10.24$, $p<0.05$; MC>RFA=Sham] and Supination 2 [$H=14.47$, $p<0.01$; MC>RFA=Sham and CMC>Sham] (Fig 2.5 C, Fig 2.6, and Fig 2.7).

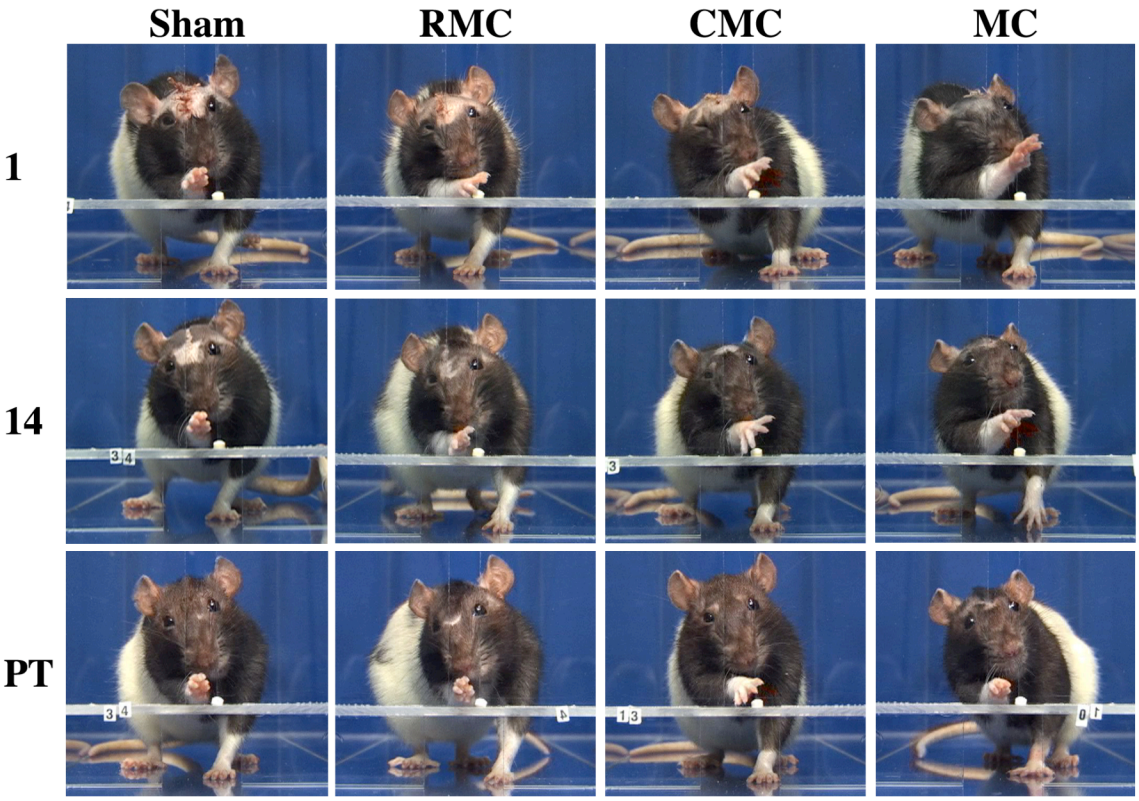


Figure 2.6. Representative still frames captured during limb transport in which the limb is advanced through the slot and the digits are extended. On the first postoperative day (1), impairments in limb advance were evident by an exaggerated limb transport supported by trunk twisting in the caudal motor cortex group (CMC) and by trunk raising in the motor cortex group (MC). Improved posture is evident in the CMC group on postoperative day (14) and in the MC group after tray reach rehabilitation (PT). Limb transport was comparable in the rostral motor cortex (RMC) and sham operated groups.

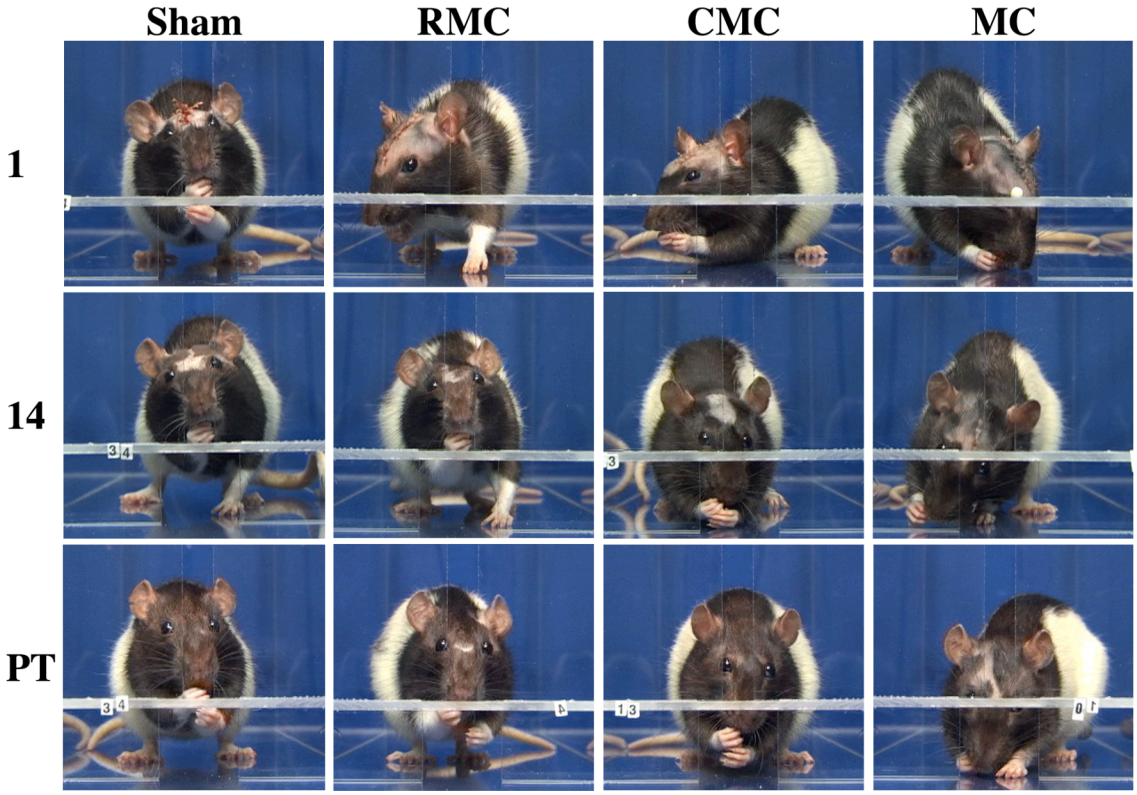


Figure 2.7. Representative still frames captured during limb withdrawal in which the paw is supinated (supination II) to present the food to the mouth and the digits extend to release the food into the mouth. On the first postoperative day (1), impairments in supination II and digit extension were evident in the rostral motor cortex (RMC) and caudal motor cortex (CMC) groups as evident by rats chasing their paw to extract the food pellet from it. Limb withdrawal could not be assessed in the motor cortex group (MC) because it did not successfully grasp a food pellet. Limb withdrawal recovered in the RMC group to resemble the sham operated group two weeks after injury (14). Chronic impairments as evident by dropping of the forequarters to the floor and paw chasing were evident in the both the CMC and MC groups even after tray reach rehabilitation (PT).

Experiment 2. The role of motor cortex remnants in sustaining skilled reaching after partial motor cortex damage

The experiment investigated the contribution of perilesion tissue within the MC in mediating recovery after caudal or rostral motor cortex injury. After the second lesion, CMC+RMC and RMC+CMC groups showed additional quantitative and qualitative reaching impairments. Performance improved after tray reach rehabilitation and the reaching impairments of both groups were not distinguishable from those of the MC group.

Quantitative scoring of single pellet reaching

Because the same rats from experiment 1 were used in the present experiment, the quantitative scores obtained at the end of experiment 1 served as the preoperative scores for the present experiment.

(1) Trials attempted. Rats attempted to reach on 100% of the trials prior to the second surgery. Although the number of trails attempted by the RMC+CMC group declined in the first three postoperative days, it did not reach significance. The second surgery did not affect the number of trials attempted by the other groups.

(2) Total success. Group differences existed before the second surgery and persisted after it was administered [$F(3,21) = 4.33$, $p<0.05$]. But follow up Fisher's LSD indicated a shift in group contribution to the main effect after the second surgery such that the RMC+CMC and the CMC+RMC groups were the ones significantly different from the Sham+Sham group (Fig 2.8 A). The absence of a day effect and lack of a day X group interaction suggests that performance was consistent within group across recovery days. Nevertheless, it is apparent from the recovery curves (Fig 2.8 A) that the second

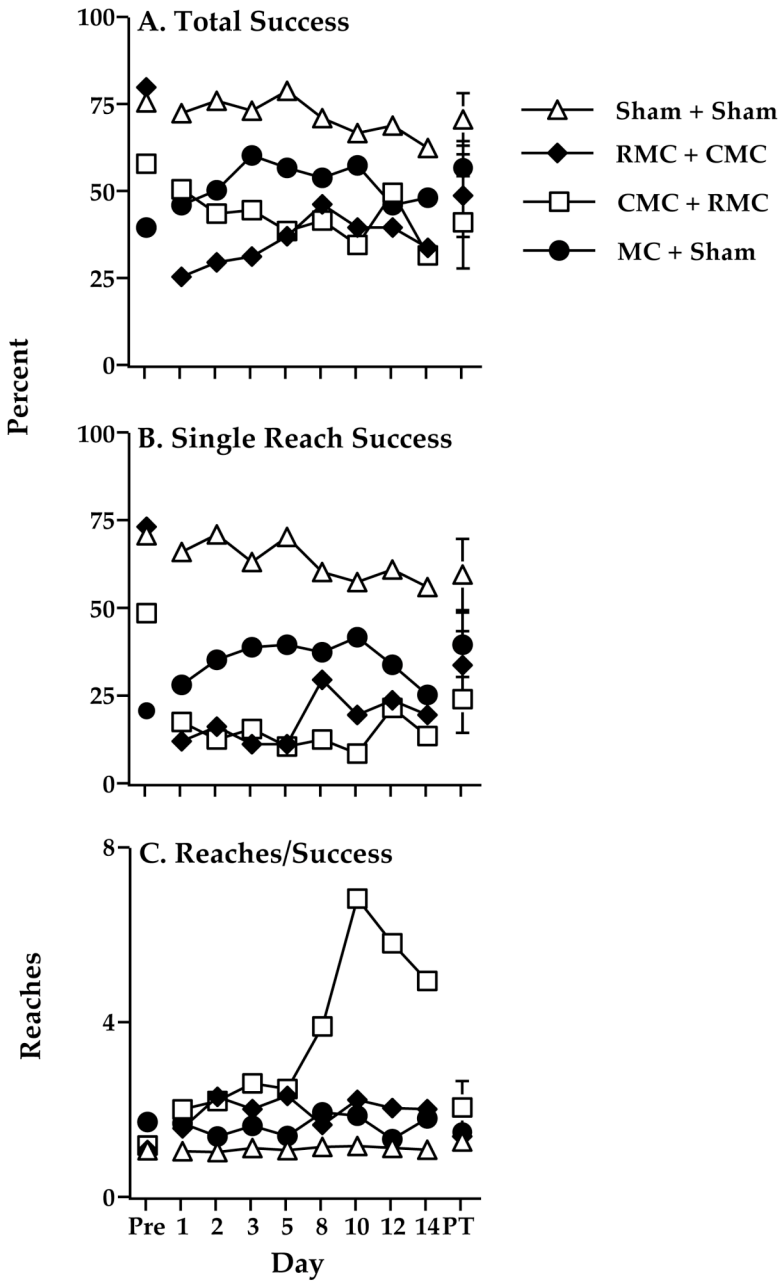


Figure 2.8. Performance was assessed on three quantitative measures of skilled reaching (A) total success, (B) single reach success, and (C) reaches/success. Scores after the rehabilitative training that succeeded the first lesion are represented here as the preoperative scores for the second surgery (Pre). After the second lesion, postoperative testing was conducted on days 1, 2, 3, 5, 8, 10, 12, and 14. Performance after tray reach rehabilitation is summarized in one time point (PT). The experimental groups: rostral motor cortex lesion (RMC), caudal motor cortex lesion (CMC), and motor cortex lesion (MC) were differentially impaired after receiving the second lesion when compared to one another and to the Sham+Sham group. Nevertheless, the experimental groups were comparable to one another after tray reach rehabilitation.

lesion most adversely affected the RMC+CMC group especially during the first postoperative week.

(3) Single reach success. The MC group was impaired in relation to the other groups before the second surgery. Additional group differences emerged after the second lesion [$F(3,21) = 7.56$, $p<0.01$] and Fisher's LSD showed that all three lesion groups were significantly impaired in relation to the Sham+Sham group (Fig 2.8 B). A significant effect of day suggested that performance improved across test days [$F(9,189) = 2.68$, $p<0.01$] and this was particularly evident in the improvement after tray reach rehabilitation as compared to performance on postoperative day 14. A significant day X group interaction [$F(27,189) = 2.53$, $p<0.01$] suggested that performance varied between groups across test days. Post hoc tests indicated that the CMC+RMC and RMC+CMC groups scored consistently poorly in the first 10 postoperative days as compared to the Sham+Sham group.

(4) Reaches/success. The group differences that existed prior to the second surgery were lifted after its administration (Fig 2.8 C). A significant effect of day [$F(9,189) = 2.80$, $p<0.05$] may account for the increased number of reaches/success in the second postoperative week.

Qualitative scoring of single pellet reaching

Postoperative day 1. Significant impairments were evident on: Aim [$H=7.88$, $p<0.05$; post hoc RMC+CMC>Sham+Sham], Supination 1 [$H=17.48$, $p<0.01$; post hoc RMC+CMC=CMC+RMC>MC+Sham=Sham+Sham], Supination 2 [$H=13.90$, $p<0.01$; post hoc all lesion groups>Sham+Sham], and Release [$H=11.12$, $p<0.05$; post hoc RMC+CMC=CMC+RMC>Sham+Sham] (Fig 2.9 A).

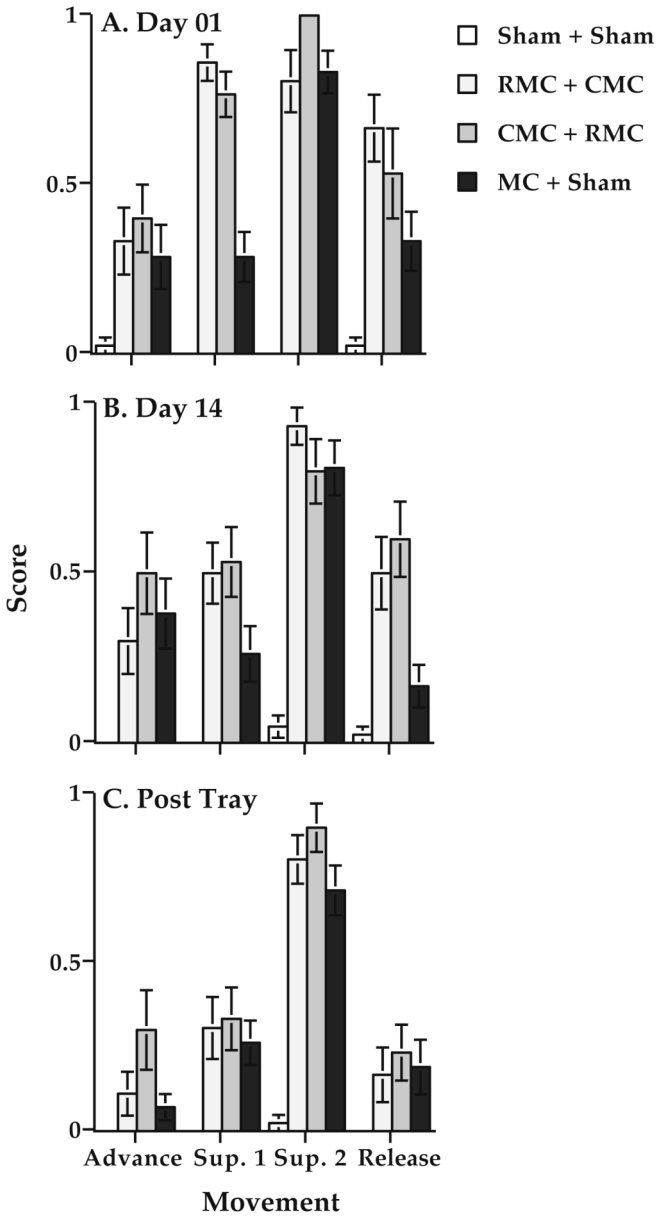


Figure 2.9. Qualitative scores on four of the elements that comprise a reach: Advance, Supination 1, Supination 2, and Release. The experimental groups: RMC+CMC, CMC+RMC, and MC+Sham were compared to each other and to a Sham+Sham group. (A) On postoperative day 1 (Day 1), the experimental groups were equally impaired on the advance, supination 2, and release. The two-stage lesion groups (CMC+RMC and RMC+CMC) were more impaired than the MC+Sham group on supination 1. (B) On postoperative day 14 (Day 14), the two-stage lesion groups showed some improvement on supination 1. Nevertheless, the MC+Sham group appeared less impaired on the supination 1 and release. (C) Tray reach rehabilitation (Post tray) improved the advance, supination 1, and release in the two-stage lesion groups such that their scores were similar to those of the MC+Sham group. All three experimental groups were chronically impaired on supination 2.

Postoperative day 14. Significant impairments were evident on: Supination 1 [H=11.22, p<0.05; post hoc RMC+CMC=CMC+RMC>Sham+Sham], Supination 2 [H=12.54, p<0.01; post hoc all lesion groups >Sham+Sham], and Release [H=9.87, p<0.05; post hoc RMC+CMC=CMC+RMC>Sham+Sham] (Fig 2.9 B).

Post tray reach rehabilitation. Performance improved after tray reach rehabilitation but significant impairments were still evident on: Supination 2 [H=15.11, p<0.01; post hoc all lesion groups>Sham+Sham] (Fig 2.9 C).

Experiment 3. The role of the ipsilateral and the contralateral hemispheres in sustaining skilled reaching after complete motor cortex damage

The experiment investigated the contribution of both hemispheres to recovery from complete MC damage. Inducing damage in the MC opposite to the first MC injury (MC+MC group) did not have an appreciable effect on skilled reaching when compared to sham operation (MC+Sham). In contrast, damage in the neocortex lateral to the first MC injury (MC+LC) devastated skilled reaching.

Quantitative scoring of single pellet reaching

The present results section will focus on skilled reaching scores after the second lesion because the effects of MC injury have already been described in the previous experiments. The quantitative single pellet reaching scores obtained from MC rats after tray reach rehabilitation in the present experiment served as the preoperative score for the second lesion.

(1) *Total success*. The three groups were differentially impaired at reaching for food after the second surgery [$F(2,17) = 9.24$, $p < 0.01$]. Follow up Fisher's LSD showed that the main effect of group was due to the poor performance of the MC+LC group as

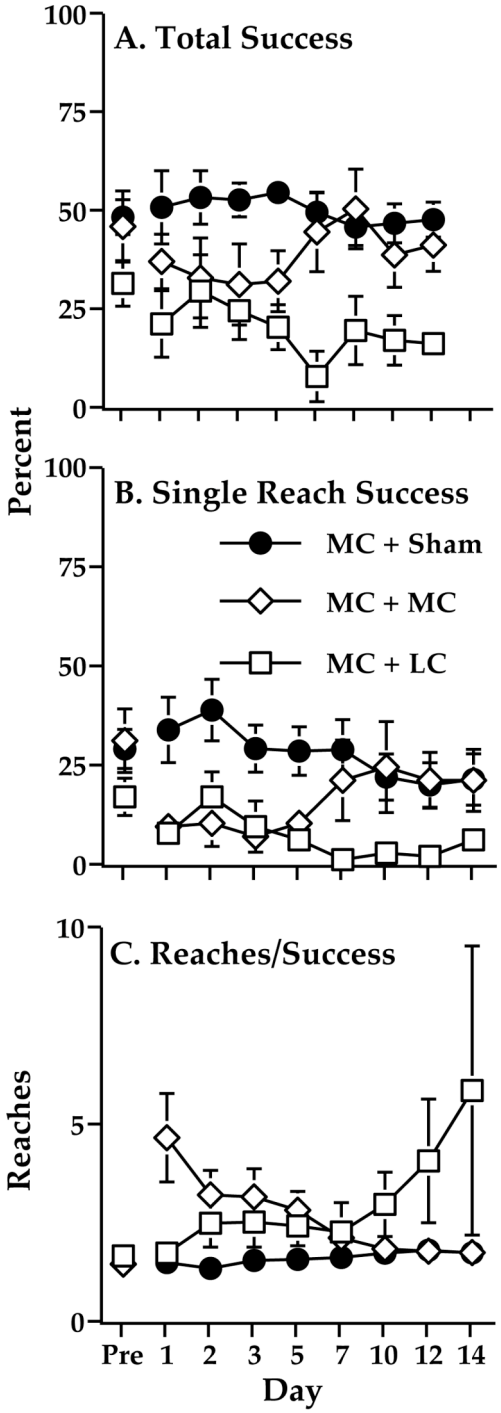


Figure 2.10. Performance was assessed on three quantitative measures of skilled reaching (A) total success, (B) single reach success, and (C) reaches/success. Scores after the rehabilitative training that succeeded the first lesion that damaged the motor cortex are represented here as the preoperative score (Pre). After the second lesion, postoperative testing was conducted on days 1, 2, 3, 5, 8, 10, 12, and 14. The MC+MC group was similar to the MC+Sham group, whereas the MC+LC group was severely impaired and showed no signs of improvement.

compared to the MC+Sham and MC+MC groups (Fig 2.10 A). The MC+MC and MC+Sham groups had comparable success scores. The lack of a day effect and absence of a day X group interaction, suggested that performance was consistent within group across recovery days.

(2) *Single success*. The three groups were differentially impaired after the second surgery [$F(2,17) = 5.11$, $p < 0.05$]. Follow up Fisher's LSD showed that the effect was due to the poor score of the MC+LC group in relation to the MC+Sham group (Fig 2.10 B). A day X group interaction [$F(14,119) = 2.48$, $p < 0.01$] might reflect the improvement in the performance of the MC+MC group during the second postoperative week.

(3) *Reaches/success*. Group differences were not detected here perhaps because of the large variability in the number of reaches/success by the MC+LC group (Fig 2.10 C). A significant day X group interaction [$F(14,119) = 2.49$, $p < 0.01$] captured the group differential across test days however.

Qualitative scoring of single pellet reaching

Postoperative day 1. Significant impairments were evident on: Supination 1 [$H=7.24$, $p<0.01$; post hoc MC+LC>MC+MC=MC+Sham] and Release [$H=9.28$, $p<0.01$; post hoc MC+LC>MC+MC=MC+Sham] (Fig 2.11 A).

Postoperative day 14. Significant impairments were evident on: Aim [$H=7.03$, $p<0.05$; post hoc MC+LC>MC+MC=MC+Sham], Supination 1 [$H=8.13$, $p<0.05$; post hoc MC+LC>MC+MC], and Release [$H=6.83$, $p<0.05$; post hoc MC+LC>MC+MC=MC+Sham] (Fig 2.11 B).

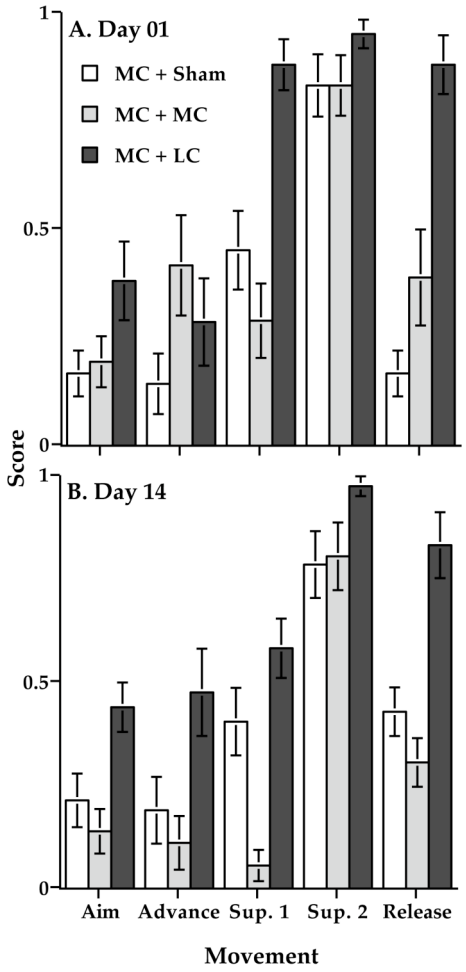


Figure 2.11. Qualitative scores on five of the elements that comprise a reach: elbow aim (Aim), Advance, Supination 1, Supination 2, and Release. (A) On postoperative day 1 (Day 1), the MC+LC group was impaired as compared to the other two groups on: Aim, supination 1, and release. (B) On postoperative day 14, the movement scores and group differences were mostly unchanged except that the MC+MC group showed some improvement in the Advance and Supination 1 elements.

Limb transport trajectory. The MC+LC group showed a characteristically inaccurate limb transport trajectory that prompted additional analysis. On any given trial an MC+LC rat transported its limb numerous times (typically ≥10) through the slot before the digits contacted the pellet regardless of whether contact resulted in grasping of the pellet or in its displacement. In contrast, a misguided limb transport by the MC+MC

or MC+Sham groups was corrected in one or two additional reaches before the pellet was either successfully grasped or knocked off the shelf. The total number of reaches in a test session best exemplifies the group difference [$F(2,17) = 3.44$, $p < 0.05$] and follow up Fisher's LSD showed that the MC+LC had more reaches than the MC+Sham group.

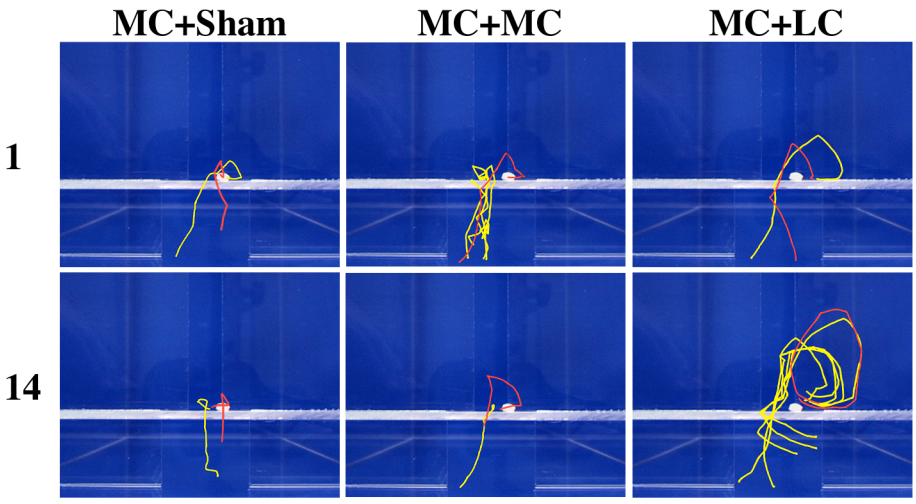


Figure 2.12. Representative tracings of limb transports that preceded successful grasping of the pellet. Each panel has tracings from one representative rat from the MC+Sham, MC+MC, or MC+LC groups performing a single trial. On postoperative day 1 (Day 1), the MC+MC rat transported its limb too medially to the pellet to grasp (yellow) several times before correcting its aim or adjusting its body to accurately target the pellet and grasp it (red). In contrast, the MC+LC group had a laterally misguided limb transport (yellow) followed by a correct trajectory that ended in a pellet grasp (red). After sham surgery, the limb transport trajectory of the (MC+Sham) was relatively direct. On postoperative day 14, the trajectory of the MC+MC rat was direct and similar to that of the MC+Sham group, whereas the trajectory of limb transport of the MC+LC showed severe impairments and an exaggerated limb transport lateral to the pellet. Only after several misguided limb transports (yellow) was the trajectory corrected (red) to allow grasping.

The misguided limb transport trajectories had a directional bias in two of the lesion groups. On the first postoperative day 1, 52.56 $\pm$ 14.10% (mean $\pm$ SE) of the missed reaches by the MC+LC group were lateral to the pellet (Fig 2.12 right) and the remainder of the misses were either medial to the pellet or too short to reach the pellet. On postoperative day 14, the number of misses increased and the directional distribution

shifted such that 81.02 +7.63 % (mean ± SE) of the missed reaches by the MC+LC group were lateral to the pellet and the remainder were either medial to the pellet or too short to reach it. The distribution of the misguided reaches by the MC+MC group was biased medially to the pellet (Fig 12 center). On postoperative day 1, 83.29 ± 8.18 % (mean ± SE) of those misses were medial to the pellet and the remainder were either lateral or too short to reach the pellet. Although the number of misses declined for the MC+MC group by postoperative day 14, the distribution of the misses did not change because 77.01 ± 8.93 % (mean ± SE) of them were medial to the pellet and the remainder were either lateral or too short.

5. Discussion

The present study examined the contribution of several neocortical regions giving rise to corticospinal projections to skilled reaching and its recovery. The most severe skilled reaching impairments followed motor cortex (MC) lesions, while caudal motor cortex (CMC) lesions were less severe than were rostral motor cortex (RMC) lesions. Additional injury to the CMC or rostral MC produced impairments equivalent to those of single stage MC lesions, and injury lateral to MC further impaired skilled reaching. The effects of motor cortex injury in the opposite hemisphere were slight. Taken together the results suggest that impairments in a forelimb used for skilled reaching are related to the extent of injury to corticofugal fibers controlling that limb while recovery is proportional to spared corticofugal fibers controlling that limb. This conclusion suggests that recovery of function following MC stroke is in the main mediated by the ipsilateral-to-injury hemisphere and the capacity of that hemisphere's corticofugal projections to undergo plastic changes.

There are several competing views about the neural basis of the recovery from motor deficits induced by neocortical injuries. One view is that the recovery is mediated by peri-infarct tissue within the same hemisphere (Glees and Cole, 1950; Nudo et al., 1996a; Liu and Rouiller, 1999; Frost et al., 2003; Werhahn et al., 2003; Fridman et al., 2004; Jaillard et al., 2005). Support for this view comes from studies that show reorganization of function with intracortical microstimulation (ICMS) or transcranial magnetic stimulation after focal lesions to the hand area of motor cortex or its descending projections. A second perspective is that neocortical areas in the opposite hemisphere mediate recovery (Cramer et al., 1997; Marshall et al., 2000; Carey et al., 2002; Schallert et al., 2003; Werhahn et al., 2003). This view is supported by functional MRI studies that show bilateral neocortical activation in response to hand movements after stroke. A third view suggests that the size of injury guides the extent of involvement of each hemisphere (Ward et al., 2004; Biernaskie et al., 2005; Gerloff et al., 2006; Nair et al., 2007). This view is supported by deficit reinstatement with temporary deactivation of one hemisphere but not the other as well as bilateral neocortical activation in response to movements of the affected hand. Despite the challenge of evaluating views based on studies with different: lesions, stages of recovery, behavioural tests, and procedures for identifying the area mediating the recovery, the views share a common ground in suggesting recovery is mediated by neocortical areas from which corticospinal projections originate.

The objective of the present study was to test the hypothesis that behavioral deficits in skilled forelimb use are related to the extent to which regions of the neocortex that support corticofugal, and especially corticospinal projections, are damaged. According to Nudo and Masterton (1990 a,b), each neocortical hemisphere has three

zones of crossed neocortical projections. A smaller rostral and a larger caudal zone in motor regions of the neocortex (M1 and M2) that project into interneuron and motoneuron pools in spinal cord lamena V to IX of Rexus. Also a lateral zone in parietal neocortex (P2) that projects predominantly into the dorsal column nuclei. In addition, the contraleral neococortex contributes a small (about 5%) uncrossed projection. If forelimb control is dependent upon corticofugal projections and their allied circuits, then behavioral deficits should be proportional to the extent to which the origin of these projections is damaged. According to this notion, therefore, behavioral deficits should be related mainly to the extent of damage in crossed corticofugal projections.

Skilled reaching was the behavioural measure of choice because the behavior is dependent upon motor cortex and its corticofugal projections (Castro, 1972b, a; Whishaw et al., 1991; Whishaw et al., 1993). Furthermore, the quantitative and the qualitative assessments have been standardized on a single pellet reaching task (Whishaw et al., 1993). The task is also ideal for studying recovery because daily test sessions can be administered as early as the first postoperative day and until performance reaches asymptote. In the present study, rehabilitative reach training was administered on an easier reaching for food task before retesting in the single pellet reaching task. The objective was not to evaluate the efficacy of rehabilitative training but to provide the rats with additional opportunities to improve their reaching performance before drawing any links between reaching deficit and lesion after retesting on single pellet reaching.

The behavioral deficits of rats with partial motor cortex lesions further our understanding of the role of the MC in skilled reaching. First, they show that as in primates, the CMC is perhaps most critical to skilled reaching (Friel et al., 2005). CMC

lesions impaired reaching on both quantitative and qualitative measures, whereas RMC lesions only mildly impaired reaching movements. Although concurrent damage to both the CMC and the RMC as in the case of the MC group produced the most pronounced reaching impairments, similarities in reaching deficits between the MC and CMC lesions suggests that the CMC injury was central to the deficits of the MC injury. Second, that the RMC lesion produced mild reaching impairments shows that it makes a contribution to skilled reaching that is perhaps independent from that of the CMC (Barth et al., 1990; Friel et al., 2005). The two subdivisions are likely involved in mediating the same submovements of skilled reaching but perhaps to different extents because damaging them had proportional effects on the same submovements of skilled reaching. A similar argument has been presented from single unit recording findings, which show that the CMC and the RMC were concurrently active and quiescent during various phases of the reach (Hyland, 1998).

The recovery rates of rats with partial motor cortex also further our understanding of the role of motor cortex remnants in sustaining skilled reaching. Rats with RMC lesion had a more favourable recovery rate than rats with CMC lesion, which in turn showed better recovery than rats with MC lesions. Such disparity may have been related to the severity of the impairments that ensued immediately after injury. This interpretation implies that the neural region damaged was central to shaping the recovery rate. Furthermore, it also implies that the structures spared in each lesion group had differential capacities for sustaining skilled reaching. Accordingly, the CMC promoted rapid restoration of success scores and limb kinematics after RMC lesions, whereas the capacity of the RMC to restore skilled reaching after CMC lesions was limited. This

position is supported by the results of the second experiment, which showed that the initial impairments from the additional caudal lesion (RMC+CMC group) were more severe than the initial impairments from the additional rostral lesion (CMC+RMC group). Nevertheless, after tray rehabilitation the quantitative and qualitative scores of the two groups were not distinguishable from one another nor from the one-stage motor cortex lesion.

According to previous work showing behavioural savings from two-stage lesions when compared to one-stage lesions of the same neural zones (McIntyre and Stein, 1973; Simons et al., 1975; Finger and Simons, 1976; Stein et al., 1977; Shanina et al., 2005), the expectation was that the present two-stage MC lesions would have a favourable outcome as compared to the one-stage MC lesion. The histological results do not support any anatomical savings in the two-stage lesion groups (Shanina et al., 2005). Also, the long-term behavioural savings of two-stage MC damage were not apparent under the present testing conditions. The benefit of damaging the MC in two stages as opposed to one might be reflected in the better course of recovery from two-stage lesions, however. For example, rats with two-stage MC lesions attempted nearly all the trials of a test session after the first and after the second lesions, whereas there was a steep decline in the number of trials attempted after one-stage lesions during the first three postoperative days. Also, the reaching success asymptote achieved by the two-stage lesion group after the second lesion was quantitatively similar to that of the one-stage lesion group but was attained more rapidly in the two-stage lesion group.

Nevertheless, interpreting the proportionality of the impairments caused by CMC and RMC lesions is complicated by the potential size discrepancy between the two

injuries. The stereotaxic parameters for the caudal and rostral lesions were designed to include the neurophysiologically defined caudal forelimb area (CFA) and rostral forelimb area (RFA) respectively and minimize overlap. At the same time the areal parameters were designed to damage the same amount of neocortical tissue in the two groups. Thus, the majority of the neocortical tissue damaged by the caudal lesion was likely dedicated to the CFA, but because the RFA is smaller than the CFA, the rostral lesion likely encompassed the RFA as well as other body representations such as the whisker, jaw, or neck. Arguably then, the CMC lesion damaged more of the neocortex that is critical to forelimb function than the rostral lesion. In that sense the CMC lesion may be considered larger than the RMC lesion, which is consistent with the more severe impairments caused by the CMC lesion. An alternative interpretation of the severity of the impairment from the CMC lesion is that the CMC is inherently more critical to skilled reaching than the RMC counterpart. This possibility can be explored in a separate study by scaling the CMC lesion such that it matches the size of the RFA. If the deficits are still more severe from the CMC lesion, then it may be assumed that the area is intrinsically more critical to skilled reaching that the RMC. The idea is substantiated by studies showing neural adaptations specific to the CFA after reach training (Remple et al., 2001; Kleim et al., 2004; Monfils and Teskey, 2004).

MC lesions chronically compromised certain limb and body movements as evident by poor qualitative scores. But the survival of other limb movements and adoption of compensatory strategies allowed rats with MC lesions to eventually achieve success scores close to their pre-operative baseline. The involvement of other neocortical areas in sustaining skilled reaching after motor cortex injury was investigated by

administering additional lesions in the neocortex lateral to the damaged motor cortex or in the opposite motor cortex. The lesion ipsilateral to the injured MC devastated success scores to the point of no recovery, whereas the consequences of MC damage in the opposite hemisphere were minimal. Damaging the neocortex lateral to the injured MC produced chronic aiming impairments during limb transport that did not abate, and even deteriorated during the two weeks of testing. This impairment was central to the poor success scores. Furthermore, after lateral cortex lesions, even when the pellet was grasped, severe impairments in the second paw supination to present the food pellet to the mouth and digit extension to release the food pellet into the mouth, were apparent. Rather than the paw being brought to the mouth, the mouth "chased" the paw to extract the pellet from it.

Because symptoms were so severe and persistent after damage to the neocortex lateral to the MC, it is probably safe to assume that they were caused by the additional damage and not due to unmasking of impairments from the first lesion. Nevertheless, the limb withdrawal impairment may also reflect the limited practice in grasping and withdrawing the paw because the group was rarely successful in retrieving a food pellet. Although the severity of this group's impairments may also be related to a synergistic effect of administering two large lesions to a single hemisphere, it likely reflects the important contribution of the lateral neocortex to skilled reaching (Gharbawie et al., 2005).

Although there has been extensive documentation of the role one hemisphere plays in controlling skilled reaching in the ipsilateral paw (Bury and Jones, 2002; Vergara-Aragon et al., 2003; Gonzalez et al., 2004), the present study confirms that this

role is secondary to the contralateral hemisphere as far as both controlling skilled reaching and mediating recovery after injury. That is, brain areas giving rise to corticofugal projections ipsilateral to an injured MC seem comparatively more important to skilled reaching; damaging one those regions as in the present study resulted in severe reaching deficits whereas damage to the contralateral hemisphere resulted in only a slight secondary impairment. This conclusion is consistent with other work (Shanina et al., 2006). Thus the severity of the behavioral deficits produced by contralateral vs ipsilateral hemisphere lesions seem proportional to the extent of corticofugal fiber projections available to contribute to the function of the affected forelimb.

The present most parsimonious explanation for recovery of function is neural adaptation after injury. At the level of the cortex, this involves alterations in inhibitory horizontal connections. The resulting expansion and contraction in motor movement representations are apparent with ICMS. Similarly, after pyramidal tract or dorsal column section, fibers sprout from the intact descending fiber projections to compensate for the fiber loss. In either case, remnants of the corticospinal tract and its neocortical points of origin are central to recovery. It may be expected then that the level of involvement of the intact structures and fibers in mediating recovery will be governed by their degree of alliance with the injured system. Because most (approximately 95%) of the descending corticospinal fiber projections in the rat are crossed and originate from three separate neocortical regions (Rgions A, B, and C' according to Nudo and Masterton, 1990), injury along the length of this system is bound to be compensated for primarily by the remnants of the crossed fibers as opposed to the less populous uncrossed projections. Furthermore, the degree of involvement from the crossed projections ought to depend on the density of

the fiber projection. The degrees of impairment and recovery rates presented in the current study support this position. The converging lines of evidence could direct future studies of behavioral recovery and plasticity to the ipsilateral-to-injury lateral neocortex, a brain region that has been relatively neglected in studies directed toward enhancing functional recovery as compared to the motor cortex of the opposite hemisphere.

Conclusion

In conclusion, the results of the present study show that the most salient feature that distinguishes the contribution of remaining neocortical regions to recovery of function is the density of their descending fiber projections. This finding should help in developing a theoretical framework to guide research directed toward enhancing the contribution that the remaining neocortex can make to recovery of function.

Chapter 3

The topography of three-dimensional exploration: A new quantification of vertical and horizontal exploration, postural support, and exploratory bouts in the cylinder test

Modified from a paper published in *Behavioural Brain Research,* 151 (1-2): 125-135 by Omar A. Gharbawie, Paul A. Whishaw, and Ian Q. Whishaw in 2004.

Abstract

The study of exploratory behavior in confined spaces can provide insights into both the spatial and the motor behavior of animals. In the present study the behavior of rats placed inside of a cylinder (the cylinder test) for 5-min was examined to uncover: (1) the overall organization of exploratory behavior, (2) pattern of movement on the horizontal surface, (3) pattern of movement on the vertical surface, and (4) supporting and stepping movements. The results show that exploratory behavior is organized into a number of bouts of lateral and vertical scans with each bout ending by movement arrest and face washing. Within bouts, activity consists of alternating horizontal and vertical progressions that vary systematically over the course of a bout. Horizontal progressions consist of head scans, turning, and stepping, and decrease in amplitude across a bout. Vertical progressions consist of rears, head scans, and descent with an amplitude expressed by an inverted-U across bouts. Each horizontal and vertical progression is directed toward a different portion of the cylinder and the direction of lateral movements alternate within progressions and between progressions. For each rat, bouts tended to end in the same location suggesting the establishment of a home base. Eshkol-Wachmann Movement Analysis indicates that during progressions, the forepaws are mainly used for postural support and follow forequarter movements, while upward, downward, and sideward shifts in body weight are generated from the hind limbs. These findings illustrate that in even brief exploratory tests the three-dimensional exploratory behavior of rats is organized. The results are discussed in relation to the use of the cylinder test for analyzing a number of behaviors including exploration, locomotion, and supporting reactions.

1. Introduction

The rat's natural propensity to explore provides an avenue for studying the neural basis of both spatial and motor behavior and can also be used as an assay of brain function. When placed on an open surface, rats initiate movement using "warm-up", in which lateral, forward, and vertical movements escalate until locomotion is achieved (Golani et al., 1981). In a featureless environment, rats establish a virtual home base in which they groom, rear, and turn (Eilam and Golani, 1989). Progression away from the home base is slow, interrupted by a number of stops, and ends with a rapid return to the home base (Eilam and Golani, 1989; Tchernichovski and Golani, 1995; Drai et al., 2000; Whishaw et al., 2001; Wallace et al., 2002). If rats are provided with a home base, outward and homeward trips are centered around the home base (Whishaw et al., 2001; Wallace et al., 2002). Applied assays of exploratory behavior have focused on distance traveled, number of rears, and locomotor patterns, all of which can reveal distinctive effects of neurological manipulations and pharmacological treatments (Eilam and Golani, 1990).

Although rats explore vertical surfaces by rearing, and rearing is frequently featured in behavioral assays, exploratory behavior in confined spaces has received comparatively little study (Golani et al., 1979; Sullivan et al., 1992). Nevertheless, forepaw placement by rats rearing in a confined space has become a central test of forelimb function in many lines of research. In a standard test, "the cylinder test", rats are placed into a small circular container and their forepaw contacts on the vertical surface as they rear are counted over a 3-5 minute interval. Asymmetries in forepaw contact (forepaw placing) provide an index of lateralized brain injury (Schallert et al., 1997;

Schallert et al., 2000). An asymmetry is used to evaluate stroke (Bland et al., 2000; Schallert et al., 2000; Biernaskie and Corbett, 2001; Bland et al., 2001; Roof et al., 2001; MacLellan et al., 2002; DeBow et al., 2003; Karhunen et al., 2003), brain stem injury (Choi-Lundberg et al., 1998; Schallert et al., 2000; Tillerson et al., 2001; Lundblad et al., 2002; Tillerson et al., 2002; Whishaw and Metz, 2002; Vergara-Aragon et al., 2003), and spinal cord injury (Liu et al., 1999; Schallert et al., 2000; Soblosky et al., 2001; Jin et al., 2002; Murray et al., 2002). Nevertheless, despite the objectivity and ease of scoring that this test provides, the context in which rearing occurs and the pattern of paw placement are not understood.

The purpose of the present study was to describe the exploratory behavior of rats in the cylinder test. The central objective of the study was to clarify the context in which rearing and paw contacts on a vertical surface occur. Such a contextual description can not only provide a description of three-dimensional exploratory organization, but can also provide insights into rearing and supporting behavior that would strengthen the use of the cylinder test for neurobehavioral investigations. Rats were filmed for five minutes as they explored the cylinder and behavior, including surface and vertical exploration, was reconstructed using frame-by-frame video analysis.

2. Materials and Methods

Animals

General observations were made on male and female rats of a number strains of rats including Wistar, Sprague-Dawley, Dark Agouti, and Long-Evans of varying age. Given a similar pattern of behavioral organization in all subjects, the present report is confined to a detailed description of six female Long-Evans hooded rats, 120 days old

that weighed 250-300g. They were born and raised in the University of Lethbridge vivarium. The animals were housed in groups of two or three individuals in clear Plexiglas cages. All animals had ad lib access to food and water. The colony room was maintained on a 12/12h light/dark cycle (08:00-20:00 h). Experiments were conducted according to standards set by the Canadian Council on Animal Care and approved by the University of Lethbridge animal care committee.

Apparatus

The apparatus was a cylinder made of clear Plexiglas and measured 20 cm in diameter and 30 cm in height (see Fig 3.1). The cylinder was high enough so that the animal could not reach the top edge of the apparatus by rearing (Schallert et al., 1997). The width of the cylinder allowed the animals to freely turn when scanning the horizontal surface. The cylinder was sufficiently heavy that it did not move when the animal supported its weight against the wall. The cylinder was placed on a glass table, beneath which was located an inclined mirror (Pinel et al., 1992). Animals were video recorded from a lateral perspective, or from a ventral perspective through the mirror (see Fig 3.1).

Video records were made using a Cannon ZR 30 MC digital video camcorder with a shutter speed of 1000th of a second. Illumination for high shutter speed filming at 30 frames/s was provided by a cold light source. Frame-by-frame analyses were made using a Sony digital videocassette recorder DSR-11. The software package Final Cut Pro (Apple, www.apple.com) was used for grabbing frames from the video records for display. Movement from the video records were traced on overheads using a felt pen, and movement distance and direction, as described by movement of the tip of the snout, were quantified on Canvas (ACD Systems, Saanichton, BC).

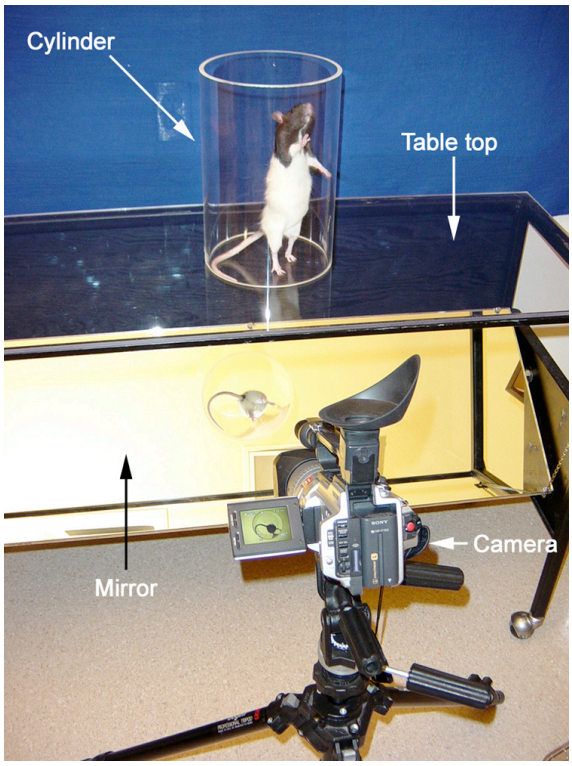


Figure 3.1. The test apparatus consisted of a transparent cylinder (20-cm diameter and 30-cm height). The cylinder was located on a clear plexiglass table-top. A mirror was positioned at an angle below the table-top for video recording the animal's behavior from a ventral perspective. Behavior was also video-recorded from dorsal and lateral perspectives. A cold light source was positioned behind the camera to allow fast shutter speed (1000th second) recording. The apparatus was set up in a test room in which many cues such as a refrigerator, a cupboard, a book shelf, a monitor, etc. were present.
2.3 Video recording and path analysis

3. Procedure

Animals that had been previously handled for about 10 min per day for two weeks but which were naïve to the apparatus were used in the experiment. Each animal was individually placed in the cylinder and filmed for five minutes. From the video records of their behavior, activity on the horizontal surface of the cylinder and on the vertical surface of the cylinder was analyzed. The following behaviors were measured in each test:

Bouts

A bout was defined by the sequence of movements performed by a rat when exploring the cylinder. Each bout consisted of alternating horizontal and vertical movements and ended with locomotor arrest and grooming.

Horizontal progression

A horizontal surface progression was defined by the animal's behavior when exploring the cylinder floor while it maintained contact with the cylinder floor with all four limbs. Measures were made of: (a) the movements made within each horizontal progression including head scans, turning movements, and forward stepping movements, (b) the direction of each lateral movement, (c) the location in the cylinder over which the snout moved, (d) the maximum angle covered by a progression as described by the tip of the snout (a tracing began when both forepaws contacted the horizontal surface and ended when a forepaw was lifted to initiate a rear) in relation to the center of the cylinder, and (e) the distance covered by a progression as defined by movement of the tip of the snout.

Vertical progression

A vertical surface progression was defined by the animal's behavior when exploring the wall of the cylinder while it maintained contact with the floor only with its hind limbs. Measures were made of: (a) the movements within each vertical progression including rearing, head scanning and turning movements, and descent, (b) the direction of movements, (c) the location on the cylinder wall over which the snout moved, (d) the maximum angle covered by a progression as described by the tip of the snout (a tracing began when either forepaw contacted the vertical surface and ended when both forepaws

contacted the floor of the cylinder), in relation to the center of the cylinder, and (d) the distance covered by a progression as defined by movement of the tip of the snout.

Limb use

The Eshkol-Wachman Movement Notation (EWMN) system (Eshkol and Wachman, 1958) was adapted in the present study to examine the relation between the movement of an animal's paws in relation to the movement of the animal's body (Whishaw and Pellis, 1990; Whishaw et al., 1992; Iwaniuk and Whishaw, 1999). EWMN is designed to express relations and changes of relation between the parts of the body. The body is treated as a system of articulated axes (i.e. body and limb segments). A limb is any part of the body that either lies between two joints or has a joint and a free extremity. These are imagined as straight lines (axes), of constant length, which move with one end fixed to the center of a sphere. The body is represented on a horizontally ruled page. Each horizontal space represents a part of the body. Vertical lines divide the manuscript page into columns that denote units of time (e.g. frames of a video). The signs for movement are read from left to right and from bottom to top (e.g. see Table 1). An important feature of EWMN is that the same movements can be notated in several polar coordinate systems. The coordinates of each system are determined with reference to the environment, to the subject's body midline axis, and to the next proximal or distal limb or body segment. By transforming the description of the same behaviour from one coordinate system to the next, invariances in that behaviour may emerge in some coordinate systems but not in others. Thus, the behaviour may be invariant in relation to some or all of the following: the rat's longitudinal axis, gravity, or bodywise in relation to the next proximal or distal segment. Using the description derived from one animal,

three horizontal and three vertical progressions were scored for each animal. Scoring consisted of rating whether: (a) a limb was used mainly for support, and (b) whether the limb was used for propulsion.

Face washing and movement arrest

Face washing and movement arrest were defined by the absence of forward progression following which a rat sat back on its haunches and made face-washing movements across its snout with its paws.

Table 1. A complete Eshkol Wachman Movement Notation (EWMN) scoring sheet in which a rat performed a vertical progression. The numbers across the top of the score indicate the number of frames from the video recording, which have elapsed (30 frames per second). The first left hand column indicates the initial or starting position of the movement. Movements of the body and right and left forepaws are notated separately throughout the score. The paired numbers in brackets refer to coordinate points on the EWMN sphere and indicate the position of the right paw (RP) and left paw (LP). Numbers without brackets indicate the magnitude of movement of the right paw (Dist RP) and left paw (Dist LP), each unit indicates one step. The orientation of the forepaws (OPP) was indicated with respect to one another, with the number on top indicating the orientation of the right forepaw and the bottom number representing the left forepaw. The orientation of the body (Fr) was notated in reference to EWMN coordinate points, whereas body weight (Wt) was notated with respect to the animal's own body with the nose at the [0] position. The symbols used are as follows: (⊤), contact with the floor; (horizontal ⊤) contact with the wall; (=) release of contact, (#), half a step.

	1	2	7	10	15	21	24	27	30	34	37	57	60	64	65	68	69	72	93	95	96	100	101	104	105	108	121	127	134	135
Fr	6									2																				4
Wt		[0]								[2]																	[4]			
Dist RP		2			1					1			1				1			1					#		#			
RP	(2 0 ⊤)	=		(4 0 ⊤)	=	(4 0 ⊤)				=	(4 1 ⊤)		=	(4 1 ⊤)			=	(4 1 ⊤)		=		(4 1 ⊤)			=	(4 1 ⊤)	=		(2 0 ⊤)	
OPP	6 2													5 3										6 2		5 3				6 2
LP	(2 0 ⊤)		=		(4 0 ⊤)		=	(4 0 ⊤)				=	(4 0 ⊤)		=	(4 0 ⊤)			=		(4 1 ⊤)		=	(4 0 ⊤)				=		(2 0 ⊤)
Dist LP			2				#					1			1				1				1					#		

4. Results

The following results are extracted from data collected from six female Long Evans rats. All six female Long Evans rats displayed similar basic components of three-dimensional explorations as other rat strains. Their behavior may be regarded representative of rat species-typical behavior.

Bouts

When placed into the cylinder, the rats explored the enclosure with alternating horizontal surface progressions and vertical surface progressions. After a number of progressions in each dimension, the rats paused and engaged in face washing. Pauses were usually preceded by short duration rears that did not involve contact with the cylinder wall. Pauses also tended to occur in the same location in the cylinder for each rat after each bout, but due to the small size of the cylinder, it was not possible to objectively measure pause location. Following face washing, exploration of the horizontal and vertical surface of the cylinder resumed initiating a new bout. The sequences of movement bounded by pausing and face washing was defined as an exploratory bout. The rats engaged in an average of 2.5 bouts within the 5-minute session. Each bout consisted of an average of 17.50 ± 2.17 vertical progressions and 17.83 ± 2.14 horizontal progressions. As each bout consisted of a similar chain of movements, the following analyses are derived from the first bout of each exploratory session.

Horizontal surface progression

Horizontal progression defines exploratory movements in which all four limbs contacted the cylinder floor and consisted of three main components: (1) lateral head scans were movements of the snout to the left or the right, associated with floor sniffing,

(2) stepping sideward with the forelimbs, and (3) pivoting or forward stepping with the hind limb. Fig 3.2 illustrates selected frames from a single horizontal progression demonstrating that the rat's center of gravity was located near the center of the container while its snout movements were confined to the periphery of the container.

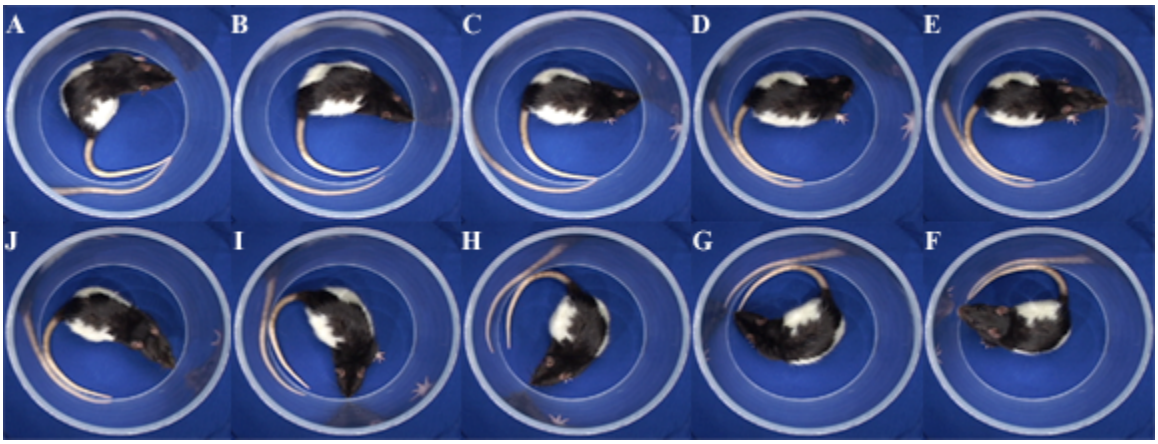


Figure 3.2. A series of frames captured from a video recording of a horizontal progression. Note small head scans, and larger pivoting movements that support movement of the snout around the outer surface of the cylinder floor.

The tracings of all of the horizontal progressions that occurred within a single bout for each rat were superimposed on a floor plan of the cylinder and are illustrated in Fig 3.3. The tracings confirmed that the horizontal progressions covered the outer region of the cylinder floor. The tracings also indicate that each rat's snout movements were distributed nearly equally around the cylinder.

In order to further examine the pattern of movements over successive horizontal progressions, the distance and direction of each head scan in each progression were represented on a Cartesian representation of the cylinder using horizontal lines to indicate distance and arrows to represent direction. A typical pattern from one rat is illustrated in Fig 3.4. This analysis showed that the distance and direction of progressions tended to alternate both between and within progressions. The analysis indicated that each

progression was directed toward a different portion of the cylinder such that the entire outer surface of the cylinder was scanned a number of times.

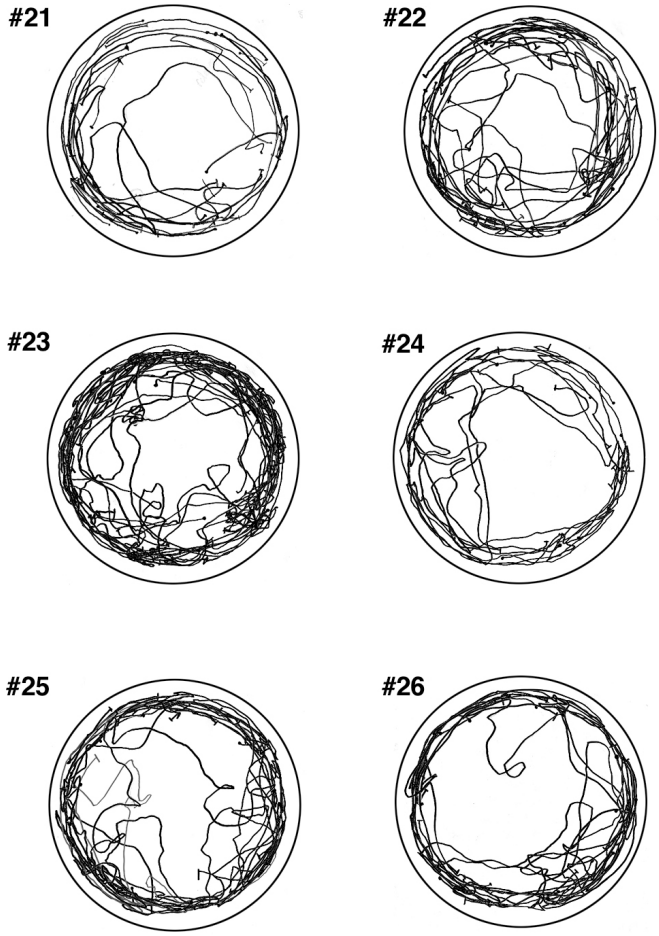


Figure 3.3. Drawing of the trajectory of the snout tip along the floor of the cylinder during horizontal progressions in a bout for each rat. Note that most tracings were restricted to the outer edge of the cylinder floor.

Successive horizontal progressions were measured with respect to (a) maximum angle covered by the horizontal progression and (b) scan distance covered by the horizontal progression as measured by movement of the tip of the snout. As illustrated in Fig 3.5, the angle covered by successive progressions tended to decrease across the bout, an observation that was confirmed by a significant linear polynomial fitted to the angle

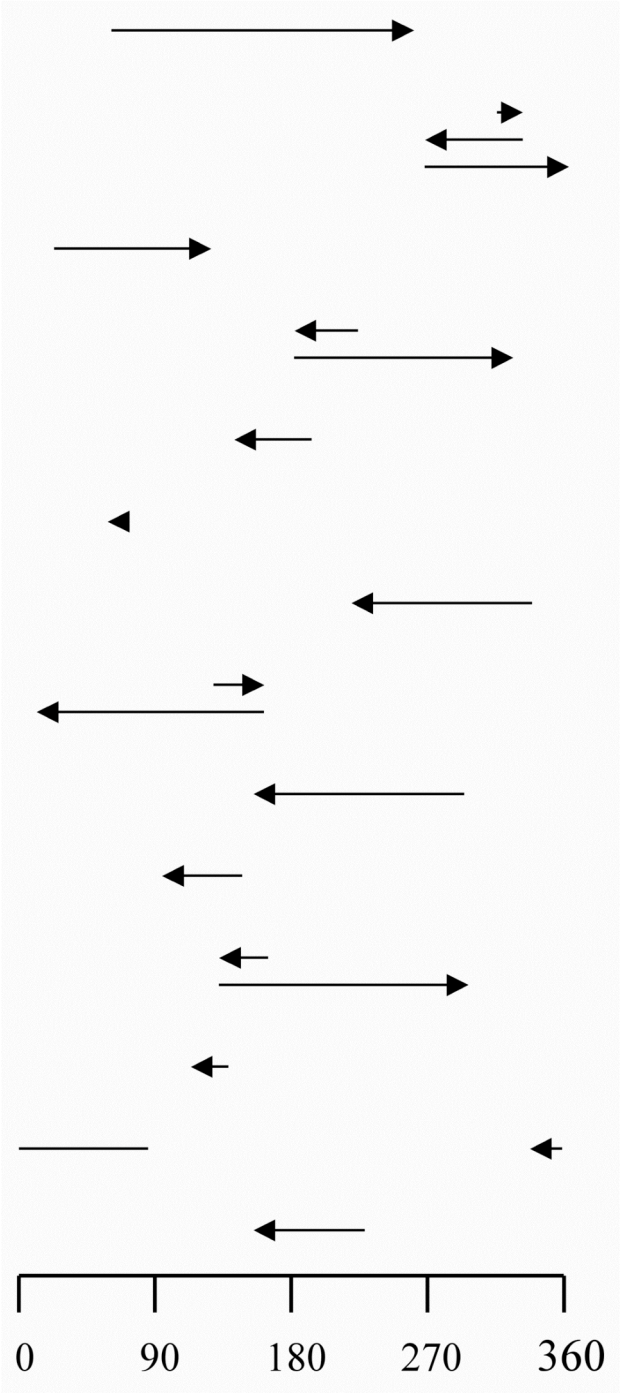


Figure 3.4. Schematic representation of the distance and direction of snout movements within and across horizontal progressions for a representative rat. The numbers on the axis represent the angular units of the internal perimeter of the cylinder. The arrows indicate the direction of movement, left = counter clockwise; right = clockwise. The length of the arrow represents the distance of the progression. Arrows close together indicate direction within a progressions, arrows further apart represent successive progressions. The sequence of horizontal progressions runs from top to bottom. Note that scans and progressions vary in location, duration, and direction across a bout.

measurements [F (1,130) = 21.64; p <0.01]. Similarly, the distance covered by successive progressions also decreased, as indicated by a significant linear polynomial fitted to the distance measurements [F (1,130) = 4.83; p < 0.05].

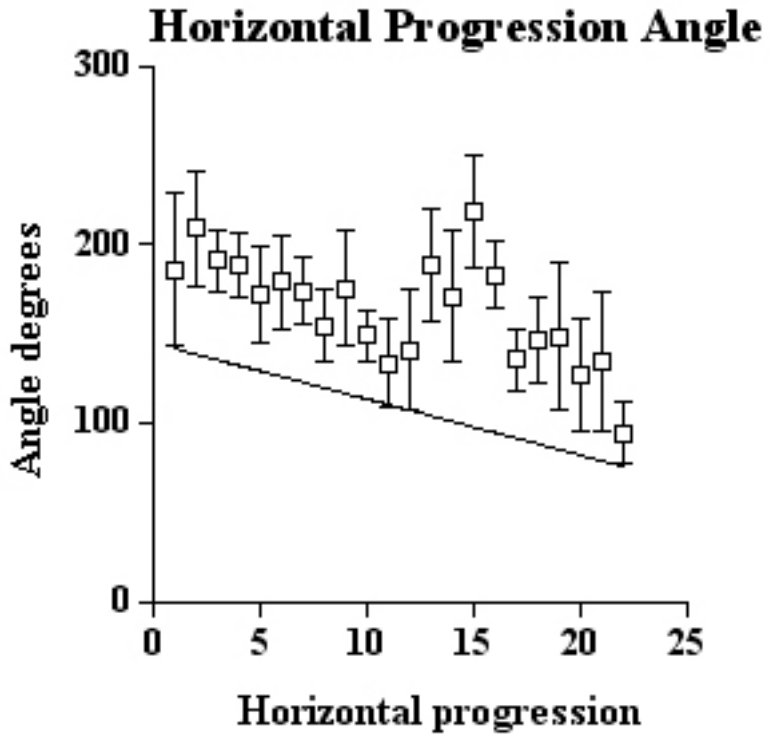


Figure 3.5. The angles (mean ± standard error) subtended by horizontal progressions in one exploratory bout represent the area of the floor investigated. A linear curve indicates best fit = -2.66 x + 194.82.

Vertical surface progression

A vertical progression consisted of three main components: (1) A rear or an ascent in which a rat raised its forequarters in a vertical direction and concurrently contacted and "climbed" the cylinder wall with one or both forepaws, (2) snout scans that were directed across and up and down the vertical surface of the cylinder, and (3)

descent, in which the animal turned to one side and lowered its forequarters ventrally and laterally until the forepaws were in contact with cylinder floor. These three phases of a typical vertical progression are illustrated in the three panels in Fig 3.6 and Appendix 1.



Figure 3.6. Components of a vertical progression. (A) Rear was an ascent in which a rat raised its forequarters and concurrently contacted and "climbed" the cylinder wall with one or both forepaws, (B) from the rearing position, the rat scanned the vertical surface of the cylinder with vertical and lateral head movements and body weight shifting, (C) Descent, in which the animal turned to one side and lowered its forequarters until the forepaws were in contact with cylinder floor. Note, frames in which the animals released forepaw contact with the wall are not shown.

The tracings of all of the vertical progressions that occurred within a single bout for each rat were superimposed on a schematic of the ventral surface of the cylinder and are illustrated in Fig 3.7. The tracings indicated that progressions as measured by snout position were reasonably distributed around the vertical surface of the cylinder. The slight tendency of progressions to be more concentrated in one portion of the cylinder for some of the rats were a result of the later progressions which tended to concentrate at a preferred location.

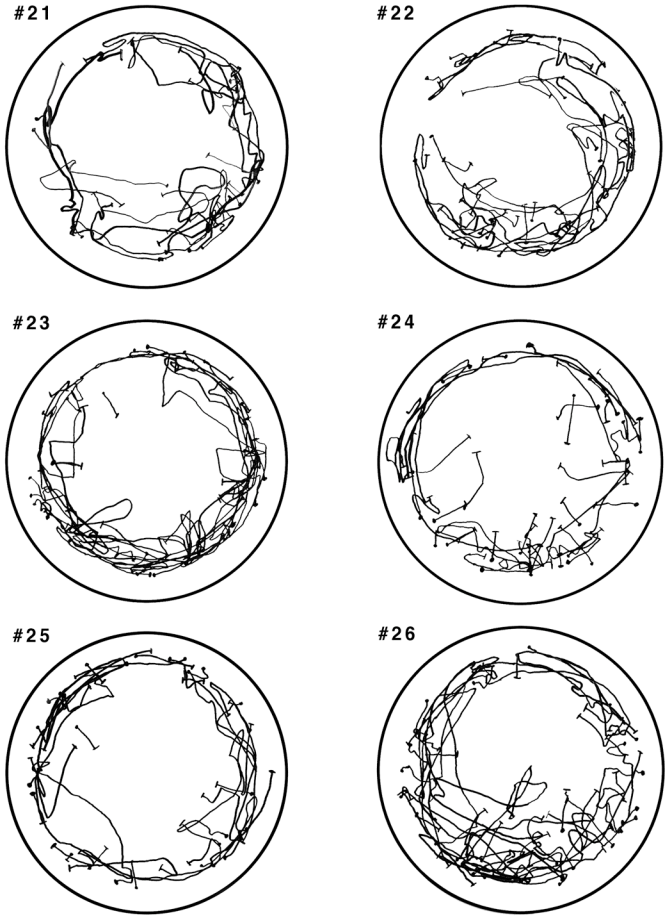


Figure 3.7. Each panel represents vertical progressions superimposed on an outline of the cylinder for one rat in one exploratory bout. Note that movements are distributed around the surface of the cylinder.

In order to further examine the pattern of movements over successive vertical progressions, the distance and direction of each head scan in each progression were represented on an unfolded Cartesian representation of the cylinder wall. A typical pattern from one rat is illustrated in Fig 3.8. The analysis indicated that each progression was directed toward a different portion of the cylinder such that the entire outer surface of the cylinder was scanned a number of times.

In order to further examine the location of vertical progressions, the location of initiation of each vertical progression was represented on an unfolded Cartesian representation of the cylinder edge. A typical pattern from one rat is illustrated in Fig 3.9. This analysis showed that the location of vertical progressions were distributed around the cylinder in such a way that successive vertical progressions were not initiated in the same location.

Vertical progressions were measured with respect to: (a) maximum angle covered by the snout on successive vertical progressions, and (b) scan distance covered by the snout on successive vertical progressions. As is illustrated in Fig 3.10, the angle subtended by successive progressions is best represented by an inverted-U, with initial and terminal progressions covering smaller angles than intervening progressions. This pattern was significant as indicated a polynomial distribution [$F(1,123) = 6.54$; $p < 0.01$]. Similarly the distance covered by the snout in successive vertical progressions had an inverted-U shaped pattern as indicated by a significant polynomial curve [$F(1,123) = 6.34$; $p < 0.01$].

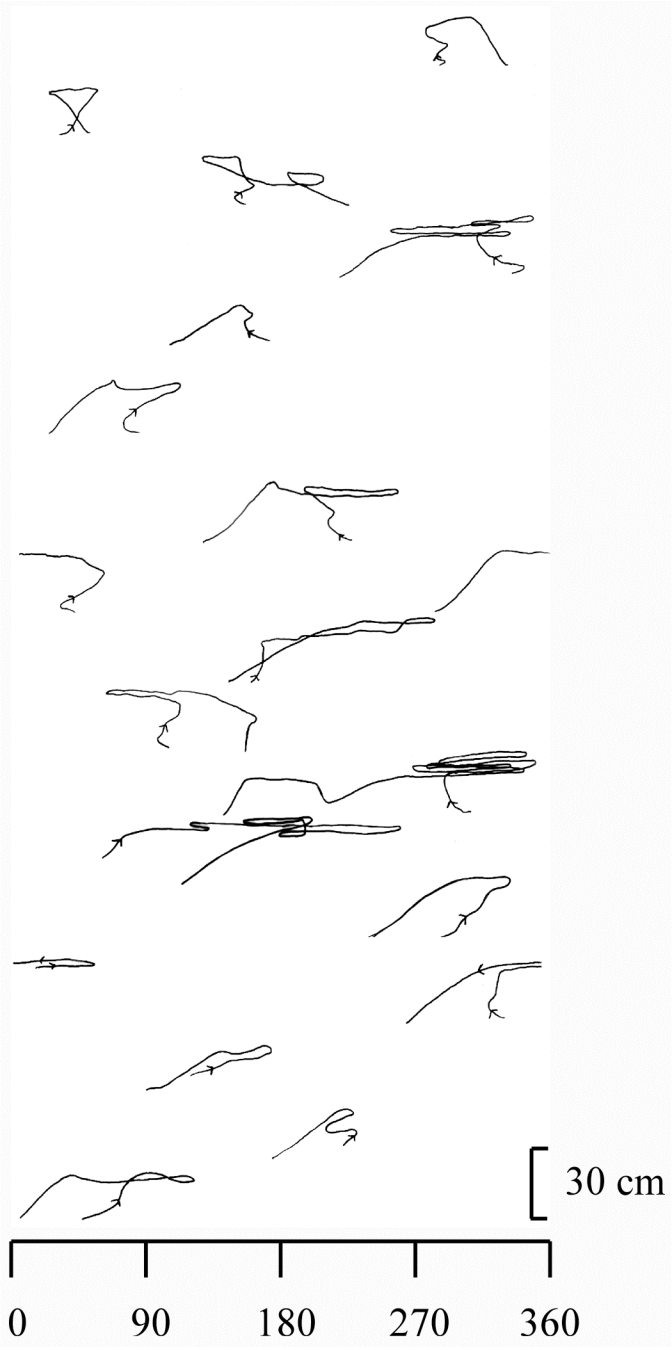


Figure 3.8. The pattern of vertical progressions reconstructed by tracing the animal's snout tip on a two-dimensional representation of the cylinder wall. The bottom scale represents the angular units of the internal perimeter of the cylinder. The vertical scale represents the height of the cylinder. Each rear is illustrated on a separate line and the arrow-head indicates the direction of the scan. Vertical progressions were initially short (top), subsequently longer and included direction change (middle), and short at the end of the bout (bottom). The sequence of vertical progressions runs from top to bottom. Note, that the scans were distributed across the vertical surface.

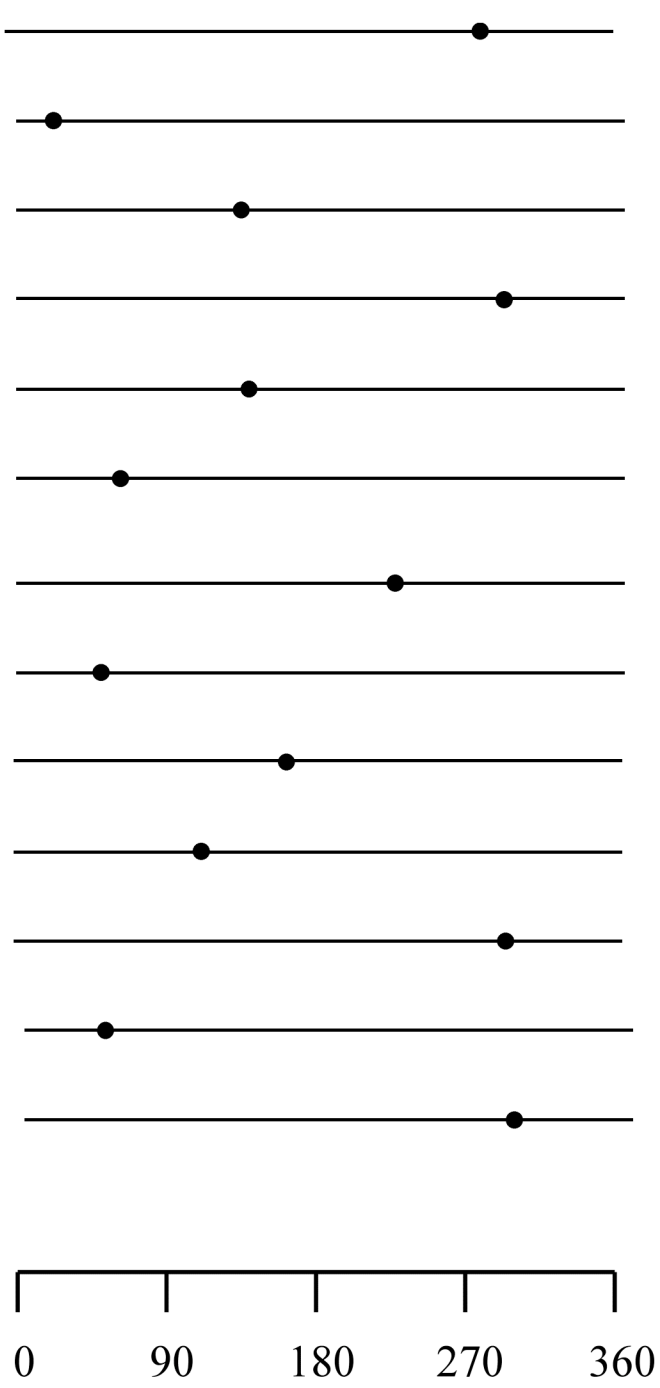


Figure 3.9. Locations of the vertical component of vertical progressions on a two-dimensional reconstructions of cylinder. The numbers on the axis represent the angular units of the internal perimeter of the cylinder. Each dots indicates the location of the nose upon initial wall contact for each vertical progression. The sequence of initial contacts runs from top to bottom. Note, successive contact points did not occur in the same location.

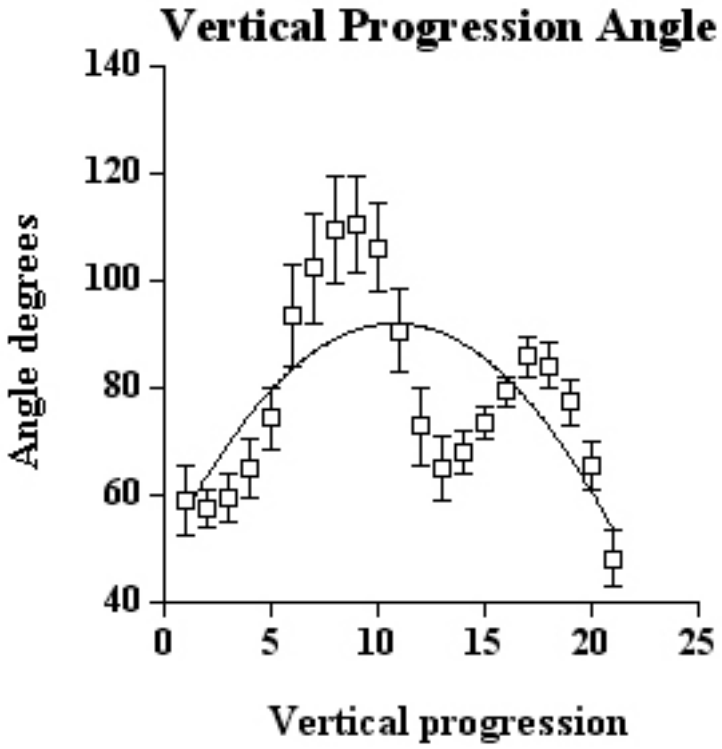


Figure 3.10. The angles (mean ± standard error) subtended by vertical progressions across an exploratory bout. A second order polynomial curve was fitted to the scatter plot $y = -0.434\ x^2 + 9.75\ x + 40.17$.

4.4 Limb use

Limb movements in relation to body position and progression sequence were notated using EWMN. A typical notation for a vertical progression is illustrated in Table 1. A drawing of forelimb movements associated with a vertical progression is shown in Fig 3.11 and Appendix 2, and shows that forelimb movements are slightly different in the three phases of a vertical progression. Associated with the rear, the forepaws step (climb) up the vertical surface as the rat is propelled upward by movement from the hind limbs. Once in the vertical position, the forepaws step to one side or to the other to accompany rotation of the forequarters. During the descent, weight is typically supported by the

forepaw closest to the cylinder wall, and this supporting paw frequently slides down the vertical wall of the cylinder as the animal descends.

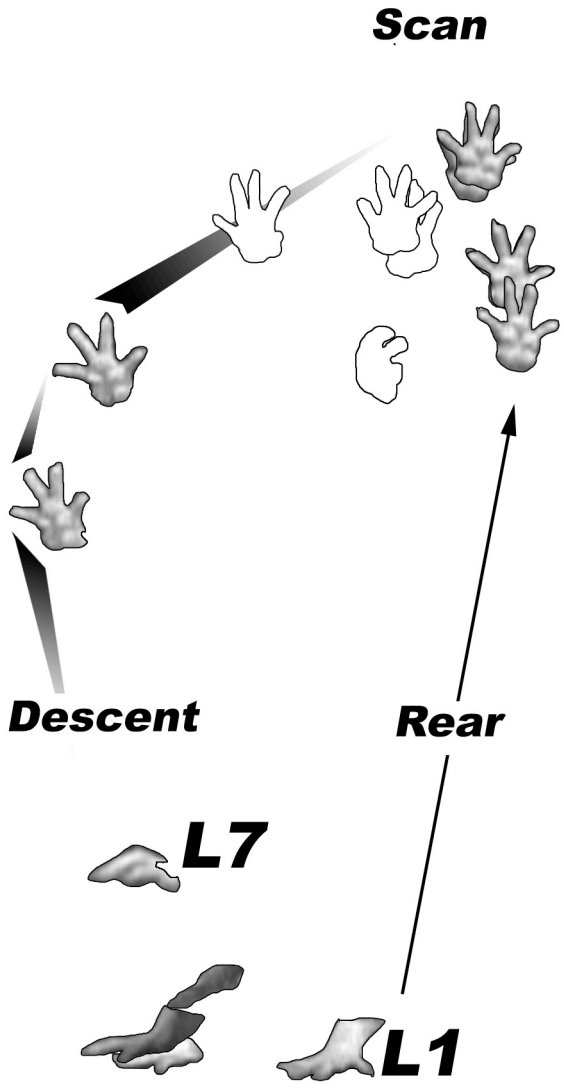


Figure 3.11: Schematic diagram of forepaw placement during a vertical progression. The right forepaw is represented in white and the left forepaw in gray. Wall contact consisted of a vertical stepping motion during rearing, side-stepping during lateral scanning, and a swipe during the descent.

In contrast to the supporting role served by the forepaws, the hind limbs stepped less frequently but provided the propulsion for the rats' movements by varying extension (Fig 3.12). For example, extension and retraction of the snout along both the horizontal

and vertical surfaces of the cylinder was driven by extension and flexion of the hind limbs.

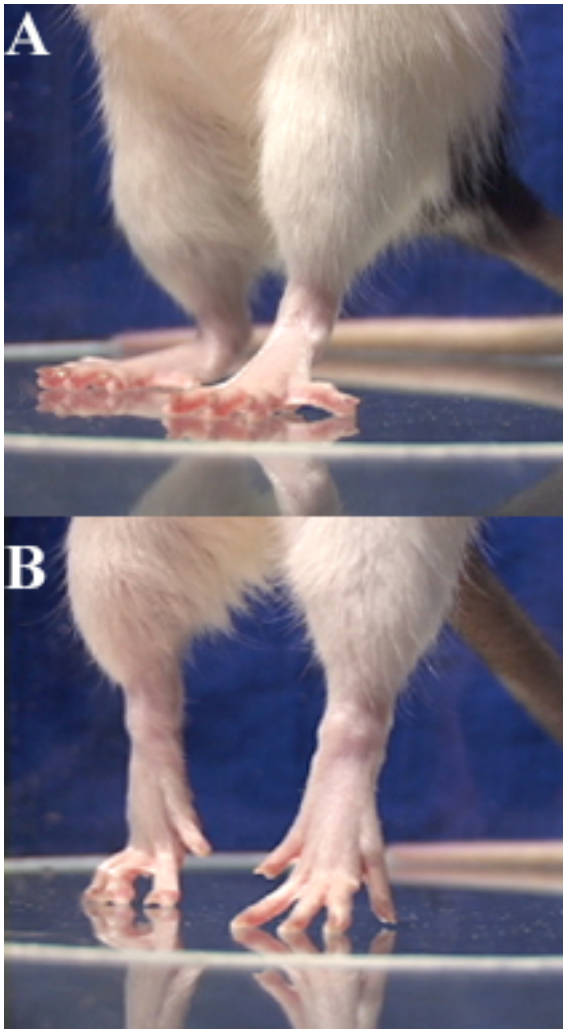


Figure 3.12: The hind limbs were used to shift weight upwards and downwards during progressions, as illustrated here for the scanning component of a vertical progression.

4.5 Face washing and movement arrest

Forward progression was interrupted by a number of non-contact rears at the end of each bout. Subsequently, the rat sat on its haunches and made face-washing movements across its snout with its paws. Pausing and grooming tended to occur in the same location of the cylinder for all rats following each bout.

5. Discussion

The objective of the present study was to describe the exploratory behavior of rats in a small three-dimensional chamber, referred to as the cylinder test. Exploratory behavior was organized into bouts lasting one to two minutes. A bout consisted of a series of alternating horizontal and vertical progressions that systematically moved the snout along the horizontal and the vertical surface of the cylinder so that by the completion of a bout the entire cylinder was explored. The forelimbs were used mainly for postural support while movement was propelled with the hind limbs. This demonstration of organization in three-dimensional exploratory behavior provides insights into the cognitive and motoric structure of rat behavior.

The present analysis is based on observations in female Long Evans rats exploring a cylinder. Our laboratory has previously tested a number of different rat strains of both genders and of varying ages in the cylinder. Female Long Evans rats displayed all the basic components of three-dimensional exploration observed in other rats. The present results should therefore be considered representative of the structure of rat exploratory behavior in a small container. Nevertheless, variations in the number of component behaviors and bout durations could be expected to vary as a function of sex, age, strain or treatment and so could be considered useful dependent measures.

There were a number of reasons that prompted a detailed analysis of rats' exploratory behavior in a three-dimensional chamber. First, exploration in the "cylinder test" is widely used as a measure of forelimb function (Jones and Schallert, 1994; Schallert et al., 1997; Choi-Lundberg et al., 1998; Humm et al., 1998; Liu et al., 1999; Bland et al., 2000; Schallert et al., 2000; Biernaskie and Corbett, 2001; Bland et al., 2001;

Roof et al., 2001; Soblosky et al., 2001; Tillerson et al., 2001; Jin et al., 2002; Lundblad et al., 2002; MacLellan et al., 2002; Murray et al., 2002; Tillerson et al., 2002; Voorhies and Jones, 2002; Whishaw and Metz, 2002; DeBow et al., 2003; Karhunen et al., 2003; Vergara-Aragon et al., 2003). In particular, asymmetry of forelimb wall contact is considered an index of motor system integrity. If forelimb movements are constrained by the overall structure of exploratory behavior, then this information could contribute to understanding movement impairments. Second, the context in which orienting movements and supporting reactions occur has not been studied. Since the structure of the animals' movements appear to represent organized spatial behavior, then the usual variables that influence spatial behavior more generally will influence behavior in the cylinder. Third, the organization of three-dimensional exploration in a small enclosure has not been examined, but many tests given to rats occur in relatively confined spaces. Possibly the behaviors described here generalize to many other situations in which rats are tested, e.g., place preference tests, tests of conditioned suppression, and maze tests. Fourth, a detailed analysis of the animals' behavior may provide new insights about the spatial and motor components of exploratory behavior. In fact, the present study shows that the rats' behavior in a small container is organized.

The exploratory behavior of the animals was organized in distinct bouts lasting about two minutes each. The bout consisted of a number of alternating horizontal and vertical progressions. Each progression occurred in a different location such that most of the surface of the cylinder was explored by the snout at the completion of a bout. Bouts tended to end by a number of non-contact rears followed by arrest of progression and face washing performed in a sitting position. Bouts tended to end in the same location for

each rat after each bout. The organization of an exploratory bout suggested that it was directed to completely examining the horizontal and the vertical surfaces of the cylinder. It is interesting that in open arenas rats set up virtual home bases in which they rear, turn and groom (Eilam and Golani, 1989). Perhaps one objective of exploration in the cylinder is to establish a home base to which ambient cues can be referenced.

There is a constraint on the conclusion that the component movements of a bout represented exploration or the establishment of a home base. Successive bouts appeared similar but it cannot be certain that the only objective was "exploration". Possibly some of the rats' activity represents a search for an escape from the cylinder. This possibility was not examined, but could be a in a somewhat different paradigm in which a rat is allowed to "escape" from an open field into a cylinder. Furthermore, the present study provides no firm direct evidence that the rats acquire "information" during exploratory bouts, but this too could be tested by probe tests in which ambient cues are manipulated.

The sequence of scans of the horizontal surface and the vertical surface progressions were organized. Animals initiated movement by scanning the horizontal surface and then alternated between horizontal and vertical surface scans. The distance and angle subtended by horizontal progressions tended to become shorter across a bout while vertical progressions had the shape of an inverted-U. This organization of exploration appears to be similar to that described for animals exploring a large two-dimensional surface. There behavior is also organized in that animals explore from a home base. Exploratory progressions are initially brief, but become successively longer (Drai et al., 2000). Thus, in both confined and open environments, exploration is organized, although obviously the various speeds of locomotor behavior punctuated by

stops characterizing open field exploration (Drai et al., 2000; Whishaw et al., 2001; Wallace et al., 2002), is not observed in a confined environment.

The organization of vertical progression is of special interest in that paw placement during rearing is frequently used as an index of nervous system integrity (Wolgin and Kehoe, 1983; Schallert and Lindner, 1990; Schallert et al., 1997; Choi-Lundberg et al., 1998; Humm et al., 1998; Liu et al., 1999; Bland et al., 2000; Grossman and Stein, 2000; Schallert et al., 2000; Biernaskie and Corbett, 2001; Bland et al., 2001; Roof et al., 2001; Soblosky et al., 2001; Tillerson et al., 2001; Bury and Jones, 2002; Jin et al., 2002; Lundblad et al., 2002; MacLellan et al., 2002; Murray et al., 2002; Tillerson et al., 2002; Voorhies and Jones, 2002; Whishaw and Metz, 2002; DeBow et al., 2003; Gharbawie and Whishaw, 2003; Karhunen et al., 2003; Vergara-Aragon et al., 2003). Here we observed that forepaw movements were largely supporting and could be grouped into three different types. During a rear, which is nearly always vertical, the forepaws step to accompany the upward movement of the forequarters. These stepping movements are similar to forward stepping on a horizontal surface. Once the animal is in the vertical position, the paws move laterally back and forth to support the lateral movements of the head as the rat scans the wall with its snout. These stepping movements are similar to the lateral stepping movements accompanying lateral scans. During a ventral and lateral descent, one forepaw typically supports the animal's weight, frequently by sliding down the wall. These supporting movements are likely unique to descent. That there are three types of paw movements suggests that in future studies in which the use of the forepaws is used as an index of neurological integrity, these different phases of movement might be analyzed separately. For example, in unpublished studies we observed that after some

time following brain injury vertical stepping movements are relatively normal whereas stepping movements accompanying lateral scans may be asymmetrical.

In the analysis of forepaw placing in the cylinder test, the context in which the paws are used is generally not considered. As shown here, the exploratory behavior of the rat is organized, and consequently impairments to other aspects of movement may be reflected in the way that the forepaws are used. That is, biases in turning, or biases in extensions and flexion of the hind limbs for balancing may of themselves influence the asymmetry of forepaw use. By the same logic, changes in the various aspects of the rat's organized exploratory behavior in the cylinder of themselves might provide interesting measures of neural integrity.

It is interesting that there is also a "spatial" component to the organization of exploratory behavior in the cylinder. At the present time, almost nothing is known about the neural structures involved in spontaneous exploration. Nevertheless, the suggestion that limbic structures, especially the hippocampus, are involved in exploration and in creating representations of the external world (O'Keefe and Nadel, 1978) suggest that a simple and quickly administered test such as the cylinder test might be exploited for spatial studies. For example, the exploratory pattern described here may provide a framework for examining the firing patterns of place cells (Best et al., 2001) and head direction cells (Taube, 1998). These cells have been associated with the behavior of rats in somewhat different "cylinder" tests (Muller et al., 1996). Similarly, the organized exploration of the cylinder suggests that process of timing and movement sequencing are required, suggesting that the cylinder test might be exploited to investigate neural structures that might be involved in these processes (Berridge and Whishaw, 1992; Welsh

et al., 1995; Aldridge and Berridge, 1998; Breukelaar and Dalrymple-Alford, 1999; Hudzik et al., 2000).

Conclusion

In conclusion, the results of the present study demonstrate that rats display organized behavior in a small three-dimensional environment. The pattern of horizontal and vertical progressions within bouts of exploration, appears designed to assist the rat in systematically investigating its environment. This organized pattern of behavior provides insights into context in which limb movements occur and may prove useful as neurological assays of limb function. In addition, a number of features of this organized behavior might be applied to investigating other motor and cognitive functions.

Chapter 4

Parallel stages of learning and recovery of skilled reaching after motor cortex stroke:

"Oppositions" organize normal and compensatory movements

Modified from a paper published in *Behavioural Brain Research,* 175(2): 249-62 by Omar A. Gharbawie and Ian Q. Whishaw in 2006.

Abstract

Forelimb/hand motor cortex injury in rodents and primates causes impairments in skilled paw/hand movements that includes a period of movement absence followed by functional recovery/compensation. Although the postsurgical period of movement absence has been attributed to "shock" or "diaschisis", the behavior of animals during this period has not been fully described. Here, rats were trained to reach for single food pellets from a shelf and then the vasculature of the forelimb region of the sensorimotor cortex contralateral to the reaching limb was removed. A control group received a posterior parietal cortex devasularization. Frame-by-frame video analysis of reaching behavior showed that the stages of the acquisition of skilled reaching and the stages of recovery after motor cortex stroke were similar. The animals sequentially learn three relationships or "oppositions" between a body part and the food target. The oppositions are invariant relationships but each can be achieved with movements that can vary from reach to reach and between rats. A snout-pellet opposition organizes the movements of orienting, a paw-pellet opposition organizes limb transport and grasping the pellet in the digits, and a mouth-pellet opposition organizes limb withdrawal and the release of the food into the mouth. The three oppositions and the movements that they recruit were disrupted after motor cortex damage, but not parietal cortex damage. The oppositions were reestablished after stroke in the order in which they were acquired prior to stroke. Enduring impairments were more noticeable in transport and withdrawal oppositions. That the stages of recovery from motor cortex stroke parallel those of initial acquisition are discussed in relation to contemporary explanations of diaschisis and the contribution of motor cortex to motor learning.

1. Introduction

Rats like many other animals are adept at reaching for and grasping food using a single paw, a behavior termed skilled reaching (Whishaw et al., 1986). Because the actions of the limb, forepaw, and the digits are in the main consistent between rats and primates (Whishaw et al., 1992), rats can serve as a model for exploring the neural basis of skilled movements, motor learning, and processes of disease (Whishaw and Miklyaeva, 1996; Whishaw, 2005). Several lines of evidence suggest that motor cortex, especially the rostral forelimb area (RFA) and the caudal forelimb area (CFA), is central to skilled reaching (Hyland, 1998; Remple et al., 2001). Within these regions, forelimb muscle contractions can be obtained with microstimulation (Hall and Lindholm, 1974; Neafsey and Sievert, 1982). Also, morphological adaptations including increased complexity of dendritic arborization (Greenough et al., 1985; Withers and Greenough, 1989) and synaptogenesis (Kleim et al., 2002; Kleim et al., 2004), neurophysiological adaptations such as facilitation of synaptic efficacy for long term potentiation (LTP) (Rioult-Pedotti et al., 1998; Monfils and Teskey, 2004), and reorganization of motor movement representations (Kleim et al., 1998; Kleim et al., 2004) are localized in the forelimb regions of motor cortex in association with the acquisition and performance of skilled reaching. Finally, damage to motor cortex by trauma, disease, or stroke results in impairments of skilled reaching (Castro, 1972; Whishaw et al., 1991; Whishaw, 2000; Gharbawie et al., 2005; Metz et al., 2005). For an initial period of hours or days after injury, animals may make no attempts to use the contralateral-to-lesion limb and can be considered to be in a state of behavioral depression or shock. During the next two weeks postsurgery, reaching success again approximates presurgical levels (Whishaw et al.,

1991; Whishaw, 2000; Gharbawie et al., 2005), but now includes compensatory movements including the substitution of body rotation for lost limb aim, advance, pronation, and supination (Whishaw et al., 1991; Gharbawie et al., 2005; Metz et al., 2005). Together these findings show that the motor cortex is involved in both the learning and the performance of skilled reaching.

There are two distinctive features of the behavioral effects of motor cortex damage/stroke relative to the effects of damage to other portions of the motor system. First, rats with motor cortex damage display a strong tendency to change limb preference (Peterson and Gucker, 1959; Whishaw et al., 1986; Castro-Alamancos and Borrell, 1993). Although damage to other motor system regions will eventually produce a switch in limb preference as a compensatory strategy (Kartje-Tillotson and Castro, 1980; Whishaw et al., 1986; Gonzalez and Kolb, 2003), switch in limb preference is evident almost immediately after motor cortex injury. This change in limb preference allows animals with motor cortex injury to display more rapid acquisition with the non-preferred paw than occurs for control animals (Peterson and Gucker, 1959; Bury and Jones, 2002). The change in preference also necessitates the use of bracelets or other restrictions on the ipsilateral-to-lesion paw if use or "recovery" in the contralateral-to-lesion paw is to be obtained (Whishaw et al., 1986; Bury and Jones, 2002). Second, there is an initial period of depression of reaching behavior following which success only gradually improves (Whishaw, 2000). The uniqueness of these symptoms to motor cortex damage is evident as rats with basal ganglia lesions (Whishaw et al., 1986; Pisa, 1988; Miklyaeva et al., 1994), red nucleus lesions (Whishaw et al., 1990), or corticospinal tract cuts (Whishaw et al., 1993), although motorically impaired do not show similar behavior. Although there

might be numerous neural mechanisms mediating the acute depression in reaching after motor cortex damage, neural depression or diaschisis (Finger and Stein, 1982) is a potential leading explanation. In diaschisis (von Monakow, 1914), behavioural depression is proposed to be due to transient shock in neural structures connected to, but distant from, the site of injury. Recovery is proposed to be due to the abatement of neural depression in these regions. Surprisingly, although patterns of recovery have been described as primitive to mature (Teitelbaum et al., 1969; Cramer and Chopp, 2000) and proximal to distal (Twitchell, 1951; Lawrence and Kuypers, 1968a) there has been little study of the causes of behavioral depression of limb use in the immediate postoperative period. The objective of the present study was to examine skilled reaching in the acute period after motor cortex damage with the expectation that documentation of the first movements of recovery could provide insight into the organization of skilled reaching.

For the experiment, rats were trained on a single pellet reaching task and then received a stroke via blood vessel devascularization to the forelimb regions of motor cortex (experimental group) or to the parietal cortex (control group) in the hemisphere contralateral to the trained paw for reaching (Whishaw, 2000; Gonzalez and Kolb, 2003; Gharbawie et al., 2005). The area damaged in the experimental group was selected to ensure that the forelimb areas as defined by intracortical microstimulation (Hall and Lindholm, 1974; Neafsey and Sievert, 1982; Kleim et al., 1998), behavioural (Peterson and Gucker, 1959; Castro-Alamancos and Borrell, 1993; Whishaw, 2000) and anatomical (Donoghue and Wise, 1982; Wise and Donoghue, 1986) studies, which could vary from rat to rat, were consistently damaged in all subjects. An area comparable in size but posterior in location was devascularized in the control group with the objective of

damaging the same amount of neocortical tissue but at the same time sparing as much of the forelimb motor areas as possible. In order to directly compare initial learning with post surgical recovery, all behavior was video recording for subsequent frame-by-frame study. Because the behavior of animals when first re-exposed to the task can consist of a variety of behaviors both related to and unrelated to the act of skilled reaching, an analysis based on movement notation (Eshkol and Wachman, 1958) was used to identify invariances in behavior. This approach has been successful in decomposing complex behaviors such as play (Pellis, 1983), courtship (Yaniv and Golani, 1987), and aggression (Pellis and Pellis, 1990) into simpler goals of maintaining oppositions between a body part of one animal and a target body part of a conspecific.

2. Materials and Methods

Animals

Twelve female Long-Evans hooded rats, 120 days old, and weighing 290 - 320 g from the University of Lethbridge vivarium were used. The experiments were conducted in compliance with the guidelines of the University of Lethbridge animal care committee and the Canadian Council for Animal Care. Rats were housed in Plexiglas cages (36 cm long, 20 cm wide, and 21 cm deep) with sawdust bedding, in groups of three in a colony room maintained on a 12/12 h light/dark cycle (08:00-20:00 h).

Feeding

For initial and subsequent training, the rats were gradually food deprived to 90-95% of their body weight by once a day feeding of a measured quantity of Purina rat chow. For the week prior to reach training, each rat received twenty 45 mg dustless precision banana-flavoured pellets (product #F0021, Bioserve Inc., Frenchtown, NJ,

USA) one hour prior to the daily chow ration. The objective was to introduce rats to the pellets, which would later serve as reaching targets, in a familiar environment. Once reach training began and until the end of the study, only rat chow was served in the home cage once a day in sufficient amounts to maintain all rats at 90-95% of their original body weight. The number of banana-flavoured pellets a rat received on a given training day depended on the rat's reaching accuracy, but it did not exceed 25 pellets (1.125 g).

Surgery

Motor cortex devascularization. Lesions were made in motor cortex to ensure that forelimb areas as defined by previous behavioural (Peterson and Gucker, 1959; Castro-Alamancos and Borrell, 1993; Whishaw, 2000), anatomical (Donoghue and Wise, 1982; Wise and Donoghue, 1986), and electrophysiological (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Neafsey and Sievert, 1982; Kleim et al., 1998) studies were damaged. Animals received an injection of atropine nitrate (0.1 mg/kg i.p.; Sigma-Aldrich, St. Loius, MO) to facilitate respiration throughout surgery and were then anesthetized with sodium pentobarbital (65 mg/kg, i.p.; Sigma-Aldrich, St. Loius, MO). Four holes were drilled using a fine dental burr in the skull overlaying the motor cortex at stereotaxic coordinates measured from Bregma (anterior (A) and lateral (L)) were A = -1.0 mm, L = +1.0 mm; A= +4.0 mm, L= +1.0 mm; A= -1.0 mm, L= +5.0 mm; A= +4.0 mm, L= +5.0 mm. The rectangular area enclosed by the four points was then trephinated and the dura was removed. The underlying tissue was devascularized by gently wiping away the pia mater and blood vessels with a saline-soaked cotton swab (Sofroniew et al., 1983; Kolb et al., 1997). The incision was closed and the animal's condition was

monitored in a recovery room for 24 hours, at which time it was returned to the colony room.

Parietal cortex devascularization. Procedures were identical to motor cortex surgery but the coordinates measured from Bregma were A= -3.5 mm, L= +1.0 mm; A= -3.5 mm, L= +4.0 mm; A= -8.5 mm, L= +1.0 mm; A= -8.5 mm, L= + 4.0 mm.

Reaching boxes

Single pellet reaching boxes (Whishaw and Pellis, 1990) were made of clear Plexiglas, with the dimensions 45 x 14 x 35 cm. In the center of each front wall was a vertical slit 1 cm-wide, which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-deep shelf. Two indentations on the surface of the shelf were located 2 cm from the inside of the wall to hold the food targets. The indentations were aligned with the edges of the slit where rats could reach.

Video recording

Reaching performance was video recorded using a Sony 3CCD camcorder (1000th of a second shutter speed) and a cold light source (Whishaw and Pellis, 1990). A Sony videocassette recorder DSR-11 was used for subsequent frame-by-frame analysis. Representative still frames were captured from digital video recordings with Final Cut Pro HD (V.4.5 www.apple.com).

Bracelets for limb restraint

A bracelet made of Elastoplast fabric adhesive tape (Smith & Nephew Inc., Lachine, Quebec) was wrapped around wrist of a particular paw to enlarge it such that it could not inserted through the slot of the reaching box (Whishaw et al., 1986). The

bracelet did not cover the digits of the paw or otherwise impede the rat's movement during stepping, grooming, or supporting the other paw during reaching and could be easily slipped off by the experimenter without discomfort to the rat's forelimb or denuding its fur.

Reach training

The first week of training consisted of daily, 10 min sessions, for each rat. In the second week, rats received daily training sessions but a session now consisted of 25 pellets, also considered 25 trials, per session. A session began when a food-deprived rat was individually placed in a reaching box and ended when the rat was removed from the box. Training involved the achievement of three stages, in which the rat learned to orient to the food pellet through the slot, transport its limb through the slot to grasp the food pellet, retract its paw through the slot to release the food into its mouth.

For a naive rat, a number of food pellets were placed on the shelf and directly in front of the slot. The objective was to make the pellets accessible to the tongue or paw through the slot. In addition, crushed food pellets were positioned on the shelf as well to intensify the scent of the pellets to prompt the rat's interest. Once a rat was successfully taking food from the shelf, pellets were moved further from the slot to encourage use of a paw. Once a rat demonstrated a preference for one paw by making more reaching attempts with it, individual pellets were placed into the indentation contralateral to that paw. Pellets so placed are accessible to that paw because the reaching paw pronates medially to grasp [35]. A delay preceded the presentation of successive pellets. During the delay, the rats were shaped to leave the slot, go to the back of the box, and then return to the slot (Gharbawie et al., 2005). Once this aspect of training was completed, food was

placed on the shelf on a random schedule and the rats were shaped to return to the back of the box if they could not confirm by sniffing that food was located on the shelf. At asymptote, rats retrieved pellets successfully with a single paw on more than 50% of trials.

Behavioral Analysis

The behavioural analysis used a conceptual framework derived from Eshkol-Wachman Movement Notation (EWMN) (Eshkol and Wachman, 1958). In brief, EWMN is designed to express relations and changes between the parts of the body or between the parts of the body and an external frame of reference. For the present reaching task, the position of individual limbs, the trunk, the snout, and the head, was described in relation to the food pellet. The body was treated as a system of articulated axes (i.e. limb segments, trunk axis, and snout axis). A scoring system was adapted from EWMN to record the following aspects of the rats' behaviour.

(1) Temporal relations. Relevant parts of the body (e.g., snout tip, reaching paw) are represented in the first column on a page, and additional columns are used to denote units of time (e.g. video frames, 30 frames/sec). The signs for movement are read from left to right and from bottom to top. Thus, the system allows the possibility of tracking the movement of one body segment over time in comparison to other body parts or posture. These methods have been previously adapted to rat reaching (Whishaw and Pellis, 1990).

(2) Body and target (oppositions). The descriptions of complex actions can be simplified by describing oppositions, which are relations between a body part and a target (Pellis, 1983; Yaniv and Golani, 1987). (In social interactions, an animal attempts to

maintain a constant relationship between a part of its body and a part of the body of a conspecific. For example, in rat play behavior two participants may engage in many complex movements of jumping, running, and tumbling, but the behavior can be descriptively simplified by understanding that one animal is attempting to maintain snout contact with the neck of the other while the second animal is trying to evade that contact. The opposition between the snout and target thus comprise a subgoal of the behavior (Pellis and Pellis, 1983).) In the present study, skilled reaching was examined in order to describe potential oppositions between body parts of the rat and the target food pellet.

(3) Cartesian reconstruction. The spatial trajectory of movement was reconstructed with Cartesian tracings from the start to the end of a movement (Whishaw et al., 1992). The distance covered by the movement was obtained by superimposing a grid composed of 5 mm squares onto each tracing and counting the number of squares intersected by the tracing. Reconstruction of the spatial trajectory allowed objective measurements of the (a) distance traveled by a particular body segment during an opposition, (b) location of the box over which the opposition occurred, and (c) frequency of movement.

(4) *Success.* A successful reach is one in which the food was grasped and transferred into the mouth using the designated paw, which was measured using the following formula (Whishaw et al., 1986):

Success percent = (number of pellets retrieved/20) x 100.

Histology

Rats were euthanized with sodium pentobarbital and intracardially perfused with saline (0.9%) followed by 4% paraformaldehyde. Brains were removed, post-fixed and

cryoprotected in 30% sucrose and 4% paraformaldehyde at 4°C for coronal sectioning (40 μm) and cresyl violet staining. Digital images were captured from the following planes as measured from Bregma: +2.70, +1.70, +0.70, -0.30, -1.30, -2.30, -3.30, -4.30, -5.30, -6.30, -7.30 mm, for subsequent analysis. The area of undamaged tissue on the ipsilateral-to-lesion hemisphere and the entire area of the contralateral hemisphere were independently estimated for each group. Using ImageJ (V 1.36 http://rsb.info.nih.gov/ij/download.html), motor cortex damage was estimated from the first six coronal planes, whereas parietal cortex damage was estimated from the last six coronal planes with the following formula:

Tissue damaged = [[area of contralateral-to-lesion hemisphere ($pixels^2$) - undamaged area of ipsilateral-to-lesion hemisphere ($pixels^2$)]/area of contralateral-to-lesion hemisphere] x100.

Procedure

Rats were trained to reach for single food pellets using one forepaw for two weeks. In anticipation that the rats might switch paw preference after motor cortex injury (Peterson and Gucker, 1959; Whishaw et al., 1986), a bracelet was applied to the non-reaching forelimb during the last week of reach training and the objective was to habituate the rats to the bracelets prior to injury. Reaching performance was video recorded on the last day and records from this time point were used for the presurgical demonstration in the figures and videos to follow. The rats were randomly assigned to the motor cortex group (n=6) or the parietal cortex (n=6) group. Surgeries were conducted in one day in counterbalanced order. Skilled reaching was tested and video recorded on postoperative days 1, 2, 3, 5, 7, 10, and 14. During post surgical testing, a bracelet was

applied for the duration of the reaching session only in the event of a rat reaching with the ipsilateral-to-lesion forelimb for two pellets at any point during the test session. Rats were euthanized several weeks later and their brains extracted for histological analysis.

3. Results

Acquisition prior to motor cortex stroke involves learning three oppositions

Analysis of the acquisition of reaching suggested that the rats sequentially learned three actions: orienting to locate the food by sniffing, transporting a limb to the food to grasp it, and withdrawing the limb to the mouth so that it can take the pellet. Thus, learning to reach for food appears to be guided by learning three subgoals, each consisting of an opposition between a body part and the food pellet. These oppositions provide an invariant structure to skilled reaching, while the movements used could vary from trial to trial and from rat to rat.

(1) Orientation. During initial acquisition, a control rat when placed into the reaching box spends a considerable period of time exploring it. The rat typically rears at either end of the apparatus, but might on occasion rear in the centre. During a typical rear, the rat shifts its body weight onto its toes and sidesteps for lateral movement while placing its forepaws onto the walls of the apparatus. It also walks around the apparatus scanning and sniffing the floor along the way. Although the rat might sniff the food pellets through the slot at the front of the apparatus with its initial traverse through the apparatus, it typically does not orient to the food before it has traveled around the apparatus a number of times. Thereafter, behavior could be described as bouts of activity each ending with attention (sniffing) to the food on the shelf. With training, the rat located the food more rapidly and other activites decreased in duration and extent. Thus,

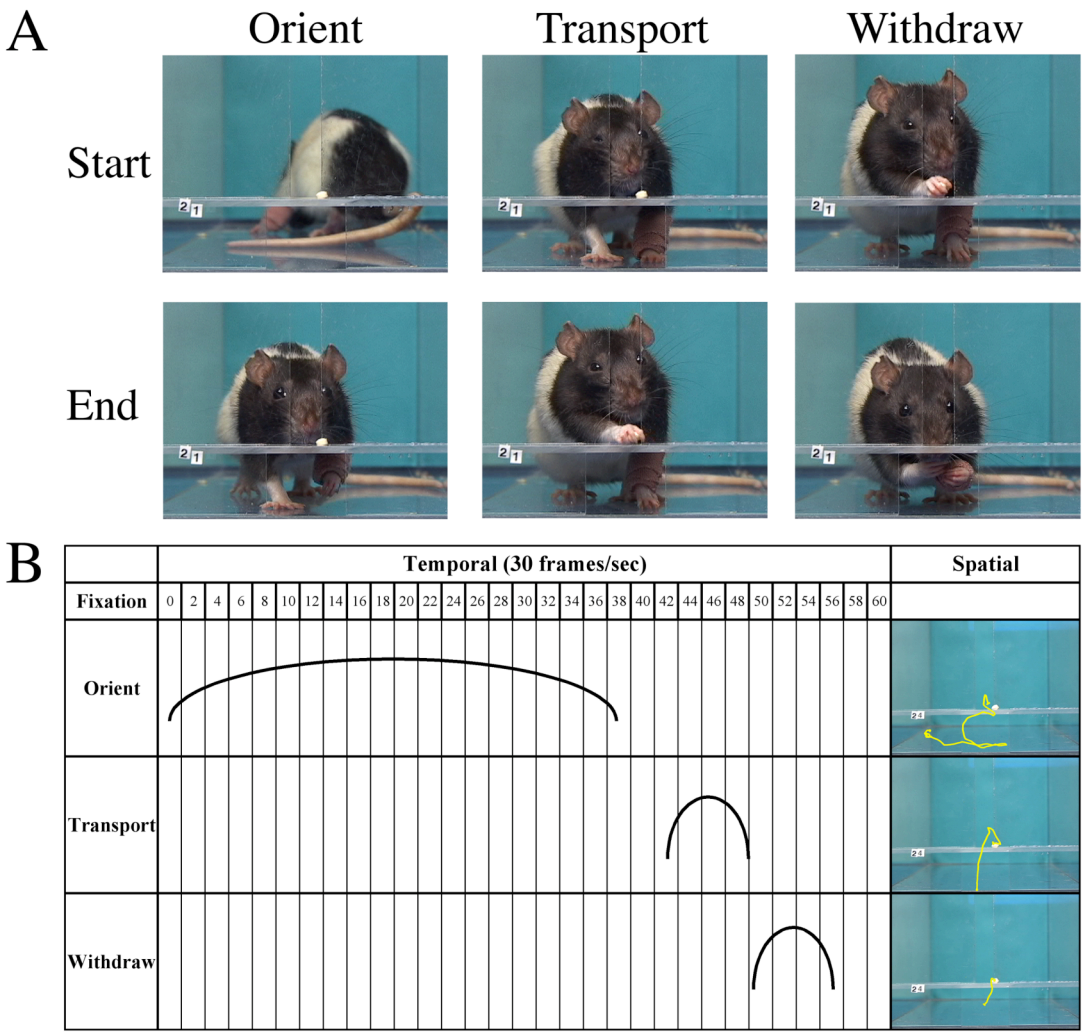


Figure 4.1: Reaching for a single food pellet is comprised of three actions each involving an opposition between a body part and the food pellet. (A) The start and end frames of the orient, limb transport, and limb withdraw were captured from video records. (B) Left panel: temporal distribution of the three oppositions of the reach indicated by inverted arcs and referenced to video frames. The snout-pellet is longest, whereas the paw-pellet and mouth-pellet are shorter and comparable in duration. The three oppositions are completed in less than 60 video frames (2 sec). Right panel: spatial distribution of the trajectory of the snout, paw, pellet for the three respective oppositions.

over the training period, the amount of time spent "exploring" the box decreased until eventually the rats walked to the slot and sniffed the food pellet immediately upon being placed into the box.

Fig. 4.1 (orient) illustrates movement (A), its duration and spatial trajectory (B) in a trained rat. The movement can be described as being directed by an opposition between the snout (nostrils) and the food pellet. In trained rats, the movement was economized to the most direct route from the rear of the apparatus to the food pellet. Nevertheless, in leaving the back of the box and walking to sniff the food pellet, rats might turn to the right or to the left, although in general they had a preferred direction. A rat might also approach the slot at an angle or approach it head on. Additionally, the animal's movement speed and the number of steps taken could vary from trial to trial.

(2) Limb transport. Once the rats were reliably orienting to the food location and sniffing to locate the food, their behavior was modified from first lapping the food pellets from the shelf to extending a single paw through the slot to grasp. In this transition, a rat might alternate between licking and biting the slot and transporting both forelimbs to the food, all the while tilting its head and shifting its body weight to maximize the movement of its mouth and paw toward the pellet. Eventually, the rat became successful in transporting a forelimb through the slot to grasp the food.

Fig. 4.1 (transport) illustrates limb transport (A) and its duration and trajectory (B). Limb transport began when the reaching paw released contact with the floor and ended with the digits flexing around the pellet to grasp it. Limb transport can be described as being guided by an opposition between the paw and the pellet that is completed when the food pellet is grasped. Although the opposition between the paw and the food pellet is invariant, posture and movements of stepping that occurred during limb transport could vary from trial to trial and from rat to rat. For example: on some trials a rat might transport its limb more than once through the slot before successfully grasping a

pellet, on other trials it might lift both forelimb during the initial part of the reach, on other trials it might move its reaching forelimb and its contralateral hind limb forward together, and on still other trials the three non-reaching limbs might remain on the floor.

(3) Limb withdrawal. Before learning to grasp food pellets, the rats typically raked the food into the box and lapped it off the floor. Upon first successfully grasping a food pellet, none of the rats brought it directly to the mouth. They lowered their paw to the floor and dropped the food as they attempted another reach or walked away. After several successful pellet grasps and releases onto the floor, the rats began to search for the dropped food pellets. This eventually led to checking the paw for the pellet. During the checking phase, a rat might check its paw on reaches on which it had not successfully grasped a food pellet as well as on reaches that were successful. Eventually, the rat learned that it grasped the food because it brought the paw with the food towards the mouth by adducting the limb and supinating the paw then extending the digits to release the food into the mouth.

Fig. 4.1 (withdraw) illustrates limb withdrawal (A) and its duration and trajectory (B). Limb withdrawal can be described as opposition between the mouth and the pellet that is complete when the food pellet is transferred from the paw to the mouth. Although the opposition between the food pellet and the mouth is invariant, the movements used could vary. A rat might bring the food pellet to the mouth with little movement of the body, or it might sit back on its haunches to take the food, or it might use its non reaching forepaw to assist the movements of the reaching paw.

(4) Variation. Although rats learned the three actions necessary for skilled reaching in a consistent order, acquisition rate varied between rats. Thus, in the time it

took one rat to learn to orient to the food pellet, another rat might learn to orient to the pellet and rake it with its paw, and yet a different rat might learn to orient to the food pellet, reach for it and grasp it, and even withdraw the paw with the pellet and release it in its mouth. For the session or two after a rat acquires the three actions, the rat remains prone to committing errors in any of the three actions. For example, it might make an orientation error by reaching in the absence of a pellet, or it might not transport its limb sufficiently to contact the pellet and knock it away, thus committing a limb transport error, or it might drop the pellet from its paw as it withdraws the pellet from the shelf thus committing a limb withdrawal error.

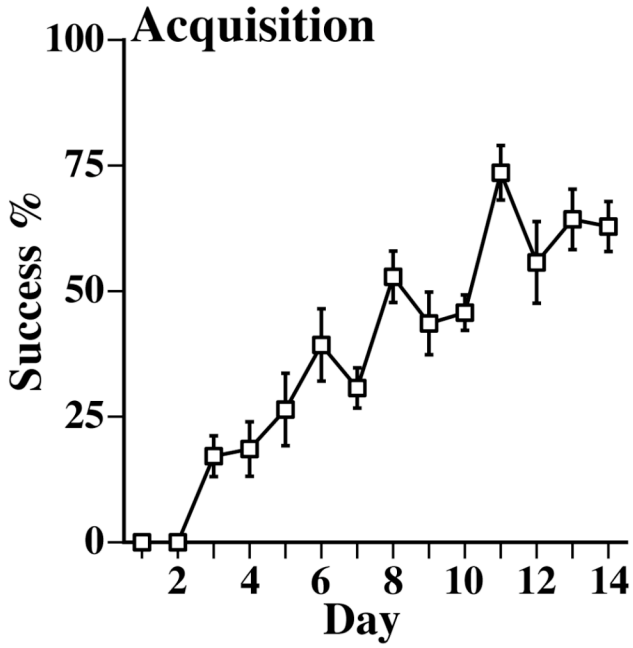


Figure 4.2: Success scores for single pellet acquisition gradually improved and reached asymptote by the tenth day of presurgical training.

(5) Success. Once a rat learned all three actions, it was possible to measures success. Thus, the gradual improvement in reaching performance during task acquisition is related to rats learning the three oppositions as well as learning rules imposed by the

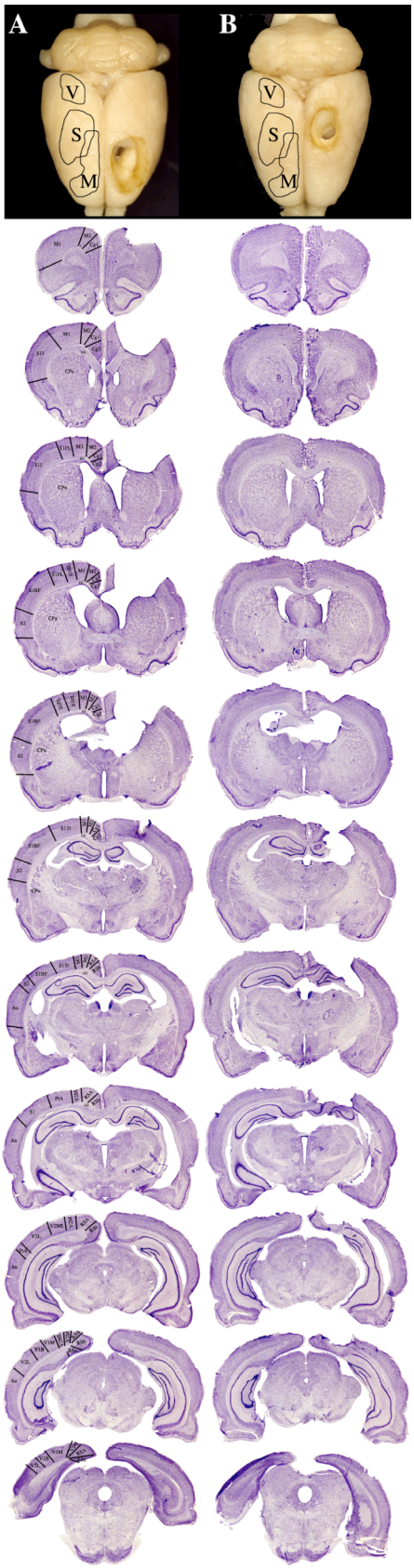

A
V
S
M
B
V
S
M

Figure 4.3: Brains extracted from (A) a motor cortex rat and (B) a parietal cortex rat. The motor (M), sensory (S), and visual (V) neocortical areas are schematically outlined for reference. Coronal sections from the respective brains were stained with cresyl violet. The lesions were anatomically distinct except for overlap evident in sections 5 and 6.

experimenter such as returning to the rear of the apparatus after reaching for a food pellet. Asymptotic performance for the group was reached by day 10 of training (Fig 4.2). There were no differences in success scores for rats that were to receive motor cortex lesions and rats that were to receive parietal cortex lesions [ANOVA: $F(1,10) = 1.83$, $p > 0.05$].

Histology

Devascularization damaged dorsal aspects of the neocortex in both groups (Fig 4.3) as has been previously described (Kolb et al., 1997; Whishaw, 2000; Gonzalez and Kolb, 2003). Coronal sections demonstrate that damage extended into all areas of motor cortex for the motor cortex group (Fig 4.3A), whereas somatosensory, parietal association, and visual cortex were damaged in the parietal cortex group (Fig 4.3B). The coronal sections show some overlap between the caudal aspects of the motor cortex lesion and the rostral aspects of the parietal cortex lesion. Corpus callosum fibers displayed degeneration directly beneath both lesions. Disfiguration in the dorsal aspects of the striatum in the motor cortex group and the dorsal aspects of the hippocampus in the parietal cortex group was probably due to swelling and ventricular enlargement. Otherwise, subcortical structures were intact in both groups. The percentage of damaged tissue was comparable for the motor cortex group (21.26 ± 3.16 %; mean $\pm$ SEM) and parietal cortex group (19.19 ± 4.65 %; mean $\pm$ SEM).

Recovery after motor cortex stroke involves reacquiring three oppositions

(1) Success. In the initial day or two after surgery, the rats with motor cortex lesions failed to make reaching attempts. When they did begin to reach, they made a few unsuccessful reaches with the contralateral-to-lesion paw and/or attempted to reach with the ipsilateral-to-lesion paw. To prevent use of the ipsilateral-to-lesion paw a restraining bracelet was wrapped around it to ensure that rats reached with the contralateral-to-lesion paw. Over testing days the rats began to use their contralateral-to-lesion paw and its number of successful reaches increased to asymptote by day 14, as confirmed by a significant effect of test day [repeated measures ANOVA: $F(11,55) = 6.86$, $p < 0.01$, Fig 4.4].

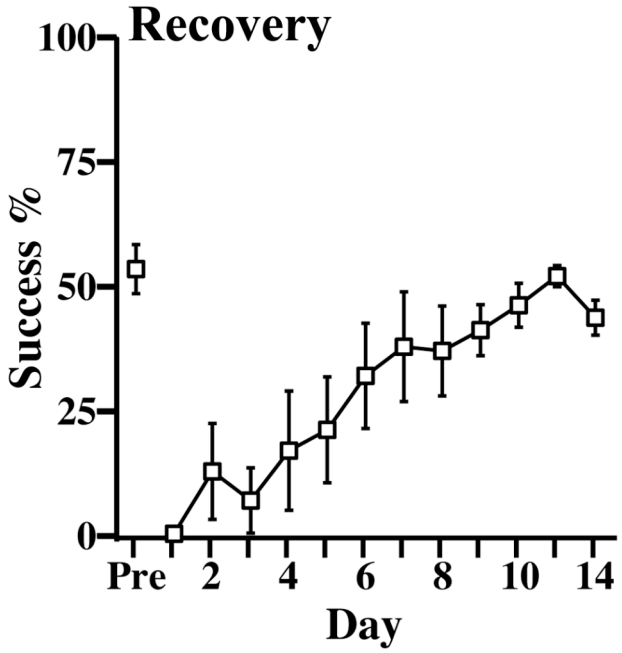


Figure 4.4: Success scores for single pellet reaching declined severely after motor cortex damage but recovered to presurgical levels over a two-week postoperative period.

(2) Oppositions. After motor cortex but not parietal cortex damage, all three oppositions were disrupted but they displayed a temporal recovery in the order snout-pellet, paw-pellet, and mouth-pellet.

(i) Orientation. At asymptote presurgery, the rats oriented to the pellet immediately upon introduction into the reaching box. Immediately postsurgery, the rats behaved as they did when training first began: they explored and scanned the apparatus with their snout and then during each test session and on subsequent test sessions their movement towards the food became more direct (see Appendix 3). The initial impairment followed by improvement was evident from the distance the snout traveled before sniffing the food pellet [repeated measures ANOVA: $F(5,25) = 15.84$, $p < 0.01$, Fig 4.5 A]. Fig 4.5 B illustrates the snout trajectory of one rat over 14 postsurgical days: each arc represents a snout scan that ended with a pause or change in direction and the end of the sequence of arcs represents a sniff at the pellet. For the rat illustrated in Fig 4.5 B, the first sniff directed toward the food pellet occurred on the second postsurgical day. Thereafter, the number of head scans preceding a sniff decreased over days until by postsurgical day 14, this rat made one head scan to reach the food pellet (as it did during presurgical asymptote). Examples of movement of the snout on each test day from the same rat across the two-week recovery period are demonstrated in Fig 4.5 C. The tracings of the snout movement indicates that the rat, although not reaching, was not inactive and was exploring the entire test apparatus even on the first postsurgical day. The extent of its exploratory movements decreased across postsurgery days until it moved directly to the food pellet by postsurgical day 14.

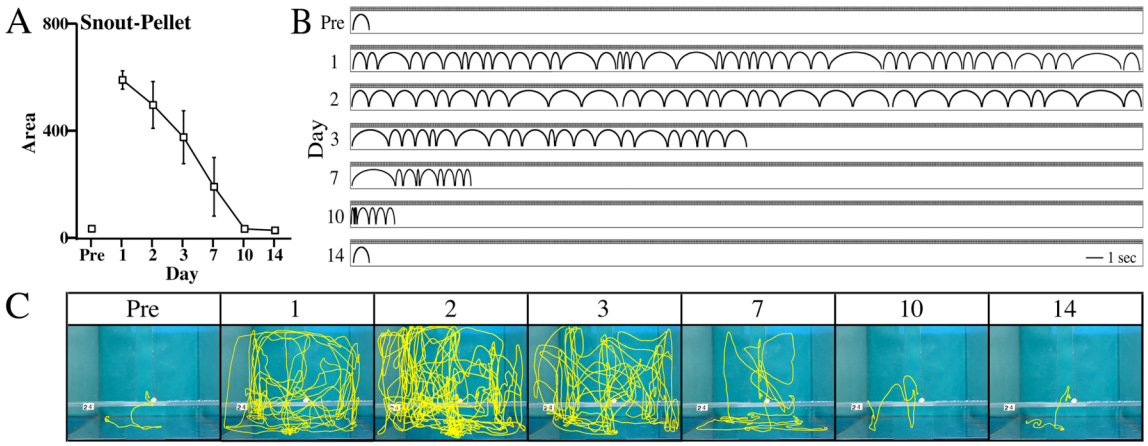


Figure 4.5: A profile generated from tracings of the tip of the snout of motor cortex rats during snout-pellet opposition. A tracing started as soon as the animal turned its forequarters at the rear of the apparatus to start walking towards the front and ended when the rat sniffed the food pellet or after two minutes. (A) The area covered by the snout was estimated by counting the number of squares intersected on a grid superimposed onto a two-dimensional snout tracing. The snout covered a much larger area after motor cortex damage and did not return to presurgical levels until post surgical day 10. (B) A representation of the temporal distribution of snout scans preceding a pellet sniff from a motor cortex rat. Snout scans increased in frequency and duration after injury but presurgical distribution was restored at the end of the two-week testing period. Note, this rat did not sniff the food pellet on postsurgical day 1 but did sniff on postsurgical day 2 and on subsequent days. (C) The spatial representation (yellow) of the snout paths preceding a sniff from the same motor cortex rat. The snout path was direct before surgery (Pre) but became circuitous in the initial days after surgery and gradually declined to normal by postsurgical day 14.

(ii) Limb transport. After surgery most motor cortex rats did not attempt any reaches on the first day. On subsequent days, motor cortex rats attempted to retrieve the food pellet by biting the edge of the slot and with short limb advances that frequently did not contact the food pellet or else knocked it away. The number of contralateral-to-lesion limb transports that preceded each successful grasp increased after injury (Fig 4.6 A). In addition, the ipsilateral-to-lesion limb appeared to become involved either in attempting to grasp the food pellet or assisting the contralateral-to-lesion forelimb out of the slot, as it might get stuck when the rat attempted to withdraw it. Together, movements of the

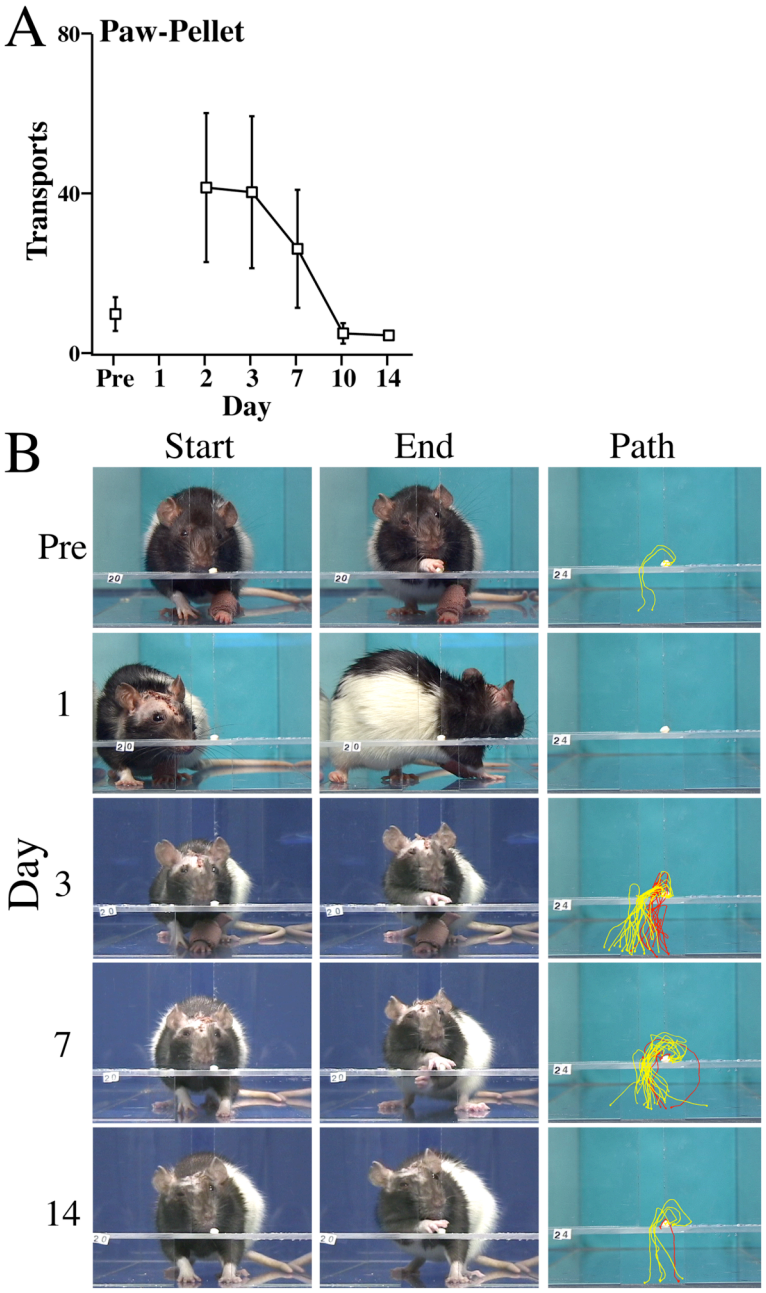


Figure 4.6: A profile generated from tracings of the tip of the second digit of motor cortex rats during the paw-pellet opposition. (A) The number contralateral-to-lesion and ipsilateral-to-lesion limb transports increased after motor cortex damage but recovered to presurgical levels (Pre) by postsurgical day 10. (B) The spatial representation of the contralateral-to-lesion paw (yellow) and the ipsilateral-to-lesion paw (red) before lesion (Pre) and on postsurgical days 1, 3, 7 and 14 from one motor cortex rat. Tracings (path) began on the video frame in which a paw released contact with the floor to be transported towards the slot (start) and ended on the frame in which the digits began to flex to grasp the food pellet (end). Note, that there is no path traced on postsurgical day 1 because this motor cortex rat did not transport either limb towards the pellet on that test day.

contralateral-to-lesion and ipsilateral-to-lesion limbs contributed to a significant increase in the number of limb transports after motor cortex injury (see Appendix 4). A decline in the number of limb transports that preceded a successful grasp was observed across test days until presurgical levels were restored by about postsurgical day 10 [repeated measures ANOVA: $F(4,20) = 3.17$, $p < 0.05$]. Topographic examples of movement of the contralateral-to-lesion paw and the ipsilateral-to-lesion paw for the two-week recovery period are demonstrated in Fig 4.6 B.

(iii) Limb withdrawal. Once the rats were able to successfully grasp a food pellet postsurgically, they did not bring it to their mouth. Pellet release from the paw into the mouth did not uniformly occur in all rats until postsurgical day 7 (Fig 4.7 A). Even when they did eventually manage to release the food to the mouth, the action was not normal (see Appendix 5). Although the trajectory of the food pellet from the shelf to the mouth declined across test days [repeated measures ANOVA: $F(2,10) = 9.64$, $p < 0.01$], it did not approach presurgical levels. As is illustrated in Fig 4.7 B (Day 3) for one rat, upon grasping the food, the paw dropped to the floor and released the pellet and the rat seemingly ignored that that it had brought a pellet inside the reaching box. The pellet was consumed directly off the floor when it was accidentally encountered when the rat was traversing the box. After a few pellets were released onto the floor, the rat began to actively search for dropped food pellets. Eventually, the rats began to retrieve the food from the paw with their mouth, but they did so by "chasing" the paw with the mouth and prying the food pellet out of the digits. The first chasing movements might involve a complete turn of the forequarters and hindquarters, but eventually the size of the chasing

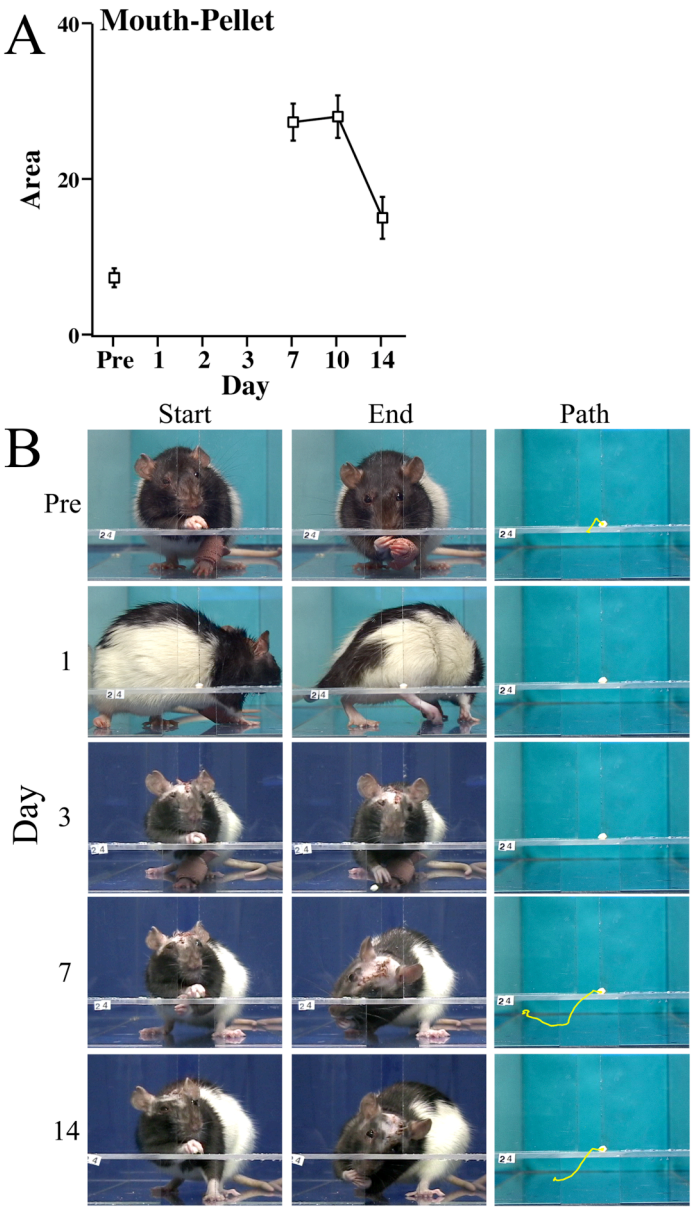


Figure 4.7: A profile generated from tracings of the pellet during mouth-pellet opposition for motor cortex rats. (A) The area covered by the pellet was estimated by counting the number of squares intersected on a grid superimposed onto a two-dimensional tracing of the pellet path from the shelf until it was released into the mouth. The pellet covered a larger area of the box after motor cortex damage and did not return to presurgical levels. (B) The spatial representation (path) of the pellet trajectory before surgery (Pre) and on postsurgical days 1, 3, 7, and 14. Tracings (path) began on the video frame in which the digits completely flexed around the pellet (start) and ended on the frame in which the digits extended to release the food pellet into the mouth (end). Note that the pellet is not grasped on post surgical day 1 and is released onto the floor instead of into the mouth on post surgical day 3.movement decreased until a rat only had to turn its head for the mouth to contact and the food and retrieve it from the paw.

(iv) Variation. Although the recovery of the oppositions or subgoals returned in the same sequential order in all rats, the recovery rate varied amongst rats. For some rats the oppositions occurred as early as the first postoperative day (Fig 4.8 A, rat A) while for other rats the behaviors reemerged as late as postsurgical day 7 (Fig 4.8 A, rat C, D, F). For example, some rats sniffed the food pellet after traversing the length of the box multiple times while other rats made many more trips over a number of days before sniffing the food pellet. Once an opposition reemerged there was variance in the time taken before the next opposition was observed. For example, a rat might successfully transport its forepaw and grasp the pellet soon after it started sniffing it (Fig 4.8 A, rat A and D) while for another rat many limb transports (at least 30) occurred before a pellet was grasped (Fig 4.8 A, rat B, C, E, and F). Furthermore, the behaviour of each rat varied from one recovery day to the next. An opposition that recovered on a particular test day did not remerge immediately in the following test session or even on the subsequent test session suggesting that each opposition recovered gradually across test days. The variance generally reflected recovery of oppositions as demonstrated by a progressive decline in the size of the coloured bars from one recovery day to the next (Fig 4.8 B).

Three oppositions are maintained after parietal cortex stroke

(1) *Success*. Limb preference and reach accuracy were unaffected by parietal cortex damage. A day x group interaction [repeated measures ANOVA: $F(11, 110) = 3.96$, $p < 0.01$] suggested that improved performance was limited to the motor cortex group.

(2) Oppositions. All three oppositions were unaffected by parietal cortex damage.

(i) Orientation. At presurgery asymptote, rats in both groups oriented to the food

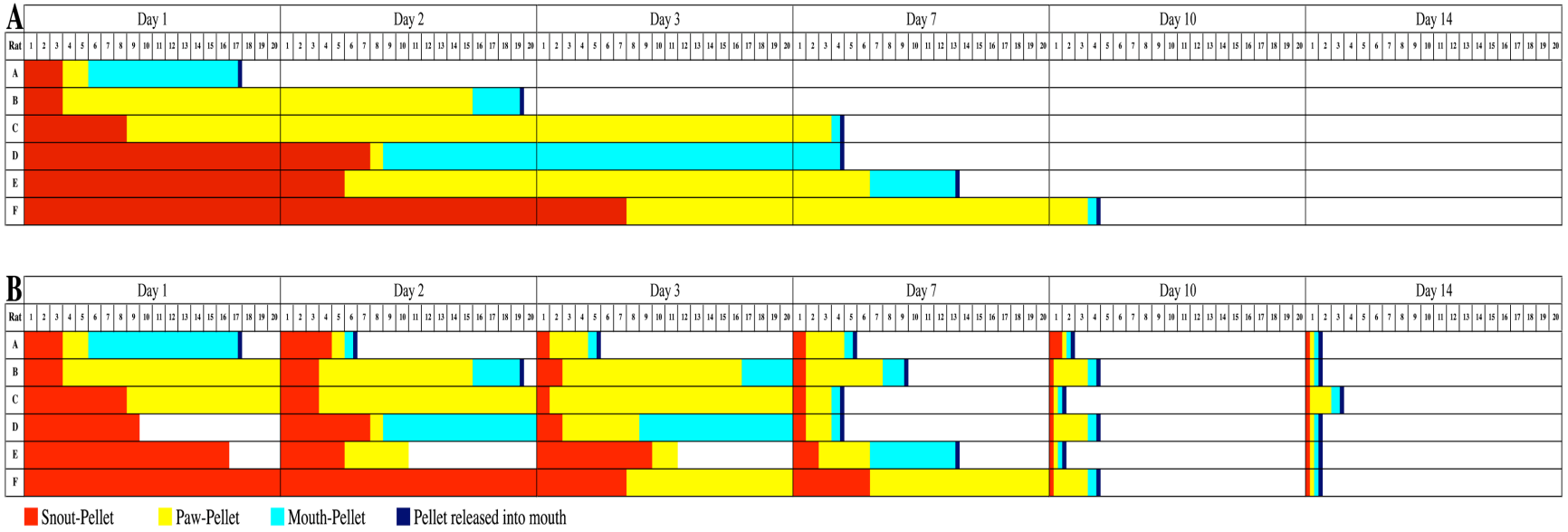

A
Day 1
Day 2
Day 3
Day 7
Day 10
Day 14
Rat
A
B
C
D
E
F
B
Day 1
Day 2
Day 3
Day 7
Day 10
Day 14
Rat
A
B
C
D
E
F
Snout-Pellet
Paw-Pellet
Mouth-Pellet
Pellet released into mouth

Figure 4.8: Variation in the pattern of recovery from motor cortex damage. Individual motor cortex rats are listed in the first column (A, B, C, D, E, and F). The number of pellets (trials) presented is indicated across the top for postsurgical test days 1, 2, 3, 7, 10, and 14. The oppositions (snout-pellet, paw-pellet, mouth-pellet) and pellet release from the paw into the the mouth are depicted with coloured bars. The length of each bar indicates the number of trials completed before each opposition was first achieved. A bar extended to the twentieth trial on a particular day indicates that the opposition was not achieved on that day. The score for the snout-pellet opposition was determined from the number of times the animal traversed the reaching box (a round trip from the rear of the apparatus to the front and then the back again without sniffing the food pellet counted for one pellet). The sequence of recovery of snout-pellet, paw-pellet, mouth-pellet was similar for all rats, but variance was observed between rats in the number of trials before a specific opposition was observed (A). For example snout pellet opposition was achieved after several trials on postsurgical day 1 for rats A, B, and C, but was not achieved until a later period for the other three rats. Also the oppositions differentially recovered for each rat. (B) Variance was observed in the number of trials that preceded the achievement of an opposition from one day to the next. The progressive decline in bar length for each opposition reflects a decrease in the number of pellets for achieving an opposition on successive postsurgical days.

pellet immediately upon introduction into the reaching box. The corresponding tracings of the snout paths during snout-pellet opposition covered similar areas for both groups [ANOVA: $F(1,10) = 0.93$, $p > 0.05$]. However, snout paths were significantly elongated and covered more regions of the reaching apparatus after motor cortex damage but not after parietal cortex damage [repeated measures ANOVA: $F(1,10) = 50.91$, $p < 0.01$]. The area covered by snout paths declined across test days for motor cortex rats but were consistent for parietal cortex rats, which is confirmed by a day x group interaction [repeated measures ANOVA: $F(5,50) = 14.84$, $p < 0.01$].

(ii) Limb transport. At presurgical asymptote, rats from both groups transported their forelimb through the slot, and pronated and flexed their digits around the pellet. The accuracy of the forelimb trajectory on a successful grasp prior to surgery was reflected in a direct limb transport path from the floor, through the slot, which ended with pellet contact. The number of limb transports did not differ between groups prior to surgery

[ANOVA: $F(1,10) = 1.22$, $p > 0.05$]. Despite a significant increase in the number of limb transports necessary to successfully grasp a food pellet after motor cortex injury, it did not differ from that of the parietal cortex group [repeated measures ANOVA: $F(1,10) = 3.30$, $p > 0.05$]. The performance of the parietal cortex group was consistent across test days.

(iii) Limb withdrawal. At presurgical asymptote, both groups withdrew the pellet from the shelf and presented it directly to the mouth. Tracings of the course of the pellet prior to injury covered a marginal area of the box for both groups [ANOVA: $F(1,10) = 0.06$, $p > 0.05$]. The area covered by the pellet path after surgery was significantly larger for motor cortex rats as compared to parietal cortex rats [repeated measures ANOVA: $F(1,10) = 60.63$, $p < 0.01$]. Limb withdrawal was consistent throughout testing for the parietal cortex group, however a day x group interaction showed a steady decline in the pellet path for motor cortex rats [repeated measures ANOVA: $F(2,20) = 7.99$, $p < 0.01$] although presurgical levels were not recovered.

4. Discussion

The present study provides a novel description of the way in which skilled reaching acutely recovers following motor cortex damage by comparing the reemergence of movements after damage to the sequence of movements observed during initial learning. The results show that skilled reaching is composed of three subgoals: (1) locating the pellet by sniffing, (2) transporting a paw to the pellet, and (3) withdrawing the pellet and releasing it into the mouth. Each subgoal is guided by an opposition, or a relationship between a body part and the food pellet, that is consummated when that body part contacts the food pellet. Although the oppositions are invariant, the movements used

to complete them could vary from trial to trial and from rat to rat. Recovery of skilled reaching after motor cortex injury involved sequential reemergence of oppositions that paralleled initial learning. Nevertheless, there was substantial variation in the rate of recovery from rat to rat and from one opposition to the next. Thus, skilled reaching can be decomposed into three learned oppositions. The loss and reacquisition of oppositions may underlie acute postsurgical behavioural depression and recovery following motor cortex lesions.

As is summarized in the introduction, the impairment in skilled reaching in rats with motor cortex lesions is distinctive in that the animals display an acute depression of behavior (Whishaw et al., 1991; Gharbawie et al., 2005) followed by recovery that features the substitution of compensatory movements for lost rotatory movements of the reaching limb (Whishaw et al., 1991). Animals with damage to other parts of the motor system do not show the same acute depression of behavior (Whishaw et al., 1986; Pisa, 1988; Whishaw et al., 1990; Whishaw et al., 1993; Miklyaeva et al., 1994). To date, however, there has been no examination of the behavior of motor cortex rats in the immediate postsurgical period other than measures of success and compensation. Thus, the behavioural symptoms during this transient depression as well as the pattern of recovery remain unclear. The present study proceeded by comparing the sequence of submovements learned during initial acquisition of a skilled reaching for food task to its reacquisition following motor cortex stroke produced by devascularization. In order to provide a control for surgical procedures, a group of rats received parietal cortex stroke, which were unimpaired.

The behavioral analysis indicated that skilled reaching can be described as consisting of three actions, each of which brings a body part to the food target. In previous work such relations between a body part and a target that direct the body part to the target have been referred to as oppositions (Golani, 1976). An opposition is invariant but the movements used to complete it can vary from instant to instant and from rat to rat. The utility of describing actions in terms of oppositions is that behaviors that are otherwise too complex for basic analysis can be decomposed into simpler actions. For example, the play or aggressive behavior of animals consists of many movements that occur in a varying order but which can be simplified into a structure in which one animal targets a body part of another animal that attempts to avoid that contact (Pellis and Pellis, 1990).

Studies of primate and human reaching have recognized that reaching consists of three components: orienting of the head and eyes to the target, limb transport during which the digits are shaped to the target to grasp it, followed by limb withdrawal with the target (Jeannerod, 1984). Thus, for rats as for primates, reaching is organized by three oppositions, although the rat sniffs to locate the target rather than visually orienting to it (Whishaw and Tomie, 1989). In the present study, the oppositions are imposed in part by the training regime and in part are adopted by the rats. For example, the rats are trained to return to the back of the apparatus when the food was absent, or after successful and failed reaches. Orientation to the food pellet therefore requires walking to the front of the apparatus and positioning the snout through the slot to detect the food pellet by sniffing. They are also required to use a specified paw because the position of the food on the shelf obscures retrieval with the tongue or the other paw. They are not, however, required to

bring food to the mouth. During initial learning, all of the rats dropped the food out of their paw onto the floor and then lapped it off the floor before they spontaneously learned to bring the paw with the food to the mouth. Because the sequence of subgoals is in part an artifact of training, different training setups could abbreviate the sequence. For example, if food was always present in a tray (Whishaw et al., 1986) or on a staircase (Montoya et al., 1991), the rats might not have to learn to detect it and so could reach "blindly" and use either paw. For these rats, task demands and oppositions learned would be partly different from those required in the present study.

The three reaching oppositions were disrupted or perhaps even lost after motor cortex damage and recovered across a number of postsurgical days. When introduced into the reaching box, motor cortex rats initially scanned the floor and walls of the test box with the snout and even bypassed the pellet a number of times without sniffing. Eventually they did sniff the food but then might not attempt to retrieve it. Retrieval attempts initially consisted of swiping a paw, often the ipsilateral-to-lesion paw, through the slot and biting the edge of the slot. After a few successful grasps, the pellet was withdrawn through the slot and released onto the floor. The rats seemed unaware that they had grasped the pellet and only later learned to hold on to it and to chase it with the mouth and extract it from the paw. Furthermore, performance at the start of each test session did not directly progress from the previous test session. Thus, an opposition that was reacquired on one test session was not immediately expressed at the beginning of the subsequent test session, suggesting that the recovery of that opposition, or its integration into the act of reaching is an ongoing progress. Thus, the sequence of recovery from motor cortex damage paralleled initial learning of the task suggesting that recovery

recapitulates initial learning (Twitchell, 1951; Teitelbaum and Stellar, 1954; Lawrence and Kuypers, 1968b; Cramer and Chopp, 2000) and is likely more complicated than unmasking of a depressed behaviour or overcoming limb use impairments.

Parallels between recovery and initial learning are also reflected in the similar progression of success scores during acquisition and recovery. Mean success scores even reached asymptote by the tenth day of preoperative training as well as during recovery. That the preoperative success scores were restored in the present study supports previous work demonstrating that rats will achieve baseline success within two weeks of motor cortex damage albeit substituting trunk movements for limb transport, wrist rotation, and supination; chasing the limb to pry food out of the digits as opposed to releasing food into the mouth (Whishaw et al., 1991; Gharbawie et al., 2005; Metz et al., 2005). Although there is probably no disagreement at present that rats use compensatory movements for reaching after motor cortex damage, the impairment as assessed by success scores ranges from being chronically below preoperative success scores (Papadopoulos et al., 2002; Gonzalez and Kolb, 2003; Emerick and Kartje, 2004), to restoring (Whishaw, 2000; Gharbawie et al., 2005), or even exceeding preoperative success scores (Bury and Jones, 2002; Metz et al., 2005). The differences might be related to animal training procedures, lesion procedures, or individual reaching abilities prior to lesion.

That skilled reaching consists of a number of learned subgoals that can be lost and relearned following motor cortex injury provides some insights into how the motor acts used in accomplishing each goal are initially learned and reacquired after injury (Fig 4.9). For a normal rat there is considerable flexibility in the selection of individual motor acts to achieve each opposition. For example, a rat can turn to the left or right to approach the

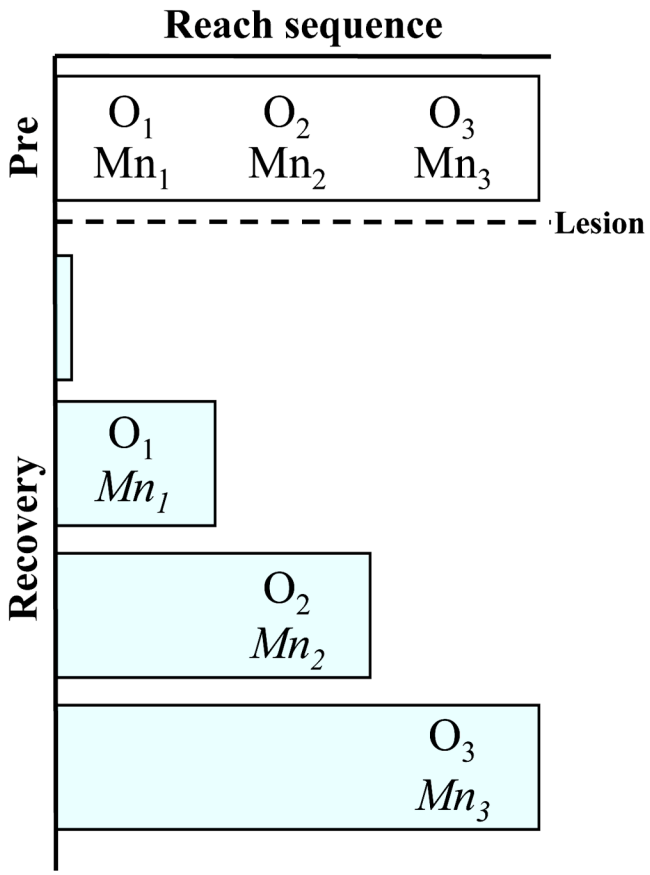


Figure 4.9: Schematic diagram representing the sequence of recovery of the three oppositions. Prior to surgery (Pre), the oppositions: snout-pellet (O_1), paw-pellet (O_2), and mouth-pellet (O_3) were achieved through a set of movements (Mn_1), where M = movement, n = number of movements, 1 = designation of movement set for a specific opposition. After motor cortex lesion, all three oppositions and associated movements might be compromised. But over the course of the recovery, animals perform alternate movements (e.g. Mn_1) to approximate an opposition (O_1). The oppositions return sequentially and reaching is not successful until all three opposition reemerge.

food, it can approach it at an angle or directly, and it can use a variety of postures and limb movements prior to and during limb transport and withdrawal. Although certain movements may be impaired by motor cortex injury, e.g., supination or pronation of the paw, the flexibility permitted by the invariance of oppositions allows an animal to use

compensatory movements, e.g., trunk rotation to substitute for lost movements (Whishaw et al., 1991; Whishaw, 2000).

The three oppositions recovered in stages that paralleled initial acquisition. This suggests that the brain internally organizes the act of reaching by serially stringing the submovement building blocks in an inflexible construct. It also suggests that the reacquisition of an opposition does not necessarily trigger the recovery of the subsequent opposition. The idea is consistent with Lashley's structure of complex movements being comprised of individual submovements (Lashley, 1951). The temporal organization of the *set* of submovements is not inherent to the submovements as the individual acts do not in themselves have a temporal valence, but the sequential order of the submovements is imposed by a generalized schemata of action. For the present study, the schemata of action would be the single pellet reaching task and the set of submovements are the oppositions necessary for achieving the task.

That the three reaching oppositions were lost after motor cortex damage suggests that skilled reaching might be hierarchically organized in the brain. In such a hierarchical representation muscular control can be envisioned on the lower end and conceptualization and rules of the task on the higher end. That motor cortex damage disrupted all three oppositions in the present study and did not simply impair limb control, suggests that motor cortex might be situated in a relatively high order of the hierarchy. The binding of the oppositions into a purposeful action might therefore reside in motor cortex, while other structures presumably lower in the hierarchy encode motor actions. This is consistent with John Hughlings Jackson's ideas about hierarchical organization in the brain in which an action is represented at each level of the hierarchy

with added complexity in the higher levels of the hierarchy. In such a model, lesions to the highest centers would produce a loss of movements and not just impair muscle control, arguably like the present loss of the reaching oppositions. Furthermore, Graziano's demonstration that complex movements of ethological significance such as limb transport and hand-to-mouth actions, which are similar to the limb transport and withdrawal oppositions in present reaching task, can be artificially derived in primates with a train of low amplitude electrical pulses delivered over 500 msec into motor cortex (Graziano et al., 2002; Graziano, 2006), suggests that motor cortex encodes behavioural repertoires as opposed to, or in addition to, being an apparatus of muscle control. The idea that motor cortex lesion damages the higher order representation of movement might well account for the acute behavioral depression in skilled action that follows motor cortex damage. A similar idea is evident in Brodal's [1] self-report on recovery from internal capsule (major cortifugal pathway of motor cortex) stroke, which relates that the loss of the schematic representation of movements was more handicapping than digit paresis.

Although the recovery sequence of oppositions was the same for each rat, the rate of recovery varied between rats. There was also variation in the time taken by individual rats to progress from the reacquisition of one opposition to the next opposition. The variation might be due to sparing of some portion of motor cortex. Although the neocortical area devascularized was purposely outlined to include both forelimb movement representations, the representations vary in location from one rat to the next (Hall and Lindholm, 1974) and are also directly linked to the acquisition of skilled movements (Kleim et al., 1998)**,** and thus a small portion of them could have survived in

some rats. Alternatively, the variation might stem from differences in the neural representation of skilled reaching, which might be under the control of a widely distributed circuit including: the damaged motor cortex contralateral-to-the reaching paw, the neocortex medial and lateral to it, and the opposite motor cortex, all of which contribute to the corticospinal tract, as well as subcortical structures, and the cerebellum (Wise and Donoghue, 1986). The variation might also be due to differences in the way that animals learned the task or to fortuitous events in relearning such as accidentally encountering the food pellet during orientation or successfully raking a food pellet into the reaching box that might have been otherwise displaced beyond reach. Chance successes might have been sufficient for rats to adapt their behaviour such that they reached more carefully to improve their chances of retrieving a food pellet. Indeed all these possibilities are supported by the observation that the reacquisition of skilled reaching was more rapid than naive learning. Whatever the source of the variation in recovery, it followed the same sequence in each rat with some variance at each stage.

Conclusion

In conclusion, the objective of the present experiment was to investigate the cause of the impairment of skilled reaching in the acute period following motor cortex stroke. The study provides a novel demonstration that skilled reaching is organized by three oppositions; submovements that are linked to construct the act of skilled reaching. The study also demonstrates that after motor cortex damage, the oppositions are lost, but are relearned sequentially in a manner that closely paralleled initial learning. In addition to providing a description of the recovery of skilled reaching, the present results suggest that the loss of the sequentially organized submovements contribute to the disability in skilled

reaching following motor cortex damage. Finally, in suggesting that motor cortex damage results in the loss of learning as well as the ability to perform skilled actions, the study provides new insights into the diaschisis of the acute postsurgical period after motor cortex lesions.

Chapter 5

Skilled reaching impairments from the lateral frontal cortex component of middle cerebral artery stroke: A qualitative and quantitative comparison to focal motor cortex lesions in rats

Modified from a paper published in *Behavioural Brain Research,* 156(1):125-37 by Omar A. Gharbawie, Claudia L. R. Gonzalez, and Ian Q. Whishaw in 2005.

Abstract

The classical approach to investigating brain contributions to behaviour has been to localize function to a region. In clinical investigations, however, injury is frequently multifocal, raising the question of how individual brain regions contribute to a resulting behavioural syndrome. For example, middle cerebral artery (MCA) ischemia in humans can concurrently damage a number of cortical and subcortical areas and the same areas are damaged in rat models of MCA stroke. In the rat, MCA occlusion produces severe motor deficits, but the cortical area of damage is the lateral neocortex, sparing motor cortex. This anatomical finding raises the question of whether the rat lateral neocortex contributes to MCA-related motor impairments, a question that was investigated in the present study. Rats received unilateral neocortical lesions via electrocoagulation of the MCA and were compared to rats with standard motor cortex lesions produced by devascaulrization of the overlaying blood vessels. The MCA group was as impaired as the motor cortex group in skilled reaching movements as assessed by quantitative measures of the contralateral-to-lesion forelimb in a single pellet task and in a tray reaching task. Although there was improvement in success scores over a two-week period in both groups, the groups were characterized by distinctive and enduring qualitative impairments. The motor cortex deficit was exemplified by use of trunk musculature and head movements to assist the reaching limb while the MCA impairment included sensory abnormalities. The results are discussed in relation to the contribution of lateral frontal cortex injury to MCA stroke sensorimotor syndromes.

1. Introduction

Localization of cerebral function using behavioural measures is a traditional approach to understanding the contribution of brain regions to behaviour. The design and analysis of tests used in animal models of clinical disorders, however, are complicated by the range of neural structures that are usually compromised. For example, in rat models of middle cerebral artery (MCA) stroke, whether produced by intraluminal suture (Longa et al., 1989), reversible snare ligature (Shigeno et al., 1985), endothelin-1 infusion (Robinson et al., 1990), photochemically initiated thrombosis (Watson et al., 1985), electrocoagulation (Tamura et al., 1981; Bederson et al., 1986), a number of structures including lateral frontal neocortex, striatum, and medial forebrain bundle are concurrently damaged. Contrastingly, the rostral and the caudal representation of the forelimb regions in motor cortex are spared.

Despite the sparing of motor cortex in rat MCA stroke, the syndrome of behavioural deficits ensuing from the injury includes a large number of impairments of forelimb function suggestive of motor cortex malfunction. For example MCA ischemia impairs performance on sensorimotor tests such as paw placing (Belayev et al., 1996; Johansson and Ohlsson, 1996; Bland et al., 2000; Jolkkonen et al., 2000; Schallert et al., 2000; Roof et al., 2001; Chen et al., 2002; Lindner et al., 2003), forelimb use for postural support (Schallert et al., 2000; Biernaskie and Corbett, 2001; Bland et al., 2001; Roof et al., 2001; Gonzalez and Kolb, 2003; Karhunen et al., 2003; Lindner et al., 2003), staircase reaching for food (Marston et al., 1995; Sharkey et al., 1996; Colbourne et al., 2000; Virley et al., 2000; Biernaskie and Corbett, 2001), tray reaching for food (Chen et al., 2002; Gonzalez and Kolb, 2003), and limb coordination (Ohlsson and Johansson,

1995; Stroemer et al., 1995, 1998; Bland et al., 2000; Jolkkonen et al., 2000; Quartermain et al., 2000; Virley et al., 2000; Biernaskie and Corbett, 2001; Bland et al., 2001; Ding et al., 2002; Lindner et al., 2003). Thus, it would seem that even though motor cortex is spared in MCA stroke, the functions thought to be supported by motor cortex are not. This raises the question of which cortical or subcortical structures compromised by MCA stroke produce motor-cortex like deficits. One approach to answering this question is to fractionate the MCA stroke damage by making more focal lesions and comparing the resulting behavioural deficits to those deficits produced by motor cortex lesions.

The purpose of the present study was to begin such a fractionated analysis by damaging the lateral frontal neocortex and comparing the resulting deficit to that from standard motor cortex injury. That is, the experimental model is similar to that used in pharmacology in which an unknown, the effects of MCA occlusion damaging lateral frontal cortex, is compared to a known, the effects of motor cortex devascularization. Lateral frontal neocortex in the rat includes the face and the barrel (vibrissae) region of sensory neocortex (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Neafsey et al., 1986) and is the primary neocortical zone of MCA stroke-induced damage. Vessel electrocoagulation was chosen for MCA ischemia because it is a procedure used for inducing stroke and damage can be directed at the lateral frontal cortex. Focal motor cortex injury was produced by devascularization of the overlaying blood vessels because the procedure and behavioural syndromes associated with it have been previously characterized (Kolb et al., 1997; Whishaw, 2000). The primary behavioural assessment in the present experiment was the quantitative and qualitative measures of forelimb function as used in two skilled reaching for food tasks.

2. Materials and Methods

Animals

Experiments were conducted according to standards set by the Canadian Council on Animal Care and approved by the University of Lethbridge animal care committee. The subjects, raised in the University of Lethbridge vivarium, were 22 female Long-Evans hooded rats, 120 days old, and weighing 250-300g when the experiments began. The animals were housed in groups of two or three individuals in Plexiglas cages in a colony room maintained on a 12/12h light/dark cycle (08:00-20:00 h).

Female rats were used in the present study because they are more suitable for the single pellet reaching apparatus due of their size dimorphism as compared to their male counterparts. Our laboratory has previously investigated recovery from motor cortex injury in both female and male rats in the tray task (Whishaw, 2000). Both sexes displayed a similar recovery pattern, suggesting that the present results derived from female rats may be representative of Long Evans rats' performance in both single pellet and tray reaching.

Feeding

One week prior to commencement of skilled reaching training, the rats were gradually food deprived to 90-95% of their original body weight by providing once a day feeding of a measured amount of Purina rat chow. Rats remained on food deprivation for the rest of the experiment. When reaching for single food pellets, rats received 45 mg dustless precision pellets (product #F0021, Bioserve Inc., Frenchtown, NJ). When reaching in the tray task they received 20-30 mg chick feed.

Surgery

Animals were assigned to three groups: control (n = 8), MCA occlusion (n = 8), motor cortex devascularization (n = 6). Surgeries were performed in the hemisphere contralateral to the paw used for reaching in the single pellet task.

Middle cerebral artery occlusion. The MCA was occluded using a procedure similar to Tamura et al (Tamura et al., 1981). Briefly, animals were anesthetized with ketamine hydrochloride (70 mg/kg, ip; Ayerst Veterinary Laboratories, Guelph, ON) and xylazine (20 mg/kg, i.p.; Bayer Inc., Toronto, ON). The bone lateral to the temporal ridge and overlaying the MCA was removed using a dental drill. The dura was opened and retracted. The vessel and its main branches were occluded by bipolar electrocoagulation dorsal to the rhinal fissure. The incision was closed and the animal's condition was monitored in a recovery room for 24 hours, at which time it was returned to the colony room.

Motor cortex devascularization. The blood vessels overlaying sensorimotor cortex were removed using a procedure similar to Kolb and colleagues (Kolb et al., 1997). Briefly, animals received an injection of atropine nitrate (0.1 mg/kg i.p.; Sigma-Aldrich, St. Loius, MO) to facilitate respiration throughout surgery and were then anesthetized with sodium pentobarbital (65 mg/kg, i.p.; Sigma-Aldrich, St. Loius, MO). Four holes were drilled using a fine dental burr in the skull overlaying the motor cortex at stereotaxic coordinates measured from Bregma (anterior (A) and lateral (L)) were A = -1.0 mm, L = +1.0 mm; A= +4.0 mm, L= +1.0 mm; A= -1.0 mm, L= +5.0 mm; A= +4.0 mm, L= +5.0 mm. The rectangular area enclosed by the four points was then trephinated and the dura was removed. The underlying tissue was devascularized by gently wiping away the pia mater and blood vessels with a saline-soaked cotton swab. The incision was

closed and the animal's condition was monitored in a recovery room for 24 hours, at which time it was returned to the colony room.

Reaching boxes

Animals were trained in two reaching tasks, the single pellet task (Whishaw and Pellis, 1990) (Fig 5.1 A) and the tray task (Whishaw et al., 1986) (Fig 5.1 B).

Single pellet reaching boxes were made of clear Plexiglas, with the dimensions 45x14x35 cm. In the center of each front wall was a 1 cm-wide slit which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-wide shelf. Two indentations on the floor of the shelf were located 2 cm from the inside of the wall and were centered on the edges of the slit where rats could reach.

The tray boxes were 26 cm high, 28 cm deep, and 19 cm wide. The front of the boxes was constructed of 2mm bars separated from each other by a 9 mm gap. Clear Plexiglass tops allowed access to the inside of the box. A 4 cm wide and 0.5 cm deep tray containing food was mounted in front of the bars. Drinking water was accessible at the rear of the box.

Reaching training and testing

For single pellet reaching, food pellets were initially placed in both indentations, but once rats displayed a paw preference, a pellet was only placed in the contralateral-to-reaching limb indentation (Whishaw and Pellis, 1990). Reaching was reinforced by immediate replacement of any successfully retrieved or displaced food pellets. Once an animal was reliably reaching for food, a short pause preceded the presentation of the next pellet, and an animal was required to return to the back of the box and then approach the

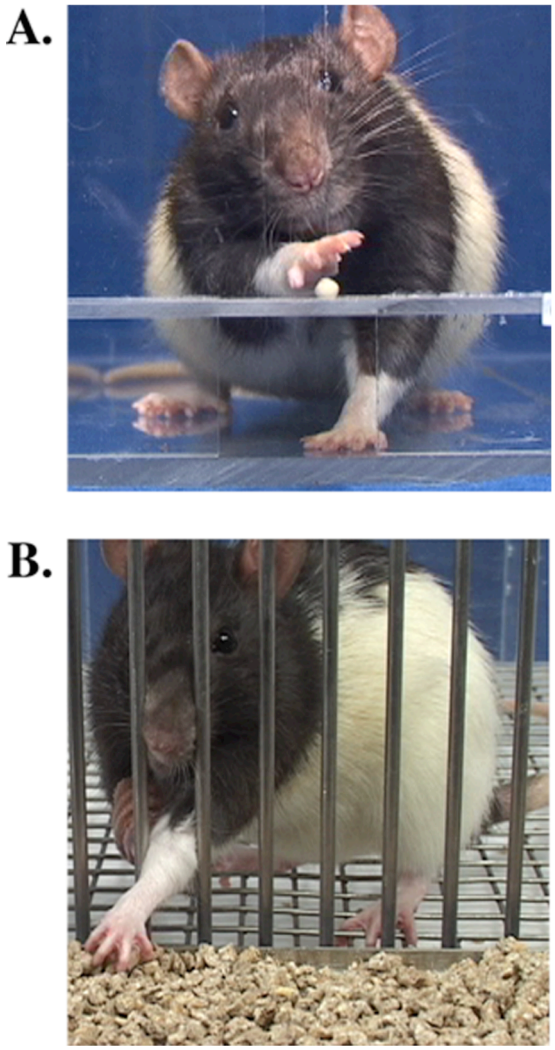


Figure 5.1: Skilled reaching tasks. (A) Single pellet reaching: a rat reaches through a slot for a single food pellet located on a shelf. (B) Tray reaching: a rat reaches through bars into a tray filled with grains of chick food.

front and reposition itself for the next reach. For the first week of training, animals were presented with pellets for 10 min/training session because a distinct separation between reaching attempts is typically not clear during initial training. For subsequent training, animals received 20 pellets/session for two weeks. Rats received one training session per day.

For tray reaching, animals were allowed to reach through the bars, grasp the food and retract it (Whishaw et al., 1986). Animals were given access to the reaching boxes for two 1-hr sessions per day, for 7 days.

Bracelets for limb restraint

To force a rat to use a particular limb for reaching, a bracelet made of Elastoplast fabric adhesive tape (Smith & Nephew Inc., Lachine, Quebec) was wrapped around one forelimb (Whishaw et al., 1986). The bracelet prevented the animal from inserting its bandaged paw through the aperture of the reaching boxes. The bracelet did not impede the subject's movement and could be easily slipped off by the experimenter without damaging the animal's forelimb or denuding its fur. Once habituated to the bracelet for a day or two, animals generally ignored it and did not attempt to remove it.

Video recording

Reaching performance was video recorded for all testing sessions of the single pellet and tray reaching. Video records were made using a Cannon ZR 30 MC digital video camcorder with a shutter speed of 1000th of a second. Illumination for high shutter speed filming was provided by a cold light source. A Sony digital videocassette recorder DSR-11was used for a frame-by-frame analysis at 30 frames/s. The software package Final Cut Pro (www.apple.com) was used for capturing still frames from the video records.

End point measures of reaching

In the single pellet reaching task, animals were successively presented with 20 food pellets and success was measured using the following formulae:

Percent Success = (number of pellets retrieved/20) x 100.

In the tray reaching task, animals were given a 10-minute test. Each time the rat placed its forelimb towards the tray, a "reach" was scored. If the animal was successful in obtaining food, a "hit" was scored. Reaching success was assessed as follows:

Percent Success = (number of hits/number of reaches) x 100.

Qualitative scoring of skilled reaching

For a qualitative analysis of single pellet reaching, a reach was subdivided into 10 components (Whishaw et al., 1993). (1) *Digits to midline*: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body. (2) *Aim*: using the upper arm, the elbow is adducted so that the forearm is aligned along the midline of the body, with the paw located just under the mouth. This movement involves fixation of the distal portion of the limb, so that digits remain aligned with the midline of the body. This is likely produced by a movement around the elbow that reverses the direction of movement of the paw to compensate for the adduction of the elbow. (3) *Digits semiflexed*: as the limb is lifted, the digits are semiflexed and the paw is supinated so that the palm faces the midline of the body. (4) *Digits open*: as the limb is advanced the digits are extended. (5*) Advance*: the head is slightly lifted to allow the limb is advanced directly forward above and beyond the food pellet. (6) *Pronate*: using a movement of the upper arm, the elbow is abducted, pronating the paw over the food while the digits open. Pronation of the paw onto the food is aided by a movement of the paw around the wrist. (7) *Grasp*: as the pads of the palm or the digits touch the food, the food is grasped by closure of the digits. This can occur as an independent movement, or the grasp can occur as the paw is withdrawn. (8) *Supination I*: as the limb is withdrawn, the paw is dorsiflexed and is supinated 90^0 by a movement around the wrist and by adduction of the

elbow. These movements can occur as soon as the food is grasped or can occur as the limb is withdrawn. (9) *Supination II*: as the rat sits back with food held in the paw, the paw is further supinated by 90^0 and ventroflexed to present the food to the mouth. (10) *Release*: the digits are opened and the food transferred to the mouth.

Posture was assessed as a separate measure. A normal reaching posture was defined as one in which the opposite to the reaching forepaw and its contralateral hind paw were used for support and assisted movement toward the pellet and sitting back on the haunches to eat after the food pellet was retrieved.

Five successful reaches were analyzed frame-by-frame on the video tapes. Each movement was rated on a three-point scale. If the movement appeared normal, it was given a score of "0"; if it appeared slightly abnormal but recognizable it was given a score of "0.5"; and a score of "1" was assigned if the movement was absent or completely unrecognizable.

Histology

On completion of behavioral testing, animals were euthanized using a lethal dose of sodium pentobarbital. They were intracardially perfused, first with saline in 0.1 M PBS followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed, post-fixed and cryoprotected in a solution of 30% sucrose in 4% paraformaldehyde for three days in 4^oC. All brains were then sectioned into 40 μm using a cryostat and stained with cresyl-violet.

Six coronal brain sections were selected from the MCA occlusion and motor cortex devascularization groups from the following coordinates A = +2.70, +1.70, +0.70, -0.30, -1.30, -2.30 mm. Digital images of the stained tissue were captured using a Kodak

digital camera DCS 410 run using a Macintosh dual G4 computer. The images were opened using NIH image V.1.63 (written by W. Rasband at the NIH and available from the Internet at http://rsb.info.nih.gov/nih-image). The lesion and the intact hemispheres were separately outlined by making boundary drawings around each. The area of each hemisphere expressed in pixels^2 was recorded. Undamaged tissue in the lesioned hemisphere was expressed as a percentage of the area of the intact hemisphere: [Undamaged area of lesion hemisphere (pixels^2)/area of intact hemisphere (pixels^2)] x100.

Procedure and time line

Animals were trained on the single pellet reaching for three weeks. Following surgery, the rats were tested on the single pellet task on post surgical days 1, 3, 7, 14. Training in the tray task began on Day 20 and lasted until Day 26. Tray task testing was conducted on Day 27.

Statistical analysis

The results were assessed using repeated measures analysis of variance (ANOVA) with follow-up Fisher LSD ($p < 0.05$). Simple ANOVA was used for single-session tests. Differences in between-group comparisons were assessed with unpaired t tests ($p < 0.05$).

3. Results

Histology

Fig 5.2 shows examples of coronal sections of the lesion locations at injury midpoint. MCA occlusion produced damage in lateral frontal neocortex (somatosensory regions of jaw, oral area, and vibrissae, secondary somatosensory cortex, and in some cases insular cortex) and spared primary and secondary motor cortex, while motor cortex

devascularization damaged sensorimotor regions (primary and secondary motor cortex and in some cases somatosensory forelimb and hind limb areas) and spared lateral frontal cortex (Fig 5.3). The area spared by the respective lesions did not significantly differ for the two groups [MCA = 82.2 $\pm$ 3.4 %, M-Ctx = 90.5 $\pm$ 2.7; $F(1,12) = 3.32$, $p > 0.05$].

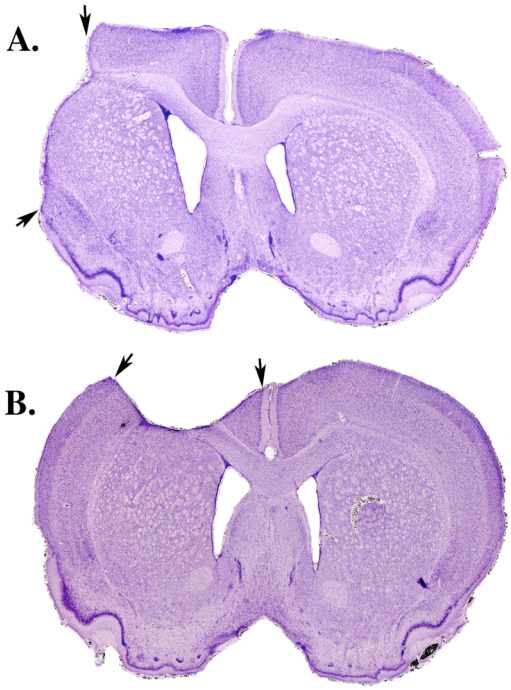


Figure 5.2: Photomicrographs of a coronal section through the middle of the lesion of (A) a middle cerebral artery occlusion and (B) a motor cortex devascularization (Cresyl violet). Arrows mark lesion boundary.

Post surgical limb preference

After surgery, all motor cortex animals attempted to reach for food with their ipsilateral to lesion forelimb and so it was necessary to bracelet that limb throughout testing in the single pellet task and training and testing in the tray task. A bracelet was necessary for only one of the MCA rats during single pellet reaching and for three rats during initial tray task training.

Success in skilled reaching

Single pellet task. Both lesion groups were impaired in single pellet reaching success but improved over the 14-day testing period (Fig 5.4 A). The effects of group [$F(2,19) = 15.39$, $p < 0.01$], day [$F(3,57) = 8.07$, $p < 0.01$], and group by day interaction [$F(6,57) = 3.51$, $p < 0.01$] were significant. Post hoc analysis ($p < 0.05$) indicated that both MCA and motor cortex groups had an overall significantly lower success rate than controls.

Tray task. After 7 days of training on the tray task, a significant group effect was detected on a 10-minute test [$F(2,19) = 4.89$, $p < 0.05$; Fig 5.4 B]. The lesion groups were inferior to the control group but did not differ from one another as confirmed by a post hoc analysis ($p < 0.05$).



Figure 5.3: Representative diagram illustrating typical lesions following (A) MCA occlusion and (B) motor cortex devascularization. Abbreviations: Insular cortex (I), primary motor cortex (M1), secondary motor cortex (M2), secondary somatosensory cortex (S2), somatosensory jaw region (S1J), somatosensory barrel cortex (S1BF), somatosensory hind limb region (S1HL), somatosensory forelimb region (S1Fl), somatosensory upper lip region (S1ULp), somatosensory trunk region (S1Tr), somatosensory jaw and oral region (S1JO), somatosensory dysgranular zone (S1DZ). Section from Paxinos and Watson (Paxinos and Watson, 1997).

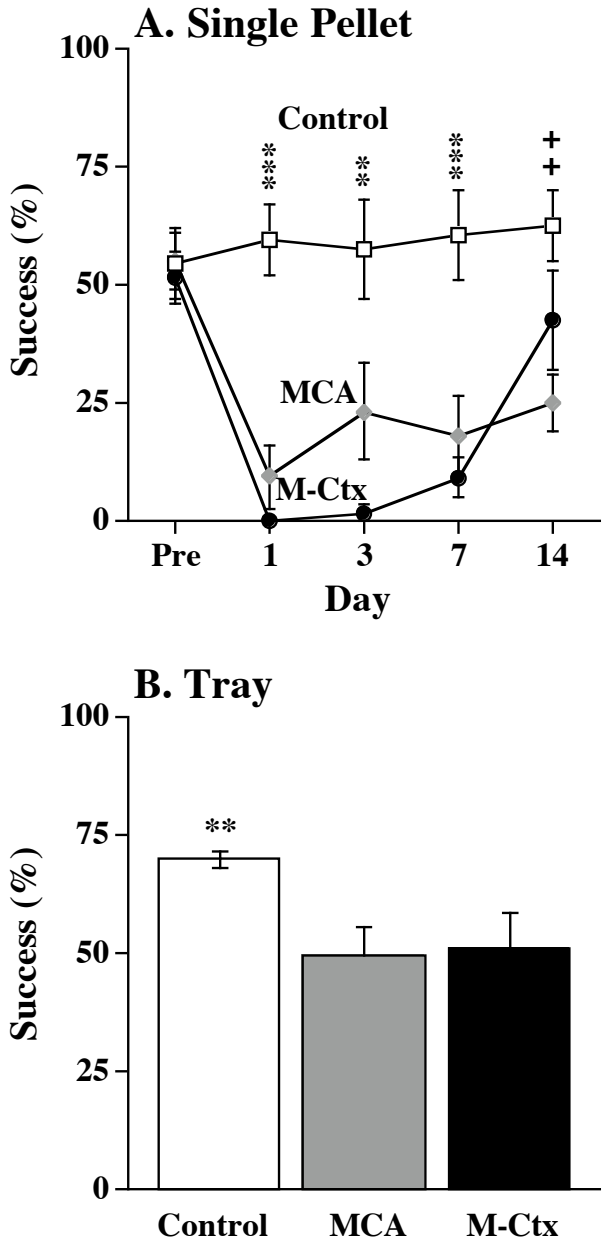


Figure 5.4: (A) Percent Success in the single pellet reaching task (number of pellets retrieved out of 20; mean ± SE) for control, middle cerebral artery occlusion (MCA), and motor cortex devascularization (M-Ctx) groups. Note: all groups were equally successful in obtaining pellets before surgery (Pre) and the lesion groups performed less successfully than the control group on post surgical days 1, 3, 7, 14. (B) Percent Success (mean ± SE) in the tray reaching task for control, MCA, and M-Ctx groups. The control group was superior to the lesion groups in reaching success. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ++ $p < 0.01$ only between MCA and control groups.

Qualitative analysis of skilled reaching

Reaching. Both groups of lesion rats were impaired on the qualitative measures of reaching relative to the control group (Fig 5.5). This was supported by significant effects of group [$F(2,19) = 79.82$, $p < 0.01$], testing day [$F(3,57) = 25.94$, $p < 0.01$], and group by day interaction [$F(6,57) = 18.98$, $p < 0.01$]. Post hoc analysis ($p < 0.05$) indicated that both MCA and motor cortex groups had significantly higher mean impairment scores than the control group on all testing days. In addition the motor cortex group had significantly higher scores than the MCA group on the postoperative days 1, 3, and 7.

A summary of scores for component movements for MCA and motor cortex groups as compared to controls on days 1 and 14 is illustrated in Fig 5.6. The analysis indicated that both groups were impaired in the lifting and aiming components of the reach and in supination of the paw upon withdrawal and in releasing the food to the mouth.

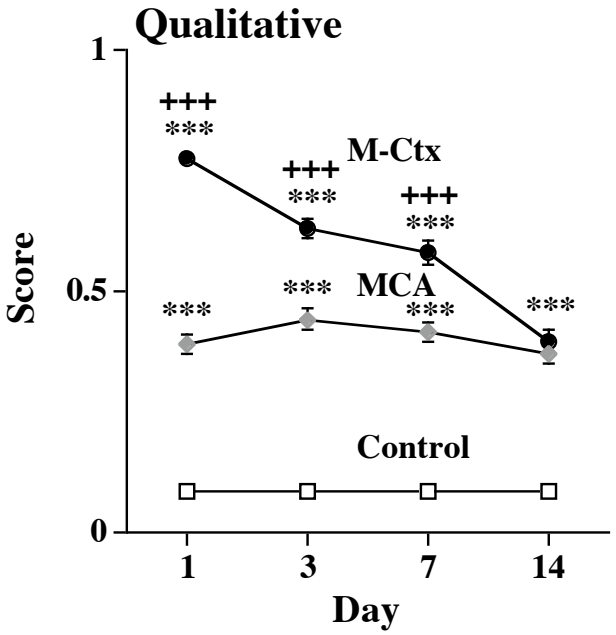


Figure 5.5: Qualitative scores of single pellet reaching movements on days 1-14 (mean ± SE). *** $p < 0.001$ (comparison between lesion groups and controls); +++ $p < 0.001$ (comparison between lesion groups).

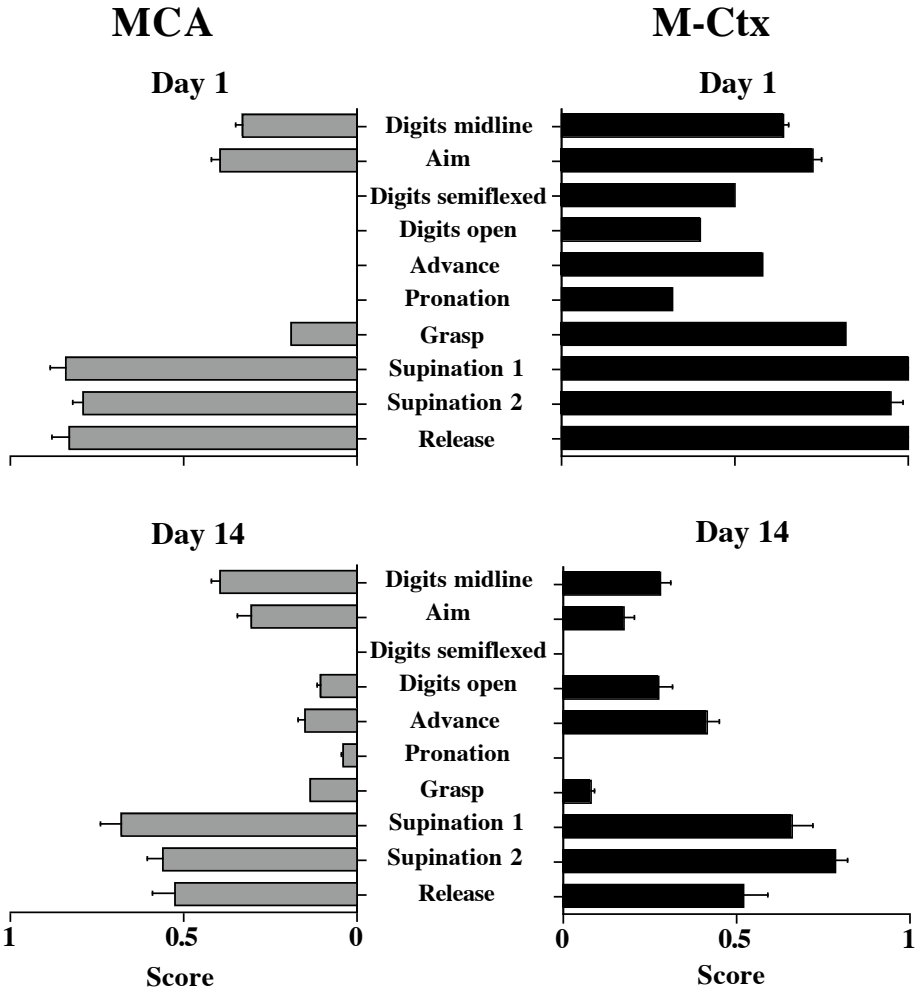


Figure 5.6: Qualitative scores of ten elements comprising a reach on post surgical test days 1 and 14. Score (mean + SE= lesion group score – control group score).

Group differences. Although the component scores of the MCA and motor cortex groups were similar, inspection of the rats' movements suggested that the respective group scores originated in part from different sources as indicated by the following differences (see Appendix 6):

(i) *Head lifts*. When control rats prepared to advance their paw toward the food pellet, they first lifted the paw so that it was partially supinated with the semiflexed digit tips aligned with the center of the body. They then quickly brought the limb to the aiming position by adducting the elbow so that it too was aligned with the center of the body. From this position the limb was advanced while the snout was slightly lifted from the position in which it was sniffing the food pellet. As indicated by the component scores, neither the MCA group or the motor cortex group had a normal aim, but rather advanced the limb on a more diagonal trajectory. In addition, the motor cortex group used more obvious body rotation to center the limb on the slot and they raised their head and forequarters presumably to assist in lifting the reaching limb to the level of the shelf (Fig 5.7 A). Consequently, when the incidences of head lifts on Day 14 were counted, there were significant group differences, $F(2,19) = 5.27$, $p < 0.05$, and post hoc tests indicated that the motor cortex group made significantly more such movements than did the MCA group ($p < 0.05$) and the control group ($p < 0.01$).

(ii) Limb withdrawal impairment. When control rats grasped a food pellet, they typically lifted and partially supinated the paw (supination I) before withdrawing it from the slot. Once the paw was removed from the slot, it was further supinated (supination II) and the digits were opened to release the food pellet into the mouth. In contrast, rats with motor cortex lesions failed to lift and supinate the paw but used it to support their weight. The paw continued to be supported on the shelf as it was supinated secondarily to adjustments of the body and the continued pressure of support. Frequently, the animals attempted to retrieve the food while the paw was still on the shelf. In addition, after performing a misguided-reach, motor cortex rats habitually grasped the front wall of the

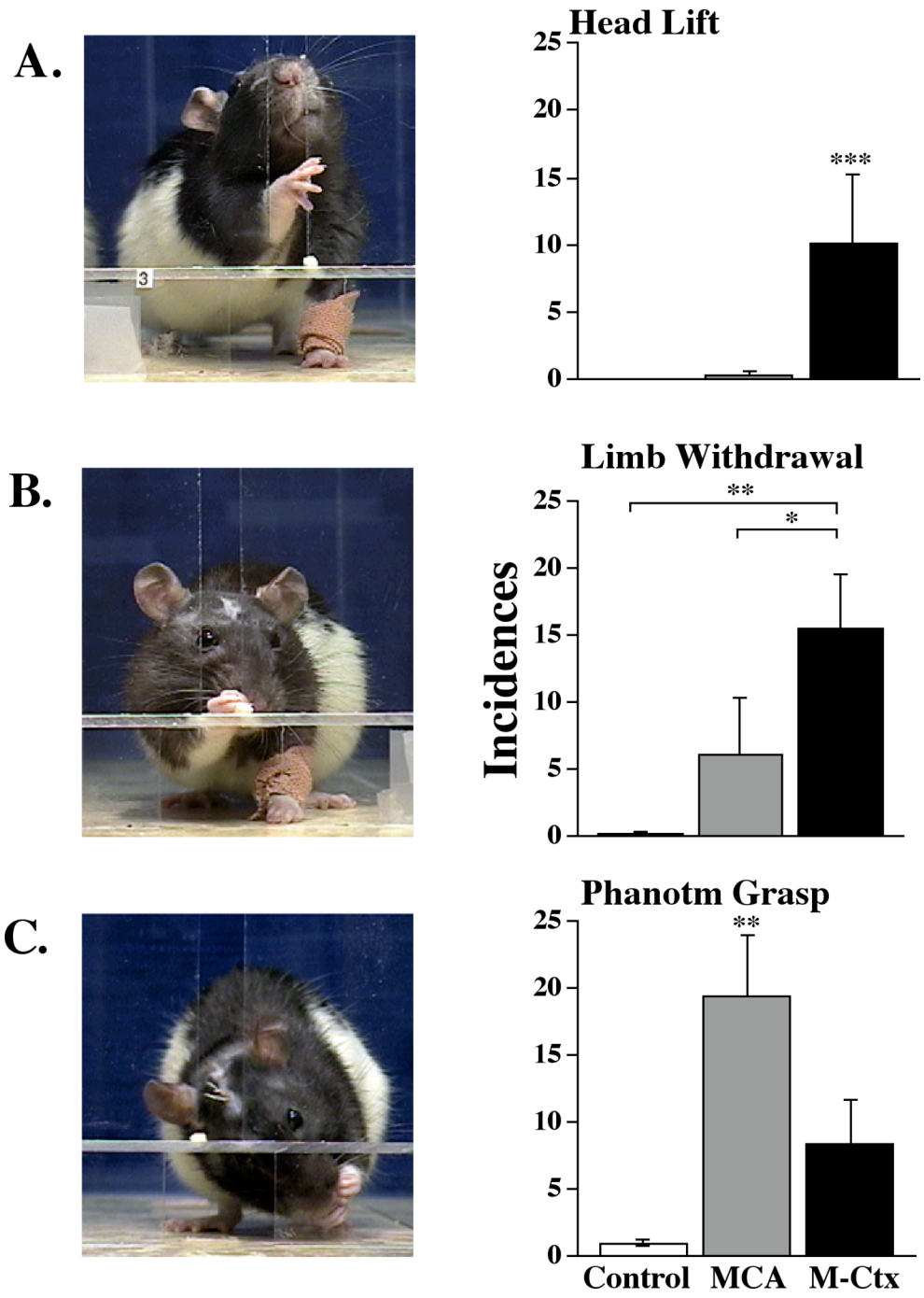


Figure 5.7: Movement abnormalities in control, middle cerebral artery (MCA) and motor cortex (M-Ctx) groups (mean incidence and SE). An incidence is defined as counts of behaviour in a 20-pellet testing session. (A) Head lift in which a rat carries the forelimb upward by raising the forequarters. (B) Limb withdrawal impairment in which the forequarters are supported on the withdrawing limb. (C) Phantom grasps in which a rat inspected its paw for a food pellet that it has missed (note food pellet on shelf). Note: head lift and support abnormalities characterize motor cortex rats while phantom grasps characterize MCA rats.

box through the slot (Fig 5.7 B). The closed-fist spastic response could not be released except with the aid of the other forelimb. Counts of this impairment on Day 14 gave a significant group effect, $F(2,19) = 4.91$, $p < 0.05$. A post hoc analysis indicated that the motor cortex group demonstrated significantly more limb withdrawal impairments than the MCA group ($p < 0.05$) and the control group ($p < 0.01$).

(iii) *Phantom grasps*. When a control rat missed a food pellet on a grasp attempt, it typically kept its digits open and adjusted its grasp or partially withdrew its paw from the slot, while semiflexing the digits, in preparation for another reach. The motor cortex rats' adjustments were similar except that they often ended up supporting their weight on their reaching paw while it was on the shelf or they withdrew the paw completely as did the control rats. Contrastingly, rats with MCA lesions rapidly closed and flexed their digits as if they had grasped a food pellet, and they then inspected their paw with their snout as if attempting to retrieve the food pellet (Fig 5.7 C). These "phantom grasps" were observed in the MCA rats on all post surgical days. Counts of phantom grasps on Day 14 gave a significant group difference, $F(2,19) = 8.54$, $p < 0.01$, and post hoc analysis indicated that the MCA group performed significantly more phantom reaches than the control group ($p < 0.01$) and the motor cortex group ($p < 0.05$).

(iv) *Pellet chasing*. When control rats withdrew their paw to present the food to the mouth, they sat back on their haunches and brought the paw to the mouth. Both the MCA and motor cortex rats were impaired in supinating the paw and so turned the snout to the paw. Rats in both groups appeared to have additional difficulty in opening the digits to release the food pellet because they "chased" the paw with the snout as is

illustrated in Fig 5.8. This retrieval deficit was observed in all of the lesion rats on almost all successful retrievals.

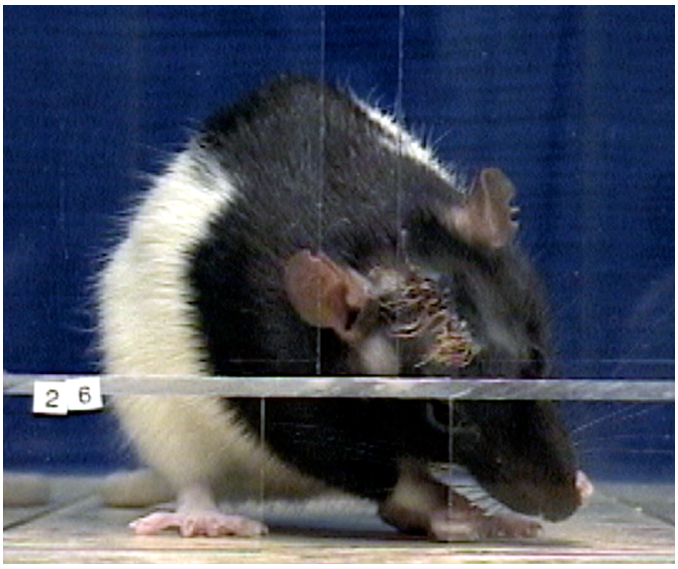


Figure 5.8: MCA rat "chases" its paw with its snout to obtain a food pellet.

(v) Posture. All of the rats in the control group supported their weight and shifted the body to and away from the slot on the limb couplet of the non-reaching forelimb and its diagonal hind limb. Usually the other hind limb moved forward with the reaching forelimb as it was advanced toward the food pellet. Instead of supporting their weight with a diagonal posture, motor cortex animals reached in primarily one of two positions. In one position, the animal's weight was supported by the non-reaching forelimb and hind limb ipsilateral to it. In this case animals supported their weight with the ipsilateral to lesion limbs. Reaches in this posture were characterized by shoulder and body rotations to advance the limb towards the pellet and to withdraw and present the food to the mouth. In the second position, weight was principally supported on both hind limbs. This was particularly evident when the rat used its non-reaching forelimb to aid the

reaching limb from the slot or during food presentation to the mouth; balance was typically compromised and consequently the animal's forequarters dropped to the floor of the apparatus. The posture of the MCA rats resembled that of the control rats. Postural

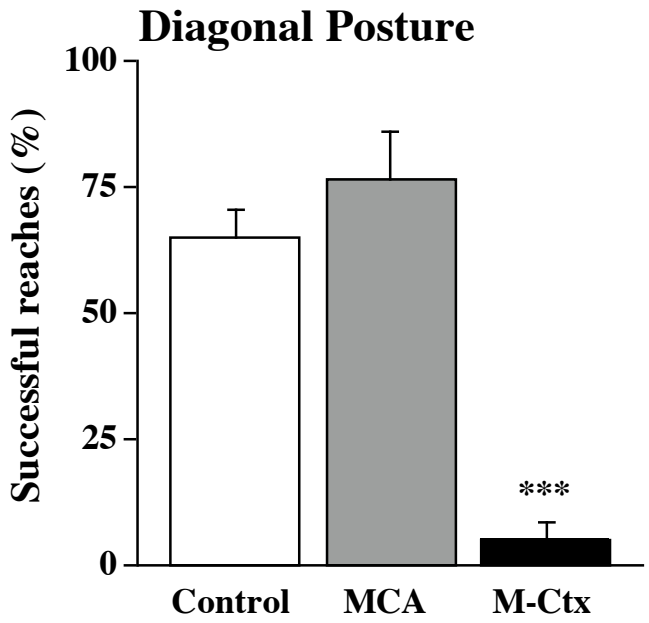


Figure 5.9: Use of a diagonal supporting pattern (percent mean and SE) by control (C), middle cerebral artery (MCA) and motor cortex (M-Ctx) groups. *** $p < 0.001$.

scoring gave a significant effect of group [$F(2,19) = 24.32$, $p < 0.01$], and a post hoc analysis indicated that control and MCA groups used a diagonal support for reaching significantly more than the motor cortex group ($p < 0.05$; Fig 5.9).

4. Discussion

The objective of the present study was to investigate the contribution of lateral frontal cortex damage to sensorimotor impairments that ensue from MCA stroke. Skilled reaching impairments were compared in rats with MCA occlusion induced lesions in

lateral frontal cortex and standard motor cortex lesions. Performance as assessed by the number of successfully retrieved food pellets was equally poor for both groups but improved over a two-week testing period. Frame-by-frame video analysis showed that both lateral frontal and motor cortex groups had enduring qualitative impairments, but abnormalities differed between the two lesion groups. The present results are the first step towards fractionating cerebral damage from MCA stroke and demonstrate the contribution of lateral frontal cortex to skilled movement impairments ensuing from MCA ischemia.

The present investigation was prompted by a number of reports that MCA occlusions produce impairments in forelimb use even though the lesions spare the forelimb representation of motor cortex. Although forelimb impairments could originate as a result of MCA damage to the striatum and lateral hypothalamus, brain regions typically associated with forelimb impairments, to date there has been no investigation of whether the region of lateral frontal neocortex damaged by MCA stroke contributes to forelimb use. The behavioral assessment used in the present study was that of skilled forelimb reaching, a behavior that has been associated with forelimb motor cortex in both electrophysiological (Dolbakyan et al., 1977; Hyland, 1998; Kargo and Nitz, 2003) and behavioral studies (Peterson and Gucker, 1959; Peterson and Barnett, 1961; Peterson and Devine, 1963; Castro, 1972, 1977; Whishaw et al., 1986; Whishaw et al., 1991; Whishaw, 2000). In the earliest of behavioral studies of skilled reaching, Peterson and colleagues (Peterson, 1934; Peterson and Fracarol, 1938) explicitly associate skilled forelimb movements with motor cortex and not adjacent frontal or parietal regions of the neocortex. Given this background, the finding in the present study that rats with MCA

occlusion-induced lesions were as impaired as rats with motor cortex lesions on two skilled reaching tasks is surprising. Furthermore an equivalent enduring impairment was obtained on qualitative measures of skilled forelimb use.

There are a number of potential explanations for the motor deficits seen in the lateral frontal cortex group. First, cortical fibers exist between lateral frontal and motor cortex and fiber deafferentation in primary motor cortex may have been disruptive. Second, neuronal reorganization can sometimes compensate for cerebral injury by assuming function of adjacent injured tissue (Nudo, 1999, 2003b, a). It is therefore possible that motor cortex may have assumed some functions of lateral cortex, thus disrupting its normal function. In the present case, forelimb representation in motor cortex may have been compromised to accommodate an expansion of face representation otherwise localized in the adjacent lateral frontal cortex. Third, it is possible that the lateral lesions may have encroached on forelimb motor cortex and in this way producing a more widespread functional impairment. Deficits from MCA occlusion were qualitatively distinct and seemed larger and more enduring than could be accommodated by any of these explanations, however.

The most parsimonious explanation of the MCA stroke related deficit is that the cortical area responsible for the organization of skilled reaching may be larger than just forelimb motor cortex. The skilled movements required for reaching are complex in the rat in that they depend upon sensory guidance from olfaction (Whishaw and Tomie, 1989) as well as from sinus hairs on the rat's forearm (Whishaw and Miklyaeva, 1996), and likely also from the vibrissae. That is, although a rat must locate the food pellet by sniffing, it also likely uses its vibrissae and other tactile information to locate the aperture

through which it must reach, to estimate the height of the shelf, and to "know" that its reach has been successful in grasping a food pellet. There are other aspects of a rat's behavior that must also be coordinated in order to execute a successful reach. The task requires that a rat must approach the food anew for each reach and accordingly it must organize its approach as well as adjust its body posture through out the reach and retrieval of the food pellet. In other words, skilled reaching requires a complex sequence of movements including approach, reaching, and food consumption that involve the whole body. Consistent with the notion that skilled reaching requires more than just forelimb motor cortex, a previous study has indicated that damage to midline cortex (anterior cingulate cortex) also impairs some aspects of skilled reaching (Whishaw et al., 1992).

Despite the finding that lateral frontal and motor cortex lesions produced similar quantitative impairments in the two reaching tasks, the qualitative analysis indicated that the contributions from the two cortical areas were not identical. The qualitative analysis indicated that rats with motor cortex lesions used a variety of rotational movements as a "crutch" to assist the movement of the limb, as has been reported (Whishaw et al., 1991). The impairments in the rats with MCA lesions were in part different. They displayed impairments in aiming, advancing, and supinating the limb, but they also displayed phantom grasps. After advancing the limb, they made a rapid grasp even though their paw had not contacted the food pellet. They then investigated their paw in a seeming attempt to obtain a food pellet. Although it is not possible to determine from the present study which region of lateral cortex contributes to this impairment, it is possible that the

impairment not only includes one in recognizing whether food has been grasped, but also includes recognizing where the limb has been directed.

One explanation of the present finding that a wider region of frontal neocortex is involved in skill forelimb use than has been expected is that skilled forelimb use may be represented as a distributed network (Whishaw, 2003). Such a network would include several brain regions that contribute: body orientation, sensory recognition, and movement execution (Fig 5.10). It is likely it is the integration of these various contributions that makes a movement distinctively "skilled". This suggestion advanced to account for the present results is consistent with a number of other lines of investigations. For example, imaging (Sanes et al., 1995), brain stimulation (Graziano et al., 2002; Cooke and Graziano, 2003; Cooke et al., 2003), and other electrophysiological studies (Schieber and Hibbard, 1993; Maier et al., 2002; Shimazu et al., 2004) all suggest that movements are represented by relatively large interconnected regions of the neocortex. Such studies also suggest that these networks include both innate and learned "synergies"; that is, movements that are biologically useful to the animal. This notion is also consistent with observations that temporary (Brochier et al., 1999) and permanent (Friel and Nudo, 1998; Schieber, 1999) focal lesions to a portion of motor cortex such as the digit representation, produce weakness that is much more extensive and includes the entire limb and many other aspects of behavior including bilateral limb coordination, attention, and memory for complex actions (Brodal, 1973).

That skilled movements may be supported by a distributed network, does not negate the possibility of regional functional localization. For example, it is interesting that in one of the earliest attempts to localize forelimb motor cortex, Peterson and

colleagues (Peterson and Gucker, 1959; Peterson and Barnett, 1961) used only paw preference as a behavioral measure. That is, rats were trained to reach and then their tendency to shift limb preference was evaluated after

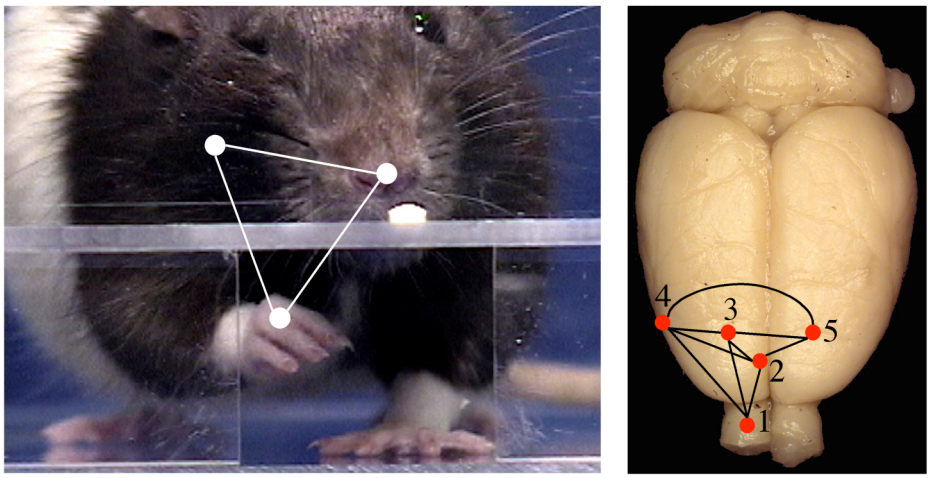


Figure 5.10: A nodal model of motor function. The elements necessary for successful skilled reaching are olfaction, sensory input via vibrissae, and forelimb motor control, which are represented in the brain by a distributed network: (1) olfactory bulb, (2) medial frontal cortex, (3) forelimb motor cortex, (4) lateral frontal cortex, (5) contralateral forelimb motor cortex.

variously placed neocortical lesions. Only forelimb motor cortex reliably shifted preference. The results of the present study are consistent, rats with motor cortex lesions had to be forced to reach with their contralateral paw through the use of bracelets whereas rats with MCA stroke continued to use their contralateral paw. Previous work also suggests that rats with striatal damage (Whishaw et al., 1986; Pisa, 1988; Jeyasingham et al., 2001) or pyramidal tract damage (Whishaw et al., 1993; Whishaw et al., 1998; Z'Graggen et al., 1998; Whishaw and Metz, 2002) maintain paw preference after lesions. Thus, “limb preference” appears to be a property of motor cortex.

Conclusion

Previous studies of MCA ischemia in rats have been largely focused on quantifying end point measures of behaviour (Marston et al., 1995; Sharkey et al., 1996; Colbourne et al., 2000; Virley et al., 2000; Biernaskie and Corbett, 2001; Chen et al., 2002; Gonzalez and Kolb, 2003; Lindner et al., 2003). The present results emphasize the importance of qualitative measures for distinguishing seemingly similar sensorimotor syndromes. We suggest that the importance of using multiple measures relates to the complexity of nervous system organization of movement. Although it is clear that some aspects of movement can be localized, it is also clear that skilled movements are likely to involve many brain regions. Although the integrity of motor cortex subsequent to MCA stroke was not directly assessed in the present experiment, qualitatively distinctive reaching deficits in the two lesion groups suggest that the neural substrates of the impairments were distinct. Nevertheless, a direct investigation of the neurophysiological and the anatomical properties of motor cortex subsequent to MCA ischemia can further our understanding of the injury. Another challenge for the present line of research is to fractionate MCA stroke to produce independent injury in striatum and in lateral hypothalamus for investigating the contribution of these structures to skilled reaching. Recognizing the complexity of nervous system organization of movement through the use of multiple measures thus assists not only in understanding nervous system organization of behavior, but also in the development of neural and behavioral approaches to treatment for brain injury.

Chapter 6

Middle cerebral artery (MCA) stroke produces dysfunction in adjacent motor cortex as detected by intracortical microstimulation in rats

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Abstract

Middle cerebral artery (MCA) stroke in the rat produces impairments in skilled movements. The lesion damages lateral neocortex but spares primary motor cortex (M1), raising the question of the origin of skilled movement deficits. Here, the behavioural deficits of MCA stroke were identified and then M1 was examined neurophysiologically and neuroanatomically. Rats were trained on a food skilled reaching task then the lateral frontal cortex was damaged by unilateral MCA electrocoagulation contralateral to the reaching forelimb. Reach testing and training on two tasks was conducted over 30 post surgical days. Later, M1 and the corticospinal tract were investigated using intracortical microstimulation (ICMS), anterograde and retrograde axon tracing. A skilled reaching impairment was observed post surgery, which partly recovered with time and training. ICMS revealed a diminished forelimb movement representation in MCA rats, but a face representation comparable in size to sham rats. Anterograde and retrograde tract tracing suggest that M1 efferents were intact. Although M1 appears to be in the main anatomically spared after MCA stroke its function as assessed electrophysiologically and behaviorally is disrupted.

1. Introduction

Human middle cerebral artery (MCA) stroke can be modeled in the laboratory rat with transient MCA occlusion using an intraluminal suture, reversible snare ligature, endothelin-1 infusion, photochemically initiated thrombosis, or permanent electrocoagulation. MCA stroke is associated with behavioural impairments as detected by tests of skilled reaching (Whishaw et al., 1986, Whishaw et al., 1991), limb coordination (Metz and Whishaw, 2002), and limb reflexes (Schallert et al., 1983, Schallert et al., 1997). Because behavioural recovery is incomplete, treatment includes methods of minimizing the extent of the lesion (Cairns and Finklestein, 2003), replacing lost tissue (Cairns and Finklestein, 2003), and behavioural rehabilitation (Nudo et al., 1996b).

Because MCA stroke spares motor cortex (M1), which includes the rostral and the caudal forelimb movement representations, it is surprising that its behavioural deficits are similar to those produced by injury to the forelimb representations of M1 (Whishaw et al., 1991, Nudo et al., 1996b, Whishaw, 2000). Following MCA stroke, rats display both quantitative and qualitative impairments in reaching for food (Colbourne et al., 2000, Biernaskie and Corbett, 2001, Gonzalez and Kolb, 2003, Gharbawie et al., 2005). In some MCA stroke models, the reaching impairment may result from subcortical damage, possibly to the lateral striatum (Whishaw et al., 1986, Pisa, 1988). Alternatively, it is possible that although M1 is anatomically spared, its function may be abnormal, although this possibility has not been investigated.

In the present study, the electrophysiological and functional integrity of M1 subsequent to MCA stroke is explored. Rats were pre-surgically trained on a skilled

forelimb task of reaching for food pellets and then the distal branches of the MCA in the hemisphere contralateral to the reaching paw were occluded. Because motor maps are influenced by post lesion experience (Nudo et al., 1996b, Friel et al., 2000), reach testing/training was conducted for two postsurgical weeks. Then the animals received additional training in a less challenging tray reaching task to ensure maximal behavioral recovery (Vergara-Aragon et al., 2003) and retested in the single pellet task. Following behavioral training/recovery, the neurophysiological properties of M1 adjacent to the injury were examined using intracortical microstimulation (ICMS) motor mapping. ICMS provides a sensitive assay of M1 function (Stoney et al., 1968, Kleim et al., 2003, Nudo, 2003, Nudo et al., 2003). In additional MCA rats, anterograde and retrograde axonal tracing techniques were used to confirm the presence of M1 pathways.

2. Materials and Methods

Animals

Twenty-two female Long-Evans hooded rats, 120 days old, and weighing 250-300 g from the University of Lethbridge vivarium were used. The number of animals was kept to a minimum and they were treated in a humane manner in compliance with the guidelines of the University of Lethbridge animal care committee and the Canadian Council for Animal Care. Rats were housed in Plexiglas cages in groups of two or three in a colony room maintained on a 12/12 h light/dark cycle (08:00-20:00 h).

Feeding

The rats were gradually food deprived to 90-95% of their body weight by once a day feeding of Purina rat chow. When reaching, the rats received 45 mg dustless

precision pellets (product #F0021, Bioserve Inc., Frenchtown, NJ, USA) or 20-30 mg chick feed (New Life Feeds, Lethbridge, AB, Canada).

Surgery

Middle cerebral artery occlusion. Rats were anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). The bone lateral to the temporal ridge, overlaying the MCA, was removed and the dura was retracted. The vessel and its three main branches dorsal to the rhinal fissure were occluded by bipolar electrocoagulation using a procedure modified from Tamura et al. (1981), and the incision was closed. Sham operation included the same procedures except for dura removal and vessel electrocoagulation.

Intracortical Microstimulation. Rats were anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). Electrophysiologists blind to the purpose of the study, performed a craniotomy, retracted the dura, and covered the pia with inert silicon oil (37 ^{o}C). To relieve edema, a puncture to the cisterna magna drained cerebrospinal fluid. A grid (500 μm^2) was superimposed onto a digital image of the cortex to guide microelectrode penetrations (Remple et al., 2001).

A tungsten filament, in a glass microelectrode (15-30 μm tip diameter) filled with concentrated saline (3.5 M), delivered current for stimulation trains of thirteen, 200 μs, 350 Hz cathodal pulses, from a stimulation isolation unit. At each site, current intensity was increased from 0 μA to 100 μA. A hydraulic microdrive was used to sample various cortical depths to accommodate potential cortical thickness aberrations that may have shifted the location of layer V pyramidal neurons from 1550 μm. An experimenter supported the rat's forelimb from underneath the elbow and recorded forelimb

movements as proximal (elbow/shoulder) or distal (wrist/digit). In the case of two simultaneous movements, the one obtained at the lowest threshold was recorded. Movements of hind limb, vibrissae, jaw, head/neck were also recorded. Area representations were analyzed using the software Canvas (version 3.5, ACD Systems, http://www.deneba.com/)

Axon tract tracing

The anterograde axon tract tracer biotynilated dextran amine (BDA 10%; 10,000 MWT; 2.0 µl; Vector labs, Burlington, ON, Canada) was microinfused into M1 [A (anterior) = + 0.5 mm; L (lateral) = 2.5 mm; V (ventral) = 2.2 mm (skull)] and [A = + 1.7 mm; L = 2.5 mm; V = 2.4 mm (skull)]. The retrograde tracer true blue (2.5%; 1.0µl; molecular probes Inc.; Burlington, ON, Canada) was microinfused into the pyramidal decussation [A = - 13.8 mm; L = 0 mm; V = 11.1 mm (skull)]. Microinfusions were delivered under isoflurane anesthesia (1.5%) via a 30-gauge cannula over 5 min and an additional 5 min for diffusion. Rats survived 7 days to allow for transport of the tracers.

Reaching boxes

Animals were trained on a single pellet task (Whishaw et al., 1991) and a tray task (Whishaw et al., 1986). Single pellet boxes were made of clear Plexiglas, with the dimensions 45 x 14 x 35 cm. In the center of each front wall was a vertical slit 1 cm-wide, which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-deep shelf. Two indentations on the surface of the shelf were located 2 cm from the inside of the wall and were aligned with the edges of the slit where rats could reach.

The tray boxes were 26 x 28 x 19 cm. The front of the boxes was constructed of 2 mm vertical bars separated from each other by a 9 mm gap. A 4 cm-deep tray containing chick feed was mounted in front of the bars. Water was accessible at the rear of the box.

Video recording

Reaching performance was video recorded using a Cannon ZR 30 MC camcorder (1000th of a second shutter speed) and a cold light source. A Sony videocassette recorder DSR-11 was used for frame-by-frame analysis.

Reaching training

Single pellet task. Food pellets were placed in the indentation contralateral to each rat's preferred limb. Once an animal was reliably reaching for food, a short delay preceded the presentation of the next pellet, until the animal walked to the back of the box and then approached the front in order to reposition itself for the next reach. Animals received 20 pellets/day for two weeks. On test days, success was measured using the following formula:

Success percentage = (number of pellets retrieved/20) x 100.

Tray task. Animals were allowed to reach through the bars, grasp the food and retract it, for two 1-hr sessions (10 a.m. and 5:00 p.m.) per day, for 7 days.

Qualitative scoring of skilled reaching

For a qualitative analysis of single pellet reaching, a reach was subdivided into 10 components (Whishaw et al., 1991). (1) *Digits to midline*: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body. (2) *Aim*: using the upper arm, the elbow is adducted so that the forearm is aligned along the midline of the body, with the paw located just under the mouth. This movement involves fixation of the

distal portion of the limb, so that digits remain aligned with the midline of the body. This is likely produced by a movement around the elbow that reverses the direction of movement of the paw to compensate for the adduction of the elbow. (3) *Digits semiflexed*: as the limb is lifted, the digits are semiflexed and the paw is supinated so that the palm faces the midline of the body. (4) *Digits open*: as the limb is advanced the digits are extended. (5*) Advance*: the head is slightly lifted to allow the limb to be advanced directly forward above and beyond the food pellet. (6) *Pronate*: using a movement of the upper arm, the elbow is abducted, pronating the paw over the food while the digits open. Pronation of the paw onto the food is aided by a movement of the paw around the wrist. (7) *Grasp*: as the pads of the palm or the digits touch the food, the food is grasped by closure of the digits. This can occur as an independent movement, or the grasp can occur as the paw is withdrawn. (8) *Supination I*: as the limb is withdrawn, the paw is dorsiflexed and is supinated 90^0 by a movement around the wrist and by adduction of the elbow. These movements can occur as soon as the food is grasped or can occur as the limb is withdrawn. (9) *Supination II*: as the rat sits back with food held in the paw, the paw is further supinated by 90^0 and ventroflexed to present the food to the mouth. (10) *Release*: the digits are opened and the food transferred to the mouth.

The first five successful reaches were analyzed frame-by-frame on the videotapes by two experimenters blind to animals' group membership. Each component was rated on a three-point scale as previously described (Whishaw et al., 1993). If the movement appeared normal, it was given a score of "0". If it appeared slightly abnormal but recognizable it was given a score of "0.5"; for example incomplete supination (supination 1) of the paw. A score of "1" was assigned if the movement was absent or completely

unrecognizable; for example the limb is withdrawn from the slot without any paw rotation (supination 1).

Procedure and timeline

Rats were pre-surgically trained on single pellet reaching for three weeks, matched for success scores, and assigned to sham (n = 8) or MCA groups (n = 12). Post surgical testing was conducted on days 1, 3, 7, and 14. Additional training on the tray task was given on post surgical days 20-28. Single pellet testing resumed on days 29 and 30. Qualitative single pellet analysis was conducted on sham (n = 8) and randomly chosen MCA (n = 8) rats. From this pool of rats, sham (n = 6) and MCA groups (n = 6) received ICMS. A separate axon tract tracing study was conducted with BDA (n = 3) or true blue (n = 3) at least 50 days post injury on MCA rats that did not receive ICMS. All animals were euthanized for histological analysis on approximately post surgical (lesion) day 60.

Histology

Animals were euthanized with sodium pentobarbital and intracardially perfused with saline (0.9%) and 4% paraformaldehyde. Brains were removed, post-fixed and cryoprotected in 30% sucrose and 4% paraformaldehyde at 4°C for coronal sectioning (40 μm) and cresyl violet staining. Alternate sections from the true blue-infused brains were not stained so that they can be examined for fluorescence. BDA sections were processed with a DAB Elite-kit (Vector Laboratories, Burlington, ON, Canada) for light microscope examination.

Six cresyl violet sections [A= +2.70, +1.70, +0.70, -0.30, -1.30, -2.30, from Bregma (Paxinos and Watson, 1997)] from each MCA animal in the ICMS and axon

tracing studies were used for lesion size assessment. Digital images were captured, opened in NIH image V.1.63, and the areas of the lesion and the intact hemispheres were measured. Lesion size was estimated using the formula: [Undamaged area of lesion hemisphere (pixels^2)/area of intact hemisphere (pixels^2)] x100].

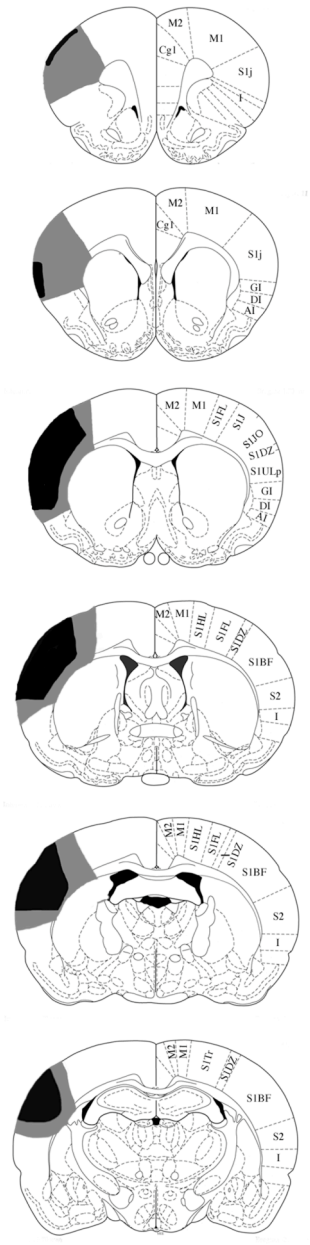


Figure 6.1: Coronal sections (Paxinos and Watson, 1997) illustrating MCA stroke that spares M1. Grey and black shadings respectively demonstrate the largest and smallest lesions.

Statistical analysis

Success scores were analyzed using repeated measures ANOVA with follow up t-tests to compare performance before and after tray reaching. Qualitative scores were analyzed with non-parametric tests: Mann-Whitney *U*-test for between-subject comparisons and Wilcoxon-signed rank for within-subject comparison. Motor maps and current thresholds for ICMS were analyzed with ANOVA.

3. Results

Histology

MCA occlusion damaged the lateral frontal neocortex including primary somatosensory cortex of the face and the vibrissae and secondary somatosensory cortex (Fig 6.1). M1, secondary motor cortex, and subcortical structures were spared. Within the six predefined coronal sections, the area of the injured hemisphere was 82.2 $\pm$ 3.4 % (mean $\pm$ SE) of the intact hemisphere.

Single pellet reaching

Success. MCA rats were impaired on post surgical testing [repeated measures ANOVA: $F(1,14) = 17.37$, $p < 0.01$, Fig 6.2 A]. Success scores improved across test days [repeated measures ANOVA: $F(4,56) = 2.69$, $p < 0.05$]. A follow up unpaired t-test indicated that the MCA group was inferior to shams on post surgical day 14 ($p < 0.01$) but not on post surgical day 30 ($p > 0.05$). In addition, separate paired t-tests indicated that MCA success improved on post surgical day 30 from day 14 ($p < 0.05$) but performance of the sham group was unchanged on those days ($p > 0.05$).

Qualitative performance. MCA rats were impaired on the qualitative measures of reaching [Mann-Whitney *U* –test: $z = -22.93$, $p < 0.01$, Fig 6.2 B]. Scores of the MCA rats

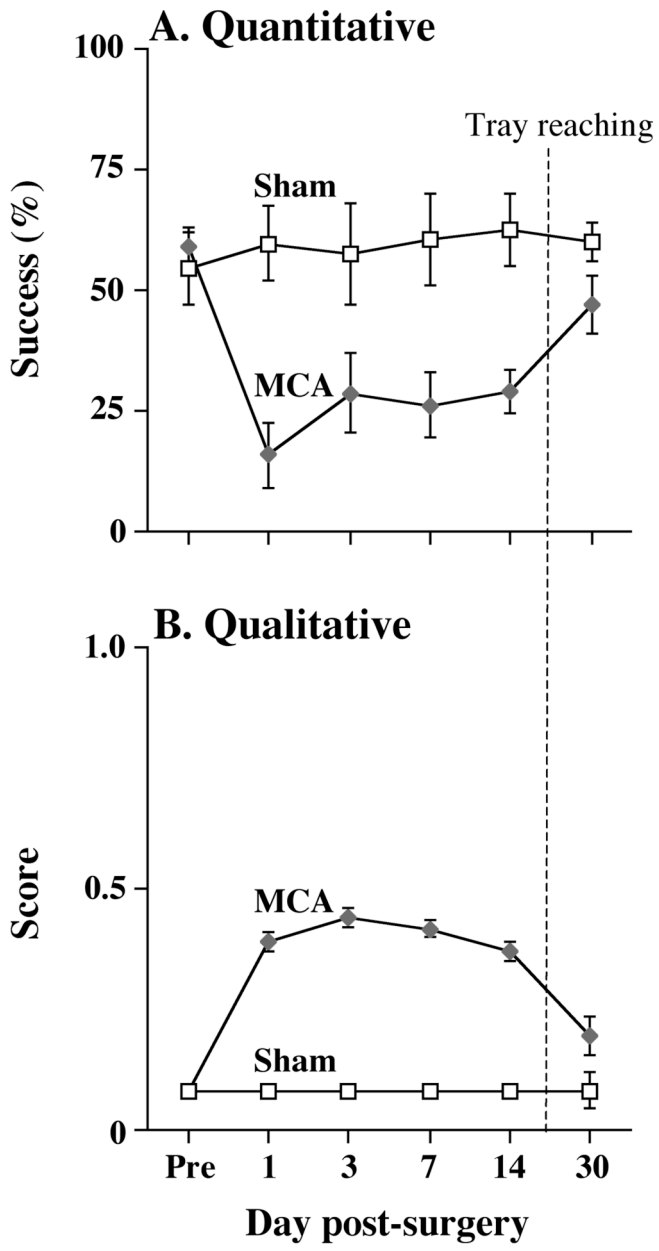


Figure 6.2: Single pellet task. (A) Success (mean ± SE) for sham and MCA groups. Both groups were equally successful in obtaining pellets before surgery (Pre) but the MCA group was impaired on post surgical days 1, 3, 7, and 14 but not after tray task training (day 30). (B) Qualitative scores were higher (impaired) on all post surgical days for the MCA group.

improved after tray task training (day 30) from day 14 [Wilcoxon-signed rank: $z = -7.29$, $p < 0.01$] but control levels were not regained as evident by significant deficits in aligning the digits with the midline of the body, supinating the paw, and grasping and releasing the food [Mann-Whitney *U* –test, $p < 0.05$, Fig. 6.3].

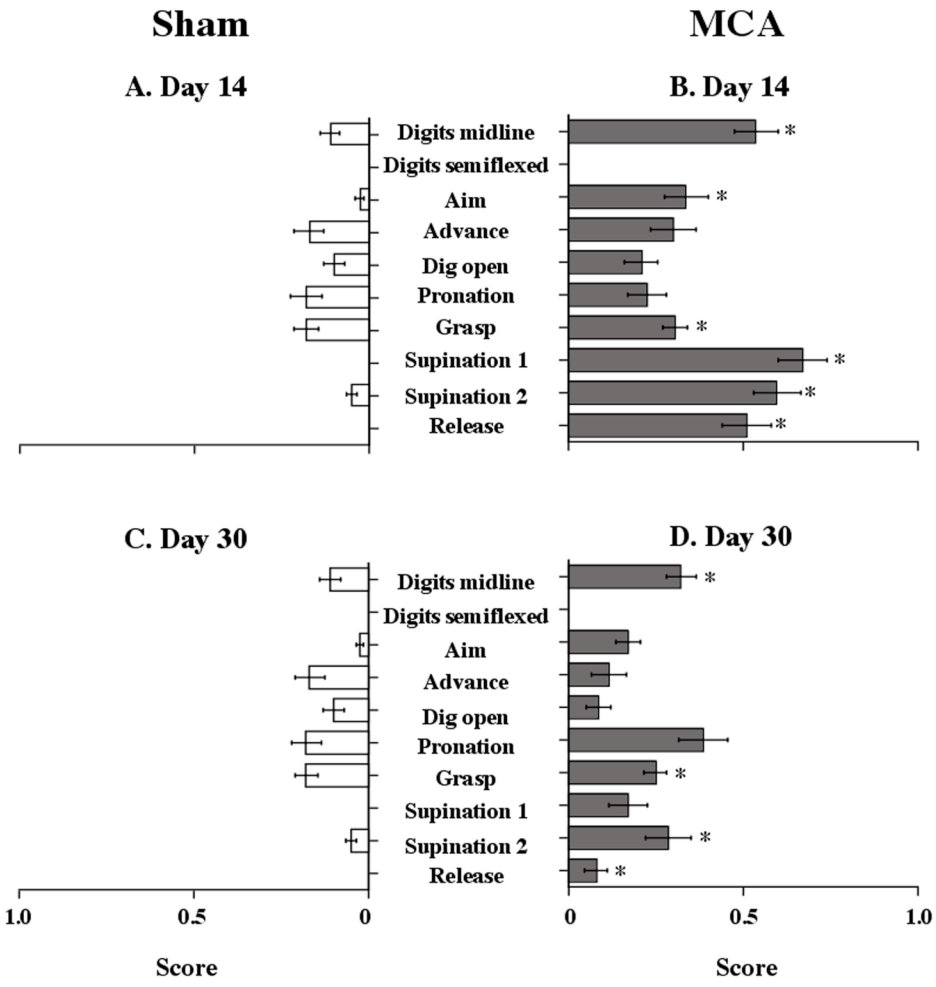


Figure 6.3: Scores of the individual reaching elements of sham (A, C) and MCA (B, D) rats on post surgical days 14 and 30 (following tray task training). Enduring deficits were detected on post surgical day 30 in aligning the digits with the midline, supinating the paw, and grasping and releasing the food. * $p < 0.05$

Performance of the MCA rats was also characterized by "phantom grasps" after a failed reach attempt, in which the digits closed and flexed as if a food pellet was grasped, and the paw was inspected as if attempting to retrieve food. Sham rats typically

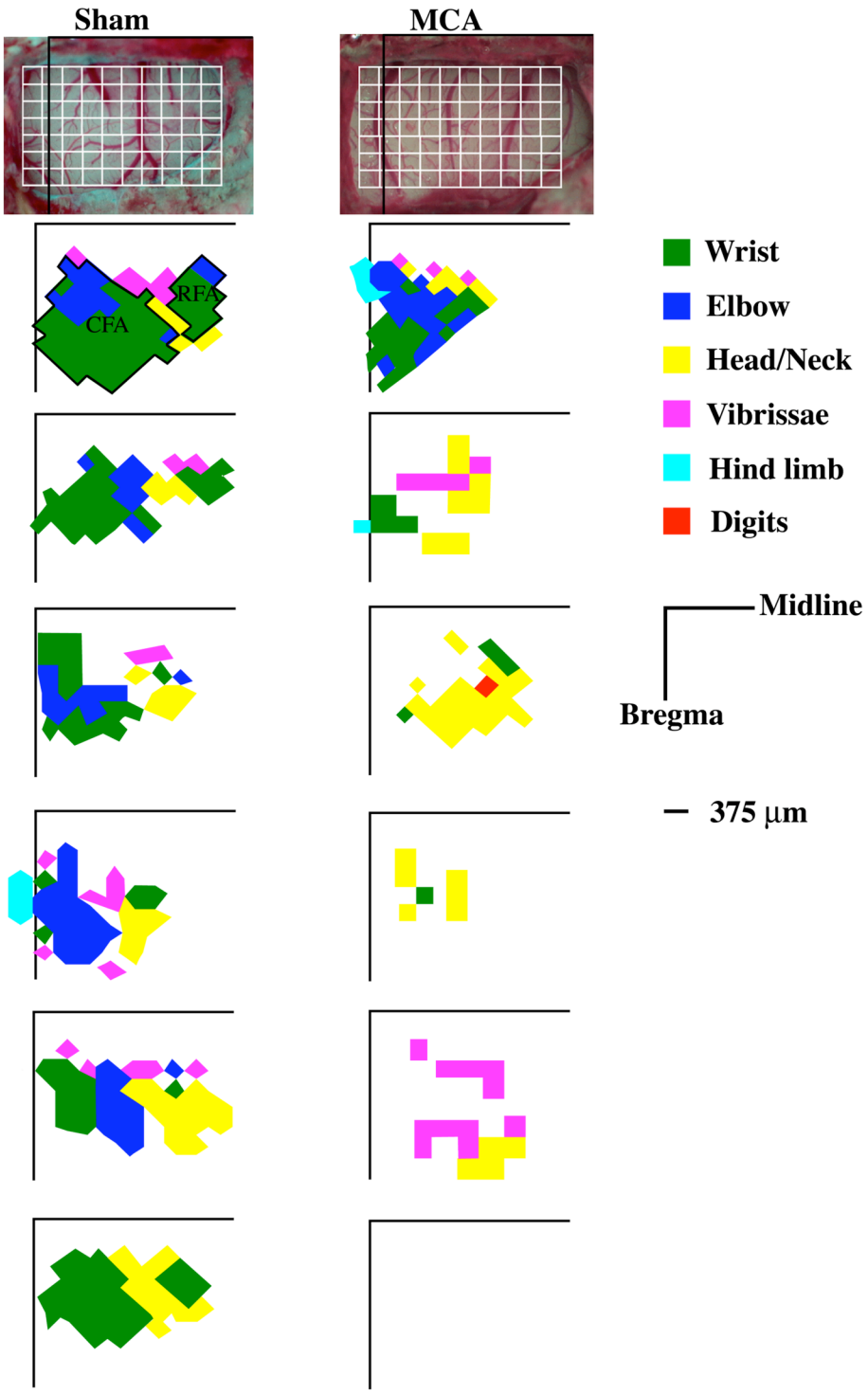


Figure 6.4: Motor maps derived from (A) sham and (B) MCA rats. A superimposed white grid guided electrode penetration sites. Sham maps included two forelimb movement representations (CFA and RFA) separated by head/neck and vibrissae responses, whereas a forelimb representation in MCA rats, if at all present, was restricted to the CFA. Visible cortical damage is lateral to the area stimulated.

repositioned their elbow to aim the limb and reattempted the reach, presumably "knowing" they failed to retrieve the pellet. This was confirmed by comparing the number of "phantom grasps" that occurred on post surgical day 30 [ANOVA: $F(1,14) = 15.83$, $p < 0.01$].

Organization of movement representations

Forelimb movement representations were organized into a caudal forelimb area (CFA) and a rostral forelimb area (RFA) separated by head/neck responses. The cortical area stimulated in sham (9.99 ± 0.76 mm^2; mean $\pm$ SE) and MCA (9.18 ± 1.0 mm^2; mean $\pm$ SE) animals was comparable. But within the stimulated area, significantly fewer sites evoked a response to ICMS in MCA animals [ANOVA: $F(1,10) = 13.42$, $p < 0.01$, Fig 6.4]. This was due to a significantly smaller forelimb movement representation [ANOVA: $F(1,10) = 12.27$, $p < 0.001$, Fig 6.5], because other representations including (vibrissae, jaw, head/neck) were not different between the groups [ANOVA: $F(1,10) = 3.26 \times 10^{-37}$, $p = 1.00$, Fig 6.5]. Similar thresholds were required to evoke forelimb movements (sham = 42.32 ± 3.08 μA, MCA = 42.88 ± 6.96 μA; mean $\pm$ SE).

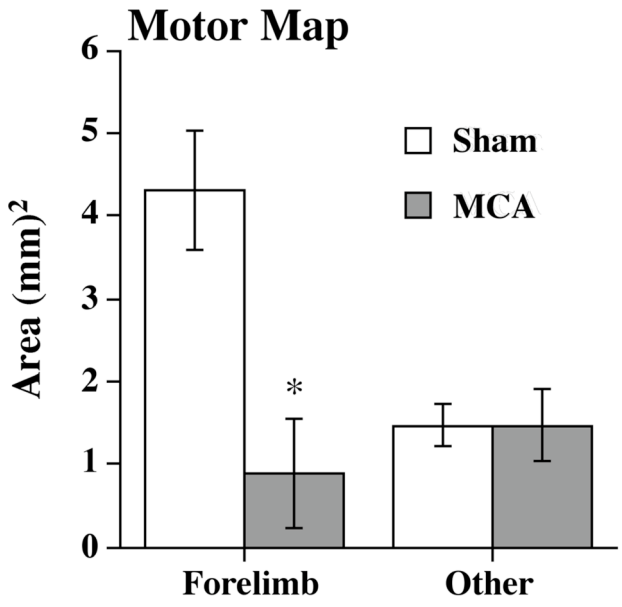


Figure 6.5: Smaller forelimb movement representations were detected in the MCA group as compared to the sham group. Other representations including hind limb, vibrissae, jaw, head/neck were comparable in both groups.

Axon Tracing

Anterograde tract axon tracing. BDA infused in M1 (Fig 6.6 A, B) labeled corticofugal fibers projecting through the medial caudate-putamen (Fig 6.6 C) and through the corpus callosum (Fig 6A). Cell bodies in the homotopic M1 were labeled as well (Fig 6.6 D).

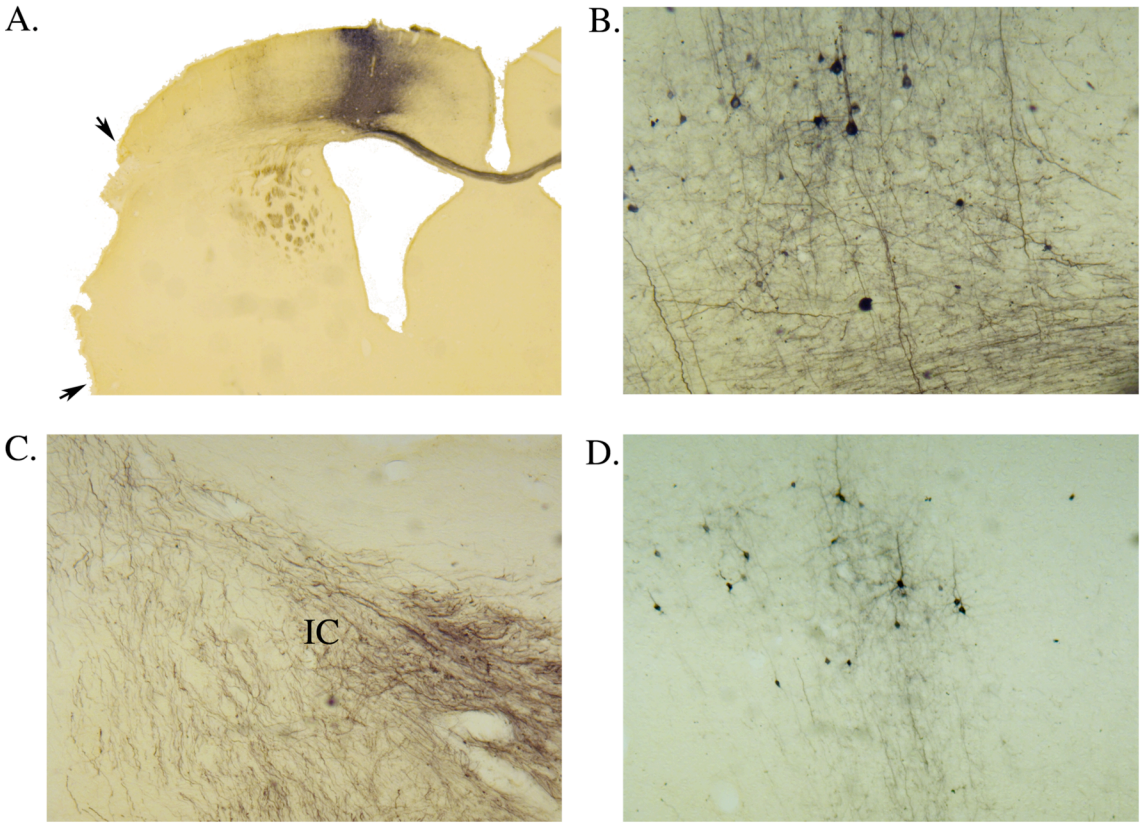


Figure 6.6: Photomicrographs of anterograde axon tract tracing in an MCA rat. Coronal sections show: (A) Infusion site of BDA in M1, corticofugal fibers are labeled in the internal capsule and corpus callosum. Arrows mark lesion boundaries. (B) Magnification (x10) of infusion site demonstrating labeled cell bodies, dendrites, and axons. (C) M1corticospinal fibers passing through the internal capsule (IC). (D) Cell bodies and dendrites labeled in the homotopic cortex.

Retrograde cortical cell labeling. True blue infused in the pyramidal decussation (Fig 6.7 A, B) retrogradely labeled layer V cell bodies in M1 and somatosensory cortex in the undamaged hemisphere (Fig 6.7 C, D, E). Similarly, cell bodies were labeled in M1 of the damaged hemisphere but labeling was sparse in the adjacent somatosensory cortex (Fig 6.7 F, G, H).

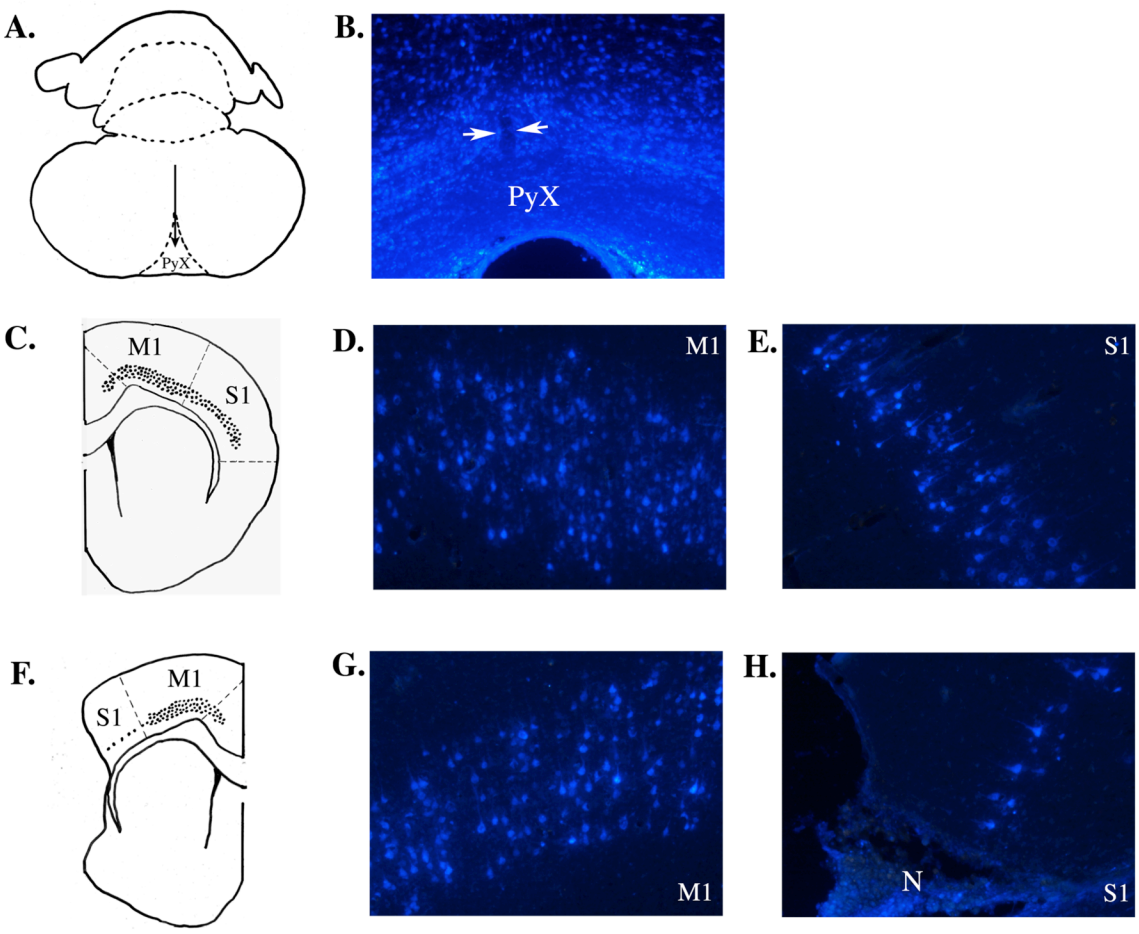


Figure 6.7: Retrograde axon tract tracing in an MCA rat. (A) Line drawing of a coronal section at the level of true blue infusion, arrow illustrates syringe tract into the pyramidal decussation (PyX). (B) Photomicrograph of the infusion site, arrows depict cannula tract. Line drawings of coronal sections and layer V pyramidal cells (dots) in the undamaged (C) and the damaged (F) hemispheres. Cell body labeling in M1 was comparable in the undamaged (D) and damaged (G) hemispheres. Distinctly more cells were labeled in somatosensory cortex (S1) in the undamaged (E) than in the damaged (H) hemisphere. Necrotic tissue (N) is visible in panel (H).

4. Discussion

The experiment was motivated by the puzzling finding that behavioural functions thought to be under the control of M1 are reported to be impaired following MCA stroke that spares M1 (Colbourne et al., 2000, Biernaskie and Corbett, 2001, Gonzalez and Kolb, 2003, Gharbawie et al., 2005). ICMS was used to examine the functional integrity of M1 and anatomy and axon tract tracing were used to confirm that the motor cortex and its corticospinal tract were present. Subjects received extensive pre-surgical and post surgical reach training and displayed substantial post surgical behavioral improvement after their initial MCA stroke-induced impairments. Nevertheless, ICMS revealed abnormally small forelimb movement representations in M1, suggesting that M1 dysfunction might contribute to initial and chronic behavioural deficits.

The present study confirms previous work indicating that distal MCA stroke damages the lateral neocortex while substantially sparing M1 and subcortical regions (Gonzalez and Kolb, 2003, Gharbawie et al., 2005). The integrity of M1 was further confirmed by axonal tracing that indicated a seemingly normal complement of corticofugal fibers leaving M1. It must be noted that although gross anatomical features suggest that M1 is largely intact, the possibility of dispersed ischemia-induced cell death in M1 cannot be ruled out using the present histological analysis. It can be examined in future work in which subjects can be sacrificed shortly after MCA stroke for dead neuron labeling.

The novel finding of the study is that ICMS mapping indicated that the forelimb movement representations of M1 were abnormally small. The decrease in motor map size was not related to the extent of M1 stimulated or to changes in thresholds required to

elicit movement as comparable areas in control and MCA rats were stimulated with similar electrical current intensities. It is unlikely that the reduced map size was due to changes in cortical thickness induced by the lesion, as a variety of depths were sampled within the stimulation tracts. Finally, the reduction in motor map size was limited to the representations of the forelimb because face and vibrissae zones were comparable in both groups.

Motor map size is influenced by behavioral experience (Friel and Nudo, 1998), but it seems unlikely that insufficient behavioral experience contributed to reduced map size in the present experiment. The rats were given pre surgical experience of reaching for food, a manipulation that increases ICMS map size (Nudo et al., 1996a, Kleim et al., 1998), and were given extensive training/testing post surgically on two different skilled reaching tasks, experience that contributes to maintaining ICMS map size (Nudo et al., 1996b). Indeed the post surgical behavioral training/testing resulted in considerable behavioral improvement. The rats improved to sham levels in the number of food pellets that they successfully obtained, although on qualitative assessments their movements remained impaired.

Forelimb movement representations may be abnormal in the MCA rats because M1 is partially deafferentated by damage to the lateral neocortex. ICMS evokes movement via direct (Stoney et al., 1968) and indirect (Jankowska et al., 1975) activation of corticospinal tract neurons, the majority of which are trans-synaptically activated (Cheney and Fetz, 1985, Lemon et al., 1987). Although projections from lateral frontal cortex to M1 were not directly investigated, sparse retrograde labeling of cell bodies in lateral frontal cortex suggests a loss of efferent projections. Presumably, projections from

the damaged lateral frontal cortex to M1 could have been damaged as well. In addition, degenerating descending projections from lateral frontal cortex may have disturbed functional properties in the neighboring fibers emerging from M1 despite their apparent preservation.

The reduction in size of the forelimb movement representations of M1 did not appear to be due to a complete disruption of all motor cortex function. The area of neocortex representing the face and the neck was comparable in sham and MCA rats. Face and neck representation may be accounted for by a conservation of face and neck representations normally present around the borders of CFA and RFA. Alternatively, reorganization of function may have developed in M1 to assume the face representation of the former lateral frontal cortex. A number of studies have shown that brain injury can result in an adopted functional representation in adjacent cortical areas (Nudo et al., 1996b, Frost et al., 2003, Kleim et al., 2003). The preserved face representation within the forelimb region of M1 is suggestive of the significance of facial sensory contributions to skilled reaching (Gharbawie et al., 2005).

The behavioural results showing impairments in skilled movements following lateral neocortex damage are consistent with previous studies (Biernaskie and Corbett, 2001, Gonzalez and Kolb, 2003, Gharbawie et al., 2005). It is possible that damage to lateral neocortex also contributes to skilled movement impairments via its effects on sensory function. For example, skilled reaching may depend upon somatosensory information from the vibrissae (Gharbawie et al., 2005) as may accurate limb placement (Jones and Schallert, 1992). In addition, haptic information from the paw may contribute to proper grip formation (Glendinning et al., 1992) and successful identification of

retrieved food (Ballermann et al., 2001). Nevertheless, the present finding of reduced map size in forelimb M1 as revealed by ICMS leaves open the possibility that dysfunction in this region of the cortex following more lateral cortical damage also contributes to impairments in skilled reaching.

Conclusion

The novel finding of the study is that ICMS revealed neurophysiological dysfunction in M1 subsequent to MCA stroke that spares M1. Potentially the electrophysiological abnormalities in M1 may contribute to the behavioural deficits that follow MCA stroke. Because M1 appears anatomically intact, although malfunctioning, it may be a promising target for therapeutic efforts.

Chapter 7

Evidence for bilateral control of skilled movements: Enduring ipsilateral skilled forelimb reaching deficits in rats follow motor cortex and lateral frontal cortex lesions

This chapter is modified from a paper published in the *European Journal of Neuroscience* 20(12):3442-52 by Claudia L. R. Gonzalez, Omar A. Gharbawie, Preston T. Williams, Jeffrey A. Kleim, Bryan Kolb, Ian Q. Whishaw in 2004

Abstract

It is well known that contralateral sensorimotor deficits follow unilateral motor cortex damage. A growing body of evidence suggests that the undamaged hemisphere undergoes significant functional and structural plasticity that might be responsible for the recovery/compensation that follows the lesion. It also has been suggested that these plastic changes might enhance motor performance in the unaffected ipsilateral forelimb. The objective of the present study was to make a systematic examination of the motor skills of the ipsilateral forelimb after frontal cortex lesions to either the motor cortex, by devascularization of the surface blood vessels (pial stroke), or to the lateral cortex, by electrocoagulation of the distal branches of the middle cerebral artery (MCA stroke). Plastic processes in the intact hemisphere were documented using intracortical microstimulation analysis. Although tests of reflexive responses in forelimb placing identified a contralateral motor impairment following both cortical lesions, quantitative and qualitative measures of skilled reaching identified a severe ipsilateral impairment from which recovery was substantial but incomplete. The finding of an enduring ipsilateral impairment in skilled movement is consistent with a large but more anecdotal literature in rats, nonhuman primates, and humans and suggests that plastic changes in the intact hemisphere are related to that hemisphere's contribution to skilled movement instead/in addition to the recovery of the damaged hemisphere's functions.

1. Introduction

Most of the efferent pathways of the forebrain are crossed and so damage to frontal neocortex or to the basal ganglia of one hemisphere results in impairments in movement on the contralateral side of the body (Whishaw et al., 1986; Kolb, 1995; Hesse and Werner, 2003). In rat models of motor cortex injury (Castro, 1972; Whishaw et al., 1991; Schallert et al., 1997; Napieralski et al., 1998; Gonzalez and Kolb 2003) or stroke (Hossmann 1998), contralateral impairments may be displayed in using a paw to reach for food (Whsishaw and Miklyaeva, 1996; Kolb 1999), in making placing responses (Schallert et al., 2000), in walking over complex surfaces such as an elevated horizontal ladder (Metz and Whishaw 2002), and in detecting tactile stimulation to the limbs (Schallert et al., 1983; 2000; Schallert and Whishaw, 1984). Immediately after surgery impairments may be pronounced but with time and/or experience, animals display substantial compensation/recovery (Whishaw 2000; Virley et al., 2000; Reglodi et al., 2003; Gonzalez and Kolb 2003).

Despite evidence supporting a preferential role for a hemisphere over sensory/motor process in the contralateral half of the body, there are many reports in laboratory animals and humans (see Table 1) of impairments on the ipsilateral side of the body. For instance, although the arm controlled by the intact side of the brain after middle cerebral artery (MCA) stroke is often referred as being "unaffected", there are reports of patients complaining about loss in strength, speed of movement, and complex action sequences with that arm (Hermsdorfer et al., 1999; Hermsdorfer and Goldenberg 2002; Jung et al., 2002). Similarly, in rodent studies in which function of the ipsilateral forelimb is included as a "control" for the contralateral limb, there are also reports of

impairments, especially in the immediate postoperative period (Marston et al., 1995). To date, there has been little systematic study of the ipsilateral impairments that might follow neocortical injury in the rat, even though such work might be helpful in understanding the nature of movement impairments and in aiding rehabilitation.

The purpose of the present study was to determine whether there are impairments in skilled movements in the forelimb ipsilateral to two kinds of neocortical injury in the rat: motor cortex injury produced by devascularization of the surface blood vessels over the forelimb regions of motor cortex (Sofroniew et al., 1983), and the neocortex lateral to it, by electrocoagulation of the distal branches of the middle cerebral artery (MCA) with a procedure similar to the one described by Gonzalez and Kolb (2003). The rats were pretrained in a skilled reaching task in which they had to learn to retrieve single food pellets located on a shelf and their behavior was studied postoperatively for a total of four weeks. Impairments were documented by end point measures of success in obtaining food pellets (Whishaw and Pellis, 1990), and the qualitative changes in reaching movements were analyzed frame by frame from video records (Whishaw et al., 1993). To enhance post-surgical recovery, animals were also given intensive training on a less demanding reaching task (Whishaw et al., 1986; Vergara-Aragon et al., 2003), between the third and fourth post-surgical weeks and later retested on the single pellet reaching task. At the completion of the behavioral tests, the forelimb movement representations in the intact hemisphere were exmained using intracortical microstimulation mapping (ICMS) of motor cortex (Kleim et al., 1998) and topographic organization was used as an index of plastic changes.

2. Materials and Methods

Subjects

Subjects were 24 female Long-Evans hooded rats, 4 months old weighing 250-300g at the beginning of the experiment. Animals were raised in the University of Lethbridge vivarium and were housed in groups of two or three individuals in clear plexi-glass cages. The colony room was maintained on a 12/12h light/dark cycle (08:00-20:00 h) and the temperature regulated at 22 ^{o}C. Experiments were conducted according to standards set by the Canadian Council on Animal Care and approved by the University of Lethbridge animal care committee.

Feeding

For initial and subsequent training, the rats were gradually food deprived to 90-95% of their body weight by once a day feeding of a measured quantity of Purina rat chow. For the week prior to reach training, each rat received twenty 45 mg dustless precision banana-flavoured pellets (product #F0021, Bioserve Inc., Frenchtown, NJ, USA) one hour prior to the daily chow ration. The objective was to introduce rats to the pellets, which would later serve as reaching targets, in a familiar environment. Once reach training began and until the end of the study, only rat chow was served in the home cage once a day in sufficient amounts to maintain all rats at 90-95% of their original body weight. The number of banana-flavoured pellets a rat received on a given training day depended on the rat's reaching accuracy, but it did not exceed 25 pellets (1.125 g). Purina rat chow feeding in the home cage was reduced on days of tray reaching to ensure that rats did not gain too much weight because animals were consuming sufficient amount of the food target (chicken feed) during the two hours of daily reaching.

Surgery

Motor cortex devascularization

Animals received an injection of atropine nitrate (0.1 mg/kg, i.p.; Sigma-Aldrich, St. Louis, MO) to facilitate respiration throughout surgery. Animals were then anesthetized (sodium pentobarbital 45 mg/kg i.p.) and placed in a stereotaxic apparatus. The lesions were produced by unilateral devascularization of the motor cortex (Sofroniew et al., 1983; Kolb et al., 1997). In brief, a flap of bone and underlying dura were removed at coordinates corresponding to the overlapping motor and sensory representation of the forelimb, areas Fr2, Fr1, Fr3, FL and HL (Zilles, 1985). A rectangular hole in the skull was produced at stereotaxic coordinates anterior (A), A= +4.0 to -1.0 mm and lateral (L), L= 1.0 to 4.0 mm using an electric dental drill. All vessels and pia matter were rubbed away with sterile, saline-soaked cotton swabs. The incision was closed and animals were observed for 24 hours before being returned to the colony.

Middle cerebral artery occlusion

Animals were anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) and xylazine (5 mg/kg). The distal MCA was occluded according to a procedure similar to the one used by Gonzalez and Kolb (2003) to produce damage to the lateral part of the frontal cortex without damaging the striatum. In brief, a rectangular hole lateral to the temporal ridge was made using an electric dental drill whilst avoiding traumatic brain injury. The opening was enlarged with rongeurs by removal of additional temporal bone to expose the MCA. At this point the dura mater was carefully removed and the MCA (and branches) distal to the inferior cerebral vein were permanently occluded by bipolar

electrocoagulation (Howard Instruments Inc.) The incision was closed and animals were observed for 24 hours and then returned to the colony.

Intracortical microstimulation (ICMS)

For this procedure animals were anesthetized with ketamine hydrochloride (70 mg/kg i.p.) and xylazine (5 mg/kg i.p.). A craniotomy was performed over the sensorimotor cortex in the hemisphere opposite the lesion with an electric dental drill. To relieve edema, a small puncture wound to the cisterna magna provided a drain outlet for excess cerebrospinal fluid. The dura was deflected without damaging underlying pia mater and the exposed cortex was covered with an inert silicon oil (37 ^{o}C). A digital picture was taken over the exposed cortex, and a calibrated high-resolution grid was superimposed onto the picture to serve as a guide for microelectrode penetration sites.

A glass microelectrode, pulled and beveled (15-30 μm tip diameter) was filled with concentrated saline (3.5 M). A tungsten filament was inserted into the glass microelectrode to deliver current from an isolation stimulation unit. Stimulation trains consisted of thirteen 200 μs cathodal pulses delivered at 350 Hz. At each penetration site, current gain was progressively increased from 0 μA to a maximum of 60 μA.

Electrode placement was controlled by a hydraulic microdrive (Kopf) to depths corresponding to layer V of motor cortex (~1550 μm). Rats were maintained in a prone position and the forelimb was elevated from underneath the elbow. At each penetration site, forelimb movements were classified as either proximal (elbow/shoulder) or distal (wrist/digit) by two experimenters. In the case of two or more simultaneous forelimb movements, the single movement persisting at the lowest stimulation intensity was

recorded and deemed representative of that particular cortical point. Movement thresholds were determined for each site evoking forelimb movements.

An image analysis program (Canvas v3.5) was used to calculate the extent of proximal and distal representations in both the caudal forelimb (CFA) and rostral forelimb (RFA) representations (Kleim et al., 1998). Mean thresholds for forelimb representations were calculated for analysis.

Behavioural testing

Cylinder

Forelimb use for weight support during explorative activity was examined by placing the rats in a transparent cylinder 20 cm in diameter and 30 cm high (Schallert et al., 1997). A mirror was placed underneath the cylinder at an angle to allow the experimenter to videotape the animal's activity from a ventral view (Pinel et al., 1993). The cylindrical shape encouraged vertical exploration of the walls with the forelimbs, but the walls were high enough so that animals could not reach the top. The animals were individually placed in the cylinder for three-minutes test sessions.

Single pellet reaching box

The box was constructed of clear Plexiglas, with the dimensions 45 cm deep by 14 cm wide by 35 cm high. In the center of each front wall was a 1 cm-wide slit which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-wide shelf. Two indentations on the floor of the shelf were located 2 cm from the inside of the wall and were centered on the edges of the slit where rats could reach. Food pellets (45 mg Rodent Chow food pellets, Bioserve Inc.) were placed in the indentation contralateral to the limb with which

the rat reached (Whishaw and Pellis, 1990). Following each reach, a short pause preceded the presentation of the next pellet. This encouraged animals to return to the back of the box after each reach, which forced them to reposition themselves and prepare for the next reach. The first week of training consisted of daily, 10 min sessions, for each rat. In the second and third weeks, rats received daily training sessions but a session now consisted of 25 pellets, also considered 25 trials, per session. The first five trials were considered practice trials but the succeeding 20 trials counted towards the score for the session.

Tray reaching box

The box was constructed of Plexiglass with dimensions 26 cm high, 28 cm deep, and 19 cm wide. The front of the boxes was constructed of 2 mm bars separated from each other by a 9 mm gap. Clear Plexiglass tops allowed access to the inside of the box. A 4 cm wide and 0.5 cm deep tray was mounted in front of the bars. The tray contained food fragments weighing approximately 30 mg each. Animals had to reach between the bars, grasp the food and retract it where they were able to freely eat.

Video recording

Video records were made using a Cannon ZR 30 MC digital video camcorder with a shutter speed of 1000th of a second. A cold light source provided illumination for high shutter speed filming. Frame-by-frame analysis at 30 frames/s was produced by a Sony digital videocassette recorder DSR-11 as well as with Final Cut Pro software (www.apple.com).

Behavioural Scoring

Cylinder

Forelimb use was measured during vertical exploration. Each forepaw contact with the cylinder wall was counted. The asymmetry score of forelimb use in wall exploration was calculated as the percent preference for the paw ipsilateral to the lesion, or for the control rats, the paw that they used for reaching: Preference = ipsilateral paw/(ipsilateral paw + contralateral paw).

Single pellet reaching (Quantitative)

(1) *Total success.* A successful reach is one in which the food was grasped and transferred into the mouth using the designated paw. Success was measured using the following formula:

Percent of total success percent = (number of pellets retrieved/20) x 100.

(2) *Single reach success.* A single reach success is one in which the designated paw grasped the pellet with a single limb transport through the slot and was then withdrawn to transfer food to the mouth. Single reach success was calculated using the following formula.

Percent of single reach success = (number of successful single reaches/20) x 100

Single pellet reaching (Qualitative)

Reaching movements made during the single pellet task were analyzed using a rating scale derived from Eshkol-Wachmann Movement Notation (EWMN: Eshkol and Wachmann 1958; Whishaw, 1993). A reach was subdivided into ten components: (1) Digits to the midline: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body. (2) Digits close: as the limb is lifted, the digits are semiflexed and the paw is supinated so that the palm faces the midline of the body. (3) Aim: using the upper arm, the elbow is adducted so that the forearm is aligned along the

midline of the body, with the paw located just under the mouth. This movement involves fixation of the distal portion of the limb, so that digits remain aligned with the midline of the body. This is likely produced by a movement around the elbow that reverses the direction of movement of the paw to compensate for the adduction of the elbow. (4) Advance: the head is lifted and the limb is advanced directly forward above and beyond the food pellet. (5) Digits open: as the limb is advanced the digits are extended and opened. (6) Pronation: using a movement of the upper arm, the elbow is abducted, pronating the paw over the food. Full pronation of the paw onto the food is aided by a movement of the paw around the wrist. (7) Grasp: as the pads of the palm or the digits touch the food, the food is grasped by closure of the digits. This can occur as an independent movement, or the grasp can occur as the paw is withdrawn. (8) Supination I: as the limb is withdrawn, the paw is dorsiflexed and is supinated 90^0 by a movement around the wrist and by adduction of the elbow. These movements can occur as soon as the food is grasped or can occur as the limb is withdrawn. (9) Supination II: as the rat sits back with food held in the paw, the paw is further supinated by 90^0 and ventroflexed to present the food to the mouth. (10) Release: the digits are opened and the food transferred to the mouth.

Five successful reaches were analyzed frame-by-frame from the videotapes. Each movement component was rated on a one-point scale. If the movement appeared normal, it was given a score of "0"; if it appeared slightly abnormal but recognizable it was given a score of "0.5"; and a score of "1" was assigned if the movement was absent or completely unrecognizable.

Tray reaching

If the rat made a reaching movement (forepaw inserted through the bars, but no food was grasped or the food was dropped), the movement was scored as a "reach", whereas if the rat obtained the food and consumed it, the movement was scored as a "hit". Success was calculated as follows:

Success percent = ("hit" / "reach + hit") X 100

Statistical analysis

Analyses of variance (ANOVA) were used for all measures and Fisher's LSD (P<0.05 or better) was used for *post hoc* evaluations.

Histology

Animals were sacrificed using a lethal dose of sodium pentobarbital. They were intracardially perfused, first with saline in 0.1 M PBS followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed, post-fixed and cryoprotected in a solution of 30% sucrose in 4% paraformaldehyde solution for three days in 4°C. All brains were then sectioned into 40 μm using a cryostat (2800 Frigocut, Reichert-Jung). Every seventh section from the control and lesion groups was mounted into glass slides and stained for cresyl violet.

Infarct measurements

Images of mounted cresyl violet-stained at standardized levels (6 different planes, Fig 7.1) were captured digitally. The cross-sectional area of each hemisphere was measured using the NIH Image software, Ver.1.62. The data was expressed as a percentage of the intact side of the brain.

Procedures and time-line

Rats were trained to reach for food in the single pellet reaching task for two weeks. Their performance was video recorded on the last day, which served as the preoperative baseline. The animals were then divided into three groups such that the mean success score was similar for the three groups: control (n = 8), motor cortex devascularization (n = 8), and MCA occlusion (n = 8). After surgery, the groups were tested in the single pellet task on postoperative days 1, 3, 7, 14, and 30. Cylinder testing was conducted on postoperative days 1, 3, 7 and 14. Rehabilitation in the tray reaching task began on postoperative day 15 and lasted for 8 days. Rats were given access to the tray reaching apparatus for one hour in the morning and one hour in the afternoon. On the last day, rats received a 10 min test in the tray reaching task and their performance was video recorded. They were retested and video recorded in the single pellet task on postoperative day 30. At the completion of the behavioral testing, four animals from each group underwent ICMS motor mapping.

3. Results

Infarct size

Infarct size was determined by measuring areas of non-infracted tissue in both the damaged and the undamaged sides of the brain. Measures are represented as percentage of the normal hemisphere. When the infarct size was calculated as a ratio of the intact hemisphere, a simple ANOVA revealed that overall animals with lateral frontal damage had larger lesions than animals with motor cortex damage [$F(1,14) = 9.19, P < 0.05$; Motor = 91.35 $\pm$ 1.19 %; Lateral Frontal = 82.42 $\pm$ 2.29 %]. Figure 7.1 illustrates an example of an animal with motor cortex damage and an animal with lateral frontal cortex

damage produced by the occlusion of the MCA.

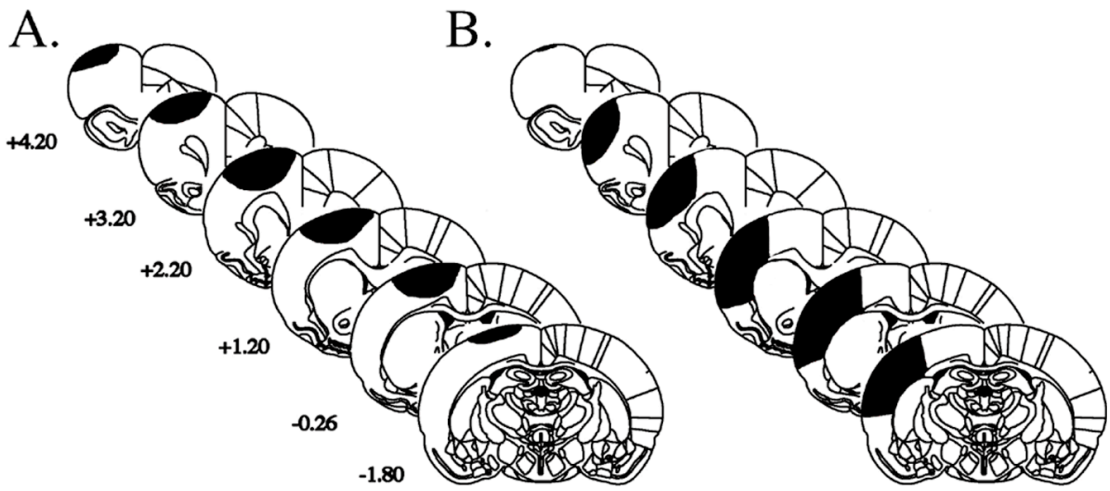


Figure 7.1: Representative diagrams illustrating the six different planes where the measurements for estimating the infarct size were taken. (A) Illustrates a typical infarct after motor cortex lesion. (B) Illustrates a typical infarct after lateral frontal cortex damage.

Cylinder test

All groups actively explored the cylinder and they reared and supported their body against the walls with their forelimbs (Fig 7.2 A). The asymmetry score of forelimb use was calculated for each group and it showed that both types of lesions produced an impairment. Control animals placed both forepaws on the cylinder wall during vertical exploration whereas animals with lesions relied on the paw ipsilateral to the lesion and had fewer contacts with the contralateral paw. A repeated measures ANOVA showed a significant effect of lesion group [$F(2,21) = 22.02$, $P < 0.01$], no main effect of test day, [$F(3,21) = 1.86$, $P > 0.05$], nor an interaction of group by day [$F(6,21) = 1.12$, $P > 0.05$] (Fig 7.2 B). Follow-up tests (Fisher's LSD) showed that the control group differed form

the motor cortex and the lateral frontal cortex group ($P < 0.01$), which in turn did not differ between themselves ($P > 0.05$).

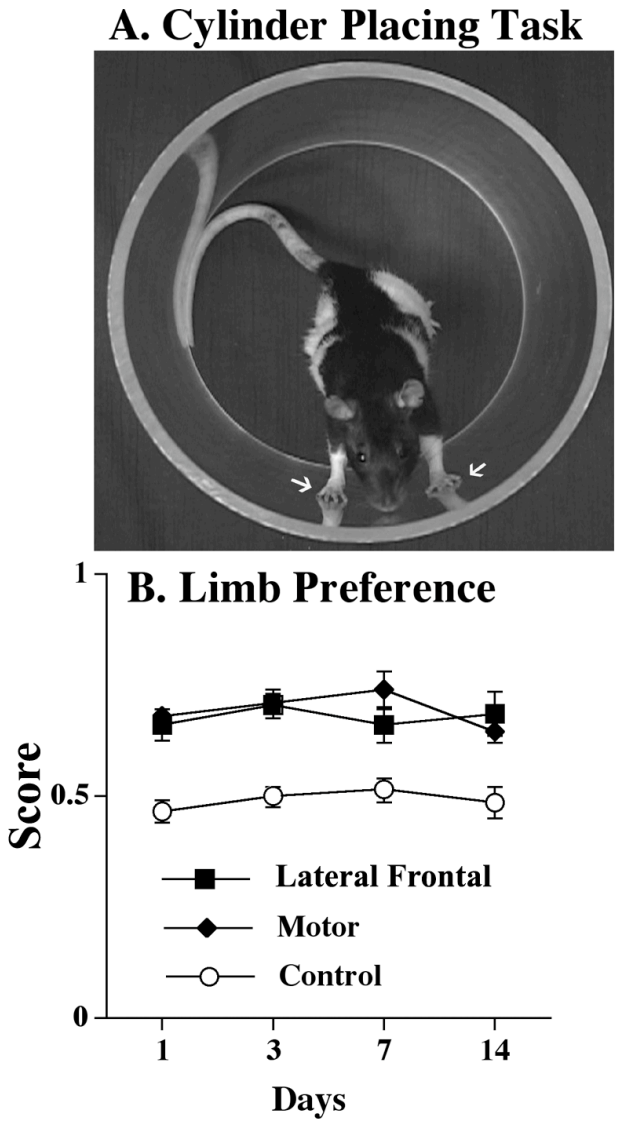


Figure 7.2: Top panel shows a picture of a control animal in the cylinder. Arrows show use of both forepaws for support during rearing. Bottom panel shows performance on this task before the lesion (BL) and on days 1, 3, 7, and 14 post-stroke (mean ± SE). A preference score of 0.5 indicates no preference on the use of left or right forepaw, a score of 1 indicates complete use of the ipsilateral forelimb. Note that both groups relied more on the ipsilateral forelimb for postural support during vertical exploration.

Single pellet reaching (Quantitative)

Animals learned to reach for the food pellets (Fig 7.3 A) and before the lesions all animals achieved a baseline of at least 50% success. Animals were tested 1, 3, 7 and 14 days after the lesions and their performance was analyzed. Two kinds of analysis were performed: total success and success on first reach. When total success was analyzed, a repeated measures ANOVA showed a significant effect of group [$F(2,21) = 6.21$, $P < 0.01$], test day, [$F(3,21) = 8.33$, $P < 0.01$], but no significant interaction [$F(6,21) = 2.11$, $P > 0.05$] (Fig 7.3 B). Follow-up tests (Fisher's LSD) showed that the control group differed form the motor and the lateral frontal groups ($P < 0.05$), which in turn did not differ between themselves ($P = 0.14$). When success on first reach was analyzed, a repeated measures ANOVA showed a significant effect of lesion group [$F(2,21) = 4.71$, $P < 0.05$), test day, ($F(3,21) = 4.25$, $P < 0.01$], but not an interaction [$F(6,21) = 1.85$, $P > 0.05$] (Fig 7.3 C). Follow-up tests (Fisher's LSD) showed that the control group differed form the motor cortex and the lateral frontal groups ($P < 0.05$), which in turn did not differ between themselves ($P > 0.05$). Subsequent to training in the tray-reaching task no significant differences in total success and first reach success were detected among the groups: [$F(2,21) = 1.64$, $P > 0.05$ and $F(2,21) = 1.46$, $P > 0.05$] respectively.

Single pellet reaching (Qualitative)

A summary of scores for component movements for motor and lateral frontal cortex groups on days 1 and 30 are illustrated in Figures 7.4 A and 7.4 B respectively. A repeated measures ANOVA showed a significant effect of lesion group [$F(2,21) = 29.52$, $P < 0.01$], test day, [$F(3,21) = 18.89$, $P < 0.01$], and an interaction of group by day [$F(6,21) = 17.25$, $P < 0.01$] (Fig 7.4 C). Follow-up tests (Fisher's LSD) showed that the control group scored better (lower) than both lesion groups ($P < 0.01$). Animals with

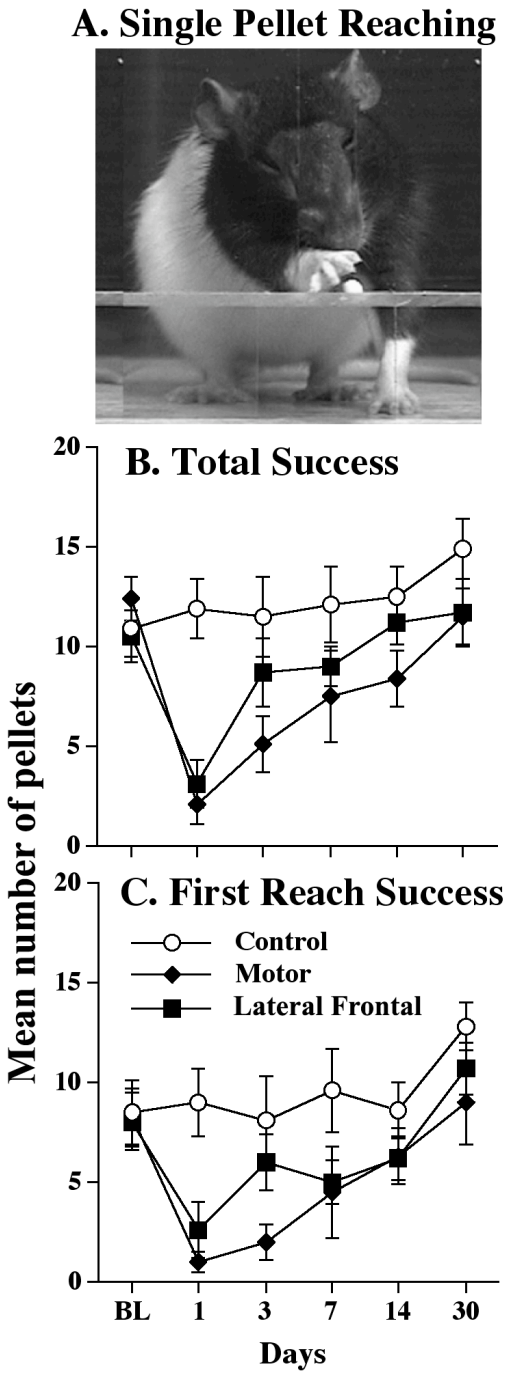


Figure 7.3: (A) Single pellet-reaching task: a rat reaches through a slot for a single food pellet located on a shelf. (B) Total reaching success and (C) first reach success in the single pellet task (number of pellets retrieved out of 20 ±SE) for control, motor cortex lesion (motor) and lateral frontal cortex (lateral frontal) groups. Note that before the lesion (BL) all groups were equally successful in obtaining pellets in both measures. Both lesion groups were significantly different than controls on days 1, 3, and 7 post-lesion. On day 30, after training in the tray-reaching task animals in both lesion groups scored comparable to controls.

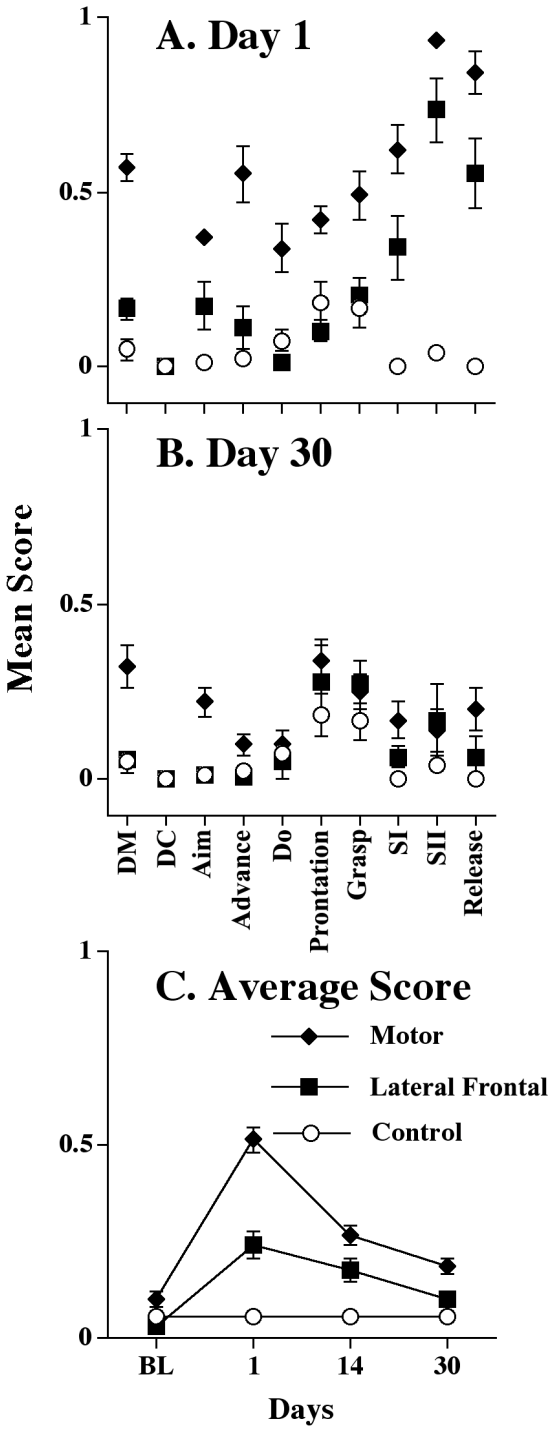


Figure 7.4: Qualitative scores of ten elements comprising a reach on post surgical days 1 (A) and 30 (B). Note that even at 30 days post-lesion animals with motor cortex damage displayed deficits in some of the components of the reach. (C) Qualitative scores of single pellet reaching movements before the lesion (BL) and on days 1, 14 and 30 after the lesions. Digits to the midline (DM); Digits close (DC); Aim; Advance; Digits open (DO); Pronation; Grasp; Supination I (SI); Supination II (SII); Release.

lateral frontal lesions scored better (lower) than animals with motor cortex lesions ($P < 0.01$). With recovery time, and after tray training, there were substantial improvements in both the motor cortex and lateral frontal cortex groups, but residual deficits could still be observed in the animals with motor cortex lesions.

The analysis of day 1 performance indicated that animals with motor cortex lesions were impaired in all the elements that comprise a reach. By day 30 they showed significant recovery although there were persistent impairments in the lifting, aiming, and advance components of the reach and in supination of the paw upon withdrawal and in releasing the food to the mouth. Some of the movement impairments that contributed to their poor qualitative scores are illustrated in Figure 7.5. As the animals approached the slot in order to locate the food pellet, rather than pointing the nose at the food pellet, as did the control rats, they sniffed in an opposite direction to the location of the pellet (Fig 7.5 a). The motor cortex lesion rats also failed to use a normal supporting posture of the contralateral-to-reach forelimb and its ipsilateral hind limb. Rather they used the wall of the cage as a crutch in order to support their body weight (Fig 7.5 b,c). The contralateral-to-reach forepaw crossed the midline of the body (and slot; Fig 7.5 d) instead of adopting a normal lateral position. Once the animals grasped a food pellet, they shifted the weight of their forequarters onto the grasping limb. Thus, once they withdrew it from the slot, the forelimb and the paw grasping the food pellet dropped to the floor (Fig 7.6 a). They were also impaired in supinating the paw and oriented their snout to the back of the grasping paw (Fig 7.6 b).

Animals with lateral frontal cortex damage also displayed poor alignment of the nose to the food pellet (Fig 7.5 e) and displayed a wider base of support in the hind

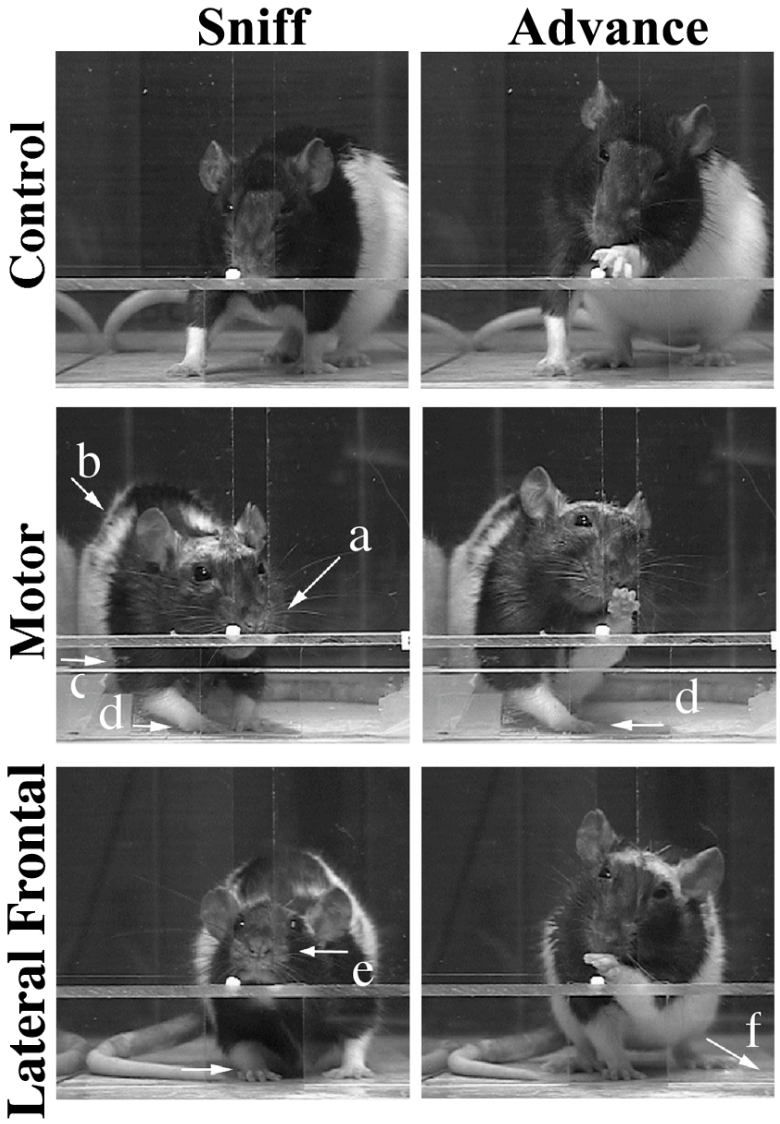


Figure 7.5: Illustration of some of the early components of the reach (Sniff and Advance) by a control rat (top), a motor cortex lesion rat (middle), and lateral frontal cortex lesion rat (bottom) 7 days after the lesions. Note (arrows) that in the motor cortex animals the sniff of the pellet is opposite to the food, their body rests against the wall of the box and the forepaw contralateral to the reaching paw is crossed to the midline. Animals in the lateral frontal cortex group also exhibited deficits orienting to the pellet and in supporting their weight with their hind limbs

limbs, typified by a more lateral placement of the good hind limb (Fig 7.5 f). They were also impaired in retrieving the food pellet from the paw after grasping. Rather than supinating the paw in order to bring the pellet to the mouth, the rat's mouth came in contact with the dorsal surface of the paw (Fig 7.6 c). On some occasions, consumption occurred because the pellet was dropped and retrieved from the floor. The rats were also impaired in using the contralateral forepaw to assist the good forepaw (Fig 7.6 d).

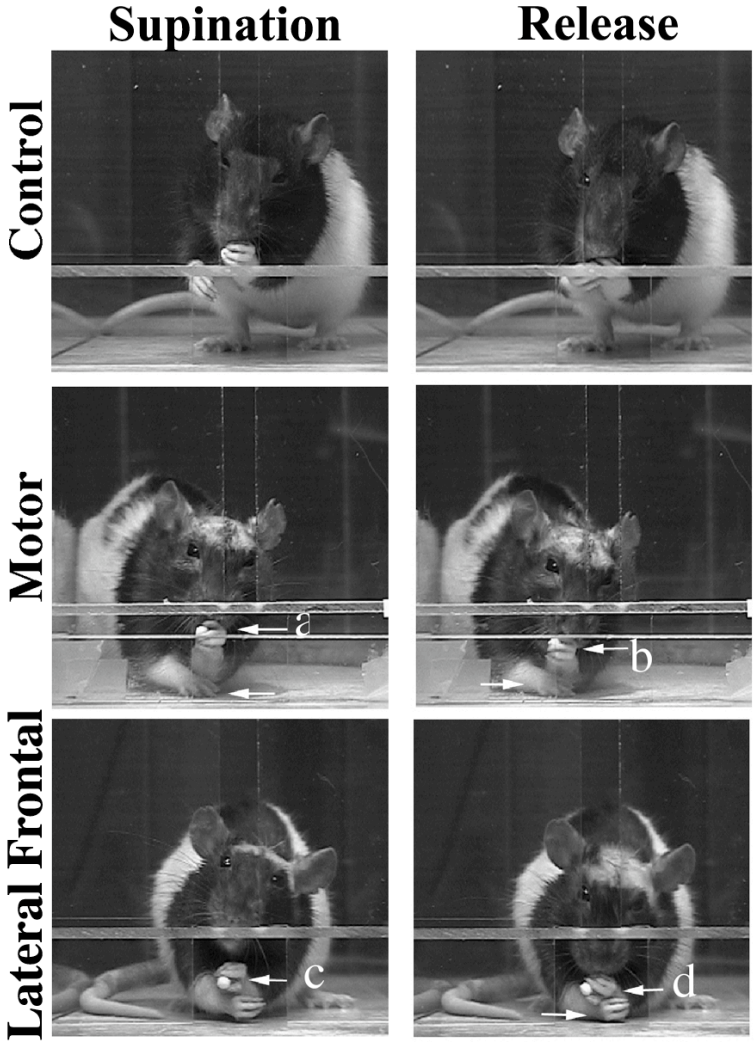


Figure 7.6: Illustration of the late components of the reach (Supination II and Release) by a control rat (top), a motor cortex lesion rat (middle), and lateral frontal cortex lesion rat (bottom) 7 days after the lesions. Note (arrows) that animals in both groups have to drag the paw through shelf to retrieve the pellet and the release is not helped by the contralateral forepaw.

Tray Reaching

All animals quickly learned to reach through the bars and successfully retrieved the chicken food pellets. At the end of the seven days of training (day 22) animals were videotaped for 10 minutes and their performance analyzed. Lesion animals performed to similar levels of accuracy as controls, which is supported by a simple ANOVA ($F(2,21) = 1.46$, $P = 0.25$; Control = 69.5 $\pm$ 1.8 %, Motor = 66.3 $\pm$ 3.7 %, Lateral Frontal = 62.4 $\pm$ 2.8 %).

Intracortical microstimulation

Movements elicited contralateral to the stimulation were recorded and the means and standard errors for the area representation of CFA and RFA of the intact hemisphere are shown in Figure 7.7 D. All groups displayed contralateral forelimb movements at similar thresholds (data not shown). An ANOVA showed no significant differences in the representation of CFA [$F(2,9) = 1.02$, $P > 0.05$], nor of RFA [$F(2,9) = 0.247$, $P > 0.05$] among the injured and control groups. This result suggests that the electrophysiological properties of the forelimb cortical area of the intact motor cortex (the one supporting the skilled movements) remained functional after either lesion. Representative motor maps for control, motor cortex lesion and lateral frontal lesion are shown in Figures 7.7 A, 7.7 B, and 7.7 C.

4. Discussion

It is well known that unilateral motor cortex lesions produce impairments in movements of the contralateral side of the body. Nevertheless, there are anecdotal reports of ipsilateral motor impairments, especially in the performance of skilled movements.

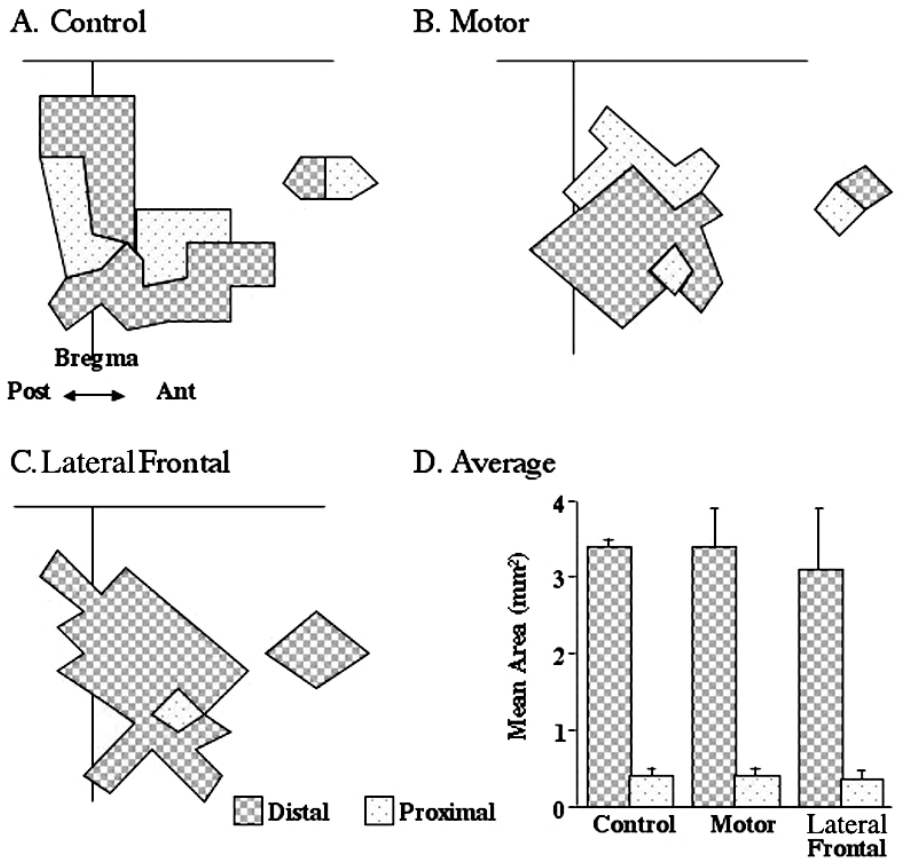


Figure 7.7: Representative maps of control (A), motor cortex lesion (B), and lateral frontal cortex lesion (C) showing distal (digit and wrist) and proximal (elbow/shoulder) areas. (D) Mean are (mm^2) of movement representations.

The objective of this study was to assess the effects of frontal cortex injury on ipsilateral movements; i.e., movements mainly controlled by the intact hemisphere. Forelimb use was evaluated by spontaneous limb preferences in exploration, as well as in quantitative and qualitative measures of skilled reaching for food. Plastic changes were assessed in the intact hemisphere by intracortical microstimulation (ICMS) mapping. Although the intact hemisphere displayed normal rostral and caudal forelimb motor maps, there were enduring deficits in both quantitative and qualitative measures of skilled reaching. Similar acute and chronic impairments followed both motor cortex lesions and lateral frontal cortex lesions that spared the motor cortex. This demonstration that cortical injury

can impair ipsilateral-to-lesion limb skilled movements suggests that these movements normally involve some degree of bilateral cortical control not seen in more reflexive limb use.

Some evidence suggests that recovery/compensation may be mediated by intact tissue surrounding the injury (Jenkins and Merzenich 1987; Stroemer et al., 1993; Castro-Alamancos and Borrel, 1995; Nudo et al., 2001 and Nudo 2003). Other evidence suggests that changes in other brain areas, including the intact hemisphere, mediate compensation/recovery (Jones and Schallert 1992; Schallert et al., 1997; Jones 1999; Biernaskie and Corbett 2001; for reviews see Witte et al., 1997; Nudo 2003). That the intact hemisphere may be involved in compensation/recovery is supported by findings of time-dependent metabolic and neural changes in that hemisphere (Jones and Schallert 1992, 1994; Szele et al., 1995; Napieralski et al., 1996; Jones 1999; Uryu et al., 2001). In fact, there is evidence showing that small electrolytic lesions to the motor cortex induce plastic processes in the intact hemisphere and these processes could be activated to such an extent that the hemisphere becomes functionally supernormal, supporting an increase in movement skill in its contralateral forepaw (Bury and Jones 2002; 2004). The idea is that these plastic changes in the undamaged hemisphere enhance motor function under the control of that hemisphere. Nevertheless, there is substantial, but perhaps more anecdotal, evidence in animals and humans indicating that bilateral motor deficits can follow unilateral damage (see Table 1). Such findings suggest that the plastic changes in the intact hemisphere may be compensatory processes that include that hemisphere's function instead of (or in addition to) functions associated with the lost tissue of the other hemisphere. It was this possibility that was examined in the present experiments. Using

lesions made to the motor cortex and "control" lesions to the lateral frontal cortex, the function of the intact hemisphere was assessed by tests of spontaneous limb use and skilled limb use. To ensure maximum recovery, animals were studied for two weeks following surgery and were then given additional training in a less demanding reaching task (Whishaw et al., 1986; Vergara-Aragon et al., 2003) before being reassessed. To explore if plastic changes occurred in the intact hemisphere, the motor cortex was mapped with ICMS.

The surprising finding of the present study was that there were severe and enduring deficits in skilled reaching in the ipsilateral forelimb following both motor cortex and lateral frontal cortex damage. This finding is consistent with multiple reports of patients showing persistent loss in strength, speed, and difficulty in making complex movements with the ipsilateral arm after a unilateral stroke and with similar incidental reports in animals (see Table 1). The impairment in skilled reaching could stem in part from shock or postural abnormalities, but its persistence suggests that it is more likely related to the complexity of movements required for skilled reaching. This idea is further supported by the contrasting results obtained in spontaneous limb use in the cylinder task, which seemingly demonstrated normal use of the ipsilateral limb for more reflexive support during vertical exploratory movements. Further evidence for the idea that it is task complexity that involves the ipsilateral limb was the finding that intensive training in the tray-reaching task facilitated compensation/recovery although it did not completely ameliorate the impairment.

Nevertheless, there was also significant recovery as seen in improvements in both quantitative and qualitative scores. This suggests that the plastic changes reported in the

Table 1. Published studies in rats and humans reporting motor impairments with the ipsilateral limb after unilateral brain damage.

Species	Type of injury	Reference
Rat	Cortex	Montoya et al., 1991; Price and Fowler, 1981
	Basal Ganglia	Dobrossy et al., 2000; Dunnett et al, 1988; Jeyasingham et al., 2001; Miklyaeva et al., 1994; Montoya et a;., 1990; Olsson et al., 1995; Whishaw et al., 1987
	Stroke	Butovas et al., 2001; Cregan et al., 1997, Grabowski et al., 1993; Hudzik et al., 2000; Marston et al., 1995; Moyanova et al., 2003; Sharkey et al., 1996; Virley et al., 2000
	Other	Henderson et al., 1999
Human	Stroke	Andrews and Bohannon, 2000; Baskett et al., 1996; Brodal, 1973; Carey et al., 1998; Debaere et al., 2001; Desrosiers et al., 1996; Dickstein et al., 1993; Fisk and Goodale, 1988; Haaland and Delaney, 1981; Haaland and Harrington, 1989; Hanna-Pladdy et al., 2002; Hermsdorfer and Goldenberg 2002; Hermsdorfer et al., 1999 a,b; Jones et al., 1989; Laufer et al., 2001; Marque et al., 1997; Pohl et al., 2001; Steenbergen et al., 1996; Sugarman et al., 2002; Sunderland 2000; Sunderland et a., 1999; Swinnen et al., 2002; Titianova and Tarkka, 1995; Winstein and Pohl, 1995; Winstein et al., 1999; Yelnik et al., 1996

intact hemisphere (Jones and Schallert, 1992; Witte et al., 1997; Jones, 1999; Gonzalez and Kolb 2003) may be mediating the recovery of the ipsilateral side of the body instead of (or in addition to) the contralateral side of the body. That the plasticity is related mainly to the function of the intact hemisphere's movements is suggested by the finding that the motor map of the intact hemisphere was largely unchanged. Substantial evidence suggests that the size and conformation of the motor map reflects plastic processes related to behavior (Nudo et al., 1996; Kleim et al., 1998), and therefore, if the intact hemisphere assumed functions largely controlled by the damaged hemisphere, more

dramatic changes in the map should be expected. Of course, it is possible that because the mapping portion of the experiment was performed at the apex of recovery/compensation, map changes had dissipated (Friel et al., 2000; Nudo and Milliken, 1996). Although we only looked for movements of the forelimb contralateral to the electrical stimulation, bilateral movements elicited by ICMS after aspiration of the motor cortex have been reported (Kartje-Tillotson and Castro, 1985; Emerick et al., 2003). It is possible then that lesions to the motor or the lateral frontal cortex, although did not change the overall CFA and RFA representations on the intact hemisphere, have produced overall electrophysiological alterations beyond the CFA and RFA representations.

Previous work has shown that skilled reaching performance in the single pellet task improves with training on a simpler reaching task (Vergara-Aragon et al., 2003). To maximize performance on the single pellet task, here animals received two hours a day of training in the tray task for one week. Although significant improvements were observed when returned to the single pellet task, detailed inspection of the movement components revealed persistent deficits. The improvement in the rats with motor cortex lesions was found in posture, in the supination of the forelimb, and in releasing the pellet to the mouth.

One of the strengths of the present study was the use of qualitative measures of skilled limb use in addition to assessments of success. Because skilled reaching for food is a complex act, measures of success can be dissociated from measures of how the limb is used. That is, following an injury it is possible that an animal can recover its ability to grasp food (Whishaw et al., 1991; Whishaw, 2000), while still being unable to recover normal movements. Just such a dissociation may be responsible for reports of normal or

improved performance on end point measures that have been used to assess performance (Bury and Jones 2002; 2004). An additional strength of the present study was the examination of more reflexive limb movements in juxtaposition to skilled limb movements. This examination confirmed that paw placing by the ipsilateral limb during rearing in a small cylinder appeared unaffected by the lesions at the same time that pronounced impairments occurred in skilled reaching. Thus, together, these findings suggest that assessment of the undamaged hemisphere's contribution to behavior requires a comprehensive movement evaluation.

It is interesting that damage to the lateral frontal cortex was also associated with impairments in use of the ipsilateral limb (although this impairment was not as severe as the one seen with motor cortex lesions). This area of the neocortex represents the face region of sensorimotor cortex (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Neafsey et al., 1986; Remple et al., 2003) and has not previously been implicated in skilled forelimb movements. Nevertheless, the lateral frontal and not the motor cortex per se, is the region of the cortex that is usually damaged following middle cerebral artery (MCA) stroke in rats and this form of stroke is reported to be associated with contralateral limb impairments on a wide variety of tests. Thus, the present findings suggest that lateral frontal cortex can contribute to forelimb control.

In conclusion the present results highlight the significance of the ipsilateral motor system to skilled movements and are consistent with many reports in both humans and animals indicating that skilled movements are under bilateral control. The findings should serve as a cautionary note against using the performance of the ipsilateral-to-lesion paw as a "control" for contralateral-to-lesion paw performance.

Chapter 8

Subcortical middle cerebral artery (MCA) ischemia abolishes the digit flexion and closing used for grasping in rat skilled reaching.

Modified from a paper published in *Neuroscience* 137(4):1107-18, by Omar A. Gharbawie, Roland N. Auer, Ian Q. Whishaw in 2006

Abstract

That rats reach for and grasp a food item using a single paw has prompted their use in neurobiological studies of skilled movements and modeling neural injury including middle cerebral artery (MCA) stroke. Although motor system lesions have been shown to disrupt various qualitative aspects of the transport of a limb to a food target and withdrawal of the limb with the food, no lesion has been found to abolish digit flexion for grasping. Here, rats received unilateral transient MCA ischemia that was restricted mainly to subcortical tissue of the forebrain (caudate-putamen, globus pallidus, and associated fibers) or a sham operation. Both paws were later trained and evaluated on skilled reaching using a rating scale for digit use. MCA rats did not flex and close their digits to grasp food when using their contralateral-to-lesion limb. The grasp impairment was not due to a failure to learn the task as MCA rats used the ipsilateral limb as successfully as control rats and they were reinforced for reaching by raking food into the test box using an open paw. The impairment was also not due to an inability to move the digits, as they were flexed and closed in other phases of the reach. The results are discussed in relation to the idea that digit closure may be controlled by the joint action of a number of neural systems that converge in the basal ganglia and so are susceptible to injury via MCA stroke.

1. Introduction

Rats are skilled in the use of their forelimbs when handling food (Whishaw et al., 1992b, Whishaw and Coles, 1996) and have been shown to be able to reach for and grasp food with a single forelimb in a number of laboratory tasks (for a review, Whishaw, 2005). The transport, grasp, and withdrawal movements of limb use in reaching provide insights into the evolution of skilled movements, their neural organization, and also provide animal models of a number of neurological conditions (for a review Whishaw and Miklyaeva, 1996). For example, reaching for and grasping food has been used as an analogue of human reaching in studies of Parkinson's disease (Dunnett et al., 1986, Miklyaeva et al., 1994), Huntington's chorea (Fricker-Gates et al., 2003), middle cerebral artery (MCA) stroke (Marston et al., 1995, Colbourne et al., 2000, Biernaskie and Corbett, 2001, Chen et al., 2002, Gonzalez and Kolb, 2003, Lindner et al., 2003, Gharbawie et al., 2005), concussion (Whishaw et al., 2004) and spinal cord injury (McKenna and Whishaw, 1999). A profile of the movement components and the movement sequence of limb transport towards a food target, grasping, and limb withdrawal with the food shows that the acts of transport and withdrawal mainly use movements of the upper arm, whereas pronation and grasping use more distal musculature (Whishaw and Pellis, 1990). Nevertheless, these actions are complex as most limb musculature is active during most phases of the reach (Hyland and Jordan, 1997). Furthermore in the many tasks that have been developed for the study of rat skilled reaching, the limb movements displayed by the animals in the different tasks are very similar (Peterson, 1934, Castro, 1972a, Whishaw et al., 1986, Whishaw and Pellis, 1990, Montoya et al., 1991, Ballermann et al., 2001).

Despite an interest in paw use in skilled reaching, few studies examined the use of the digits in skilled reaching (Castro, 1972b, Castro, 1972a). Subsequent work using high-speed filming or video analysis shows that the digits make a number of movements during skilled reaching. When the limb is lifted to initiate reaching, the digits are loosely flexed, the digits extend as the paw is advanced towards the food target, and the digits make an arpeggio movement in which digits five (outer digit) through digit two are successively placed over and around the target to grasp (Whishaw and Gorny, 1994). After the limb is withdrawn, the digits are extended to release the food to the mouth. Although arpeggio and digit extension to release food to the mouth are impaired by some motor system lesions, the grasp response survives corticospinal (Whishaw et al., 1991, Whishaw et al., 1992b, Whishaw et al., 1993, Z'Graggen et al., 1998, Weidner et al., 2001, Gilmour et al., 2004, Gharbawie et al., 2005, Metz et al., 2005), caudate-putamen (Pisa, 1988), red nucleus (Whishaw and Gorny, 1996), corticospinal and red nucleus (Whishaw et al., 1998), entire neocortex (Whishaw and Kolb, 1988), substantia nigra (Dunnett et al., 1986, Miklyaeva et al., 1994), neocortical MCA stroke (Gharbawie et al., 2005), and spinal cord (McKenna and Whishaw, 1999) lesions. The present study describes a fortuitous observation that food grasping can be severely impaired by nonspecific basal ganglia lesions.

In the present study, rats received unilateral transient MCA ischemia that produced subcortical damage in: caudate-putamen, globus pallidus, and associated fibers. MCA rats were later tested with both forelimbs on a single pellet reaching task. Successful performance required rats to advance their forelimb through a slot and grasp a small food pellet on a shelf, and then withdraw their paw and transfer the food into the

mouth. In order to evaluate the effects of the MCA stroke-induced lesion, paw and digit movements were video recorded and using frame-by-frame analysis a rating scale was devised to assess digit movements throughout the course of a reach. After reaching for food testing of both paws, the regions of brain damage were histologically inspected to correlate the locus of damage with grasping impairment.

2. Materials and Methods

Animals

Twelve male Long-Evans hooded rats, 120 days old, and weighing 350-400 g from Charles River were used. The experiments were conducted in compliance with the guidelines of the University of Lethbridge animal care committee and the Canadian Council for Animal Care. Rats were housed in Plexiglas cages in groups of two in a colony room maintained on a 12/12 h light/dark cycle (08:00-20:00 h).

Feeding

Three weeks post surgery the rats were gradually food deprived to 90-95% of their body weight by once a day feeding of Purina rat chow. When reaching, the rats received 45 mg dustless precision pellets (product #F0021, Bioserve Inc., Frenchtown, NJ, USA).

Surgery

Unilateral transient MCA occlusion was achieved with the intraluminal suture procedure (Longa et al., 1989). Rats were intubated under 3-4% halothane in a mixture of 30% O_2 and 70% N_2O. Rats were maintained on a ventilator for the duration of the procedure and halothane levels were adjusted to 1%. The ventral tail artery was cannulated with polythene tubing and connected to a Statham transducer. Blood pressure

was automatically recorded by a PC computer running Labtech software V.12. Blood samples were drawn throughout the surgery for pH and gas analyses.

An incision was made in the midline of the neck. The common carotid artery (CCA) was isolated, then the occipital, superior, and thyroid branches of the external carotid artery (ECA) were isolated and electrocoagulated. A 6-0 silk suture was loosely tied around the ECA and a microvascular clip was temporarily placed at the origin of the ECA. The distal end of the ECA was cut with bipolar electrocoagulation. A hole was cut into the ECA near its origin using fine surgical scissors. A 26-mm long, 3-0 monofilament nylon suture, with a tip tapered on fine grade sandpaper was inserted through the hole cut into the ECA. The silk thread around the ECA and intraluminal suture was tied to prevent bleeding and the vascular microclip was removed. The monofilament was advanced through the ECA, then the ICA, until a faint resistance was encountered along with a sharp drop in blood pressure, indicating the tip of the filament was introduced into the proximal segment of the anterior cerebral artery. Halothane was used to regulate blood pressure at 75 mm Hg. The suture was withdrawn after 80 min and the ECA was permanently ligated and the end of the stump electrocoagulated to prevent bleeding. All wounds were sutured and blood pressure was restored by discontinuing halothane. Sham surgery involved all the above procedures except for advancing the monofilament suture into the ICA.

The present ischemic parameters of blood pressure and duration of MCA occlusion were chosen because similar parameters have been reported to primarily damage subcortical structures and largely spare neocortical areas (Zhu and Auer, 1995). Subcortical structures are primarily irrigated by proximal MCA branches whereas

neocortical zones are irrigated by MCA branches. Transient MCA ischemia under relatively high blood pressure allows collateral blood flow to irrigate the distal branches of the MCA ipsilateral to the occluding suture. This sustains neocortical areas but at the same time is not sufficient to maintain blood flow in subcortical areas irrigated by the proximal branches of the MCA.

Reaching boxes

Single pellet boxes (Whishaw and Pellis, 1990) were made of clear Plexiglas, with the dimensions 45 x 14 x 35 cm. In the center of each front wall was a vertical slit 1 cm-wide, which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-deep shelf. Two indentations on the surface of the shelf were located 2 cm from the inside of the wall and were aligned with the edges of the slit where rats could reach.

Reach training

For initial training sessions, a number of food pellets were placed on the shelf directly in front of the slot where a rat could easily obtain the food pellet with its tongue or forelimbs. Pellets were gradually moved further from the slot to encourage the rat to reach. Once a rat demonstrated preference for a particular forelimb, individual pellets were placed in the indentation contralateral to the preferred limb. After a rat was reliably reaching for food, a short delay preceded the presentation of the next pellet, until the rat walked to the back of the box and then approached the front in order to reposition itself for each next reach.

Bracelets for limb restraint

To force a rat to use its non-preferred limb for reaching, a bracelet made of Elastoplast fabric adhesive tape (Smith & Nephew Inc., Lachine, Quebec) was wrapped around the preferred forelimb (Whishaw et al., 1986). The bracelet prevented the rat from inserting its bandaged paw through the slot of the reaching box. The bracelet did not impede the rat's movement and could be easily slipped off by the experimenter without damaging the rat's forelimb or denuding its fur. Once habituated to the bracelet for two or three reaching sessions, rats generally ignored it and did not attempt to remove it.

Video recording

Reaching performance was video recorded using a Canon ZR 80 MC camcorder (1000th of a second shutter speed) and a cold light source. A Sony videocassette recorder DSR-11 was used for subsequent frame-by-frame analysis. Representative still frames were captured from digital video recordings with Final Cut Pro (www.apple.com).

Reaching success

Rats received 20 pellets/day on all days in which their reaching success was scored. A successful reach is one in which the food was grasped and transferred into the mouth using the designated paw, which was measured using the following formula (Whishaw et al., 1986):

Success percentage = (number of pellets retrieved/20) x 100.

Digit movement rating

During the act of reaching for single food pellets, the digits underwent a number of transitions from being flexed (digits bent towards the palm) to being extended and from being closed (digits adjacent to each other) to opened (digits spread apart). The movements of flex/extend and open/close could occur independent of each other. To

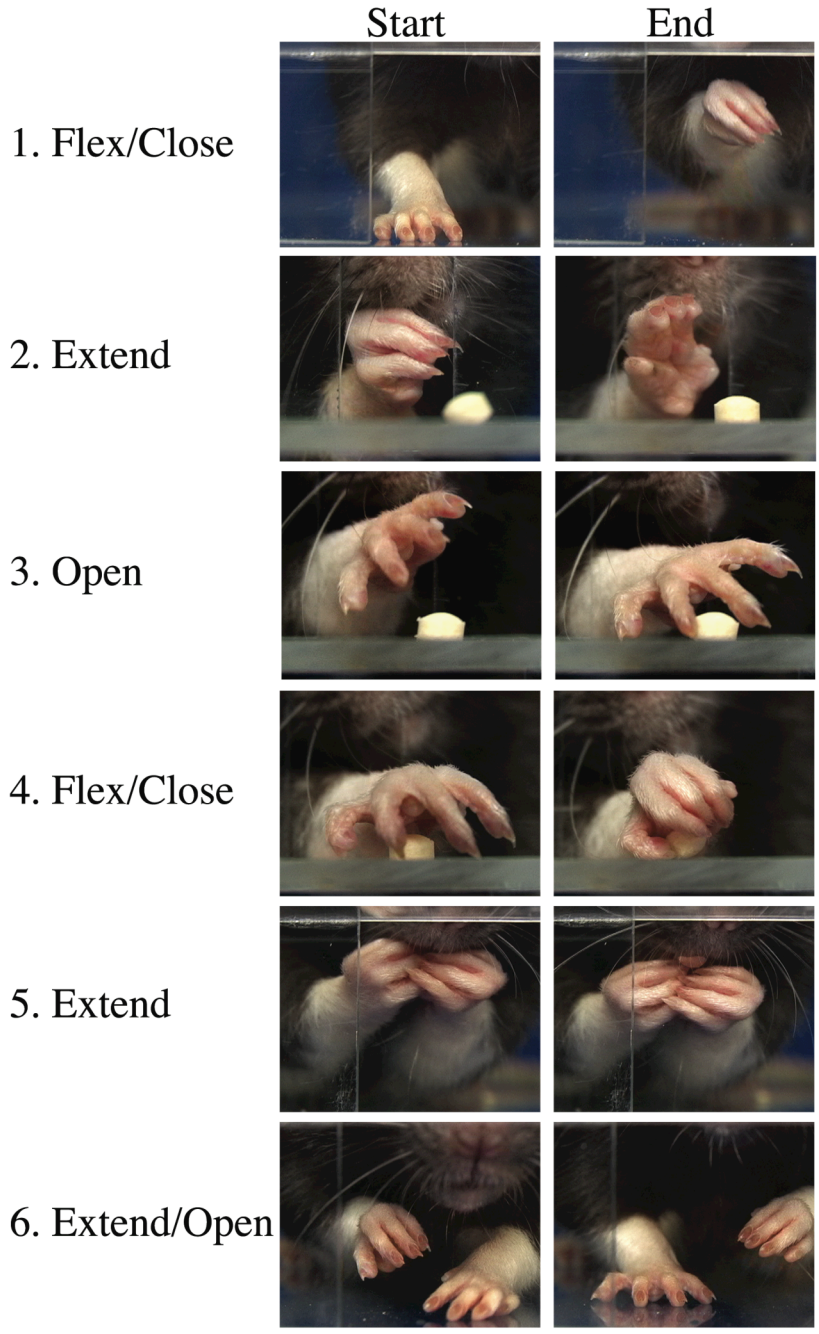


Figure 8.1: Still frames captured from a representative reach by a control rat. The first (Start) and last (End) frames of six independent digit movements.

evaluate digit function, these movements were described sequentially from the time the paw was raised from the floor to initiate a reach until it was replaced after completing the reach (Fig 8.1):

(1) *Digits close/flex*: as the limb is lifted, the digits are semiflexed and closed and the paw is supinated so that the palm faces the midline of the body.

(2) *Digits extend*: the digits extend as the limb is advanced through the slot.

(3) *Digits open*: the digits open as they contact the shelf sequentially from lateral to medial and surround the pellet.

(4) *Digits flex/close*: after the pellet is palpated by the palm or the digits, they flex and close to grasp the pellet. This can occur as an independent movement, or the grasp can occur as the paw is withdrawn.

(5) *Digits extend*: the snout contacts the pellet and the digits extend to release food into the mouth.

(6) *Digits extend/open*: the digits contact the floor from lateral to medial and spread as they contact the floor.

Five reaches were analyzed frame-by-frame on the videotapes by two experimenters blind to rat's group membership. Each component was rated on a three-point scale (Table 1). If the movement appeared normal, it was given a score of "0". If it appeared slightly abnormal but recognizable it was given a score of "0.5"; a score of "1" was assigned if the movement was absent or completely unrecognizable.

Histology

Rats were euthanized with sodium pentobarbital and intracardially perfused with saline (0.9%) and 4% paraformaldehyde. Brains were removed, post-fixed and cryoprotected in 30% sucrose and 4% paraformaldehyde at 4°C for coronal sectioning (40 μm) and cresyl violet staining. Digital images were captured from six planes as measured

Table 1. Digit movement rating scale

Behaviour	Normal	Slightly Abnormal	Impaired
(1) Close/Flex	0	0.5	1
(2) Extend	0	0.5	1
(3) Open	0	0.5	1
(4) Close/Flex	0	0.5	1
(5) Open	0	0.5	1
(6) Extend/Open	0	0.5	1

from Bregma: +1.70, +0.70, -0.30, -0.80, -1.30, -1.80 mm (Fig 8.2). The areas of striatum (caudate-putamen), ventral pallidum, globus pallidus, and neocortex were measured from both hemispheres using NIH image (v. 1.63). Damage was estimated using the formula: Percentage of structure damaged = [(Area contralateral to lesion) – (undamaged area ipsilateral to lesion)]/ (Area contralateral to lesion) X 100.

Statistical analysis

Success scores were analyzed using repeated measures ANOVA. Qualitative scores were analyzed with Mann-Whitney U-test for non-parametric data.

Procedure and timeline

Rats were randomly assigned to the unilateral transient MCA ischemia group (n=7) or sham surgery group (n=5). Two rats in the MCA group died within 24 hrs of surgery. Single pellet training commenced after a three-week recovery period. In the first block, all rats were trained/tested on single pellet reaching for at least 14 days. In the second block, forelimb use was switched to the non-preferred limb. A restraining bracelet was applied to the preferred forelimb at the beginning of the first session to encourage reaching with the non-preferred forelimb. For all subsequent sessions, rats were allowed

to voluntarily reach with their non-preferred forelimb. Bracelets were only applied in the event of an animal reaching with the originally preferred forelimb for at least three pellets within a test session. Training/testing with the non-preferred forelimb lasted for at least 14 days.

The present experimental design was selected based on the assumption that the sham group would not have impairments in either paw. To standardize the order in which the paws were trained/tested after surgery, rats in both groups were allowed to reach for food with either paw in the first block of training/testing. In the second block, both MCA and sham rats had to reach with their "non-preferred forelimb".

Table 2. Physiological parameters

	Stage of MCA ischemia		
	Before	During	End
Sham			
pH	7.51 $\pm$ 0.01	7.51 $\pm$ 0.01	7.49 $\pm$ 0.02
PO_2 (mm Hg)	95.82 $\pm$ 5.23	114.04 $\pm$ 7.74	123.4 $\pm$ 5.81
PCO_2 (mm Hg)	32.2 $\pm$ 1.36	28.44 $\pm$ 0.99	29.48 $\pm$ 1.81
MCA			
pH	7.49 $\pm$ 0.02	7.45 $\pm$ 0.02	7.39 $\pm$ 0.01
PO_2 (mm Hg)	101 $\pm$ 10.16	110.98 $\pm$ 10.19	118.88 $\pm$ 8.05
PCO_2 (mm Hg)	33.58 $\pm$ 2.11	33.6 $\pm$ 1.98	39.16 $\pm$ 0.93

3. Results

Surgical physiological parameters

Blood gases and pH were monitored before, during, and after MCA ischemia (Table 2). Lower pH was recorded for MCA rats [ANOVA: $F(1,8) = 11.50$, $p < 0.01$]. This was largely due to a significant decrease in pH after ischemia [unpaired t-test: $t = -3.81$, $p < 0.01$]. Blood O_2 levels increased equally for both groups throughout the surgery

[ANOVA: F (1,8) = 16.11, $p < 0.01$] but CO_2 levels were elevated only in MCA rats [ANOVA: F (1,8) = 10.62, $p < 0.05$]. A significant group x time interaction was also recorded [ANOVA: F (1,16) = 4.910, $p < 0.05$].

Histology

Fig 8.2 illustrates coronal sections from rats with right MCA occlusion. In three of the five MCA rats the lesion did not produce any obvious injury to the neocortex or allocortex but damaged a region including: the caudate-putamen, the globus pallidus, and the lateral hypothalamus. In the fourth rat, the infarct spread to allocortex and in the fifth rat, the lesion included substantial portions of the lateral neocortex and allocortex. Lesion variability is also summarized in Fig 8.7.

Table 3. Lesion size measurements.

	Percentage of structure damaged		
MCA Rat ID	Caudate / Putamen	Globus Pallidus	Neocortex
21	34.9	38.5	-3.1
4	65.6	71.7	2.1
23	55.4	39.0	1.7
22	67.1	60.1	6.3
12	67.3	46.1	51.8

Fig 8.3 illustrates high magnification photomicrographs of subcortical damage, which was typical of all of the MCA rats except one. The lesion included the neostriatum (Fig 8.3 A, B), ventral striatum (Fig 8.3 A), ventral pallidum (Fig 8.3 A), and lateral globus pallidus (Fig 8.3 C). Areal measurements of neostriatum, ventral pallidum, and lateral globus pallidus indicate extensive damage to the structures ipsilateral-to-lesion as compared to contralateral-to-lesion (Table 3), whereas neocortical damage was extensive

only in one rat. Neural degeneration was evident in numerous zones outside the infracted area such as: subthalamic nucleus (Fig 8.3 D), substantia nigra reticulata (Fig 8.3 E), substantia nigra pars compacta (Fig 8.3 E), cerebral peduncle (Fig 8.3 D, E), and posterior internal capsule.

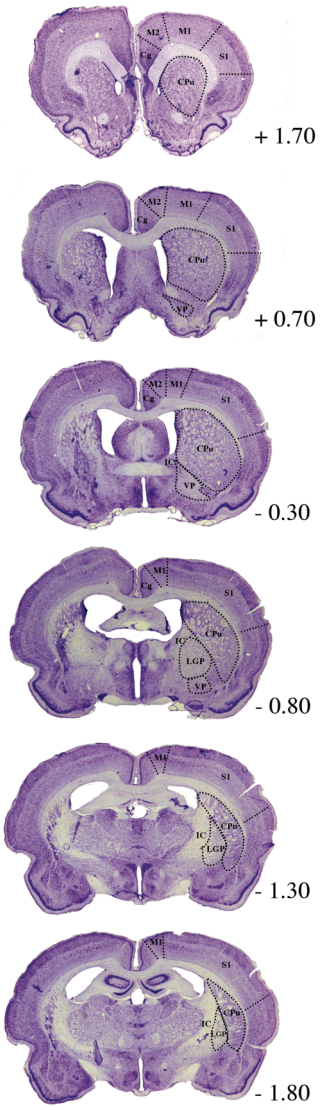


Figure 8.2: Photomicrographs of coronal sections (cresyl violet) of unilateral right middle cerebral artery ischemia. Outline of the caudate-putamen (CPu), ventral pallidum (VP), lateral globus pallidus (LGP), and neocortex were used for aerial measures. Note: damage and shrinkage of subcortical structures and enlarged lateral ventricle on the right hemisphere. Primary motor cortex (M1), secondary motor cortex (M2), primary somatosensory cortex (S1), cingulate cortex (Cg), internal capsule (IC). Section levels from Bregma after Paxinos and Watson (1997).

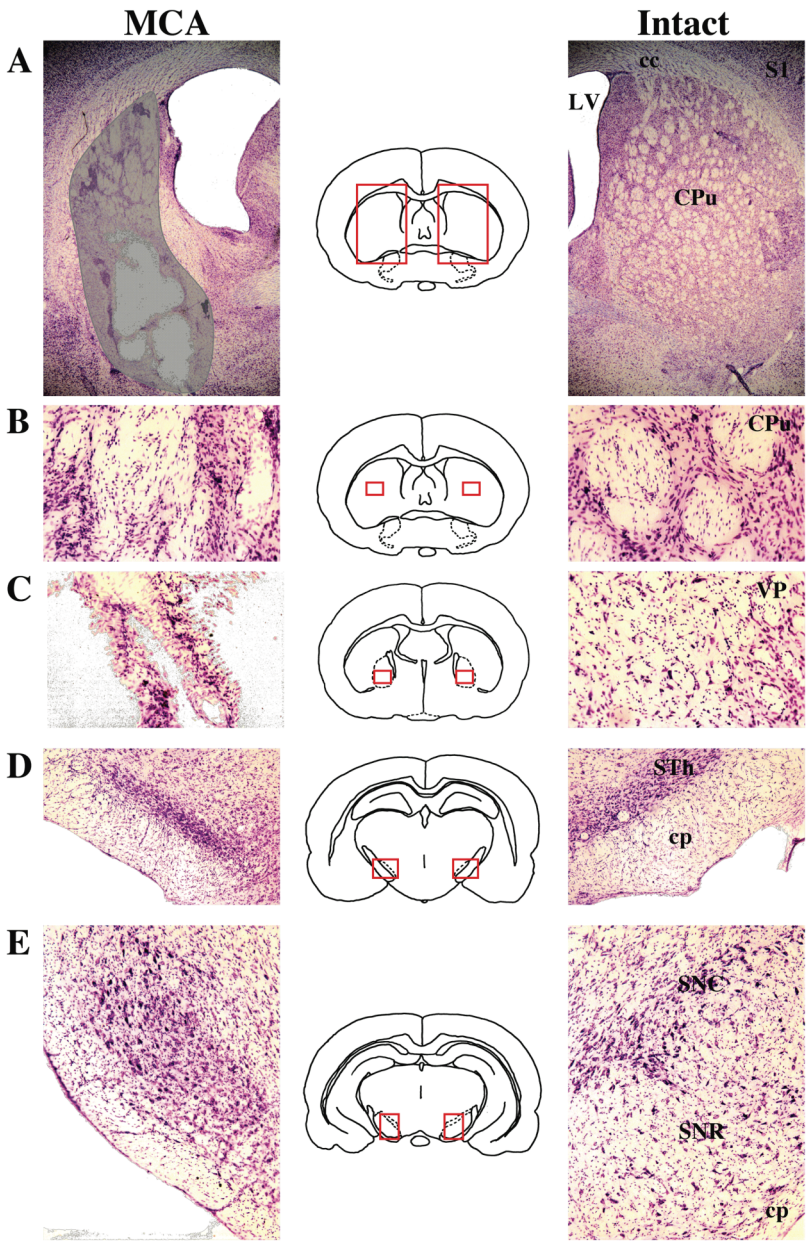


Figure 8.3: Photomicrographs of middle cerebral artery (MCA) ischemia and intact hemispheres. Rectangular box superimposed on schematic diagram specifies the region magnified in the photomicrograph. (A) Left, infarct in neostriatum and ventral pallidum are highlighted in grey (magnification 2.5x). Note, enlarged ventricles. Somatosensory cortex (SC), caudate-putamen (CPu), lateral ventricle (LV), corpus callosum (cc). (B) Striated appearance of neostriatum is disrupted because of damage to both myelinated fibers and medium spiny neurons (magnification 25x). (C) Extensive damage to fusiform neurons and myelinated fibers in lateral globus pallidus (LGP, magnification 25x). (D) Atrophy of of subthalamic nucleus (STh) and cerebral peduncle (cp, magnification 10x). (E) Atrophy of substantia nigra reticulata (SNR) and substantia nigra pars compacta (SNC, magnification 10x).

Skilled reaching

Reaching success

All MCA rats demonstrated a preference for the ipsilateral-to-lesion forelimb. After at least 14 days of training/testing, sham and MCA rats were encouraged to reach with the non-preferred forelimb by restraining the preferred forelimb with a bracelet. Paw preference was switched in sham rats within the first week of training and limb restraint was discontinued thereafter. In contrast, MCA rats were resistant to switch from reaching with the ipsilateral-to-lesion to reaching with the contralateral-to-lesion forelimb. Three MCA rats reached with the ipsilateral forelimb for the first three pellets of each session and often retrieved pellets using this strategy. For these cases bracelets were applied to the ipsilateral forelimb in every session. Even then, these rats attempted to advance their bandaged forelimb through the slot as if reaching for the pellet. Only the tips of the digits entered the slot however because the bracelet obstructed further advance. With the ipsilateral digits on the shelf, rats reached for the pellet with their contralateral forelimb. Limb restraint was discontinued for the other two MCA rats during the second week of training/testing because reaching with the contralateral-to-lesion forelimb was established and rarely did they attempt to reach with the ipsilateral-to-lesion forelimb.

Success scores of the sham rats and the MCA rats were comparable for the preferred and ipsilateral forelimbs respectively as shown by an ANOVA: Group [$F(1,8) = 4.23$, $p > 0.05$]; Days [$F(1,6) = 2.97$, $p < 0.05$, Fig 8.4 A].

MCA rats were severely impaired with the contralateral forelimb as evident by their poor success scores in relation to the sham's non-preferred forelimb, which was confirmed by an ANOVA: Group [$F(1,8) = 10.92$, $p < 0.05$]; Days [$F(1,6) = 0.22$, $p >$

0.05, Fig 8.4 B]. The variance illustrated in the MCA rats is related to the fact that three rats could not grasp and so had no successes, one rat could make partial grasps and so had occasional success, and one rat could grasp. All of the rats could insert their paw through the slot to contact the food pellet, and although failing to grasp, the unsuccessful rats were occasionally able to drag the food through the slot with the palm of the paw.

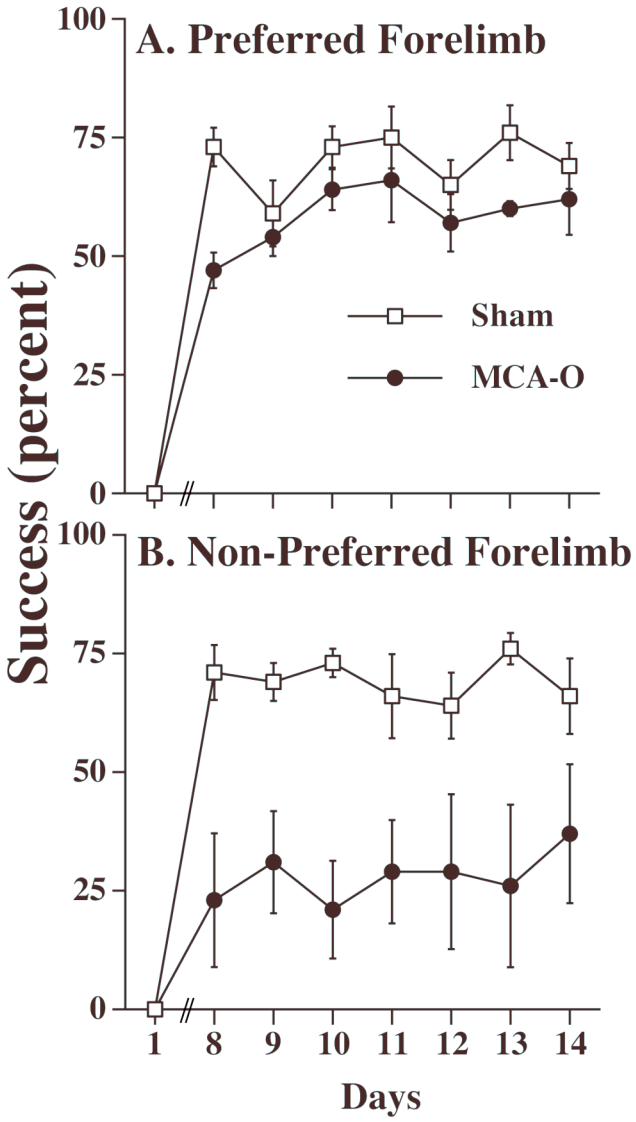


Figure 8.4: Success (mean ± SEM) of skilled reaching for sham and MCA rats assessed on single pellet reaching. (A) Limb ipsilateral to the lesion. (B) Limb contralateral to the lesion. Note: impairment in the contralateral limb.

Digit movement rating

Most of the movements of flexing/extending and opening/closing the digits were intact, but there were selective movement impairments in both paws (Table 4). As illustrated in the following descriptions, using the ipsilateral-to-lesion paw MCA rats were impaired in releasing the food pellet once it was grasped, whereas grasping was impaired while using the contralateral paw.

Table 4. Digit function evaluation

Digit movement	Sham (Both limbs)	MCA (Ipsilateral limb)	MCA (Contralateral limb)
(1) Close/Flex	0	0	0
(2) Extend	0	0	0
(3) Open	0	0	0
(4) Close/Flex	0	0.08 $\pm$ 0.04	0.68 $\pm$ 0.08 *
(5) Open	0	0.52 $\pm$ 0.08 *	0.40 $\pm$ 0.14
(6) Extend/Open	0	0.14 $\pm$ 0.05	0.14 $\pm$ 0.05

* $p < 0.05$

Sham. After orienting to the food pellet by sniffing, the reaching forelimb released contact with the floor. As the limb was lifted and the paw supinated, the digits were closed and semiflexed with tips aligned to the midline of the body. As the limb advanced through the slot, the digits extended, and as the paw was pronated/lowered onto the food pellet, the digits were opened. Once the food pellet was palpated with the digits or pads of the palm, the digits flexed and closed to grasp the food pellet. The limb withdrew and the paw was supinated, and the digits extended to release food into the mouth. Then the digits further extended and opened as they successively contacted the floor. There were differences in the digit movements of the ipsilateral paw of the MCA rats and differences in the digit movements of the contralateral paw of the MCA rats.

MCA ipsilateral forelimb. Digit extension and opening during limb transport and digit flexing and closing during grasping (Fig 8.5 A) were similar for the ipsilateral forelimb of the MCA rats and the sham rats. Once the food pellet was withdrawn from the slot, however, the rats failed to supinate the paw and extend the digits to release the food pellet. Rather the limb was flexed medially and the mouth was extended across the wrist (Fig 8.5 B). This resulted in significantly impaired scores on digit extension for

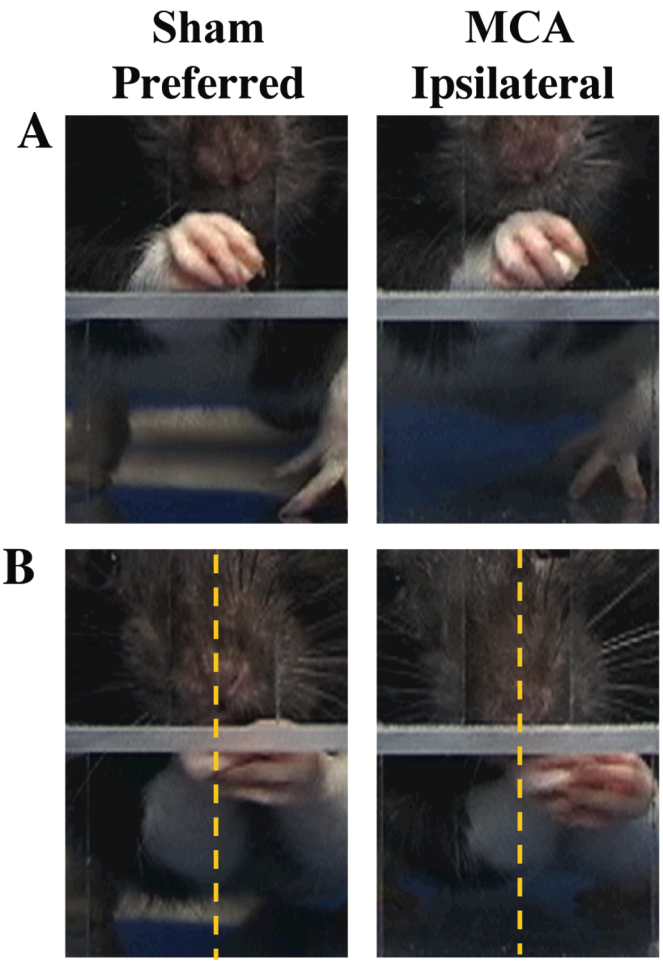


Figure 8.5: Still frames captured from representative reaches by sham (preferred forelimb) and middle cerebral artery (MCA) ischemia (ipsilateral-to-lesion forelimb) rats. (A) Grasping is similar between rats. (B) Supination to present the food to the mouth (supination 2) is normal in the control rat but impaired in the MCA lesion rat. Note: the snout of the MCA rat is oriented to the wrist and not digits.

food release as compared to shams [Mann-Whitney U: $z = -2.35$, $p < 0.05$]. Thereafter, the rats made a variety of twisting movements of the snout and limb in order to align the paw with the mouth to extract the food pellet. After a pellet was successfully grasped, rats used their non-reaching limb for assistance. In the case of MCA rats, the contralateral-to-lesion forelimb was lifted and adducted and the digits semiflexed such that the tips contributed to holding the pellet or supporting the reaching paw (Fig 8.5 B).

MCA contralateral forelimb. Digit extension and opening during transport were comparable between the contralateral-to-lesion forelimb of the MCA rats and sham rats (Fig 8.6 A). Once the digits or palm of the paw contacted the food pellet, however, the MCA rats failed to flex and close their digits to grasp the food pellet, which resulted in knocking the pellet away [Mann-Whitney U: $z = -2.36$, $p < 0.05$, Fig 8.6 B, C]. Rather, when the food pellet was obtained at all, it was hooked with one digit or was palpated with the palm against the shelf and dragged toward the slot. The pellet was raked to within lapping range or dropped onto the floor (Fig 8.6 D) where it could be retrieved by mouth. A significant correlation was detected between grasping impairment and success score [$R^2 = 0.87$, $p < 0.05$].

Relation between grasping impairments and MCA lesion

A comparison between the size/location of the MCA stroke lesion and the grasping impairment in the contralateral-to-lesion paw suggested that the rat with the smallest lesion had a grasp indistinguishable from sham rats (Fig 8.7 A-B). The remaining four MCA rats displayed little to no digit closure or flexion (Fig 8.7 C-F). However, no significant correlation was detected between the percentage of caudate/putamen, globus pallidus, nor neocortex damaged and grasp score.

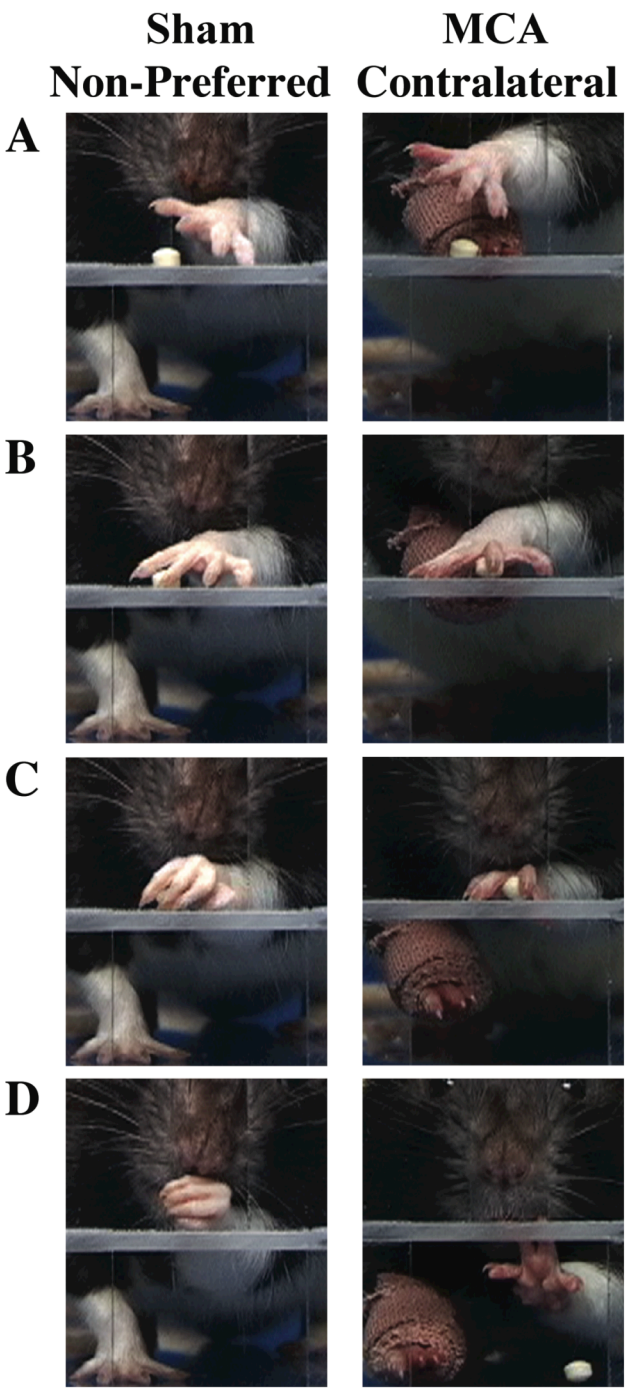


Figure 8.6: Still frames captured from representative reaches by sham (non-preferred forelimb) and middle cerebral artery (MCA) ischemia (contralateral-to-lesion forelimb) rats. (A) The digits extend and open in both sham and MCA rats. (B) The digits flex onto the pellet in the sham rat but remain extended in the MCA rat. (C) The digits further flex in the sham rat to complete pellet grasp but only a slight flex is observed in the MCA rat and the pellet is dragged against the shelf. (D) The digits close and the paw is supinated in the sham rat but the pellet is not grasped and is dropped onto the floor in the MCA rat.

4. Discussion

In rats that received MCA stroke that produced extensive subcortical damage including, caudate-putamen, globus pallidus, and associated fibers a rating scale focused on digit movement revealed impairments in food grasping. For the paw contralateral to the lesion, the movements of flexing and closing the digits to grasp the food pellet were abnormal to absent when the paw reached and contacted the food pellet but the digits moved more normally in other phases of the reach. The digits of the ipsilateral limb were used successfully to grasp the food, but an impairment in supination prevented the rats from extending the digits to present the food to the mouth. The grasping impairment seemed not due to a lack of training, loss of motivation, or inability to move the digits. The results suggest that grasping might be controlled by multiple subsystems within the affected region of the basal ganglia.

Rats were tested on a single pellet reaching task that has previously mainly been used to describe whole limb transport and withdrawal movements used in reaching for food (Whishaw and Pellis, 1990). The study focused on digit movements using a rating scale that disentangles digit movements from any other movements/impairments of the forelimb. The reaching task is optimal for digit analysis in part because it has previously been standardized for studying the neural basis of skilled limb movements and rat models of neurological injury. In addition, it is repetitive and therefore rats receive extensive training and numerous independent reaches are obtained so that individual movement components can be analyzed on separate trials. In keeping with previous work (see introduction), the present study used frame-by-frame video analysis but focused on digit movements in both paws as rats reached for food.

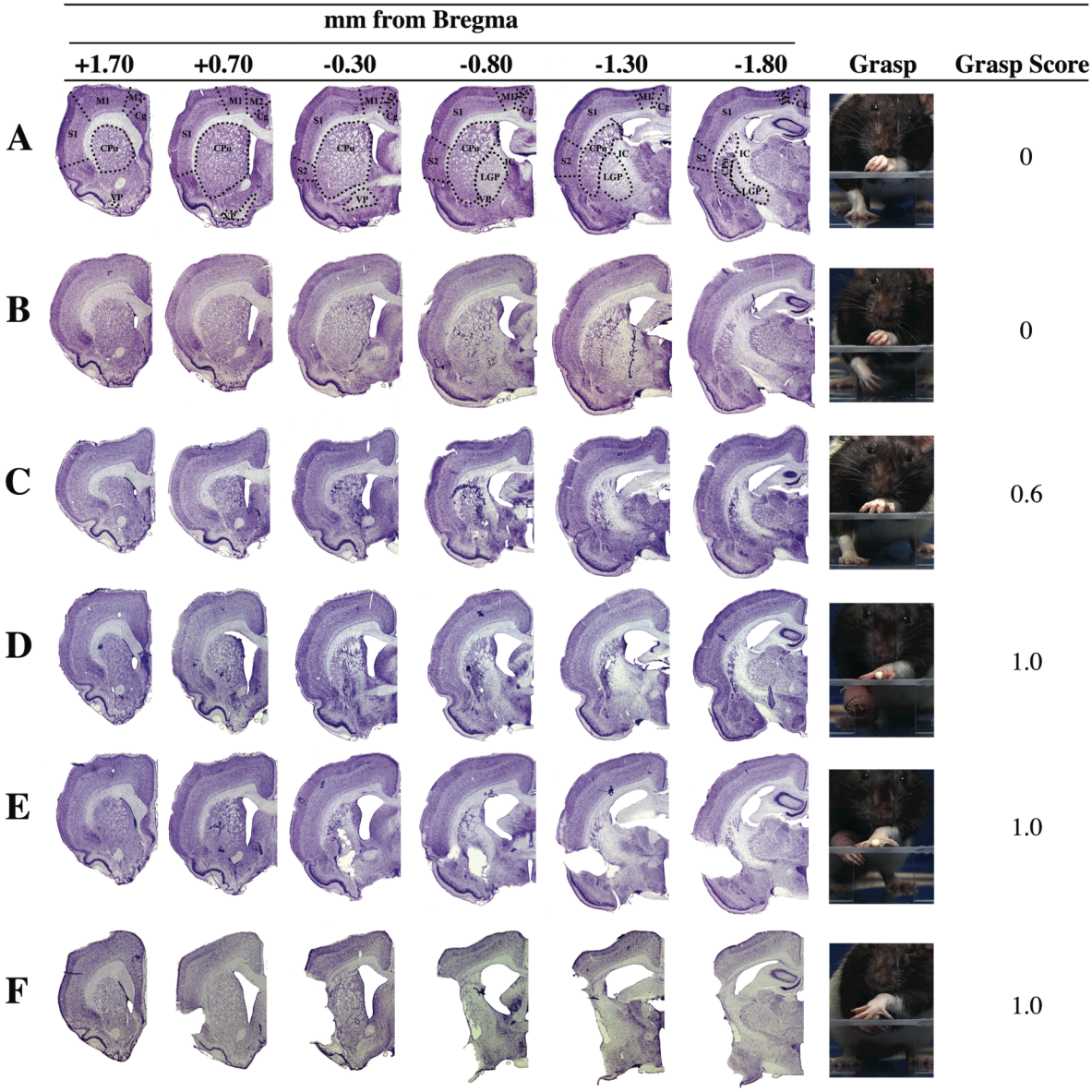


Figure 8.7: Summary of anatomical variability and corresponding digits flex/close score during grasping. Note: damage and shrinkage of subcortical structures including: caudate-putamen (CPu), ventral pallidum (VP), lateral globus pallidus (LGP) and enlarged lateral ventricle on the right hemisphere. Section levels from Bregma after Paxinos and Watson (1997). Also shown are still frames of a representative reach and mean grasping scores for (A) sham (non-preferred paw) and (B-F) middle cerebral artery (MCA, contralateral-to-lesion paw). Note: only the MCA rat with the smallest lesion (B) grasps the food pellet while the remaining MCA rats display little to no digit closure after the palm and digits contact the food pellet. Primary motor cortex (M1), secondary motor cortex (M2), primary somatosensory cortex (S1), secondary somatosensory cortex (S2), cingulate cortex (Cg), internal capsule (IC).

The present lesion was modified from a standard transient MCA ischemia procedure (Longa et al., 1989). Ischemic parameters such as blood pressure and occlusion duration were regulated to primarily damage subcortical regions but minimize neocortical injury (Zhu and Auer, 1995). Focal subcortical lesions have been successfully produced with transient MCA ischemia in the Long Evans rat strain as well (Bland et al., 2001). The objective for minimizing neocortical damage was in part because rats with extensive MCA ischemia-induced damage to both the neocortex and striatum are severely impaired in reaching to the point that they may not learn to reach (Chen et al., 2002). Also, impairments from the neocortical component of MCA stroke have been reported elsewhere (Gonzalez et al., 2004, Gharbawie et al., 2005). The intraluminal suture model was favoured over microinfusion of a vasoconstrictor such as endothelin-1 to avoid neocortical disturbance with multiple electrode or cannula penetrations.

The most striking finding in the present study was that the MCA rats were impaired in the use of their contralateral digits in retrieving food pellets and the rating scale of digit use indicated that the impairment was mainly due to failure to flex and close the digits over the food pellet once it was contacted by the paw. The deficit was not due to a lack of training because the MCA rats learned to reach successfully with the ipsilateral forelimb with which they achieved success scores comparable to their control group. Also, during training with the contralateral limb, the MCA rats occasionally grasped successfully with the ipsilateral to lesion limb before a restraining bracelet was applied to it. Furthermore, the rats did learn to advance and withdraw the contralateral limb, sometimes raking a pellet back into the test box. Thus, it is unlikely that learned non-use was causative in the deficits. The rats were also motivated to perform the task as

confirmed by the observation that when using the contralateral to lesion paw they attempted to retrieve every food pellet that was presented. Motivation was likely maintained by reinforcement from the pellets that they did drag into the chamber with an open paw. The deficit was also not due to paralysis of the digits because the rats flexed, extended, and closed the digits during transport of the limb to the food and when holding the food for eating. Handedness was not determined prior to surgery and therefore the lesion might have been delivered to the hemisphere contralateral to the rat's preferred paw for reaching or non-preferred paw. But rats are equally proficient in reaching for food with both paws (Whishaw, 2000) despite individualized paw preference for reaching (Whishaw, 1992). Nevertheless, the possibility of better digit function in the event of pre-surgical reach training cannot be ruled out. The deficit might in part be due to hyperextension of the digits as the limb was advanced towards the food target, or an impairment in digit sensation required to trigger the grasp response, or due to the loss of the motor response specifically associated with skilled grasping.

The MCA stroke-induced impairment in grasping is novel when compared with previous studies of skilled reaching following brain injury in rats. Although some work has inferred grasping impairments after motor cortex (Castro, 1972a) and pyramidal tract (Castro, 1972b) lesions, subsequent video analysis has shown that these deficits were not due to a loss of digit movements for grasping (see introduction). Other studies define "grasp score" as the number of pellets an animal picked up, carried into the box, and placed in its mouth (Emerick and Kartje, 2004) but again impairments on this measure have not been specifically linked to an inability to flex and close the digits around a food pellet. Given that previous frame-by-frame video analysis reports spared grasping with

the contralateral paw after many different lesions, including sensorimotor cortex (Whishaw et al., 1991, Whishaw et al., 1992b, Gilmour et al., 2004, Gharbawie et al., 2005, Metz et al., 2005), lateral frontal cortex (Gharbawie et al., 2005), corticospinal tract (Whishaw et al., 1993, Z'Graggen et al., 1998, Weidner et al., 2001), red nucleus (Whishaw et al., 1996), combined lesions of the corticospinal tract and red nucleus (Whishaw et al., 1998), complete neocortex (Whishaw and Kolb, 1998), caudate putamen (Pisa, 1988), globus pallidus (Gharbawie and Whishaw, 2003), and substantia nigra (Whishaw et al., 1992a, Miklyaeva et al., 1994), the present report is novel and instructive.

This previous work suggests that it is unlikely that the impairment in grasping can be attributed to damage of a single neural structure. For the same reasons, it is unlikely that the impairment in the present study was due solely to neocortical injury. Specifically, the grasping impairment was similar between animals with: no neocortical damage (Fig 8.6 D), insular neocortical damage (Fig 8.6 E), large lateral neocortical damage (Fig 8.6 F). Because the present lesions damaged a number of subcortical neural structures including: caudate-putamen, globus pallidus, and fiber pathways such as the nigrostriatal pathway and the corticospinal pathway, it is likely that the deficit stems from conjoint damage to a number of these structures. In support of the idea that grasping may have multiple neural representations, there is evidence from developmental research that grasping is linked to a number of different allied reflexes, including limb traction, palm contact, and digit contact, as well as volitional processes (Twitchell, 1958). In addition, using a pharmacological subtraction method, Pellis et al (1987) suggest that grasping is mediated by a number of parallel systems, and is not abolished unless two or more of

these systems are blocked. Furthermore, work in humans and primates has documented that some aspects of individuated finger flexion/extension can be lost with neocortical or corticospinal tract injury (Twitchell, 1951, Lawrence and Kuypers, 1968, Lang and Schieber, 2003, Lang and Schieber, 2004), while other aspects of grasping are spared. Finally, grasping may not be simply under the control of the forebrain because grasping can be elicited by electrical stimulation of the lower brainstem (Robinson, 1978).

It is not surprising that the grasping deficit might have been overlooked in previous behavioural studies of MCA ischemia. Had the rats in the present study been scored for reaching success only without a detailed video analysis of their movements, they would have simply been described as unable to reach confirming previous work (Chen et al., 2002). In addition, most of the present lesions were relatively small in comparison to standard lesions from transient MCA ischemia (Ginsberg and Busto, 1989, Zhu and Auer, 1995), and thus permitted the rats some use of their contralateral-to-lesion forelimbs for limb advancement and withdrawal. Finally, the rats were trained on the use of both limbs, a method that is helpful in maximizing performance.

In the process of describing this MCA-induced impairment, a rating scale of digit movements during reaching was developed. The utility of the rating scale is that it can be used in a way that is neutral with respect to whether a rat's reach is successful. In other words, rather than classifying reaches into those that are successful vs. those that are unsuccessful, the method lends itself to describing the movements of the digits throughout all phases of any attempted reaching movement. This is particularly useful considering the complexity of limb movements during the course of a reach, which involves at least five paw rotations and six digit actions that occur simultaneously with

other upper arm movements. Although the present study describes a deficit that is relatively specific to grasping a food pellet on only one task, it is likely that in future work use of this analytical procedure will find other impairments in paw function and therefore contribute to a taxonomy of the relation between neural systems and digit movements.

It is noteworthy that an impairment in limb use was also observed in MCA rats when they reached with their ipsilateral limb. They failed to supinate the limb and thus had difficulty releasing the food pellet to the mouth. It is unclear whether this deficit is due to an impairment in digit use or if it is indirectly caused by a failure to supinate the paw to the mouth. A previous study has reported a very similar deficit in the ipsilateral limb in rats that had received unilateral dopamine depletions. When the deficit was treated with behavioral therapy, supination was improved and the digits then released the food pellet to the mouth (Vergara-Aragon et al., 2003).

Conclusion

The present study describes impairment in digit flexing/closing for grasping following subcortical MCA stroke and also describes a rating scale of digit movements used in a skilled reaching task. Because a number of structures were damaged by the MCA stroke, it is likely that the deficit in grasping results from the conjoint damage to a number of structures related to digit use. The present results also indicate that the impairments produced by MCA ischemia are more complex than can be documented by studies using end point measures of performance such as success in reaching (Grabowski et al., 1993, Biernaskie and Corbett, 2001, Lindner et al., 2003) or single measures of performance such as limb placing (Stroemer et al., 1995, Johansson, 1996, Kawamata et

al., 1997, Bland et al., 2001). Finally, the digit rating scale described here should be useful in both basic and applied future studies of motor system function.

Chapter 9

Transient middle cerebral artery ischemia disrupts the forelimb movement representations of motor cortex derived with intracortical microstimulation

Modified from a paper submitted to the *Journal of Neurophysiology* by Omar A. Gharbawie, Preston T. J. Williams, Ian Q. Whishaw in 2006.

Abstract

Subcortical infarcts caused by proximal middle cerebral artery (MCA) stroke can produce impairments in finger movements in humans and abolish digit flexion in rats. The origin of the impairment in rats is not clear because proximal MCA ischemia damages multiple basal ganglia structures as well corticofugal fibers passing through the striatum. Although the primary motor cortex (M1) is spared, its functional integrity is uncertain because even a small proximal MCA infarct damages the ventral/medial striatum through which M1 fibers project. This motivated the present study to investigate the neurophysiological integrity of M1 after the proximal MCA was transiently occluded with an intraluminal suture. Following a three-week recovery period, the rats were trained to reach for food pellets with one paw for three weeks then the other paw for an additional three weeks. The caudal and rostral forelimb movement representations were derived from M1 ipsilateral to the lesion with intracortical microstimulation (ICMS) and an anterograde axon tract tracer was infused into the caudal forelimb area (CFA) in some cases. Digit flexion was impaired in rats with a relatively small subcortical infarct and completely abolished in rats with a comparatively medium or large subcortical infarct. Forelimb representations were derived from two areas within M1 in most MCA rats but the CFA was abnormally small. The results suggest that subcortical MCA ischemia does not spare the neurophysiological properties of M1, probably because of damage sustained to its corticospinal fibers coursing through the striatum before fusing with the internal capsule. Although transient MCA ischemia produces a focal infarct, the ensuing dysfunction is widespread because it extends beyond the boundaries of the infarct.

1. Introduction

The laboratory rat is widely used as an animal model of human stroke. The forebrain of the rat is irrigated by both the middle cerebral artery (MCA) and the anterior cerebral artery (ACA) and at the junction of their capillaries in the lateral neocortex there is extensive anastomoses (Lee, 1995; Scremin, 2004). Because of collateral routes of irrigation, ischemia in an arterial branch will produce an infarct that is ordinarily quite localized (Scremin, 2004). Nevertheless, a number of procedures have been developed in rats to model human MCA stroke including: devsasularization (Stephens et al., 1985), vessel electrocoagulation (Tamura et al., 1981), transient vasoconstriction with agents such as endothein-1 (Macrae et al., 1993), temporary ligation with a snare ligature (Shigeno et al., 1985), or transient blockade of the proximal MCA with an intraluminal suture (Longa et al., 1989). Experiments using the intraluminal suture procedure show that the infarct location is influenced by ischemic parameters such as blood pressure and occlusion duration (Cole et al., 1992; Memezawa et al., 1992; Zhu and Auer, 1995). Mild hypotension and brief occlusion duration limit the infarct to subcortical structures, especially the region of the ventral basal ganglia (Cole et al., 1992; Memezawa et al., 1992; Zhu and Auer, 1995). But the infract from severe hypotension and prolonged occlusion duration spreads laterally to include the dorsolateral basal ganglia and the lateral neocortex and also spreads medially to include the lateral hypothalamus (Memezawa et al., 1992). Thus, severe and mild ischemia anatomically spare the dorsolateral neocortex, which includes the two forelimb movement representations of the primary motor cortex (M1) (Hall and Lindholm, 1974), likely because it is sufficiently irrigated by the ACA. Nevertheless, the infarcts of proximal MCA ischemia, even when

limited to subcortical structures, are associated with severe and enduring deficits on tests sensitive to M1 injury including, paw placing in a cylinder (Bland et al., 2001; Lindner et al., 2003), paw placing on a grid (Lindner et al., 2003), reaching for food (Gharbawie et al., 2006), and somatosensory detection (Lindner et al., 2003).

The subcortical location of the infarct and the behavioral deficits resulting from proximal MCA ischemia raise a question concerning the functionality of M1 following proximal MCA stroke. Although seemingly intact with gross anatomical inspection, the neurophysiological integrity of M1 may be compromised in a number of different ways by MCA stroke. These include: (1) by direct effects of ischemia on the cells of M1 (Garcia et al., 1997), (2) via incidental effects on corticofugal fibers coursing through the striatum before fusing with the internal capsule (Iizuka et al., 1989), and/or (3) via effects on cortico-basal ganglia-thalamo-cortical loops (Alexander et al., 1986). Neurophysiological assessment of M1 layer 5 output, the point of origin of most of the corticospinal tract (Donoghue and Wise, 1982; Wise and Donoghue, 1986), offers an appropriate assessment of the functional integrity of M1 and its corticospinal fibers by generating a topographical representation of the forelimb movements with intracortical microstimulation (ICMS). The procedure has been reliably used to examine M1 after neocortical stroke within the forelimb representation (Nudo and Milliken, 1996; Kleim et al., 2003; Teskey et al., 2003) and outside the forelimb representation (Gharbawie et al., 2005b). A neurophysiological study is more practical than an anatomical study in the present circumstance because: (1) the functional integrity of M1 and its corticospinal fibers can be investigated with ICMS as opposed to neuroanatomical methods, which would be limited to examining the structural integrity alone, and (2) ICMS can be

conducted at an objectively defined area of M1 such as that of the forelimb movement representations, whereas the objective is more complicated in anatomical study because corticospinal fibers originate from a vast areas of the frontal cortex making an exhaustive investigation of their integrity a formidable undertaking. Because reach training has been shown to be central to beneficial motor map reorganization after ischemia (Nudo et al., 1996a), extensive reach training with both paws was included in the present study to ensure that the ipsilateral-to-lesion M1 has been sufficiently "trained" prior to ICMS mapping.

For the present study, rats received unilateral MCA ischemia with parameters intended to produce subcortical infarcts and limit neocortical damage (Zhu and Auer, 1995). After three weeks of recovery, rats were trained on a skilled reaching for food task for at least two weeks with one paw followed by at least two weeks of training with the other paw. Grasping was also assessed during spontaneous food handling in case the reaching box imposed constraints on limb use that might have contributed to forepaw use impairments. The forelimb representations of M1 ipsilateral-to-lesion were later mapped with ICMS. In some cases the anterograde axon tract tracer BDA (biotinylated dextran amine) was microinfused into M1 at the termination of ICMS mapping. The procedure was only intended to confirm that the subocortical infarcts did indeed interrupt the course of descending neocortical projections arising from the forelimb movement representations.

2. Materials and Methods

Animals

Fourteen male Long-Evans hooded rats, 120-140 days old, and weighing 400-450 g from Charles River were used. The experiments were conducted in compliance with the guidelines of the University of Lethbridge animal care committee and the Canadian Council for Animal Care. Rats were housed in Plexiglas cages in groups of two in a colony room maintained on a 12/12 h light/dark cycle (08:00-20:00 h).

Feeding

Two weeks post surgery the rats were gradually food deprived to 90-95% of their body weight over seven days. During this week, each rat received twenty 45 mg dustless precision banana-flavoured pellets (product #F0021, Bioserve Inc., Frenchtown, NJ, USA) one hour prior to the daily Purina rat chow ration. The objective was to introduce rats to the banana-flavoured pellets, which would later serve as reaching targets, in a familiar environment. Once reach training began, only rat chow was served in the home cage. The number of banana-flavoured pellets a rat received on a given training day depended on the rat's reaching accuracy, however it did not exceed 25 pellets.

Surgery

Unilateral transient MCA occlusion was achieved in the right hemisphere with the intraluminal suture procedure (Longa et al., 1989). Rats were intubated under 3-4% halothane in a mixture of 30% O_2 and 70% N_2O. Ten of the rats were maintained on a ventilator for the duration of the procedure and halothane levels were adjusted to 1%. The ventral tail artery was cannulated in these ten rats with polythene tubing and connected to a Statham transducer. Blood pressure was automatically recorded by a PC computer

running Labtech software V.12. Blood samples were drawn throughout the surgery for pH and gas analyses (Zhu and Auer, 1995). Blood pressure and gases were not monitored in the remainder of the animals. To prevent hypothermia, all rats were placed on a heating pad during surgery.

Surgery was conducted with the rat in a supine position. An incision was made in the midline of the neck. The common carotid artery (CCA) was isolated, then the occipital, superior, and thyroid branches of the external carotid artery (ECA) were isolated and electrocoagulated. A 6-0 silk suture was tied around the ECA to prevent bleeding and to be used for gripping the ECA for manipulation. A microvascular clip was temporarily placed at the origin of the ECA for additional security against bleeding. The distal end of the ECA was cut with bipolar electrocoagulation. Another silk suture was loosely tied around the ECA and positioned as close to the ECA-ICA bifurcation as possible. A hole was cut into the ECA stump close to the ECA-ICA bifurcation. A 26-mm long, 3-0 monofilament nylon suture, with a tip tapered on fine grade sandpaper was inserted through the hole cut into the ECA. The loose silk suture around the ECA was tied to secure the tip of the intraluminal suture that has been advanced into the ECA in place and to prevent bleeding. Once the vascular microclip was removed, the monofilament was advanced through the ECA, then the internal carotid artery (ICA), until a faint resistance was encountered along with a sharp drop in blood pressure, indicating the tip of the filament has been sufficiently advanced to occlude the origin of the MCA. Halothane was used to regulate blood pressure at 75 mm Hg. The suture was withdrawn after 80 min and the ECA was permanently ligated and the end of the stump electrocoagulated to prevent bleeding from the hole that was cut into it for introducing

the intraluminal suture. All wounds were sutured and halothane was discontinued to restore blood pressure. Sham surgery involved all the above procedures except for advancing the monofilament suture into the ICA.

Reaching boxes

Single pellet boxes were made of clear Plexiglas, with the dimensions 45 cm long x 14 cm wide x 35 cm high. In the center of each front wall was a vertical slit 1 cm-wide, which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-deep shelf. Two indentations on the surface of the shelf were located 2 cm from the inside of the wall and were aligned with the edges of the slit where rats could reach.

Reach training

The first week of training consisted of daily, 10 min sessions, for each rat. In the second week, rats received daily training sessions but a session now consisted of 25 pellets, also considered 25 trials, per session. A session began when a food-deprived rat was individually placed in a reaching box and ended when the rat was removed from the box. Training involved the achievement of three stages, in which the rat learned to orient to the food pellet through the slot, transport its limb through the slot to grasp the food pellet, retract its paw through the slot to release the food into its mouth.

For a naive rat, a number of food pellets were placed on the shelf and directly in front of the slot. The objective was to make the pellets accessible to the tongue or paw through the slot. In addition, crushed food pellets were positioned on the shelf as well to intensify the scent of the pellets to prompt the rat's interest. Once a rat was successfully taking food from the shelf, pellets were moved further from the slot to encourage use of a

paw. Once a rat demonstrated a preference for one paw by making more reaching attempts with it, individual pellets were placed into the indentation contralateral to that paw. Pellets so placed are accessible to that paw because the reaching paw pronates medially to grasp. A delay preceded the presentation of successive pellets. During the delay, the rats were shaped to leave the slot, go to the back of the box, and then return to the slot. Once this aspect of training was completed, food was placed on the shelf on a random schedule and the rats were shaped to return to the back of the box if they could not confirm by sniffing that food was located on the shelf.

Bracelets for limb restraint

To force a rat to use a specific limb for reaching, a bracelet made of Elastoplast fabric adhesive tape (Smith & Nephew Inc., Lachine, Quebec) was wrapped around the preferred forelimb (Whishaw et al., 1986). The bracelet prevented the rat from inserting its bandaged paw through the slot of the reaching box but did not impede the rat's movement and could be easily slipped off by the experimenter without denuding the forelimb fur. Once habituated to the bracelet for two or three reaching sessions, rats generally ignored it and did not attempt to remove it.

Reaching success

Rats received 20 test pellets/day on all days in which their reaching success was scored. A successful reach is one in which the food was grasped and transferred into the mouth using the designated paw and was measured using the following formula:

Success percentage = (number of pellets retrieved/20) x 100.

Spontaneous food handling box

Spontaneous food handling boxes were made of clear Plexiglas, with the dimensions 35 cm long x 14 cm wide x 35 cm high.

Spontaneous food handling

The box was bated with either a 300 mg food banana-flavoured food pellet (a larger form of the reaching target) or a 10 cm long uncooked piece of angel hair pasta (Whishaw and Coles, 1996). The use of two food pieces of different sizes and shapes may be envisioned as two levels of difficulty of the same task. Manipulating the strand of pasta for consumption likely requires skilled movement of the digits and the paws, whereas the skills for manipulating the much larger 300 mg food pellet are by comparison less demanding. Each rat was individually placed into the box and was not removed from the box until the end of the trial signified by complete consumption of the food item. The box was then bated with the other food type and the rat reintroduced into the box for the next trial. The behaviour of the animal on each trial was video recorded. After the animal completed one trial of each food type, it was placed into a holding cage with access to water and a different rat was tested. Each rat was filmed on three trials for each food type and all testing was conducted over two days.

Video recording

Reaching performance was video recorded using a Sony 3CCD camcorder (1000th of a second shutter speed) and a cold light source. The camera was position orthogonally to the test boxes such that animal's behaviour was filmed from the front. Representative still frames were captured from digital video recordings with the video editing software Final Cut Pro (www.apple.com).

Intracortical Microstimulation

Mapping was conducted with the objective of investigating the topography of the caudal forelimb area (CFA), the rostral forelimb (RFA), and their borders (Kleim et al., 1998). Rats were anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). Electrophysiologists blind to group membership, performed a craniotomy, retracted the dura, and covered the pia with inert silicon oil (37 ^{o}C). To relieve edema, a puncture to the cisterna magna drained cerebrospinal fluid. A grid (500 μm^2) was superimposed onto a digital image of the cortex to guide microelectrode penetrations (Remple et al., 2001).

A tungsten filament, in a glass microelectrode (15-30 μm tip diameter) filled with concentrated saline (3.5 M), delivered current for stimulation trains of thirteen, 200 ms, 350 Hz cathodal pulses, from a stimulation isolation unit. At each site, current intensity was increased from 0 μA to 100 μA. A hydraulic microdrive was used to lower the tip of the electrode to 1550 μm beneath the surface of the neocortex, which is the approximate location of layer V pyramidal neurons that give rise to corticospinal fibers.

An experimenter supported the rat's forelimb from underneath the elbow and recorded forelimb movements as proximal (elbow/shoulder) or distal (wrist/digit). In the case of two simultaneous movements, the movement obtained at the lowest threshold was recorded along with the current intensity. Movements of hind limb, vibrissae, jaw, head/neck were also recorded. Area representations were analyzed using the software Canvas (version 3.5, ACD Systems, http://www.deneba.com)

Because it was readily apparent at the end of the mapping session that the forelimb motor maps of the MCA rats were abnormally small, the non-responsive sites surrounding the map were reinvestigated. The sites were stimulated with current

intensities up to 100 μA with the tip of the electrode lowered to multiple depths between 1000-1800 μm beneath the cortical surface. Various depths were stimulated to accommodate potential cortical thickness aberrations that may have shifted the position of layer V.

Axon tract tracing

The objective was to label some of the corticospinal fibers that originate from the caudal forelimb area of M1 to determine if the infarct interrupts their course. Six of the rats (sham, n=3; MCA, n=3) that received ICMS mapping were used for axon tract tracing. At the completion of ICMS mapping, the anterograde tracer biotinylated dextran amine (BDA 10% in sterilized saline; 10,000 MWT; Vector labs, Burlington, ON, Canada) was microinfused into the centre of the CFA. A single microinfusion measuring 2.0 μl was delivered via a 30-gauge Hamilton syringe. The tip of the needle was lowered approximately 1550 μm below the surface of the cortex to label layer V pyramidal neurons. The tracer was delivered over 5 min and an additional 5 min was allowed for diffusion. The needle tip was slowly retracted out of the brain, the incision was sutured, and the animal was monitored in a recovery room for 24 hrs before returning to the animal colony. Seven days were allowed for BDA to be transported at least past the and then the rats were sacrificed.

Histology

Rats were euthanized with an overdose of sodium pentobarbital and intracardially perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were removed, post-fixed and cryoprotected in 30% sucrose and 4% paraformaldehyde at 4°C for coronal sectioning (40 μm). Alternate sections were mounted onto glass slides and

stained with cresyl violet or collected into a vial and later processed with a DAB Elite-kit (Vector Laboratories, Burlington, ON, Canada) to visualize BDA labeling under a light microscope examination. Six cresyl violet sections [A= +2.70, +1.70, +0.70, -0.30, -1.30, -2.30 mm, from Bregma] from each MCA rat were digitally photographed for lesion reconstruction.

Statistical analysis

Success scores were analyzed using repeated measures ANOVA with Group and Paw as between group variables and Day of testing as within group variable. Motor maps and current thresholds for ICMS were analyzed with ANOVA.

Procedure and timeline

Figure 9.1 shows an overview of the experimental timeline. Rats that previously (Gharbawie et al., 2006) received unilateral MCA ischemia (n=5) or sham operation (n=5) were used in the present study. Additional rats randomly received MCA ischemia or sham operation, such that each group included a total of seven subjects. Single pellet training/testing commenced after a three-week recovery period. For the first three weeks of training/testing, rats were allowed to reach for single food pellets with their preferred paw. For the following three weeks, rats were encouraged to reach with their non-preferred paw. This was achieved by applying a restraining bracelet to the preferred paw on the first day, for subsequent days rats were allowed to voluntarily reach with the non-preferred paw and bracelets were applied only in the event of a rat reaching with the originally preferred paw for three trials in a session. Because MCA rats demonstrated digit flexion impairment during grasping in the single pellet task, they were assessed on a spontaneous food-handling task over two days in the following week. This testing was

conducted to ensure that the digit flexion impairment was not imposed by the design of the reaching apparatus or the size of the food target. The present experimental design would make certain that each paw was extensively trained to reach for food and would ensure that the hemisphere ipsilateral-to-lesion was sufficiently engaged in the reaching task before ICMS mapping. In the following two weeks, ICMS mapping of the forelimb movement representations in M1 ipsilateral to the lesion was conducted in all rats. Rats were either perfused at the end of the ICMS mapping session (sham n=4, MCA n=4) or received the anterograde axon tract tracer BDA within the CFA and perfused one week later (sham n=3, MCA n=3).

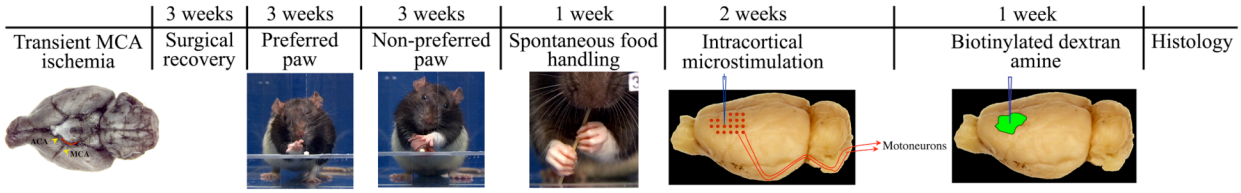


Figure 9.1: Procedural time line. The experiment started with middle cerebral artery (MCA) ischemia by advancing a suture into the anterior cerebral artery (ACA) to transiently block the origin of the MCA. Both paws were then trained/tested on skilled reaching and spontaneous food handling. The forelimb movement representations of motor cortex were then mapped with intracortical microstimulation (ICMS). Rats were either sacrificed immediately after ICMS mapping and the brain processed for histological examination or received a microinfusion of the anterograde axon tract tracer biotinylated dextran amine (BDA) into the caudal forelimb area (approximate location highlighted in green) and sacrificed for histology one week later.

3. Results

Skilled reaching

MCA rats were impaired in reaching success as compared to sham rats [$F(1,24) = 19.64$, $p < 0.01$]. Reaching was more successful with one paw [$F(1,24) = 4.36$, $p < 0.05$] and a Group x Paw interaction [$F(1,24) = 10.47$, $p < 0.01$] suggests that differential paw

performance was due to poor success scores with the contralateral-to-lesion paw of the MCA group (Fig 9.2).

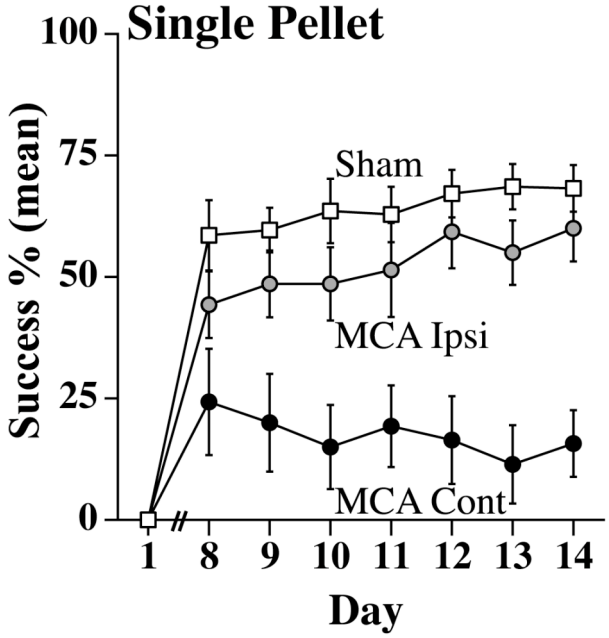


Figure 9.2: Sham rats were equally successful in reaching for and grasping food pellets with either paw, so the scores of both paws were pooled (Sham). MCA rats were equally successful with their ipsilateral-to-lesion paw (MCA Ipsi) as the sham rats. Performance was poor however with the contralateral-to-lesion paw (MCA Cont) and did not improve across training/testing days.

During initial training, all MCA rats preferred to reach with their ipsilateral-to-lesion paw and acquired the task at the same rate as sham rats (Fig 9.2). Nevertheless, qualitative impairments related to supination of the paw upon withdrawal from the shelf as well as impairments in digit extension to release the food pellet into the mouth were observed in all MCA rats. For non-preferred paw training/testing, paw preference was successfully switched in all sham rats after one week of reaching with a bracelet wrapped around the originally preferred paw. In contrast, a restraining bracelet on the ipsilateral-to-lesion paw was required on all training/testing days in which the MCA rats were required to reach with the contralateral-to-lesion paw. Poor success scores (Fig 9.2) were

primarily related to an inability to flex and close the digits around the pellet (Fig 9.3A). Of the seven MCA rats: two partially flexed their digits on all trials and therefore occasionally grasped a pellet (42% and 31% success rate), one rat partially flexed its digits on occasional trials and therefore seldom grasped a pellet (20% success), however, digit flexion was abolished in the other four rats and they did not grasp a pellet (< 3% success). Nevertheless, all MCA rats advanced their contralateral-to-lesion paw through the slot and contacted the pellet with their digits and the four rats that could not grasp advanced their paw well beyond the pellet and raked it against the shelf with an open paw (movie 1). The pellet was displaced out of reach on most occasions but at times fortuitously dropped inside the box where it was lapped off the floor. Despite poor performance with the contralateral-to-lesion paw the rats remained interested in the task as evident by their attempts to retrieve each pellet presented. Additional details on ipsilateral-to-lesion as well contralateral-to-lesion impairments are described in chapter 8 (Gharbawie et al., 2006)

Spontaneous food handling

Upon introduction into the test box, rats immediately oriented to the food pellet, sniffed it, and picked it up with their mouths. Sham rats then transferred the pellet from the mouth to the paws and it was held in the tips of the digits. The forepaws were adducted and the snout tilted forwards for each bite the rat took out of the food pellet all the while the pellet was held and manipulated by the tips of the digits (Fig 9.3 B). The actions for biting the pellet were repeated until it was completely consumed and rarely did any pellet crumbs accumulate on the floor. In contrast, MCA rats held the pellet with the ipsilateral-to-lesion digits and balanced it with the radial aspect of the second digit or

with the base of the palm, of the contralateral-to-lesion paw (Fig 9.3 B). Pellet crumbs accumulated on the floor with each bite and during chewing. The rat typically neglected the food crumbs.

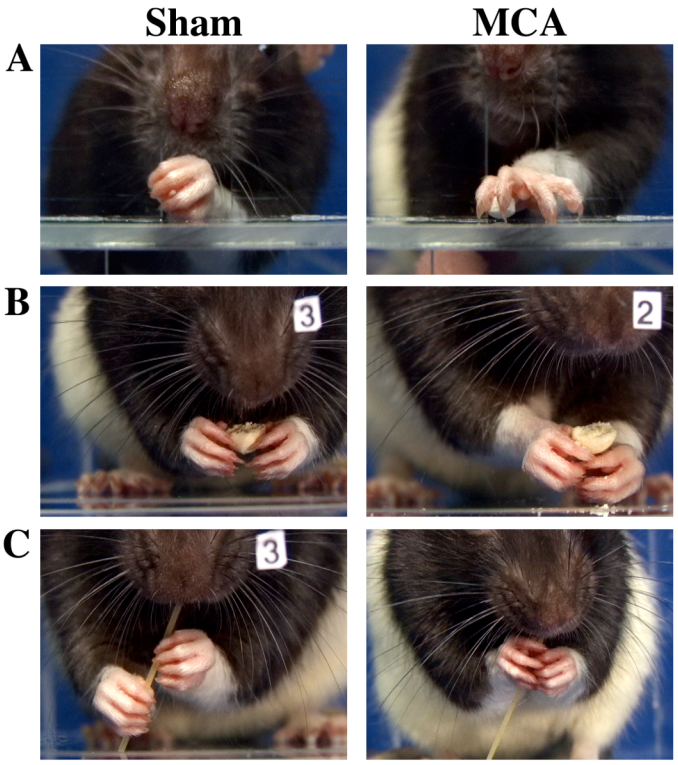


Figure 9.3: Digits flexion is contrasted between a sham and a middle cerebral artery (MCA) stroke rat in three food grasping situations. (A) The sham rat flexes its digits onto the pellet as it starts to withdraw the paw from the shelf during single pellet reaching, whereas digit flexion is abolished in the MCA rat and the pellet is raked across the shelf with an open paw instead. The sham rats holds a (B) 300 mg food pellet, (C) 10 cm piece of uncooked angel hair pasta with both paws during spontaneous food handling, whereas the MCA rat holds the food with the ipsilateral-to-lesion digits and the mouth. Note, the contralateral-to-lesion digits partially flex but do not participate in holding the food.

To consume the pasta, sham rats picked it up with their mouths and grasped it with both paws. The pasta was held in the digits of both paws in an overhand grip with one paw closest to the mouth and the other nearly immediately below it. The arms were typically oriented to one side of the rat's body midline. The lower paw maneuvered the

pasta upwards and it was chewed as it was advanced into the mouth, while the paw closest to the mouth appeared to stabilize the pasta (Fig 9.3 C). In contrast, MCA rats held the pasta in the ipsilateral to lesion paw. The contralateral-to-lesion digits were level with the ipsilateral digits and although were partially flexed did not hold the pasta (Fig 9.3 C). Instead, the pasta was pushed into the mouth with the ipsilateral-to-lesion digits and probably pulled in with the teeth at the same time. Pasta crumbs accumulated on the floor during consumption and were neglected

Axon tract tracing

Infusion of the anterograde axon tract tracer BDA into the centre of the CFA of a sham rat (Fig 9.4 A) labeled fiber projections of M1 coursing through the caudate-putamen (Fig 9.4 B) and pyramidal tract (Fig 9.4 C). The same infusion in an MCA rat (Fig 9.4 A) sparsely labeled fibers in the caudate-putamen (Fig 9.4 B) and pyramidal tract (Fig 9.4 C). Coronal sections from the sham and MCA rats are presented in Fig 9.5 (cases A and C respectively). A similar, sparse pattern of fiber labeling was seen in one MCA rat that had a comparable lesion (Fig 9.5 B) and labeling was even sparser in the case with the larger infarct (Fig 9.5 E).

Histology

Lesion variability from transient right MCA occlusion is summarized in Fig 9.5. In four of the seven rats the lesion did not produce any overt injury to the neocortex or allocortex but damaged a region including: the caudate-putamen and the globus pallidus(Fig 9.5 B, C, D, E). Minor cortical damage limited to the allocortex was observed in one rat (Fig 9.5 F). Rats with larger infarcts had extensive lateral frontal cortex and parietal cortex damage (Fig 9.5 G, H). The internal capsule and lateral

hypothalamus were spared in all MCA rats except the one with the largest infarct (Fig 9.5 H).

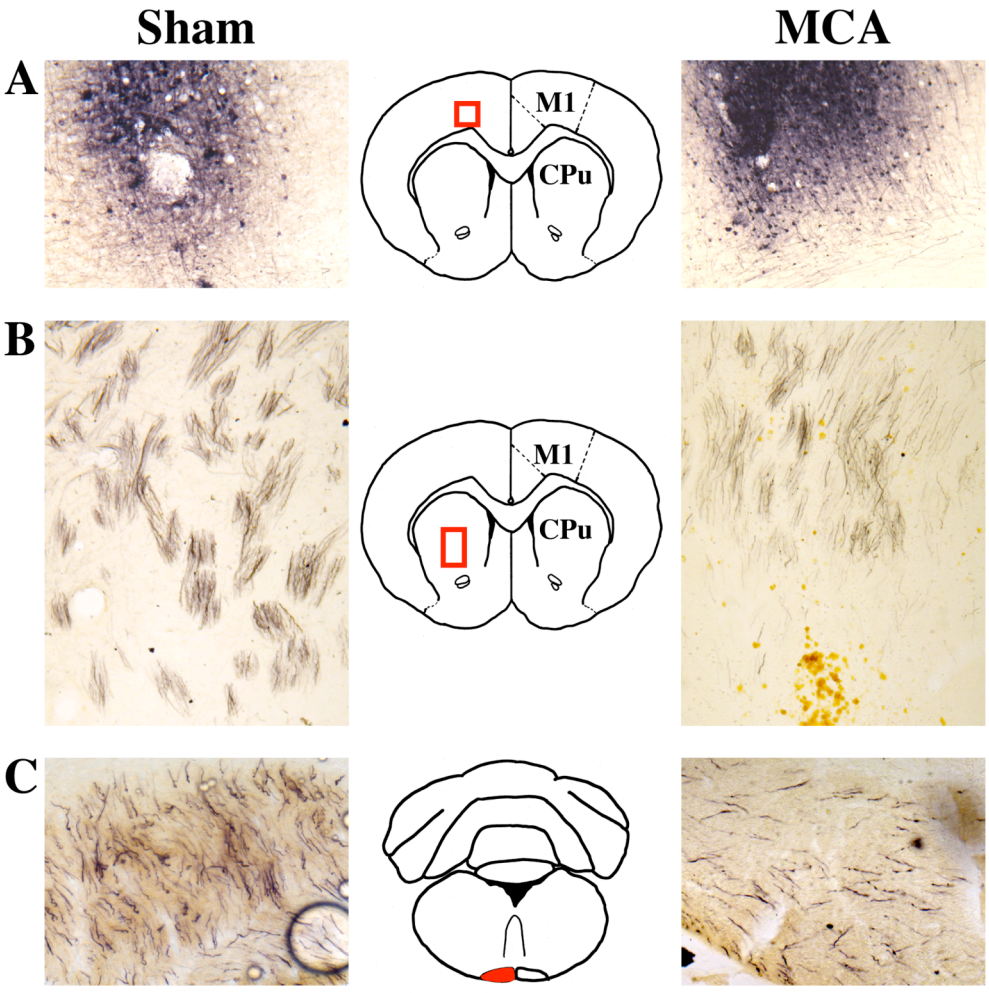


Figure 9.4: Photomicrographs of BDA anterograde axon tract tracing in a sham and a middle cerebral artery stroke (MCA) rat. Rectangular box superimposed on schematic diagram specifies the region magnified in the photomicrograph. Coronal sections show: (A) comparable infusion of BDA in primary motor cortex (M1) of a sham and an MCA rat. (B) M1 corticofugal fiber labeling was sparse in the striatum (CPu) of the MCA rat in comparison to the sham rat. Blood clotting and tissue scaring is apparent in the MCA section. (C) Fiber labeling was sparse at the level of the pyramidal tract in the MCA rat as compared to the sham rat. For representative coronal sections showing the infarct of this MCA rat see Fig 5C.

ICMS mapping

Comparable areas of M1 were stimulated in sham (7.33 $\pm$ 0.77 mm^2; mean $\pm$ SEM) and MCA rats (5.81 $\pm$ 1.20 mm^2; mean $\pm$ SEM) and a similar number of electrode penetrations were made in sham (69.00 $\pm$ 5.98; mean $\pm$ SEM) and MCA (53.71 $\pm$ 5.03; mean $\pm$ SEM) rats. Also, the current intensity necessary for eliciting forelimb movements was similar for sham (40.24 $\pm$ 2.48 μA; mean + SEM) and MCA (41.14 $\pm$ 1.41 μA; mean $\pm$ SEM) rats.

In sham rats, forelimb responses were derived from two separate regions within M1 (Fig 9.5 A). The caudal forelimb area (CFA) was predominantly composed of distal (wrist) representations and comparatively smaller proximal (shoulder/elbow) representations (Table 1), whereas the rostral forelimb area (RFA) was mostly composed of distal representations. The border separating the CFA from the RFA was primarily comprised of head/neck and vibrissae representations and fewer non-responsive sites.

In five of the seven MCA cases (Fig 9.5 B, C, D, E, G) forelimb movements were derived from a CFA and a separate RFA. The rostral border of the CFA that separates it from the RFA was mostly made up of non-responsive sites except for a limited number of head/neck responses. In the other two rats, forelimb movements were only derived from the CFA (Fig 9.5 F, H).

Although CFA size was small in MCA rats as compared to sham rats [$F(1,12) = 14.91$, $p > 0.05$, Fig 9.6], there was no evidence that the motor representations had shifted into ectopic territories as the centre of the CFA representations was appropriately situated in relation to bregma and the midline. Also, RFA dimensions were comparable [$F(1,12) = 1.38$, $p > 0.05$] for sham (0.29 $\pm$ 0.04 mm^2; mean $\pm$ SEM) and MCA (0.18 $\pm$ 0.09 mm^2;

mean ± SEM) rats.

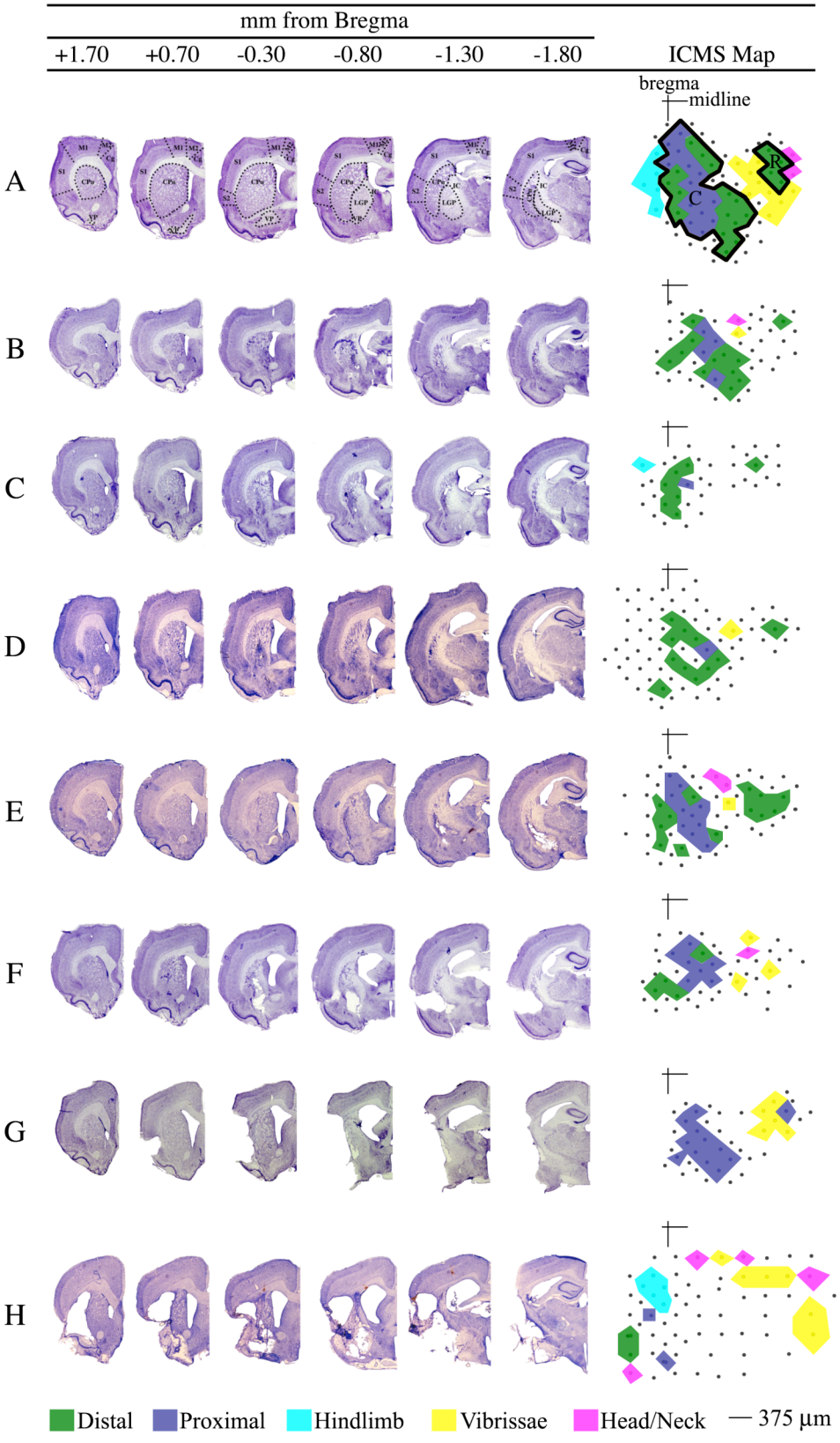


Figure 9.5: Summary of anatomical variability and corresponding M1 motor maps generated with intracortical microstimulation from (A) a sham operated rat and (B-H) seven middle cerebral artery (MCA) rats. The present labels correspond to the cases in Table 1. The order in which the cases are presented is in accordance with the mean success score of the contralateral-to-lesion paw, which can be found in Table 1. The caudal forelimb area and rostral forelimb area are labeled as C and R respectively in case A. Subsequent maps follow the same orientation. Note: damage and shrinkage of subcortical structures including: caudate-putamen (CPu), ventral pallidum (VP), lateral globus pallidus (LGP) and enlarged lateral ventricles. Primary motor cortex (M1), secondary motor cortex (M2), primary somatosensory cortex (S1), cingulate cortex (Cg), internal capsule (IC).

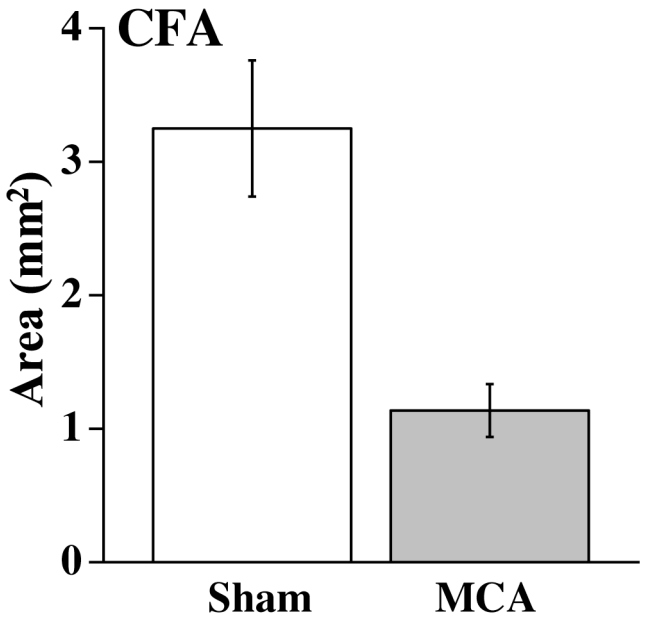


Figure 9.6: The caudal forelimb areas (CFA) were significantly smaller in middle cerebral artery stroke (MCA) rats as compared to sham rats.

Relation between map, lesion, and grasp

Regression analyses between lesion size, success score, and forelimb map size did not reveal any significant correlations. Nevertheless, a distinct pattern emerged when the MCA rats were according to their ability to flex their digits. Rats that could partially flex their digits (Table 1 B, C, and D) were distinct from the remainder of the group in that

they were occasionally successful in retrieving food pellets. But rats that could not flex their digits (Table 1 E, F, G, and H) had an extremely low success score. It follows that

Table 1. Profile of reaching success with the contralateral-to-lesion paw, size of the caudal forelimb area (CFA) and the rostral forelimb area (RFA), and ratio of distal/proximal forelimb representations within the CFA for sham (A1-A7) and MCA (B-H) rats. Rat A4 corresponds to case A in Fig 9.5 and MCA rats (B-H) correspond to cases B-H in Fig 9.5. The cases are listed in descending order of success scores.

Rat	Group	Success (%)	CFA (mm^2)	RFA (mm^2)	Ratio (Distal/Proximal) in CFA
A1	Sham	85.72	1.81	0.17	1.89
A2	Sham	85.00	4.64	0.39	1.68
A3	Sham	76.43	3.34	0.46	1.09
A4	Sham	75.00	2.70	0.25	10.25
A5	Sham	72.15	3.22	0.27	8.41
A6	Sham	59.29	2.15	0.23	1.29
A7	Sham	36.43	3.60	0.23	4.46
B	MCA	42.15	1.97	0.14	2.71
C	MCA	31.43	0.87	0.13	6.25
D	MCA	20.00	1.20	0.12	9.00
E	MCA	2.86	1.29	0.13	1.00
F	MCA	2.15	1.33	0.00	0.49
G	MCA	2.15	1.97	0.71	0.81
H	MCA	0.00	0.24	0.00	0.00

the infarct of the rats that could flex their digits (Fig 9.5 B, C, and D) were smaller than the infarcts of the rats that could not flex their digits during grasping (Fig 9.5 E, F, G, and H). Last, the topographical organization of the forelimb movements derived from rats that could flex their digits during grasping was more biased towards distal movements than proximal movements (Table 1 B, C, and D), which was also similar to sham rats (Table A1-A7). But the opposite was true of rats that could not flex their digits for grasping (Table 1 E, F, G, and H).

4. Discussion

The objective of the present study was to investigate the integrity of primary motor cortex (M1) using intracortical microstimulation (ICMS) after transient occlusion of the proximal middle cerebral artery (MCA) with an intraluminal suture. The MCA ischemia overtly spared M1 in all of the rats and produced damage to the lateral neocortex in a subset of rats. The striatum, globus pallidus, associated fibers, and M1 projections passing through the striatum were damaged in all of the rats. Digit flexion was impaired on a skilled reaching test in all MCA rats, albeit to varying degrees, and paw use impairments were evident when spontaneously handling food. ICMS indicated that the topography of the forelimb maps, especially those in the caudal forelimb area (CFA), was abnormally small. Thus, the results demonstrate that even though the present infarcts do not encroach into M1, its neurophysiological integrity is compromised. These results are discussed in relation to the anatomical and behavioural effects of subcortical MCA stroke in the rat.

The size of the infarct varied between rats, which was likely due to idiosyncratic effects of anesthesia on hypotension as well as individual variance in vasculature architecture as occlusion duration was consistent between rats (Zhu and Auer, 1995). The core of all infarcts was in the striatum and the globus pallidus. Infarcts extended laterally and medially within subcortical territories with increased ischemic severity. Yet larger infarcts included damage as far lateral as the frontal and parietal neocortex and in one case as far as medial (largest infarct) as the internal capsule. In a subset of rats with infarcts limited to the basal ganglia, anterograde labeling of M1 fibers suggested a reduction in the number of descending M1 projections. The fiber examination was only

intended to confirm that the course of the descending cortical projections was interrupted by the present infarct and thus replicated previous work (Iizuka et al., 1989). Because the sparse fiber labeling was demonstrated only in three rats, it is probably safe to assume that the rest of the MCA group, most of which had larger infarcts, suffered at least the same axonal degeneration. But to overt inspection, all of the infarcts spared M1.

When first trained in a skilled reaching task following MCA ischemia, all of the rats learned to reach successfully. The MCA rats preferentially used the ipsilateral-to-lesion paw and their acquisition rate and final success scores were comparable to those of sham rats. In contrast success with the contralateral-to-lesion paw was poor relative to sham rats, which is consistent with previous work (Biernaskie and Corbett, 2001; Chen et al., 2002; Gonzalez and Kolb, 2003; Lindner et al., 2003; Gharbawie et al., 2005a). The impairment displayed by the MCA rats was distinctive in that all of the rats transported their limb towards the food target and contacted it with their digits but only three of seven rats partially flexed the digits around the food pellets to occasionally grasp it. The remaining rats raked the pellet with an open paw against the shelf and occasionally dragged it into the box and lapped it off the floor. Additionally, during spontaneous handling of uncooked pasta strands and round food pellets, all of the MCA rats displayed impairments in holding the food with the contralateral-to-lesion digits. Abolished digit flexion in the present MCA rats might reflect a more severe digit use syndrome than digit independence impairments caused by corticospinal stroke (Lang and Schieber, 2003; Raghavan et al., 2006) or pyramidal tract lesions (Twitchell, 1951; Lawrence and Kuypers, 1968).

The ICMS results indicated that although forelimb movements could be derived from the ipsilateral-to-lesion M1 of all rats, the areas comprising the forelimb representations were abnormally small. The size reduction was mainly in the caudal forelimb area (CFA) as there was no group difference in the size of the rostral forelimb area (RFA). The reduction in forelimb map size did not appear to be due to changes in sensitivity to electrical stimulation because the mean current intensity for evoking movements was similar between groups. This is somewhat surprising because previous work shows that higher stimulation current are necessary to drive movements from M1 after pyramidal tract cuts (Mitz and Humphrey, 1986). But perhaps the reach training in the present task, even though it involved pellet dragging as opposed to grasping in the majority of the MCA rats, was sufficient to in restoring thresholds for evoking movements. Nevertheless it is unlikely that different ICMS parameters could have elicited movements from the silent sites because they were stimulated with currents up to 100 μA to accommodate the potential for increased current thresholds to elicit movement. Also, the tip of the electrode delivering the current was maneuvered approximately 400 μm above and below the normal depth of layer 5 in case its location shifted with any cortical thickness aberrations. It is also unlikely that additional training could have restored responsiveness in the silent sites surrounding the forelimb because the rats were not showing any signs of improvement after three weeks of training with each paw, which is normally sufficient for success score recovery from large M1 lesions (Whishaw, 2000; Gharbawie et al., 2005a) and motor map restoration after partial M1 injury (Nudo et al., 1996a). In addition, the residual motor maps were located in the appropriate region of M1 in relation to bregma and the midline. Thus, there is reasonable confidence that the

present maps represent the most "recovered" outcome of the infarcts induced. That the CFA was most affected is consistent with work indicating that this region of M1 is most responsive to experiential effects (Kleim et al., 1998; Teskey et al., 2002; Monfils et al., 2004) and injury (Castro-Alamancos and Borrel, 1995; Kleim et al., 2003; Teskey et al., 2003; Piecharka et al., 2005).

Although the present results clearly indicate that the functional integrity of M1 is compromised by MCA stroke, they do not unequivocally identify a neuroanatomical underpinning of M1 dysfunction. Basal ganglia damage likely interrupted cortico-basal ganglia-thalamo-cortical loops (Alexander et al., 1986), whether this affected M1 maps remains to be confirmed but dopamine depletion from the basal ganglia sufficiently disrupts the same subcortical circuits involved in the present infarct but does not alter motor map topography (Metz et al., 2004). Nevertheless, damage to circuitry infrastructure as in the present study might have a different effect on motor maps than neurotransmitter depletion. Also, M1 corticospinal neurons, their intracortical horizontal connections, and the internal capsule (except in one case), were spared by the present infarct suggesting that these components of the circuitry were unlikely involved in the small motor maps. Nevertheless, the possibility of sporadic cell death in M1 or weakening of their synaptic connections (Garcia et al., 1997) cannot be ruled out. Also, neural shock was unlikely a factor because ICMS mapping was conducted at least ten weeks after ischemia and extensive reach training with both paws that presumably restores forelimb map topography (Nudo et al., 1996a).

Thus, the most parsimonious interpretation of the diminished forelimb topography is that it reflects a loss of M1 output due to damage of corticospinal fibers coursing

through the striatum before entering the internal capsule. Even the smallest of the present infarcts would have interrupted the course of the descending M1 projections, which may have been sufficient for motor map shrinkage. That motor map shrinkage is related to the loss of the descending M1 projections is made clear by comparing the maps of the rat with internal capsule damage to the rest of any of the other MCA rats. While all MCA infarcts were associated with a reduction in the size of the forelimb motor map, the reduction to the point of almost an absent forelimb motor map was observed in the one case that sustained internal capsule damage (Fig 9.5H). The interpretation is consistent with reports in rats (Kartje-Tillotson et al., 1987; Piecharka et al., 2005) and primates (Mitz and Humphrey, 1986; Schmidlin et al., 2004) demonstrating a reduction in forelimb map size after pyramidal tract sectioning. Also consistent with clinical subcortical stroke in which motor potentials evoked by transcranial magnetic stimulation (TMS) is subject to corticospinal tract integrity (Ward et al., 2006).

That corticospinal tract damage was central to the present ICMS results raises two possibilities about how the forelimb motor map shrank. First, regions of M1 that lost their corticofugal fibers might have been silenced and the residual forelimb responses were derived from regions with spared output that either maintained their inherent forelimb representations or reorganized and adopted forelimb representations. Alternatively, forelimb responses were lost from the perimeter of the CFA and the motor maps generated are the residual centre. Both interpretations are conceivable given that the CFA is the most adaptive region of the forelimb motor map (Kleim et al., 2002; Teskey et al., 2002; Monfils et al., 2004). That the forelimb representations were not overtaken by other body representations suggests that the lesion was equally disruptive to descending M1

projections controlling other body parts and not just the forelimb. This is consistent with the idea that orderly somatotopy of M1 fibers is not clear (Schieber, 2001) and thus even focal fiber damage is bound to disrupt various M1 body representations.

That the forelimb motor maps shrank and not completely abolished may also reflect the neurophysiological status of M1. Previous work demonstrated a deteriorated indirect wave in the pyramidal tract in response to ICMS after proximal MCA ischemia despite rapid recovery in the direct wave (Bolay and Dalkara, 1998). Also, it is worth noting that forelimb responses could be elicited from pyramidotomized monkeys with surface electrodes from otherwise silent sites (Mitz and Humphrey, 1986). Surface electrodes produce more current spread than the microelectrodes used in ICMS, which fosters the idea that the indirect pathways are not responsive to normal stimulation parameters after corticospinal projection damage. Perhaps silent sites in the present maps are ones in which both the direct and indirect waves were deteriorated.

The MCA rats' poor paw use might have contributed to the small forelimb motor maps. Despite the variability in infarct size, grasping syndromes were evident in each of the MCA rats. In mild cases rats could partially grasp presumably because sufficient basal ganglia and M1 projections survived to mediate the response, whereas with more severe subcortical damage, grasping was abolished. It is interesting that the small forelimb maps of the three rats that could partially flex their digits included an overrepresentation of distal (wrist) movements on the account of proximal (shoulder/elbow) movements, whereas proximal representations predominated the forelimb representations in rats that could not grasp. Because all MCA rats contacted the pellets with their digits but only three rats occasionally grasped the pellet and consumed

it from the mouth, it is possible that the topographic pattern of these three rats that favours distal forelimb movements is shaped by the grasping and limb withdrawal actions of reaching as opposed to limb transport. A similar topographical distribution ensues from skilled reach training in rats (Kleim et al., 1998) and primates (Nudo et al., 1996b) but not after repetitive forelimb movements (Kleim et al., 2002) or strength training (Remple et al., 2001).

The present ICMS results show dysfunction in M1 output that is consistent even in the smallest subcortical MCA infarct. The evidence suggests that infarcts produced by the intraluminal suture procedure are bound to implicate M1 despite its remoteness from the injury. It would not be surprising if other neural structures not investigated in the present study were implicated as well (Neumann-Haefelin and Witte, 2000; Witte et al., 2000). Indeed the involvement of the contralesional neocortex in both the behavioural impairment and the recovery after stroke is being debated in the clinic (Fridman et al., 2004; Lotze et al., 2006) and in rat models (Biernaskie et al., 2005; Shanina et al., 2006). Nevertheless, the role of the ipsilateral-to-lesion M1, its residual descending fibers and motor map in skilled movements should be investigated with permanent or temporary lesions.

Conclusion

Transient MCA occlusion with an intraluminal suture is now a well-accepted rat model of focal MCA stroke. It is not surprising that the effectiveness of neuroprotective agents, rehabilitative therapies, and stem cell therapy is investigated in this model. The present results show that the infarct created by the procedure is complicated in that it damages numerous subcortical structures and more importantly the ensuing neural

dysfunction extends beyond the boundaries of the infarct. Thus, therapeutic efforts focused on the infarct core alone will be only partially effective because the lesion is not selective. The results should also serve as a cautionary note to interpreting behavioural syndromes of subcortical MCA ischemia as related to striatal damage only (Bland et al., 2001) as well as relying on infarct size as a measure of function. Finally, the sensitivity of ICMS to ischemia suggests that mapping is a useful measure of functional change produced by MCA stroke.

Chapter 10

General Discussion

Middle cerebral artery (MCA) stroke can damage a range of brain structures because the vessel ramifies throughout most of the forebrain including cortical and subcortical components of the motor system that are irrigated by the MCA. Because there are limited treatment options, either with neuroprotectants immediately post stroke or with rehabilitation or drug therapies later, mean that the majority of MCA stroke accidents will produce long-term motor disabilities. Not surprisingly there is therefore an urgent need for further developing rat models of stroke to provide an avenue for preclinical testing of treatments. Bridging the gap between experimental stroke and translational research by further exploring the behavioural, neurophysiological, and anatomical characteristics of different models of stroke was the objective of the present thesis. This was achieved by documenting the long-term skilled reaching impairments from three models of stroke in rats. In addition, parallel studies investigated the neurophysiological and the neuroanatomical characteristics of the tissue spared by the injury.

In the present discussion, the major findings of the thesis will first be summarized. To facilitate the summary the experiments will be organized differently from previous chapters. Because each group of rats received one of three stroke infarcts and some experiments examined more than one infarct; the groups and their respective behavioural and neurophysiological results will be assembled according to infarct and described. Infarcts that spanned: (1) the forelimb movement representations will be summarized under motor cortex stroke (Fig 10.1 A); (2) the neocortex lateral to motor cortex will be included under distal MCA stroke (Fig 10.1 B); (3) subcortical structures and fibers will be discussed under proximal MCA stroke (Fig 10.1 C). Second, the

outcome (anatomical, behavioural, neurophysiological) of the three strokes will be compared head-to-head, which may include some overlap from the previous section. Last, some of the limitations of rat models of stroke will be discussed.

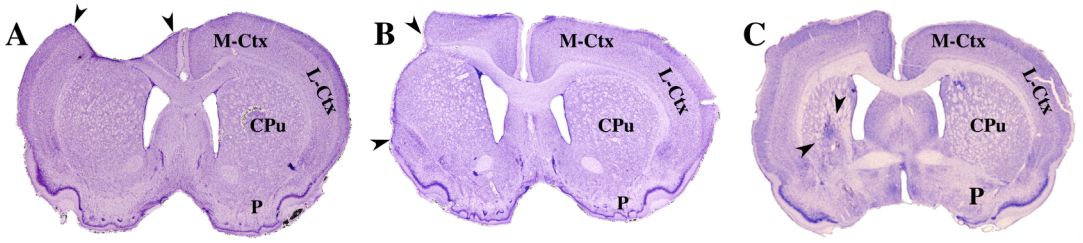


Figure 10.1. Coronal sections from representative rats that received: (A) motor cortex stroke, (B) distal MCA stroke, (C) proximal MCA stroke. The infarcts were anatomically separate in the three groups. Damage was localized: to motor cortex (M-Ctx) in the motor cortex group, to lateral cortex (L-Ctx) in the distal MCA group, and to striatum (CPu) and globus pallidus (P) in the proximal MCA group. Arrows indicate infarct boundaries.

The anatomical, behavioural, and neurophysiological effects of three MCA strokes in rats

(1) Motor cortex stroke

Anatomical. In a series of studies, the motor cortex was devascularized either in the hemisphere ipsilateral to the preferred paw for reaching or in the hemisphere contralateral to the preferred paw. The infarct extended into all layers of the agranular motor cortex and produced white matter loss in the corpus callosum. Disfiguration was evident in the ventricles and the striatum although they were not directly damaged. In some cases, the corpus callosum was severed but this was likely due to swelling pressuring the fibers as opposed to ischemic damage. In larger infarcts, the injury extended medially into the cingulate cortex and laterally into the dysgranular zone but did not encroach into the granular zone of the somatosensory cortex.

Behavioural. In the immediate postoperative days (1-3 days), rats with motor cortex stroke in the hemisphere contralateral to the reaching paw might not attempt to reach for food. Instead they spent the test session exploring the reaching box as though it was novel to them. When they did attempt to reach, they generally failed to retrieve food pellets because they could not transport their paw far enough through the slot to contact the pellet. This failure prompted some rats to stop reaching all together, whereas other rats tried to reach with the ipsilateral-to-lesion paw. Restraining the ipsilateral-to-lesion paw with a bracelet initially discouraged rats from reaching all together. Nevertheless, by the end of the first postoperative week all rats attempted to reach with their contralateral-to-lesion paw when the ipsilateral-to-lesion paw was restrained. From this point, success scores gradually improved and approached preoperative levels by the second or third postoperative weeks. Chronic impairments in limb transport, limb withdrawal, and presentation of food to the mouth were evident, nevertheless.

Motor cortex stroke in the hemisphere ipsilateral to the reaching paw impaired performance but to a milder degree than contralateral motor cortex stroke. Most rats in this group reached for food with the designated paw in the immediate postoperative period. Success scores were initially poor due to short limb trajectory (hypometria) that knocked the pellet away from the rat. Improvement was more rapid for this group such that preoperative success levels were restored by the end of the first postoperative week. Impairments in orienting the snout to the pellet, aiming the elbow, and supinating the paw to present food to the mouth remained but were also milder than in rats with contralateral motor cortex stroke.

Neurophysiological. The integrity of the motor cortex spared by the infarct was investigated with intracortical microstimulation (ICMS). Because the motor cortex infarct was inclusive of both forelimb movement representations, ICMS was conducted in M1 of the opposite hemisphere. Forelimb motor maps were indistinguishable from those of sham rats. This is surprising for two reasons: (1) unilateral sensorimotor cortex lesions are reported to produce morphological adaptations in the homotopic neocortex and the expectation was that the structural changes would be reflected in the ICMS examination; (2) chronic movement abnormalities were evident in the paw ipsilateral-to-lesion, which is also the paw primarily controlled by the mapped hemisphere. The expectation was that the kinematic aberrations of the limb would be reflected neurophysiologically in motor reorganization. The morphological adaptations and behavioural impairments may be too subtle for detection with ICMS or simply do not bare significance on motor map organization or motor cortex output.

(2) Distal middle cerebral artery (MCA) stroke

Anatomical. To induce ischemia in the distal branches of the MCA, the vessel and its main branches were occluded with bipolar electrocoagulation between the rhinal fissure and the temporal ridge. Separate groups of rats received ischemia either in the hemisphere contralateral to the reaching paw or ipsilateral to the reaching paw. Postmortem histology showed that at a minimum the infarct damaged the primary somatosensory areas of the jaw and the whiskers. Larger infarcts extended medially to include somatosensory areas of the limbs and laterally to include the secondary somatosensory cortex and insular cortex. Nevertheless, even the largest infarct did not damage the underlying subcortical structures or encroach into motor cortex.

Behavioural. Rats with distal MCA stroke in the hemisphere contralateral to the reaching paw reached for food in the immediate postoperative period. Their attempts were not successful primarily because of a misguided limb trajectory. When they did grasp a food target, they were impaired at withdrawing the limb and presenting the food to the mouth. Also, "phantom grasping", in which rats inspected their paw with their snout/mouth for a pellet that was not grasped, characterized the missed reaches of this group. Success scores recovered to preoperative levels but kinematic aberrations and phantom grasping persisted. Although the impairments of the ipsilateral-to-lesion paw were milder than those of the contralateral-to-lesion paw, they were readily detected in success scores and qualitative movement analysis during the first postoperative week. Chronic impairments in orienting the snout to the pellet and supinating the wrist to present a grasped pellet to the mouth were evident even after success scores were restored.

Neurophysiological. The integrity of motor cortex (M1) ipsilateral to the lesion was examined with ICMS 4-6 weeks after reaching scores were restored. Aberrations of M1 output manifested in smaller forelimb movement representations. This probably did not reflect generalized dysfunction in M1 because movements of the jaw, whiskers, neck, and occasionally forelimb, were derived from M1. Smaller forelimb maps were likely a side effect of deafferentation of M1 from direct cortico-cortical input that otherwise would have reached M1 from the lateral neocortex. The deafferentation may have weakened the M1 neural framework that supports the long distance signal transmission from ICMS necessary for eliciting limb movements. At the same time the M1 framework may have been still capable of supporting signal transmission for shorter distances such

to the muscles of the head. A similar investigation of M1 in the opposite hemisphere did not reveal any motor map adaptations.

(3) Proximal MCA stroke

Anatomical. Proximal MCA ischemia was transiently induced with an intraluminal suture and created a range of infarcts. At a minimum, the infarct partially damaged the striatum and the globus pallidus. Incremental increase in infarct severity involved near complete damage of the striatum and the globus pallidus, then damage in the lateral neocortex irrigated by distal branches of the MCA, then partial damage in the lateral hypothalamus and internal capsule. Corticospinal fibers coursing through the striatum were damaged regardless of infarct spectrum. It is safe to assume that larger infarcts interrupted more corticospinal fibers than smaller ones.

Behavioural. Paw dominance was not determined preoperatively as rats were not trained to reach for food before stroke. Three weeks of recovery were allowed before all rats were fit for the feeding restriction regiment necessary for reach training/testing. All MCA rats reached for the food target with the ipsilateral-to-lesion paw. They acquired the task at the same rate as sham operated rats and performance was quantitatively similar between the two groups throughout the three-week testing period. Nevertheless, video records of performance with the ipsilateral-to-lesion paw revealed impairments in supinating the paw to present food to the mouth as well as deficits in extending the digits to release the food into the mouth.

The contralateral to lesion paw was tested in the same group of MCA rats by restraining the ipsilateral-to-lesion paw with a bracelet at the start of the reaching session. Performance was extremely poor as compared to the ipsilateral-to-lesion paw and as

compared to shams. Improvement over the three-week testing period was negligible. Nevertheless, the distribution of infarct severity was reflected in varying degrees of reaching impairments. Whether MCA rats were successful in retrieving food pellets was mostly dependent on their ability to grasp. Rats able to flex their digits around a food pellet were able to compensate for impairments in aiming the elbow during limb transport, withdrawing the paw from the slot, and supinating the paw to present food to the mouth, with trunk and shoulder rotations that allowed them to retrieve the food target. Rats unable to flex their digits around a food pellet were not successful in retrieving it. Their compensatory strategy of raking the food pellet against the shelf with an open paw was occasionally successful in dragging the pellet through the slot and onto the floor of the apparatus where it was lapped. The strategy likely sustained their interest in the task but did not constitute a successful reach and received poor quantitative and qualitative scores.

Neurophysiological. The neurophysiological integrity of M1 ipsilateral-to-the infarct was examined with ICMS 10-12 weeks after ischemia and reach training. Forelimb motor maps were abnormally small in all MCA rats. Nevertheless, the topography of the forelimb motor maps reflected the digit flexion capacity. Rats that could grasp a food pellet had a higher ratio of distal forelimb representations (wrist/digits) over proximal forelimb representations (shoulder/elbow), which is reminiscent of the motor maps of rats trained on tasks of skilled reaching. The topography of the maps in rats that raked the food pellet instead of grasping it was biased to proximal forelimb representations over distal representations, which is similar to rats not trained on tasks of prehensility. The neural underpinning of the aberrant forelimb

motor maps and digit flexion impairments is uncertain because the infarct damaged a combination of basal ganglia structures and corticofugal fibers.

Head-to-head comparison of the three models of stroke

The following section will compare the anatomical, behavioural, and neurophysiological consequences of the three models.

Anatomical

Although the infarcts ensuing from the three models were for the most part anatomically separate from one another (Fig 10.1), the potential for overlap is worth noting. In comparing the infarcts ensuing from M1 devascularization and distal MCA electrocoagulation, overlap can occur in the event of inadvertently large infarcts from either procedure. This can result from the M1 infarct extending too far laterally, or distal MCA stroke extending too far medially, or both. In such cases, overlap is projected at the junction of the two infarcts in the dysgranular zone. This overlap may be within an acceptable error range, but additional overlap from the two procedures should be suspect because it likely implies that one of the infarcts was extremely large and encroached well into the territory of the other. Because the neocortex irrigated by the distal MCA and the motor cortex are reciprocally connected, both stroke procedures involve indirect overlap through deafferentation.

Overlap is also possible between the infarcts from proximal MCA occlusion with a suture and distal MCA electrocoagulation. Under severe ischemic parameters such as longer occlusion duration, hypotension, or both, proximal MCA ischemia damages neocortical regions that are irrigated by distal MCA branches that would otherwise be spared. Overlap can also be achieved if electrocoagulation of the MCA is carried out too

close to it point of origin because it is bound to damage subcortical structures irrigated by proximal MCA branches. But even when the infarcts from the two procedures are anatomically separate, they overlap indirectly through fiber deafferentation. Layer V of the lateral neocortex, which gives rise to corticospinal fibers, is lost with distal MCA stroke as are its corticofugal fibers. The subcortical infarct from proximal MCA stroke interrupts the corticospinal fibers coursing through the basal ganglia. Although it is not clear if the infarct is biased to corticospinal fibers of a particular origin, the fibers originating in the lateral neocortex are likely damaged. By the same token, ascending sensory fibers coursing through the basal ganglia en route to the neocortex are interrupted by the subcortical infarct and the infarct in distal MCA stroke ablates the layer IV neocortical destination of the same ascending fibers.

The proximal MCA infarct and motor cortex stroke seldom encroach into one another's territory even with large lesions. Nevertheless, indirect overlap is evident because corticospinal fiber damage is common ground to both procedures. The devascularization infarct extends into layer V of M1 from which the majority of the corticospinal fibers originates and not surprisingly lost. The proximal MCA infarct damages subcortical territories through which the same corticospinal fibers project before fusing with the internal capsule, which is the major corticofugal pathway for M1. In severe proximal MCA ischemia the infarct may even extend into the internal capsule. Additional overlap is probable considering that subcortical infarcts damage fibers ascending through basal ganglia to reach the motor cortex, which itself is damaged in motor cortex stroke.

In summary, the three strokes produce infarcts that are anatomically distinguishable from one another. Nevertheless, the infarcts may overlap directly when ischemic parameters are made more severe. The infarcts may also overlap indirectly when fiber deafferentation is considered.

Behavioural

The three infarcts initially devastated reaching performance but success scores recovered to control levels with the ipsilateral-to-lesion paw. Success scores also recovered to control levels with the contralateral-to-lesion paw in the motor cortex stroke and distal MCA stroke. Distinct qualitative impairments and recovery patterns characterized the three strokes, however.

The stages of recovery from M1 stroke paralleled the stages of learning the reaching task, which include learning to: (1) orient the snout to the food target; (2) transport the limb to grasp the pellet in the digits; and, (3) withdraw the paw with the pellet and release it into the mouth. After the three stages of recovery were complete and success scores were restored, impairments of limb transport and limb withdrawal were still evident but were overcome with compensatory movements of the trunk. The duration of each stage varied from rat to rat but the pattern was consistent. Paw preference switched after motor cortex injury and applying a bracelet to the ipsilateral-to-lesion paw enforced reaching with the contralateral-to-lesion paw.

In contrast, rats with distal MCA stroke oriented to the food pellet in the immediate postoperative period and attempted to retrieve the food with the designated paw but without success. Thus, the conceptualization of the task was preserved after distal MCA stroke and there was no need to relearn the snout orientation. The reaching

deficits of this group were characterized by an inaccurate limb aim during transport that resulted in numerous reach attempts before the digits grasped the food pellet or displaced it from the holding shelf. These reach attempts unveiled the most distinctive symptom of distal MCA stroke, “phantom grasping”, in which rats checked their paw for a food pellet after each limb transport even though their digits did not contact the food pellet let alone grasp it. Limb transport improved with recovery days, but the “phantom grasp” persisted even after preoperative success scores were restored.

A profile of reaching recovery for the immediate post operative period was not been obtained for proximal MCA ischemia because the health status of this group precluded testing for 2-3 weeks post surgery. Success scores for this group were distinctive in that they remained extremely poor throughout testing. The behavioural underpinning was a digit flexion impairment that prevented successful grasping of the food target. The group’s reluctance to reach with the contralateral-to-lesion paw was also characteristic because a bracelet had to be applied to the ipsilateral-to-lesion at the start of each test session.

Neurophysiological

Intracortical microstimulation (ICMS) was applied to M1 to investigate the topographical organization of movement representations after each of the three strokes as a measure of neurophysiological integrity. Because the infarcts from both distal and proximal MCA strokes do not encroach into M1, it was possible to map M1 ipsilateral to the infarct, whereas the investigation was limited to the opposite hemisphere in motor cortex stroke. Both distal and proximal MCA stroke produced neurophysiological dysfunction in M1 that manifested in the form of diminished forelimb movement

representations. Because in the main the behavioural impairments and anatomical infarcts were distinct for both groups, it is likely that the underpinnings of the diminished forelimb movement representations for the two groups were also incongruent. For example, the diminished forelimb movement representations after distal MCA ischemia might have pertained to deafferentation of M1 input, whereas small motor maps from proximal MCA ischemia might have been related to the loss M1 corticofugal fibers. Also, the distal MCA stroke maps might have reflected inaccurate limb aim during reaching but represented grasping impairments in proximal MCA infarcts. The motor cortex stroke might be envisioned as a complete removal of the motor map from the injured hemisphere but the topography was preserved in the opposite hemisphere.

The laboratory rat as a model for stroke

Most basic life science research depends on model organisms for investigating scientific questions. Some of the model organisms may seem as too distant of a relative to humans and thus less than ideal from satisfying the end goal of understanding the human body and developing methods to repair it. But the value of model organisms to research is readily apparent from the advantages they offer over exploring scientific questions in humans, even if it was ethically acceptable. Invertebrates for example may be favoured as model organisms because they are in the main simpler than humans; their genes are more limited in number and their body parts are smaller and thus have fewer cells. Conducting research in model organisms is facilitated by the fact that they are raised and maintained in controlled laboratory environments, which means they can be manipulated at any point in their lifetime and little is unknown about their past. The controlled conditions of the laboratory also facilitate research in that variables of interest can be manipulated one at a

time, promoting sound interpretation of relationships between independent variables and phenomena.

The usefulness of discoveries made in model organisms can take years until it is completely appreciated. During this time, studies are replicated, novel ideas are tested, competing views emerge and perhaps resolved. Nevertheless, this does not detract from the fact that the basis of major discoveries in the life sciences that eventually became pertinent to understanding the human and body and brain, is derived from observations made in model organisms that may be regarded as unfit models because of lack of relatedness to humans. For example, Seymour Benzer's seminal work on the link between genes and behaviour that has shaped the field of genetics as we know it today, was primarily conducted in the fruit fly *Drosophila melanogaster.* Alan Hodgkin and Andrew Huxley revolutionized our understanding of the ionic mechanisms involved in excitation and inhibition in the neuronal membrane. Their work in the 1950's in the giant squid axon shaped the field of in vitro neurophysiology until the present day. The neural basis of memory consolidation is arguably rooted in Eric Kandel's work on ganglia extracted from the marine mollusk *Aplysia californica*. The behavioural relevance of model organisms is also apparent in the work of the Konrad Lorenz demonstrating the conservation of simple learning between animals. His work on geese is considered seminal to the pertinence of early childhood in shaping adult behaviour, a line of work that is rapidly extending beyond the scientific community to becoming a household staple.

The examples given are by no means an exhaustive survey of the pertinence of model organisms to life science research, but they should be sufficient to put in

perspective the use of the laboratory rat to investigate the neural basis of movement and for modeling the motor syndromes of stroke.

Limitations of the rat model of MCA stroke

The laboratory rat has emerged as a clear favorite among laboratory animals for modeling MCA stroke. The rat shares a similar motor system and similar vasculature to humans. Surgical procedures have been specifically developed to model MCA ischemia in rats and motor tests have been developed to assess impairment and recovery. One may view the progress in so far as promising because modeling stroke in rats is approximately three decades old, a young field by most scientific standards. A narrower view may be more critical of the progress, however. From the standpoint of treatment efficacy, one may argue that progress has been limited. An extreme position within that view may contend that the bottleneck of stroke research is at the stroke-modeling phase, which may need reform either by using a species other than rats or improving research conduct in the rat models of stroke. In the next section, I will discuss some of the potential pitfalls and how they may imperil the translation of treatments from the laboratory to the clinic. A counter argument to some of the points will be presented.

(1) Procedures of ischemia

The experimental induction of ischemia is an approximation of ischemic stroke. Rats, including the stroke prone spontaneously hypertensive rat strain (SHR), rarely have spontaneous stroke accidents. An intricately connected network of blood vessels provides collateral blood flow that compensates for vascular blockage. In addition, a healthy diet probably reduces the risk of arteriosclerosis, which is a major contributor to stroke. Stroke therefore has to be artificially induced in rats with measures that overcome

collateral blood flow. Occluding the entire length of a blood vessel, occluding the vessels that provide collateral blood flow as well as the target vessel, and systemic hypotension during stroke, are all viable approaches for counteracting the collateral blood flow. Such measures are necessary for ischemia to induce any apparent infarct in rats and are obviously contrived when compared to clinical stroke.

Treatments developed in the laboratory are tested on rats that received ischemic procedures approximating clinical stroke. Translating the treatments from such an animal model to the clinic is not surprisingly challenging. It may even be the case that the effectiveness of a particular treatment is limited to the experimental lesion. For example, immediate treatments generally aim to halt cell death after ischemia. But if the cell death episodes in experimental ischemia are distinct from human stroke, then laboratory treatments may not be valuable in the clinic or at least require further modification. Long-term treatments administered after the stroke infarct matures are subject to the same concerns. The objective of such treatments is to alleviate stroke syndromes by promoting functional reorganization in tissue spared by the stroke. But if the laboratory infarct is inherently different from the clinical infarct, then it is also conceivable that the tissue spared by the experimental infarct may be morphologically and physiologically different from the tissue spared in clinical stroke. Thus, treatments effective in promoting functional reorganization in intact tissue after laboratory stroke may not be effective after clinical stroke. Although stem cell therapies are still at the laboratory stage, they do not escape the same scrutiny.

(2) Infarct volume as a measure of recovery

The relationship between infarct location/size and functional outcome is complicated and poorly understood. Using infarct volume as a primary measure of treatment efficacy is therefore misleading. For example, an infarct that damages the internal capsule, the largest collection of motor cortex output fibers, need not be too large to severely deafferent the motor cortex and devastate skilled forelimb movements. An infarct that is comparable in size but delivered to the motor cortex may have a milder consequence on limb movements as well as a more promising prospect for recovery because of the potential for compensatory functional reorganization in the intact motor cortex to mediate the functions lost to the injury. Thus, both groups are differentially impaired from infarcts of comparable size but delivered to different locations within the same system. Setting a target of infarct volume reduction is therefore at best conjectural because it is not clear what an effective target reduction may be, especially when the number of potential infarct sizes/locations is indefinite such as in MCA stroke. To relate this to the example offered, a 20% reduction in infarct volume may significantly improve function for the motor cortex group but the effects may be negligible for the more severely impaired internal capsule group. Thus, even if a treatment is effective in limiting infarct volume in any location, it is not clear whether this is of any benefit for functional outcome. Infarct volume may be useful for assessing treatments if coupled with behavioural and neurophysiological measures.

(3) Age at time of stroke

Laboratory rats receive strokes at a younger age than when most humans suffer a stroke. The incident of stroke is twice more prevalent in the second half of a person's life. Rats typically receive stroke at 3-4 months of age. Considering that the life span of a rat

is approximately two years, rats are receiving strokes months prior to their half-life and at a much younger age if compared to humans.

The neural plasticity that mediates the reorganization of function that underlies behavioural improvements is at optimal capacity at younger ages and declines with time. This is best exemplified by the remarkable spontaneous recovery demonstrated by rats that suffered a neonatal lesion and to a lesser extent by the young rats in the present thesis that received neocortical strokes. The expectation is that rats older than the ones used in the present thesis recover more modestly from the same lesions. Arguably, the relationship between age and recovery from injury is the same for humans, which implies that most stroke accidents are occurring at an age when the capacity for recovery is in decline. Yet treatments developed in the laboratory are tested on young stroke rats. It is not surprising that treatments developed under such circumstances may fail when applied to the older clinical population.

The problem is readily reformed by inducing stroke and testing treatments on rats that are approximately at their half-life, to make the model more relevant to clinical stroke. Nevertheless, this can present additional challenges because most of the knowledge base about rat behaviour and motor systems is derived from young rats. Modeling stroke in old rats means that ageing, the motor syndromes of stroke, and the interaction between ageing and the motor syndromes of stroke, have to be investigated, as opposed to just studying the motor syndromes of stroke as would be the case in young rats.

(4) Experience prior to stroke

The life history of each rat subject is known and controlled by an experimenter. Generations of rats are born in breeding facilities and closely monitored till they are terminated at the end of a study. Rats are raised in clean and climate controlled laboratory environments and eat measured quantities of a healthy diet. Rats are typically housed with at least one other rat, with which they socialize and exercise during play fighting. An experimenter also frequently handles the animals during cage cleaning, weighing, and behavioural testing. Symptoms of illness are considered grounds for exclusion from the study. Thus, stroke treatments are tested on groups of rats that aside from having a stroke are healthy and live in optimal conditions.

To the contrary, stroke sufferers come from a diverse background (ethnicity and life style). Because there is no practical way to screen people before they suffer a stroke to document their life style and history, such a profile is created in the aftermath of a stroke. Of course this can be complicated in cases in which amnesia and/or aphasia preclude the patients from recounting their history prior to the injury. But even if this is not the case, the number of factors that may have contributed to the stroke and the interaction between these factors is endless and idiosyncratic making it impossible to parse factors that may have relevant to the injury from benign ones. Thus, aside from obvious culprits such as smoking, obesity, hypertension, and lack of exercise, profiling a patient's history may be of little value for stroke research. Comparing the range of factors that may dispose a patient to suffer a stroke to the well-regulated life style of the rat before it receives a stroke, suggests that translating stroke treatments developed in the laboratory to a clinical population has the potential to fail because the treatment was not

tested on an equivalently experienced brain. Worse yet, it may be impossible to identify the underpinning of the translation failure for future improvement.

At the same time the closely monitored history and tightly regulated living conditions of the laboratory rat are some of the most important assets of model organisms in general and for the laboratory rat and stroke. Only under such conditions can the benefits of a stroke treatment be empirically investigated with little concern for factors that may interact positively or negatively with the treatment. This is at least a critical step for objective screening of promising treatments. A possibility that is prohibitive in the clinical stroke population.

(5) Homogeneity of laboratory infarcts

Laboratory studies test treatments on groups of rats with homogenous stroke infarcts. Although this experimental set up is necessary for assessing the efficacy of a treatment on multiple and sizeable groups of rats for adequate experimental control and statistical power; it is problematic for translating the findings to a stroke population with heterogeneous infarcts. The benefits of a treatment that is effective in one experimental infarct may be limited to the same clinical infarct but fail in the rest of the population. The problem can be addressed by testing experimental treatments on a number of different rat strokes (e.g. proximal and distal MCA ischemia) and only advance treatments that are robust in multiple stroke procedures to the clinic. Alternatively, a treatment proven in one type of experimental stroke should be selectively applied to human cases with similar infarcts.

(6) Duration of laboratory stroke studies

Studies that use rat models of stroke are generally brief lasting less than a week from the onset of ischemia. Testing a treatment for such a brief duration presents a major confound for translating the findings to clinical research because the benefits of the treatment may be overestimated. This may be related to inappropriate differentiation between spontaneous recovery, which is optimal in the immediate postoperative period, and the benefits of the treatment. It may also be related to an inherently limited long-term benefit of the treatment. This cautionary note does not undermine the importance of developing treatments that tackle stroke early after onset, but it does emphasize the importance of assessing the long-term benefits of all treatments including ones designed for administration immediately after stroke. Studying the effects of a treatment on rats for several weeks after stroke can circumvent the advancement of treatments with little promise for enduring benefits to the clinic where the long-term improvement is pertinent.

(7) Behavioural assessment of rat models of stroke

Functional outcome may be inadequately assessed in experimental stroke. The measures for assessing motor capacity in rats are either reflexive, or involve active participation on part of the rat. Reflexive tests such as tail hanging, tests of bracing, and vibrissae placing, identify if an animal is impaired. But the assessment scale for these tests is binary and does not capture the degree of impairment or recovery, both of which are more appropriately represented on a continuous gradient. In addition, because reflexive tests do not offer qualitative descriptions, they are unlikely to distinguish between stroke groups that are equally impaired but have varying deficits. Reflexive tests can be useful for screening the effectiveness of an ischemic procedure in damaging the

brain when visual confirmation of ischemia is not possible such as in the intraluminal suture model. But they should perhaps be avoided in the actual study.

For motor tests that involve active engagement on part of the animal, the assessment is comprised of multiple quantitative and/or qualitative measures. Rats can therefore be ranked according to the severity of their impairment, which allows separation between groups that may not be possible with binary measures. Nevertheless, the motivation underlying the task can complicate interpretation of the results. For example, in the rung walking test, a rat walks across a horizontal ladder to reach a dark goal box, which it favors over the uncovered testing apparatus. After stroke, the rat traverses the ladder but commits more slipping errors than before stroke. The impairments reflect a motor incapacity, but the possibility that the animal is not motivated to rehabilitate its stepping because it reaches the goal box irrespective of stepping errors is not addressed. Repetitive testing on the apparatus may even foster the rat's perception that slipping on the rungs is of no major consequence. The cylinder test is subject to the same pitfall. Rats are impaired at supporting their weight with the contralateral-to-lesion paw during vertical exploration. Nevertheless, long-term disuse of the contralateral-to-lesion paw may be related to a motor incapacity, a learned disuse, or a lack of motivation to use the impaired paw. After all the animal can explore the cylinder walls by lifting off from the floor with one paw, supporting its weight on the wall with the same paw, and landing on the floor with the same paw again. Thus, even if the unilateral impairment is severe it does not pose a major consequence to the rat in the cylinder test. Recovery in such tests may be implicitly driven in that neural adaptation may aspire towards restoring normal paw placing and postural support for the sake of

restoring normalcy. But its all the more reason for optimism if a treatment shows benefits on these measures.

In contrast, rats tested on reaching-for-food tasks are hungry during testing and thus motivated to perform the task to the best of their ability to maximize food retrieval. Thus, the animals may actively engage in their own rehabilitation under such testing conditions. Performance can also be measured with quantitative and qualitative measures, which makes it valuable for characterizing the motor syndromes of stroke. This was particularly constructive for purposes of the present thesis aimed at differentiating the impairments that ensue from various MCA infarcts in rats, which would not have been possible in tests that do not offer a qualitative analysis.

The potential for motivational differences between task objectives poses a challenge for understanding recovery of function because the objective of the laboratory test can influence the rat's engagement in the recovery process. Furthermore, drawing parallels between the factors that motivate a rat to perform a laboratory test and the factors that motivate patients to participate in clinical tests or engage in rehabilitative therapy is not straightforward. Treatments that promote recovery on laboratory tasks that entail a certain motivational level may yield unsuspected results in the clinic where functional outcome is measured on tests that demand a different level of motivation. Nevertheless, the use of tests that are ethologically relevant to both rats and humans at least has the potential of ameliorating the problem because both rats and humans may be equally motivated to perform the task.

There is no doubt that our implementation of the laboratory rat as an animal model of stroke continues to improve. Nevertheless, there is still plenty of room for

progress. Using rats with infarcts of different sizes and locations, studying these rats for weeks or months to understand how their impairment and recovery evolve, and testing them on behavioural measures that offer a qualitative analysis and can be readily related to humans, are but a few of the improvements that can be easily standardized in stroke research. In considering the potential return of these feasible changes that should manifest in improved understanding of the most widely used animal model of stroke, implementation delays seem unwarranted.

Conclusion

Scientists modeling stroke in the laboratory rat will continue to be partial towards specific procedures for inducing ischemia. Their choice of model may be guided by the semblance of a particular procedure to human stroke, or the reproducibility of the infarct produced by the procedure, or the lack of surgical complexity of a procedure. The debate may not relent until a treatment is discovered and successfully implemented in the clinic. It is not my intention to recommend one particular stroke procedure over the other, as there are advantages and limitations to each. But I take no reservation in asserting that aspects of experimental stroke such as the behavioural syndromes, anatomy of the infarct, neurophysiological characteristics of tissue spared, should be seriously considered when selecting a model of stroke.

The present thesis described the behavioural, anatomical, and neurophysiological consequences of three procedures routinely used to model MCA stroke in the laboratory rat. The experiments narrow the gap that separates experimental and translational research by offering neutral ground that is comprehensible to both camps. The unique contribution of the thesis is that it tested rats with different strokes on a skilled reaching

task that permitted a qualitative profiling of the impairments. The index of impairments should guide the selection of a rat model of stroke and set a clear objective for stroke-specific treatments. A contribution was also made in understanding the neurophysiological sequel of stroke, which was examined with intracortical microstimulation in the spared tissue. The experiments showed that infarcts that spared the motor cortex rendered it dysfunctional for weeks after injury. This was due to loss of input into motor cortex, loss of output from motor cortex, or both. Thus, neural dysfunction from stroke extends beyond the boundaries of the infarcts. It follows that although the three infarcts were anatomically distinct, they overlapped indirectly perhaps through deafferentation. Because tissue spared by the infarct is intact but deafferented and at the same time neurophysiologically dysfunctional, treating the neurophysiological dysfunction of the spared tissue may hold the most promise for intervention that promotes recovery as opposed to tissue directly damaged by ischemia.

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Appendix 1

Edited video clip demonstrating the three phases of rearing in a cylinder: (1) ascent, (2) wall scan, and (3) descent.

Appendix 2

Edited video clip demonstrating the pattern of paw placement on the walls of a cylinder during a rear.

Appendix 3

Edited video clips demonstrating snout-pellet opposition presurgery and on postsurgical days 1, 2, 3, 7, 10, 14 from a single motor cortex rat. The first video frame from the video clip for each day was paused for 20 video frames (0.67 sec) to show the starting position of orientation with the rat at the rear of the apparatus. The remainder of the opposition is played at normal speed or at 400% of the normal speed (FF X4). The last video frame from the video clip for each day was paused for 6 seconds. The first second shows the position of the animal when it sniffs the food pellet. In the other five seconds a tracing (yellow) of the path of the snout from the starting video frame to the last video frame was superimposed. Note, that the rat did not sniff the food pellet on the postsurgical day 1 and snout tracings were progressively shorter on subsequent recovery days as snout-pellet opposition was achieved more directly.

Appendix 4

Edited video clips demonstrating paw-pellet opposition presurgery and on postsurgical days 1, 3, 7, 14 from a single motor cortex rat. The first video frame from each attempt was paused for 20 video frames (0.67 sec) to show the starting position of the paw on the floor of the reaching box before it was advanced towards the slot. The remainder of the opposition is played at normal speed. The last frame from each failed paw-pellet opposition attempt was paused for 20 video frames to show the final position of the paw with the pellet displaced or untouched. The last video frame for the opposition was paused for 6 seconds. The first second shows the posture of the rat as well as the position of the forepaw as the digits flexed around the pellet. In the other five seconds a tracing of the path of the contralateral-to-lesion paw (yellow) and ipsilateral-to-lesion paw (red) from the starting video frame to the last video frame was superimposed. Note, paw transport was is not traced on postsurgical day 1 because the paw-pellet opposition was not achieved. Upon establishment of paw-pellet opposition, the number of paw transports increased and then progressively declined across test days. Traced movements of the ipsilateral-to-lesion paw revealed that at times the paw was attempting to retrieve the food pellet, whereas at other times it assisted in withdrawing the contralateral-to-lesion paw from the shelf where it might have been stuck.

Appendix 5

Edited video clips demonstrating mouth-pellet opposition presurgery and on postsurgical days 1, 3, 7, 14 from a single motor cortex rat. The first video frame for each mouth-pellet opposition was paused for 20 video frames (0.67 sec) to show the starting position

of the mouth and paw at the beginning of the opposition. The remainder of the opposition is played in normal speed. The last video frame of the opposition from each day was paused for six seconds. In the first second, the posture of the rat as well as the position of the mouth and paw are depicted as the pellet is released into the mouth. In the subsequent five seconds, a tracing (yellow) of the path of the pellet from the shelf until it was released into the mouth was superimposed. Note, the path of the pellet was not traced on postsurgical days 1 and 3 because mouth-pellet was not achieved.

Appendix 6

Edited video clips demonstrating the characteristic impairments from distal MCA stroke and motor cortex stroke. The clips have been synchronized to allow head-to-head comparison of the different phases of the reach.