# EXPERIENCE DEPENDENT PLASTICITY OF STROKE OUTCOME

BROOKE D. RAKAI
Bachelor of Science, University of Calgary

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## Abstract

Stroke outcome is highly variable. Experiments in this thesis test the hypothesis that experience prior to a stroke is an important variable in the manifestation of stroke. Optokinetic tracking was used to evaluate the effects of visual cortex stroke and MCA occlusion in rats. Normal laboratory rats showed a small, but significant decrease in tracking thresholds following visual cortex stroke. Animals with developmental visuomotor experience or reach training experience in adulthood, however, had tracking thresholds which were substantially increased, and the effects of visual cortex strokes were greater. MCA occlusions did not affect tracking behaviour. These data indicate that specific experiences engage neural plasticity that can alter brain function. These changes can, in turn, affect the behavioural manifestation of a stroke. Understanding the effect that environmental experience has on stroke outcome promises to enable better characterization of strokes, and set appropriate behavioural baselines for the measurement of recovery of function.

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MCA = Middle Cerebral Artery
MCAo = Middle Cerebral Artery Occlusion
PCA = Posterior Cerebral Artery
ACA = Anterior Cerebral Artery
OKT = Optokinetic Tracking
VOS = Virtual Optomotor System
VWT = Visual Water Task
SF = Spatial Frequency
c/d = cycles per degree
P15 = Postnatal Day 15
P30 = Postnatal Day 30
V1 = Primary Visual Cortex
LE = Long Evans
CCBN = Canadian Centre for Behavioural Neuroscience
CCAC = Canadian Council on Animal Care

## Chapter 1 - Introduction

Imagine you are on vacation at your favorite hot spot. You wake up in the morning, get your cup of coffee, and sit out on the patio to enjoy the view. After a few minutes you get up to refresh your cup and stumble a bit, but think nothing of it as it is still early in the day and you haven't done much moving around. Soon you start to develop a headache, and you hope it won't interrupt your plans for the day. As more time passes, your headache gets worse, and you start to feel nauseated. You head for the bedroom in hopes of snoozing the pain away. While lying in bed, you suddenly realize that you can no longer move. You want desperately to at least sit up, but your body just won't no matter how hard you try; you are completely immobile. You have had a stroke, likely a massive one, and with the way it has progressed you may not regain mobility and could even die. In less than a few hours from onset, you may be declared brain dead. This is a very scary thought, and an extreme example, but this is a true story. It happened to a very close family member of mine, who did not survive. Yet, this story really shouldn't be new to many of us. In Canada alone, 50,000 people suffer from stroke each year. This works out to one person every ten minutes (Canadian Stroke Network, 2008), and statistically speaking we all should, at the very least, know of one person who has had a stroke. The effects of stroke can be devastating, with the majority of patients showing debilitating functional losses, and like the case just described, death. In fact, stroke is a leading cause of death around the world and its prevalence is increasing with an aging population,

because the aged are most commonly, though not exclusively, affected (Canadian Stroke Network, 2008).

Not all stroke patients die, however, and not all outcomes are the same (Minn, Cho, Kim, Kwon, Kim, Oh, et al., 2008; Patel, McKevitt, Lawerence, Rudd, & Wolfe, 2007; Carandang, Seshadri, Beiser, Kelly-Hayes, Kase, Kannel, Wolf, 2006) because many variables which influence stroke manifestation and outcome naturally vary. There is not a simple relationship between factors such as the location and size of the damage, the vascular and structural organization of each individual brain, impairments, recovery, and response to treatment following a stroke. This thesis proposes that, among the multitude of factors that vary from one stroke patient to the next, individual differences in prior experience may be a key variable affecting stroke outcome. A model has been developed which is sensitive to the differential effects of specific experiences on behavioural outcome following stroke. By testing fundamental, reflexive visuomotor behaviours known as optokinetic tracking (OKT) in a rat model, the effects of specific developmental or adulthood experiences on OKT following stroke have been investigated. This model of OKT quantification has shown that the behavioural manifestation of stroke is differentially affected by specific experiences.

### 1.1 Stroke

Stroke is classified as a cerebrovasuclar accident, caused by a sudden interruption of blood flow to the brain resulting in the death of neurons (Martin, 2006). There are two main types of stroke, ischemic and hemorrhagic. Ischemic stroke comprises approximately 80% of all strokes, and is characterized by a

blood clot which interrupts blood flow to the brain (Heart and Stroke Foundation, 2008). Ischemic strokes can be further divided into thrombotic strokes, where the clot forms in an artery directly supplying the brain with blood, or embolic strokes in which a clot originating elsewhere in the body travels to the brain to cause a blockage there. Either of these forms of ischemic attack can be permanent, or temporary, the latter case being termed a transient ischemic attack (Heart and Stroke Foundation, 2008). Hemorrhagic strokes comprise the other 20% of strokes, and are characterized by a rupture of a blood vessel in the brain. Again there are two sub-classifications of hemorrhagic stroke, subarachnoid hemorrhage and intracerebral hemorrhage. As their names imply, subarachnoid hemorrhages refer to bleeding on the surface of the brain, while intracerebral hemorrhage refers to bleeding deeper within the brain (Heart and Stroke Foundation, 2008). The most common type of stroke, however, is unilateral middle cerebral artery (MCA) ischemic stroke (Heart and Stroke Foundation, 2008; Canadian Stroke Network, 2008).

### 1.2 Variability in Stroke

All types of stroke display one puzzling feature: variability. Between patients, there are deviations in the location of damage caused by stroke, the size of the damaged area, the functions that are affected by stroke, the amount of recovery that each patient undergoes, and their apparent responses to treatment. In fact, variability seems to be a defining feature of stroke, as few patients have the same lesion, outcome, or general circumstances and there is no clear relationship between variables affecting outcome.

### 1.2.1 Variability in Lesion Location

The first aspect of variability in stroke to be discussed is the location, in the brain, of the resulting lesion. The brain is a large organ supplied with blood by three major cerebral arteries, the anterior, posterior, and middle cerebral arteries, each of which has numerous divisions and communicating arteries (Martin, 2006; Liebeskind, 2003; Kolb & Whishaw, 2003). A stroke can occur in any of these arteries or their divisions, and so the location of the lesion will vary depending on which artery and which portion of an artery is blocked (Heart and Stroke Foundation, 2008). For example, the posterior cerebral artery (PCA) artery supplies numerous regions of the posterior portion of the brain including the occipital lobes, the medial and inferior temporal lobes, the inferior medial parietal lobes, as well as portions of the midbrain and thalamus (Finelli, 2008). Therefore, stroke patients with a blockage in the PCA may show damage in one or many of these regions. The middle cerebral artery (MCA) supplies nearly 70% of the brain with blood including regions of the medial cortex such as Broca's area, Wernicke's area, the pre- and post-central gyri, and the temporal and parietal lobes, as well as subcortical structures such as the basal ganglia and internal capsule (Martin, 2006). Because of the vast part of the brain that is supplied blood by the MCA and its divisions, patients with an MCA stroke show very different locations of damage as well, in all or some of the regions which are fed by this artery. Ganesan, Ng, Chong, Kirkham and Connelly (1999) discovered, in a study of childhood MCA territory strokes, that the location of damage varied greatly, as did the manifestation of symptoms and overall outcome. The results of

this study show that even when a stroke occurs in the same artery, different lesion locations can result.

Though the same arteries can be found in each individual brain, their exact arrangement differs from person to person (Kolb & Whishaw, 2003; Lee, 1995; Moody, Bell, & Challa, 1990). Individual differences in the organization of the brain's vasculature can also cause variability in the location of damage caused by a stroke event, and may also have played a role in the result of Gaesan's (1999) study. If the same branch of the MCA was blocked in his patients, but the exact location of the branch differed across patients, then different lesion locations, symptoms, and outcomes would be expected. This is often the case, as many patients with blockages of the same artery show different lesion locations within the brain (Rordorf, Koroshetz, Copen, Cramer, Schaefer, Budzik, et al., 1998).

### 1.2.2 Variability in Lesion Size

Each stroke patient also displays different lesion sizes, and this can be affected by characteristics of the blockage, the portion of the artery in which the blockage occurs, collateral blood supply, as well as conditions during and following the stroke event (Schiemanck, Post, Kwakkel, Witkamp, Kappelle, & Prevo, 2005). First, the larger the area of the brain that is deprived of blood, the larger the resulting lesion will be. For example, a blockage which completely occludes an artery vs. one which causes only partial occlusion or narrowing of the artery, will cause greater severity of damage and larger lesion size (Heart and Stroke Foundation, 2008; Martin, 2006). Also, clotting at lower portions of an artery, before extensive branching, will also cause greater damage than a blockage

further along the artery, as many more branches will be starved of blood. Variation in collateral blood supply, that is, arteries which indirectly feed an area, can also help to prevent the spread of the damage in some patients. In this sense, patients will show inconsistent lesion sizes depending on the extent of the blockage, the point along the artery at which the blockage occurs, and the collateral blood supply from other arteries. Lesion size will also fluctuate depending on the duration of the blockage. This is because there is a core region of irreversibly injured tissue which is surrounded by an area of mild damage following stroke. This region of mild damage is highly susceptible to further injury, and if it is not supplied with blood soon after the initial infarction, the damage will spread (these surrounding regions are known as the ischemic penumbra). The dimensions of this penumbra and the areas of severe and mild damage will differ depending on the length of time until reperfusion (Kolb and Whishaw, 2003). The longer the brain is deprived of blood the more damage ensues, as there is more time for the cascading effects of the damage to expand into surrounding areas (Martin, 2006; Kolb & Whishaw, 2003).

The temperature of the brain and blood during and following the initial stroke event can also strongly affect the outcome with respect to the size and depth of damage. High temperature is conducive to cell death, and the spread of the ischemic penumbra (Hong, Rha, Lee, Ha, Kwon, & Kim, 2003; Thornhill & Corbett, 2001; Reith, Jorgensen, Pedersen, Nakayama, Raaschou, Jeppesen, et al., 1996). Though the exact mechanisms of the effects of temperature and causal relationships between temperature and infarct size have not been fully elucidated,

nflammation is thought to be conducive to cell death, and encourages the expansion of severely damaged areas and the ischemic penumbra following injury (Nakase, Yamazaki, Ogura, Suzuki, & Nagata, in press; Wang & Zhu, 2008; Wang, Tang, & Yenari, 2007; Marquardt, Ruf, Mansmann, Winter, Buggle, Kallenberg, et al., 2005; Nakase, Sohl, Theis, Willecke, & Naus, 2004; Barone & Feuerstein, 1999; Haring, Berg, Tsurushita, Tagaya, del Zoppo, 1996). Though the cellular mechanisms involved in hyperthermia and inflammation are complex and not well understood, evidence has shown that both high temperature and inflammation prevent the brain from returning to a normal state where damaging processes will cease, and repairing processes can begin (Rodriguez-Yanez & Castillo, 2008). Therefore, lesion size will vary depending on the temperature and inflammation in the brain before, during, and following a stroke. (Cho et al., 2007)

### 1.2.3 Variability in Functional Impairments

There are many symptoms that may be described in stroke patients, regardless of lesion size. Functional losses are often distinct from person to person, and may be linked to lesion location (Rose, Brooks, & Rizzo, 2005; Petty, Brown, Whisnant, Sicks, O'Fallon & Wiebers, 2000; Chen, Tang, Chen, Chung, & Wong, 2000). Generally, damage to posterior portions of the brain tend to cause visual field defects, damage to the central regions of the cortex often cause

motor disturbances, and damage to anterior regions can disrupt limbic system functions as well as causing disabilities of cognitive function (Martin, 2006; Kolb & Whishaw, 2003). More specifically, certain aspects of a general function can be differentially affected by stroke between patients. For example, damage to motor areas of the brain can cause impairments in upper limb function, lower limb function, or both (Baer & Smith, 2001; Duncan, Goldstein, Horner, Landsman, Samsa, & Matchar, 1994). Certain sub-movements of the limbs can also be differentially affected (Gharbawie, Auer, & Whishaw, 2006; Rohrer, Fasoli, Krebs, Volpe, Frontera, Stein, et al., 2004; Whishaw, 2000) Finger movements and placement for grasping, wrist movements, and directional arm movements can each be affected separately or in combination following motor cortex stroke (Nakayama, Jorgensen, Raaschou, & Olsen, 1994). These studies investigating behavioural outcome following stroke have shown that specific impairments in function can vary.

### 1.2.4 Variability in "Spontaneous" Recovery of Function

Further variability in stroke occurs with recovery of lost function (for review see Kollen, Kwakkel, & Lindeman, 2006; Cramer, 2008; Cramer & Bastings, 2000). Current statistics from the Canadian population show that 10% of patients recover completely, and appear to be unimpaired by the stroke event (Heart and Stroke Foundation, 2008). Other patients recover only certain aspects of their original abilities (Bonita & Beaglehole, 1988). Of all stroke patients, 25% are said to recover with minor impairments, and 40% are left with moderate to severe disabilities. Approximately 10% of stroke patients, however, do not show

signs of recovery at all, and are so debilitated by the stroke that they need assistance with many aspects of daily care (Heart and Stroke Foundation, 2008; Rothwell, Coull, Giles, Howard, Silver, Bull, et al., 2004). The extent of recovery of function is clearly inconsistent across patients, and can also be influenced by factors other than the stroke itself.

### 1.2.5 Variability in Treatment Effects

Finally, the main problem with stroke today is that there is a lack of effective treatments for stroke patients. Those treatments that are available vary from drug administration, to surgery, behavioural therapies, or combinations of these, although none of the available treatments have proven satisfactory. Many patients have shown large increases in functional ability after treatment or therapy, while other patients have shown only minor effects of the same treatment (Mirbagheri & Rymer, 2008). For example, a recent clinical trial of Minocycline, a second generation derivative of tetracycline known to have neuroprotective effects in rodent models, showed a significant increase in behavioural outcome following treatment (Lampl, Boaz, Gilad, Lorberboym, Dabby, Rapoport et al., 2007). Though raw scores were not provided, the variability in behavioural assessment scores were more than 50% of the reported improved score, suggesting that not all patients recovered to the same degree, and a wide range of variability in outcome measures. Another study tested the effects of a behavioural treatment, constraint-induced movement therapy, and showed great variability in functional outcome as well (Wolf, Winstein, Miller, Thompson, Taub, Uswatte, et al., 2008). Patients' recovery varied depending on the measure, the amount of

time from stroke to the beginning of therapy, initial impairments, and follow-up time period.

In addition to these studies, others have shown that the amount of time it takes for treatments to show beneficial effects is inconsistent. Some patients apparently respond immediately to treatment and show signs of recovery in days or weeks following treatment onset (Stinear, Barber, Coxon, Fleming, & Byblow, 2008; Lampl et al., 2007; Celnik, Hummel, Harris-Love, Wolk, & Cohen, 2007) whereas others seem to take months or even years to show functional improvements (Kwakkel, Kollen, & Twisk, 2006; Schiemanck et al., 2005; Meldrum, Pittock, Hardiman, Ni Dhuill, O'Regan, & Moroney, 2004; Broeks, Lankhorst, Rumping, & Prevo, 1999; Duncan et al., 1994; Dam, Tonin, Casson, Ermani, Pizzolato, Iaia & Battistin, 1993). Overall, there is little consensus on the benefits of any of the available treatments and therapies, and results of treatment trials have been largely inconsistent (Kwakkel, Kollen, & Krebs, 2008; Stinear et al., 2008; Martinsson, Hardemark, & Eksborg, 2007; Merino, Latour, Todd, Luby, Schellinger, Kan, et al., 2007; McCain, Pollo, Baum, Coleman, Baker, & Smith, 2008; Lampl et al., 2007; Rose et al., 2005; Kidwell, Liebeskind, Starkman, & Saver, 2001).

Variability in stroke location, size, impairments, recovery, treatment success and overall behavioural outcome make treating stroke and developing new treatments difficult for researchers and clinicians. There is insufficient knowledge available about which treatment options will be beneficial for which patient and why (Pollock, Baer, Langhorne, & Pomeroy, 2007; Kolb & Whishaw,

2003). It is necessary to know which treatment to assign to each patient to ensure that they have the best chance for recovery, and the best possible outcome. Unfortunately, this knowledge is currently lacking. Until the factors underlying variability in stroke are better understood, progress in stroke treatment will undoubtedly remain limited.

### 1.3 Causes of Variability

Recently, researchers have attempted to determine the cause of the vast amount of variability in stroke. By holding constant many of the variables associated with stroke, such as age, demographics, infarct location and size, stroke subtype, and clinical impairments, attempts have been made to account for biologically relevant variability in stroke outcome (Ottenbacher, Campbell, Kuo, Deutsch, Ostir, &Granger, 2008; Prabhakaran, Zarahn, Riley, Speizer, Chong, Lazar et al., 2008; Broeks et al, 1999). In spite of these attempts, unexplained variability remains present in all stroke studies and the manifestation and consequences of stroke remain differential across patients, regardless of the number of variables which are controlled. Results of stroke studies suggest that there may be other sources of variability which have not yet been fully characterized.

#### 1.3.1 Individual Experience

Though our brains are slightly different at birth, for the most part, they are a blank slate prepared to respond to the world. When we are born, our brains are still developing, and this development is influenced by the environment. Therefore, it is not until we begin to grow in the environment that our brains

begin to organize themselves and function in very different ways, and we become very different people (Keverne, 2004). The gross morphology of the brain remains similar between individuals throughout life, but differences in vascular and synaptic organization and specific functioning of each individual brain become more apparent as we age (Prabhakaran et al., 2008; Kolb & Whishaw, 1998; Kolb, 1995). It has become commonly known that the brain is a plastic structure, and that changes occur in response to experience, the environment, and to injury throughout life (Gauthier, Taub, Perkins, Ortman, Mark, & Uswatte, 2008; Prabhakaran et al., 2008; Kolb, 1995; Kolb, Gibb, & Robinson, 2003). The experiences that each individual has and the brain's responses to those experiences are often unique, and so the changes that occur in the brain because of these experiences are also individually distinct (Prabhakaran et al., 2008; Kolb, 1995). Therefore, individual differences in organization, structure, and function of the brain may be related to the life experiences that help to shape our brains.

Since differences in each brain, such as vascular and structural organization, are related to differences in stroke outcome, it may be that the experiences that a person has in life are also linked to the variability in stroke location, size and depth, impairments, recovery, and overall outcome. A key to unlocking the puzzle of variability in stroke manifestation and outcome may lie in individual differences in experience.

### 1.4 Hypothesis

If, in fact, our life experiences cause the variability that occurs with stroke, then how can we use this information to help those affected by stroke? Part of the

difficulty in treating stroke patients is that we cannot immediately determine how they will be affected. Accurate estimation of a patient's prognosis soon after the stroke may allow clinicians to start the appropriate treatment at an optimal time (Canadian Stroke Network, 2008; Gilman, 2006). The earlier a patient's needs for recovery can be determined, the earlier those needs can be fulfilled through treatment. If a certain experience is known to lead to specific functional losses after stroke, we may be able to make better predictions about a patients' functional outcome based on some of the major experiences they have had. For example, if learning to play the guitar results in similar plastic changes in the brains of all individuals then information can be gathered from stroke patients who played guitar before their stroke, and knowledge about their outcome can be applied when making predictions about the prognosis of new guitar playing stroke patients. Similarly, if working in construction causes certain changes in the brain, we can also look at past construction workers who have had a stroke, and apply information about their functional outcome and recovery to new patients who have also worked in construction. Overall, understanding the effect that environmental experience before a stroke has on stroke outcome promises to enable better characterization of strokes, and may help to control some of the variability seen in stroke treatment trials. The key is to know what to expect, so that we can combat the loss of function that a patient will experience with the greatest accuracy and at the most appropriate time.

The following studies aim to investigate individual experiences as a factor influencing stroke manifestation, and hypothesize that variability in behavioural outcome is affected by individual differences in experience.

But can variability caused by experience be represented by meaningful quantitative measurements? In essence, a seemingly simple experiment is necessary. First, one must have a quantitative test of functional ability which is sensitive to plastic changes in the brain caused by experience. After looking at natural ability, a novel experience must then be introduced. With record of functional ability before and after a given experience, the effects of stroke on that function can then be examined. Final testing after the stroke can give insight into how the experience influenced the loss of function. Changes in function can also be compared to participants who have not been exposed to new experiences. This will allow for analysis of the degree of variability that can be said to be caused by the experience itself. Overall, all that is necessary is a test of function, a new experience, and a stroke, and with these three factors we can look at variability caused by experience. Implementing these factors into a model of stroke will allow researchers to gain experimental control over the effects of experience on stroke outcome.

### 1.5 Model of the Effects of Experience on Stroke Outcome

The model utilized in this thesis follows the above guidelines using rat subjects. There are many specific requirements that must also be met in developing such a rodent model, as described by Willott and colleagues (2003). First, the test of function must be observed in both rodents and humans, and must

be applicable to everyday life. Secondly, measurements must be accurate, easy, and relatively quick to obtain. More importantly for this model, the measure must be sensitive to the differential effects of specific experiences on the brain, both during development and in adulthood. The model as a whole also needs to allow for a large amount of control over experience, and the method of stroke induction should also limit variability in the location and size of damage from stroke. These conditions have been met in the following model.

### 1.5.1 A Fundamental Vertebrate Behaviour

Many potential stroke treatments have proven successful in animal models (Shukla, Khanna, Ali, Khan, & Srimal, 2008; Aldandashi, Noor, Wang, Uddin, & Shuaib, 2007; Hemmen & Lyden, 2007; Gonzalez, Gharbawie, & Kolb, 2006). However, a discrepancy remains between animal and human trials of stroke treatments, in that many of the positive results obtained in animal models have not been confirmed in humans (Shin, Moon, & Bang, 2007; Martinsson, Hardemark, & Eksborg, 2007; Lampyl et al., 2007; Wang, Sun, Simonyi, Jensen, Shelat, Rottinghaus, et al., 2005; Martinsson & Eksborg, 2004; Gladstone, Black, & Hakim, 2002). Why is it that a treatment can be successful in rodent models but then show few, if any, positive effects for humans? It's obvious that humans and rodents are not alike in many ways, physiologically, behaviourally, and even at the cellular level. Of importance here though, is the fact that certain functions which are commonly used as measures of stroke recovery in rodents may not be equivalent to measures used in humans. For example, does ladder walking in rodents relate directly to walking in humans? Clinicians certainly do not make

patients walk along a horizontal ladder to test their impairments. However, if the measure of function is essentially the same in all species, then the effects of stroke on that measure may also be similar across species.

One of the best ways to guarantee that the function being investigated is the same in different species is to use a function that has not been changed evolutionarily, one that draws on mechanisms from many regions of the brain but remains a reflexive response. The following studies take advantage of such a response, in hopes of beginning to solve the problem of non-transferability of treatments between species.

### 1.5.2 Optokinetic Tracking (OKT)

In the following studies the test of function is a visuomotor test of the optokinetic tracking (OKT) (Prusky, Silver, Alam, & Douglas, in press; Alam, Rakai, Jadavji, Metz, Douglas, & Prusky, 2008; Prusky, Alam, & Douglas, 2006; Douglas, Alam, Silver, McGill, Tschetter, & Prusky, 2005; Prusky, Alam, Beekman, & Douglas, 2004; Prusky, Douglas, 2004; Prusky, Harker, Douglas, & Whishaw, 2002; Prusky, Reidel, & Douglas, 2000; Prusky, West, & Douglas, 2000). OKT is a primitive response that can be seen in all vertebrate species (Schwartz, 1999). When a moving object passes through the visual field, the initial response of an animal is to stabilize the image on the retina. This results in movement of the head and neck at a similar speed as the movement of the stimulus (Schwartz, 1999). This motion of the head and neck is referred to in this thesis as “tracking”. Movement is a strong visual stimulus that reflexively results in this tracking motion.

### 1.5.3 Virtual Optomotor System (VOS)

The method of testing described here is not a strict test of vision (though it has been used as a test of motion vision in the past by McGill, Lund, Douglas, Wang, Lu, Silver, et al. 2007; Prusky, Alam, Beekman, & Douglas, 2004; Douglas, Alam, Silver, McGill, Tschetter, & Prusky, 2005). In fact, vision itself is not simply a single function. Vision is much more a sensory integrative process, as input to the visual system is used for many different functions, especially in humans. Visual input is almost always responded to in some way, most commonly with a motor response. For example, if you see a truck moving toward you, you move out of the way. Or if you see something you want, you reach for it. To interact with the environment, visual information is used to elicit motor responses, and this is essentially what the following virtual optomotor system (VOS) test examines. For these reasons, this test is much more applicable to real life situations than tests of visual discrimination or visual acuity (Prusky, West, & Douglas, 2000; Douglas, Alam, Silver, McGill, Tschetter, & Prusky, 2005).

Using a virtual optokinetic system (VOS) described in detail previously (Prusky, Alam, Beekman, & Douglas, 2004; Douglas, Alam, Silver, McGill, Tschetter, & Prusky, 2005), spatial frequency (SF), and contrast sensitivity OKT thresholds were measured in rats. The apparatus consists of four computer monitors which face toward each other to create a square box testing arena. The animal is placed on a platform within the testing arena, and moving sine wave gratings are presented on the monitors. The animal reflexively tracks the gratings,

and the spatial frequency and contrast of the grating is adjusted in a staircase manner until the animal no longer tracks the stimulus. The last value at which the animal tracks the stimulus is recorded as the OKT threshold. The apparatus, then, is measuring the animal's tracking response to moving visual stimuli. Overall, obtaining SF thresholds and contrast sensitivity curves for both eyes is relatively fast, and takes approximately 45 minutes or less.

### 1.5.4 Experience

Since inbred laboratory strain rats are genetically very similar, and their environment is also very similar (because they are raised in the same shoebox cages in the same colony room throughout life), it is possible to subject them to only a few novel experiences. This allows for greater control over the plasticity that occurs in response to new experiences or environments. Other animal models may allow for experience to be restricted, but the genetic differences in higher-order animals are greater, and many require more care and therefore more experience in their lifetime. The goal is to keep experiences as limited and as constant as possible, and this is most easily accomplished with a rat model.

Many experiments have shown that deprivation of visual experience in rats can result in different functional abilities and outcomes than in non-deprived animals (Maffei, Nataraj, Nelson, & Turrigiano, 2006; Prusky et al., 2006). In the following experiments, however, instead of depriving animals of experience, two specific experiences have been added to the normal experiences of the typical laboratory rat; one experience during development, and the one in adulthood. Deprivation studies provide necessity measures, whereas testing the effects of

adding new experience will provide a sufficiency measure to analyze the effects of experience on brain plasticity.

### 1.5.5 Stroke Induction

Stroke induction is the final piece of these experiments. There are many methods of inducing stroke in rodents (for review, see Hossmann, 2007). The two methods chosen for the following studies are both permanent ischemic models. The first is MCA occlusion by electrocoagulation, and the second, primary visual cortex (V1) devascularization. The method of MCA occlusion that has been chosen for this experiment was initially developed by Tamura et al. in 1980, and results in a non-reversible permanent occlusion of the MCA. By clamping the carotid arteries immediately after occluding the MCA, the time frame in which we can be certain that reperfusion did not occur can be controlled. As mentioned earlier, the duration of the blockage of blood flow can drastically change the anatomical outcome after a stroke, and with the method used here this variability is controlled to a much greater extent than with other MCA occlusion methods (Hossmann, 2007).

A stroke induction method known as "pial strip" was used to induce lesions in the primary visual cortex (V1). This method of devascularization has been used extensively as a model of cerebral damage and stroke (Gonzalez & Kolb, 2003; Kolb, Cote, Ribeiro-da-Silva, & Cuello, 1997; Goldstein & Oakley, 1987; Sofroniew, Pearson, Eckenstein, Cuello, & Powell, 1983). Devascularization is a permanent stroke model, as there is nothing left in this area to reperfuse once the vessels and arteries have been wiped away. Other methods

of inducing visual cortex damage, such as posterior artery occlusion, do not selectively damage the visual cortex alone (Finelli, 2008). Because V1, our area of interest, is located immediately under the skull and is easily accessible by removing only a small square of skull, the pial strip method is efficient, reliable, quick, and relatively non-invasive. It is also known to cause very similar damage from one animal to the next, limiting variability due to lesion location and size.

Overall, this model meets the requirements set out by Willott et al. (2003), and follows the guidelines necessary to test the effects of experience on stroke outcome. Rodent models allow for many of the factors that naturally vary in humans and other animal species to be held constant, and therefore have been used in the following experiments.

### 1.6 Introduction to the Chapters

In this thesis, Chapter 2 will examine the effects of stroke on OKT behaviour of normal rats, and rats with developmental visual experience. Chapter 3 will explore the effects of adulthood motor experience on OKT thresholds following stroke. Chapter 4 will look at the effects of combining developmental visual and adulthood motor experience on OKT function after stroke. Finally, Chapter 5 will be dedicated to a discussion of the results, and future directions for this research.

# Chapter 2 – Effects of Stroke in Rats With and Without Developmental Visual Experience

## 2.1 Enhancing OKT With Developmental Visual Experience

In order to test the effects of experience on stroke outcome it is first necessary to characterize normal function, and the effects of stroke on normal function. Such information will provide a foundation from which to compare the effects of experience on stroke. This chapter will introduce the characteristics of optokinetic tracking (OKT) behaviour in normal inexperienced animals, and the effects of stroke on their OKT behaviour. OKT thresholds have previously been characterized in normal rats (McGill, Prusky, Douglas, Yasumura, Matthes, Nune, et al., 2007; Silver, 2003). Results from the present experiment are comparable to previous studies, as will be described below, and show that normal Long Evans rats' OKT threshold measurements are consistently found to be approximately 0.52 to 0.54 cycles per degree (c/d). Studies of the effects of visual cortex aspiration and inactivation on normal animals' OKT thresholds have found that such methods deny enhancement of OKT (Silver, 2003; Prusky & Ramoa, 1999). Yet, the effects of other models of stroke on OKT behaviour have not previously been reported. The following study will examine OKT behaviour following MCAo and V1 devascularization stroke models.

The effect of developmental visual experience on OKT behaviour after stroke will also be examined here. Developmental experience often has lasting effects on the brain and behaviour (Kleim & Jones, 2008; Hooks & Chen, 2007; Gibb & Kolb, 2005; Kolb, Gibb, & Gorny, 2003; Kolb, 1995). Evidence of this

phenomenon on visuomotor behaviour originates from research in the Prusky lab, which has shown that exposing rats to approximately 30 minutes of VOS testing every day from P15 to P30 significantly enhances rats' OKT spatial frequency (SF) threshold measures, as tested by the virtual optomotor system (VOS) described earlier (Prusky, Silver, Alam, & Douglas, in press; Silver, 2003). Specifically, OKT thresholds are significantly increased by approximately 60% as a result of developmental visual experience (Silver, 2003). As changes in behaviour correlate with changes in the brain, and the OKT behaviour is changed, the assumption can be made that the brain has changed in some way as well. Therefore, less than 45 minutes of daily early life experience forms enduring changes in the brain, as OKT thresholds of experienced animals do not revert to pre-experience values over time and are considered permanent (Prusky, et al., in press; Silver, 2003).

Silver (2003), in hopes of finding the location in the brain of these plastic changes, removed the primary visual cortex (V1) of P15-P30 experience enhanced animals via aspiration lesion. He found that after removing the primary visual cortex, normal animals' OKT thresholds were unaffected. In developmentally experienced animals, however, OKT thresholds reverted to values similar to normal animals, which were not subject to the enhancing experience. In addition, animals with P14 visual cortex aspiration, just prior to the commencement of the developmental experience, never improved beyond that of a normal animal. The conclusion drawn from this experiment was that the

plasticity that allowed for the increase in OKT thresholds may have relied on the visual cortex, since its removal eliminates the effects of the experience.

The visual cortex is clearly involved in the production of OKT behaviour in visually experienced animals. However, OKT behaviour involves both a visual component (a visual stimulus) and a motor component (motor output in the form of tracking). Since it is now known that a motor task can enhance OKT (unpublished data), the prediction can be made that visuomotor, motor, or sensory association areas of the cortex may also play a role in the enhancement of OKT. In fact, it has been predicted that the majority of the synaptic connections that are activated during OKT are those that do not travel through the visual thalamocortical pathway from the retina to the visual cortex, and therefore may connect in motor or association areas of the brain (Douglas, Alam, Silver, McGill, Tschetter, & Prusky 2005).

The pathway activated by moving visual stimuli, and which produces the motor output of OKT, may have the ability to be disturbed in absence of reduced visual function. That is, without causing any damage to the visual system, there may be a way of disturbing the OKT motor response alone, without affecting the visual side of OKT. Damage to motor or association areas of the cortex may interfere with the motor output aspect of OKT, while leaving vision intact. This will also be indirectly examined in the following experiment.

### 2.2 Effects of Two Stroke Models on OKT Thresholds

In order to show how developmental visual experience affects visuomotor behavioural outcome after stroke, OKT thresholds were evaluated in normal

animals following MCA occlusion (MCAo) and V1 stroke. OKT thresholds of rats with developmental visual experience were also examined following experience and following stroke.

*Method*

Animals were housed and handled according to Canadian Council on Animal Care (CCAC) standards, under the supervision of the University of Lethbridge Animal Care Committee.

Nine male and 1 female Long Evans (LE) hooded rats were used in this experiment ($N$ = 10), as well as 12 male age-matched control rats of the same species. No sex differences were apparent in these experiments. All animals were bred in the Canadian Centre for Behavioural Neurosciences (CCBN) vivarium, with original stock obtained from Charles River. The animals were housed in polycarbonate cages (45.5cm L x 25.5cm W x 20cm H) in the University of Lethbridge colony room, which was maintained at an ambient temperature of 21 degrees Celsius, 35% relative humidity, and a light/dark cycle of 12h/12h. The animals had access to food and water *ad libitum* throughout the study. Animals were weaned at approximately 22 postnatal days, at which time males and females were separated and housed in identical conditions in the colony room.

*Virtual Optokinetic System (VOS) Apparatus*

Using a virtual optokinetic system (VOS), described in detail previously (Prusky, Alam, Beekman, & Douglas, 2004; Douglas, Alam, Silver, Mcgill, Tschetter, & Prusky, 2005) SF thresholds were measured in rats (contrast

sensitivity thresholds are available in Appendix B). The VOS is a relatively new method of testing SF thresholds and contrast sensitivity thresholds. The system consists of four computer monitors (in this study, 19 inch Dell monitors) that face toward each other forming a square box testing arena, and are held together by a black Plexiglas frame (Figure 1). In the center of the arena, a pole extends from the floor of the box to 6" above the bottom surface of the box. A circular platform sits atop this pole, and is where the animal is placed for testing. The inside top and bottom of this box are mirrored so that stimuli on the computer monitors are replicated both up and down, creating the illusion that the box continues on indefinitely from the animal's viewing position. The top of the apparatus opens to gain access to the inside, and a small Apple iCam is built into the center of the lid so when an animal is inside, the lid can be closed and the animal observed in real time on a separate computer monitor. Using the accompanying software (Optomotry 1.7.0), the image of the animal appears on a viewing monitor, and a crosshair cursor is used to center the stimuli. The computer monitors show a uniform grey screen, but the observer can change their output to resemble a rotating cylinder. The stimuli in this experiment, though it can be changed, consisted of sine wave gratings of black and white moving at 12 degrees/sec. This is much the same as the rotating cylinder method used to make types of measurements in the past (Thomas, Seiler, Sadda, Coffey, & Aramant, 2004; Harvey, De'Sperati, & Strata, 1997; Cowey & Franzini, 1979). The ability to move the center of the cylinder to the animal's head with the crosshair prevents mis-measurements, as the center of the virtual cylinder is always centered on the

animal, and the movement of the animal does not affect its distance from the stimuli, and therefore the measurement. The animal's movement need not be restricted, and because the response is reflexive, training is not necessary.

*Behavioural Testing in VOS*

To obtain a threshold, the observer must watch the animal and note its optokinetic response to the moving stimuli. A small but noticeable tracking motion is made by the animal in response to the moving grating. The tracking motion of the animal can be seen to get smaller and smaller with increasing spatial frequencies, until it is no longer detectable. The last point where the OKT can be seen is recorded as the threshold. The SF threshold is obtained by adjusting the spatial frequency of the grating while keeping the contrast between the black and white regions at 100%. Contrast sensitivity can also be measured with this apparatus, and is accomplished by adjusting the contrast of the black and white gratings at different spatial frequencies. In our lab, we use up to 9 spatial frequency points to create a contrast sensitivity curve. The points chosen (0.031, 0.064, 0.092, 0.103, 0.119, 0.272, 0.403, 0.603) are based on the contrast sensitivity curve itself. Previous testing in our lab has shown that 0.103 cycles/degree (c/d) is the peak spatial frequency for rats, and the other points are placed evenly around this peak. By adjusting the contrast of the grating at these spatial frequencies, we can obtain a reliable contrast sensitivity curve. To obtain contrast sensitivity values, a Michelson contrast is calculated from the screen luminance (maximum - minimum) / (maximum + minimum), and the reciprocal is recorded as the contrast sensitivity.

One benefit to using this system is that it allows for the stimuli to be restricted to certain portions of the animal's visual field (Figure 2). This may be useful in the following experiments because visual field defects often arise following stroke (Kerkhoff, 2001; Cassidy, Bruce, & Gray, 2001). Here, monocular field SF thresholds are also obtained for each eye (contrast sensitivity curves for the monocular visual field can be found in Appendix B). Stimuli are placed only from 35 degrees to 145 degrees from the center of the animal's visual fields. This is accomplished by using a T-shaped cursor which rotates so that the observer can center the "T" on the animal's eyes and down the length of its nose. Though the monocular field begins at 30 degrees in rats, we start the stimulus at 35 degrees to allow for 5 degrees of observer error due to cursor placement.

*Experimental Timeline*

In all experiments, in this chapter and the upcoming chapters, the experimental timeline began with baseline testing of OKT thresholds, prior to any form of novel experience (for the developmentally enhanced animals, P15-P30 testing comprised the baseline measurements). After baseline measurements had been obtained, thresholds were tested post-experience. For groups receiving both MCAo and V1 devascularization lesions, MCA stroke was induced first. Thresholds were obtained 2 days, 1 week, and 2 weeks following the stroke, after which the unilateral V1 lesion was induced contralateral to the MCAo damage. Testing resumed again for a 2 week period, and the day following the 2 week testing session, bilateral V1 damage was induced. The timeline was the same for animals without MCAo, except that the first lesion was a unilateral V1

devascularization, on a randomly assigned hemisphere, followed 2 weeks later by the bilateral V1 lesion.

*Experience*

Animals were placed in the VOS system each day from eye opening (P15) to P30. Full field SF thresholds and contrast sensitivity thresholds were obtained each day, approximately 45 minutes of experience or less. This process has been shown to enhance the animals' SF thresholds well above those of adult animals which have not been tested in this apparatus previously, referred to as "normal" animals (Silver, 2003). This enhancement has also been shown to be permanent, and thus, once testing from P15 to P30 was completed, the animals remained in their home cages until they reached adulthood, a minimum of 80 days of age.

*OKT Testing*

For each testing session in adulthood, animals were placed in the VOS system for approximately 30-45 minutes of testing. Full visual field SF thresholds and both monocular visual fields' SF thresholds were obtained as described earlier. Testing took place the day prior to stroke induction and again at three time points after each stroke; two days post-stroke (2D), one week post stroke (1W), and two weeks post stroke (2W). Spatial frequency measurements were randomly double-checked by an outside blind observer.

*Stroke*

*MCA Occlusion*

A permanent occlusion model was used for MCA stroke in this study. Animals were anesthetized with 2% isofuorane evaporated in 1.5L/min oxygen

via nose cone administration. A heating pad was used to keep animals' body temperature at 37.5 degrees Celsius throughout the surgery. Opthalamic ointment (BNP) was applied to the eyes, and the neck and top of the head were wiped with diluted hibitane and 70% alcohol. With the animal lying on its back, an incision was made from the chest bone rostrally approximately 1.5cm, and the underlying tissue exposed. The underlying connective tissue was gently cut to reveal the neck muscles, which were then gently separated and held aside using a saline dampened swab. The carotid artery could then be located. Fine curved tweezers were used to carefully isolate the carotid and separate it from the attached Vagal nerve. A soft string was then wrapped around the carotid so it could be easily located later, and the incision was pressed shut with the strings out. Saline was applied as necessary to keep the area moist.

The animal was then moved to the stereotaxic apparatus, where a midline incision was made, approximately 1.5 cm in length. The skin was held aside with small hemostats and a #11 scalpel blade was then used to detach the muscle from the sagittal ridge. The muscle was then held aside using a small thin spatula, while an opening was drilled lateral to the sagittal ridge using a 1mm dental burr. The opening began approximately 1mm anterior to bregma to approximately 5mm posterior to bregma, and extended from just above the mandible joint, 4 mm dorsally. With this section of the skull removed, the MCA could be viewed, and the dura was gently removed using fine tipped tweezers. Current conducting tweezers were then plugged into a bipolar electrocoagulator (SMT BM, Czech Republic), set at an arbitrary intensity of 2, and by placing the tweezers' tips on

either side of the MCA, current passed through the space between the tips of the tweezers to coagulate the MCA. The animal was then quickly removed from the stereotaxic apparatus, and both carotids were clamped with micro-aneurysm (ROBOZ) clamps for 30 minutes to ensure that the MCA area was not reperfused. After this time period, the clamps were removed and both incisions were sutured with standard monofilament suture material. Polysporin was applied to the incisions to aid healing, and 1ml Saline and 0.03ml buprenorphine (torbusec) were administered subcutaneously. Animals were given food and water ad libitum for 24 hours while being monitored in the surgery preparation area. Mashed food combined with water was also supplied to the animals to ensure easy intake of food. Animals were returned to their home cages in the colony room after 24 hours of monitoring their recovery from the procedure.

*V1 Pial Strip*

Induction of anesthesia was accomplished using 4-5% inhaled isofourane and was held at 2% inhaled isofuorane evaporated in 1.5L/min oxygen via nose cone administration throughout the procedure. Animals were also placed on a heating pad throughout surgery to maintain body temperature at 37.5 degrees Celsius. Once anesthetized, ear bars were used to place the animal in a stereotaxic apparatus, keeping the head immobile. Opthalamic ointment (BNP) was applied to the eyes, and the top of the head was wiped with diluted hibitane and 70% alcohol. An incision was made down the midline, approximately 1.5 cm in length. The skin was then resected and held aside with small hemostats. An opening was made in the skull using a 1mm dental drill, from 6mm to 12mm

posterior to bregma, and from 1mm lateral to bregma to the sagittal ridge, the area defined as V1 by Paxinos and Watson (1998). This square of skull was then removed, and the underlying dura was carefully peeled off using fine tipped tweezers. With the dura removed and the brain exposed, a cotton swab was dampened with Saline and was used to wipe the vasculature off of the exposed brain surface. The incision was then sutured closed using standard suture material, and Polysporin was smoothed over the sutures to aid healing. Animals were also given 0.03ml of buprenorphine (torbusec) and a 1ml injection of Saline subcutaneously before being removed from the stereotaxic apparatus. Food and water were available to the animals *ad libitum* for 24 hours while being monitored in a surgery preparation area. Animals were returned to their home cages after 24 hours of monitoring their recovery.

*Histology*

Once testing was completed, the animals were sacrificed to verify lesion boundaries. An intraperitoneal injection of 0.7ml Euthansyl was used to anesthetize the animals. Once all reflexive responses were absent, the animals were perfused with 1% buffered saline and 4% paraformaldehyde. The brains were removed and kept in 4% paraformaldehyde at room temperature for 48 hours, at which point they were placed in 30% sucrose solution until slicing. Digital pictures of the dorsal surface of the brains were taken before slicing for quantification of the surface features and lesion boundaries. Brains were sliced on a microtome (American Optical, model #860; Buffalo, NY, USA) at 40 μm

thickness, and mounted on slides. After mounting, the brains were stained using Cresyl Violet to view the depth of the damage.

*Statistical Analyses*

Repeated measures analysis of variance (ANOVA) was used to analyze the SF threshold at each testing time for each group, as well as for comparisons between each group and controls (Statview, Version 5.0.1.0, SAS Institute Inc.). Follow-up paired t-tests were used to analyze between testing sessions data, and unpaired t-tests were used to compare within testing session effects (comparisons between contralateral and ipsilateral to lesion visual fields). The alpha level for this experiment was set at 0.05.

*Results*

Results showed no effect of MCAo for all groups. V1 lesions significantly reduced full field OKT thresholds.

*Normal Rats (animals without experience) – MCAo - Full Field SF Thresholds*

Repeated measures ANOVA failed to reveal a significant interaction in inexperienced normal rats ($F(1, 3) = 0$, $p = 0.0$). There was no effect of MCA occlusion on OKT full visual field thresholds, which were recorded as $0.530 \pm 0.002$ c/d. This effect, or lack thereof, also applied to monocular visual field thresholds which remained at $0.489 \pm 0.004$ following the MCA territory lesion ($F(1, 3) = 0$, $p = 0.0$).

*Normal Rats - V1 Lesions - Full Field SF Thresholds*

Analyses showed no significant differences between baseline thresholds, and those obtained after MCA occlusion in normal rats. However, repeated

measures ANOVA did reveal a significant main effect of testing time ($F(1, 6) = 76.443$, $p < 0.0001$), as well as a significant interaction ($F(1, 6) = 80.375$, $p < 0.0001$) for the sequential V1 lesion data. Follow up paired t-tests revealed significant differences between pre-stroke measures and all post V1 stroke time points (2D post unilateral V1: $t(15) = 3.719$, $p = 0.0021$; 1W post unilateral V1: $t(15) = 3.719$, $p = 0.0021$; 2W post unilateral V1: $t(15) = 3.742$, $p = 0.0020$; 2D post bilateral V1: $t(15) = 15.056$, $p < 0.0001$; 1W post bilateral V1: $t(15) = 14.334$, $p < 0.0001$; 2W post bilateral V1: $t(15) = 12.314$, $p < 0.0001$), which suggested that the V1 lesions significantly reduced OKT thresholds. Other significant differences were apparent between 2D after unilateral V1 lesion and 2D after bilateral V1 lesion ($t(15) = 2.260$, $p = 0.0392$), as well as between 1W after unilateral V1 lesion and 2D after bilateral V1 lesion ($t(15) = 2.260$, $p = 0.0392$), which showed that unilateral V1 lesions significantly reduced thresholds measured through the eye contralateral to the lesion.

Unpaired t-tests revealed significant differences between the ipsilateral and contralateral visual fields as well. Two days, one week, and two weeks after the unilateral V1 lesion, the contralateral and ipsilateral visual fields were significantly different from each other ($t(14) = -10.954$, $p < 0.0001$; $t(14) = -10.954$, $p < 0.0001$; $t(14) = -11.124$, $p < 0.0001$, respectively). These data implied that following a unilateral lesion, thresholds obtained through each eye were significantly different, that is, thresholds from the visual field contralateral to the lesion were reduced, yet the ipsilateral visual field was not affected until induction of the bilateral lesion. These data are available in Figure 5, and lesion

boundaries for this group can be found in Figure 4. Figure 3 shows typical MCAo and V1 lesion damage for all groups.

*Normal Rats - V1 Lesions - Monocular SF Thresholds*

Repeated measures ANOVA exposed a significant interaction for monocular visual field data of normal rats as well ($F(1, 6) = 9.463$, $p < 0.0001$). Follow-up paired t-tests showed that thresholds were significantly higher before stroke than they were two days after the bilateral V1 lesion ($t(15) = 2.455$, $p = 0.0268$).

Significant differences between contralateral and ipsilateral side were also apparent with unpaired follow-up t-tests. After the bilateral lesion, significance occurred between ipsilateral and contralateral sides 2D after stroke induction ($t(14) = 3.135$, $p = 0.0073$), 1W after ($t(14) = 3.090$, $p = 0.0080$), as well as 2W after ($t(14) = 3.472$, $p = 0.0037$). These were the only significant differences found in this group, as are shown in Figure 6.

*Experienced Rats - MCAo and V1 Lesions - Full Field SF Thresholds*

As has been described previously (Silver, 2003), and as is shown in Figure 7, animals' full field SF threshold measures increased from P15 to P30 through both eyes with daily VOS testing ($M = 0.269 \pm 0.002$, and $M = 0.833\pm$, $M = 0.832 \pm 0.002$). Full field SF thresholds were also significantly greater for P15-P30 enhanced animals in adulthood than for adult controls, which did not receive developmental (P15-P30) visual experience ($M = 0.832 \pm 0.002$ vs. $M = 0.529 \pm 0.002$, $t(9) = 525.149$, $p < 0.0001$).

Repeated measures ANOVA revealed a significant interaction for the full visual field SF thresholds of animals with both MCA occlusion and V1 lesions ($F(1, 9) = 2752.295$, $p < 0.0001$). Follow-up paired t-tests showed that there was not a significant effect of MCA occlusion at any time point. However, the difference between pre-stroke/post experience thresholds and post V1 lesion thresholds were significant at all testing times (2D post unilateral V1: $t(9) = 3.010$, $p = 0.0147$; 1W post unilateral V1: $t(9) = 3.010$, $p = 0.0147$; 2W post unilateral V1: $t(9) = 3.009$, $p = 0.0147$; 2D post bilateral V1: $t(9) = 101.040$, $p < 0.0001$; 1W post bilateral V1: $t(9) = 93.123$, $p < 0.0001$; 2W post bilateral V1: $t(9) = 95.852$, $p < 0.0001$), with pre-stroke measures being significantly higher than those obtained post-stroke. Other significant differences were apparent between testing sessions 2D post unilateral V1 lesion and all three post-bilateral lesion points (2D post bilateral V1: $t(9) = 2.896$, $p = 0.0177$; 1W post bilateral V1: ($t(9) = 2.885$, $p = 0.0180$; 2W post bilateral V1: $t(9) = 2.884$, $p = 0.0181$), as well as between both 1W and 2W post unilateral V1 lesion, and all three post bilateral V1 lesion testing sessions (1W post unilateral V1 vs. 2D post bilateral V1: $t(9) = 2.431$, $p = 0.0379$; 1W post unilateral V1 vs. 1W post bilateral V1: $t(9) = 2.927$, $p = 0.0168$; 1W post unilateral V1 vs. 2W post bilateral V1: $t(9) = 2.916$, $p = 0.0172$; 2W post unilateral V1 vs. 2D post bilateral V1: $t(9) = 2.440$, $p = 0.0374$; 2W post unilateral V1 vs. 1W post bilateral V1: $t(9) = 2.938$, $p = 0.0165$; 2W post unilateral V1 vs. 2W post bilateral V1: $t(9) = 2.927$, $p = 0.0168$), again suggesting that thresholds were greatly reduced following stroke. These differences are represented in Figure 9, and lesion boundaries in Figure 8.

Unpaired t-test follow-ups unveiled a significant difference between contralateral and ipsilateral thresholds at 2D ($t(9) = -193.118$, $p < 0.0001$ ), 1W ($t(8) = -110.059$, $p < 0.0001$), and 2W post unilateral V1 lesion ($t(8) = -102.792$, $p < 0.0001$), suggesting that the first V1 lesion reduced the contralateral visual field threshold, and that the induction of the bilateral V1 lesion evened out OKT thresholds in both directions. These differences are also shown in Figure 9.

*Experienced Rats - MCAo and V1 lesions - Monocular Field SF Thresholds*

There was a significant interaction, exposed by repeated measures ANOVA, for the monocular field SF threshold data of developmentally experienced animals ($F(1, 9) = 6.762$, $p < 0.0001$). Follow-up paired comparisons show no significant differences between the SF threshold measures at each testing session.

Unpaired t-tests showed that the only significant difference between sides (contralateral vs. ipsilateral) was apparent at 2D after unilateral V1 lesions ($t(8) = -2.712$, $p = 0.0266$). This difference is small, and suggests a minor deviation from pre-stroke thresholds contralateral to a unilateral stroke, but this difference is no longer apparent one week after the lesion, as is shown by Figure 10.

*Normal vs. Experienced Rats - MCAo and V1 lesions - Full Field SF Thresholds*

With data collected using repeated measures ANOVA, a significant interaction was apparent between group, side, and full field thresholds when comparing experienced and normal rats ($F(1, 9) = 1559.099$, $p < 0.0001$). Follow-up unpaired t-tests showed that developmentally experienced animals' OKT thresholds were significantly higher than controls ($t(16) = 242.863$, $p <$

0.0001), which also showed that this enhancement raised OKT thresholds to levels significantly higher than animals with no such experience. This difference between the two groups remained significant after MCAo, as this procedure did not affect OKT thresholds. Differences between groups were significant at all times after unilateral V1 stroke (2D post unilateral V1: t(24) = 3.547, p = 0.0016; 1W post unilateral V1: t(24) = 3.600, p = 0.0014; 2W post unilateral V1: t(24) = 3.604, p = 0.0014), though these differences disappeared after the bilateral V1 stroke (2D post bilateral V1: t(24) = -1.167, p = 0.2546; 1W post bilateral V1: t(24) = -1.443, p = 0.1620; 2W post bilateral V1: t(24) = -1.327, p = 0.1969), which showed that the enhanced animals' thresholds were significantly higher than controls, and following V1 lesions were reduced from values far above controls *(M* = 0.837 ± 0.003), to values very similar to V1 damaged controls (*M* = 0.508 ± 0.009). This portion of the data illustrated how the enhancement was removed completely with damage to V1 (Figure 11).

*Experienced Rats - V1 Lesions - Full Field SF Thresholds*

A significant interaction was present for the full field SF threshold data of experienced animals with V1 lesions only ($F(1, 6) = 3898.250$, $p < 0.0001$). Follow-up paired t-tests showed that thresholds were significantly reduced after V1 stroke, and the difference between pre-stroke thresholds and all post stroke thresholds were significant (2D post unilateral V1: $t(9) = .2999$, $p = 0.015$; 1W post unilateral V1: $t(9) = .2998$, $p = 0.015$; 2W post unilateral V1: $t(9) = .2998$, $p = 0.015$, 2D post bilateral V1: $t(9) = 115.493$, $p < 0.0001$; 1W post bilateral V1: $t(9) = 96.472$, $p < 0.0001$; 2W post bilateral V1: $t(9) = 88.393$, $p < 0.0001$).

Follow-up unpaired t-tests revealed a significant difference between the contralateral and ipsilateral side at all times within the unilateral lesion 2 week period ($t(8) = -95.763$, $p < .0001$; $t(8) = -74.367$, $p < .0001$; $t(8) = -74.333$, $p < .0001$), just before the bilateral lesion. These differences suggest that there was an initial significant decrease in the visual field contralateral to the first lesion. After the bilateral lesion the SF threshold of the opposite visual field came down to the level of the first affected visual field, and therefore they were no longer significantly different (see Figure 13; for lesion boundaries, see Figure 12).

*Experienced Rats - V1 lesions – Monocular Field SF Thresholds*

As can be seen in Figure 14, repeated measures ANOVA did not disclose a significant interaction for monocular field SF thresholds of V1 stroke animals ($F(1, 6) = 2.104$, $p = 0.070$). No further follow-up tests were completed on these data.

*Normal vs. Experienced Rats - V1 lesions – Full Field SF Thresholds*

The overall trends in OKT threshold changes were similar between experimental animals and controls, although the differences were much greater for experimental animals (Figure 15). Repeated measures ANOVA was used to compare experimental groups and control groups. A significant interaction was apparent between group, side, and full field thresholds ($F(1, 6) = 3101.742$, $p < 0.0001$). Follow-up unpaired t-tests confirmed a significant difference between developmental visual experienced animals and controls prior to stroke induction ($t(24) = 402.998$, $p < 0.0001$), providing further evidence that P15-P30 testing does significantly enhance full field OKT thresholds beyond that of a naïve

animal. Following unilateral V1 devascularization, differences between the groups remained significant (2D post unilateral V1: $t(24) = 3.544$, $p = 0.0017$; 1W post unilateral V1: $t(24) = 3.592$, $p = 0.0015$; 2W post unilateral V1: $t(24) = 3.606$, $p = 0.0014$), albeit to a much lesser degree. After the bilateral V1 stroke, thresholds were no longer significantly different between the two groups which demonstrated further the sizeable reduction in enhanced animals' thresholds compared to controls. Experience enhanced animals thresholds were far above normal inexperienced animals prior to stroke *(*$M = 0.834 \pm 0.002$ vs. $M = 0.529 \pm 0.002$, respectively), and were reduced to the same level as normal animals after damage to V1 (Experienced: $M = 0.511 \pm 0.010$ vs. Normal: $M = 0.511 \pm 0.005$).

*Summary*

The first objective of this study was to determine the effects of stroke on visuomotor behaviour in normal animals. These data show that MCAo did not affect normal animals' OKT thresholds. However, there was a significant 3% decrease in full field SF thresholds following visual cortex stroke. Silver (2003) found no such decrease in OKT thresholds after aspiration lesion, providing further evidence for the finding that aspiration lesions do not cause the same effects as other stroke models in rodents (Gonzalez & Kolb, 2003; Forgie, Gibb, & Kolb, 1996).

A second objective of this study was to determine if association areas of the cortex may be involved in OKT enhancement. Thresholds obtained from experimental animals with developmental visual experience show no effect of cortical damage from MCAo, on OKT thresholds. These data suggest that the

MCA territory is not involved in the developmental induced enhancement of OKT behaviour. However, MCAo is known to have an effect on other tests of function such as reach training and beam walking (Gharbawie, Gonzalez, Williams, Kleim, & Whishaw, 2005) therefore it is important to ensure that the test of function is sensitive to the manipulations being made. Though the OKT test holds promise for detecting plastic changes in the visual cortex, this experiment suggests that it is not sensitive to lateral MCA territory cortical damage when developmental visual experience causes changes in behaviour.

The effects of sequential V1 lesions in absence of MCA occlusion were the same as the effects seen in animals with MCA occlusion followed by V1 lesions. That V1 lesion outcome does not differ when preceded by MCA occlusion suggests that the enhancement that occurs following developmental experience in the form of P15-P30 OKT testing does not rely on communication between cortical MCA territory and visual areas, and that Silver's (2003) original hypothesis that P15 enhancing takes place solely in the visual cortex has not been disproved. However, this may be the case only for visual experience, and I will return to this topic in upcoming chapters.

The main objective of this study was to determine the effects of developmental experience on OKT thresholds after a stroke. The above data shows that plasticity and enhancement that occurs in infancy has long lasting effects into adulthood, and can also affect functional outcome after cortical damage in adulthood. Developmentally enhanced animals showed a differential loss of function when compared to animals that were not subject to visual

experience. A 60% decrease in OKT was apparent in experienced animals, which is significantly greater than the 3% decrease seen in controls. These data suggest that early life experiences, which result in greater functioning than in naïve laboratory animals, may actually increase the relative severity of functional losses that accrue after stroke damage in adulthood. It also shows that there is a differential effect of experience, or lack of experience, on stroke outcome.

Monocular visual field data revealed only one minor significant difference one week after the unilateral lesion. Because this difference was apparent at no other testing time, it is likely caused by short term effects of surgery, and does not represent a permanent change in monocular visual field OKT thresholds. On the other hand, it may also be the case that minor changes do occur in the monocular visual fields, but that they are much less consistent, and much more difficult to detect with statistical significance. Further investigation of the monocular visual fields is necessary to make an imperative conclusion about the effect seen here.

Overall, this experiment provides evidence to show that 1) visual cortex stroke reduces OKT thresholds in the full visual field, 2) association areas of the cortex are not involved in the production of normal OKT, or in developmental visual experience-induced changes in OKT thresholds, and 3) further evidence showing that plastic changes in the visual cortex are linked to the enhancement of OKT resulting from developmental visual experience. A final conclusion that can be made from these data is that individual differences in experience can and do affect outcome after stroke, in that the amount of functional loss after stroke appears to vary depending on pre-stroke experiences. Developmental experience

in rodents affects stroke outcome in adulthood differently than in animals without such experience.

# Chapter 3 – Effects of Stroke in Rats With Adulthood Motor Experience

## 3.1 Enhancing OKT With Adulthood Motor Experience

The previous chapter showed that visual cortex stroke has a small effect on normal function, and that developmental visual experience has a different effect on stroke outcome than typical laboratory experience in rats. It is possible that there may be something specific about developmental experience that has caused these reported effects, and that different experiences at various times throughout the lifespan may not show similar results. In addition, the experience was the same as the measure, and therefore the specificity of the experience may be related to the significance of the effects. To show that it is not just developmental experience, or some factor specific to the task which caused a change in OKT behaviour following stroke, it is necessary to test stroke outcome using a different form of experience all together.

It is well known that plasticity occurs in the adult brain as well as in the developing brain (Kleim & Jones, 2008; Cheetham, Hammond, Edwards, & Finnerty, 2007; Karmarkar & Dan, 2006; Hofer, Mrsic-Flogel, Bonhoeffer, & Hubener, 2006; Monfils, Plautz, & Kleim, 2005). To test that this model is sensitive to other forms of plasticity inducing experience, a non-visual adulthood experience was used. The effects of this new type of experience may show that this model can be applied to plasticity and experience more generally.

The following study aims to assess the effects of adulthood motor experience, in the form of skilled reaching, on stroke outcome. Single pellet reach training, one form of skilled reaching, is a good motor task candidate for

testing the effects of adulthood motor experience on stroke outcome for many reasons. First, it is not a highly visual task, and vision is apparently not a requirement to reach success (Whishaw & Tomie, 1989). In Whishaw's (1989) study, vision was completely occluded using eye patches, and these animals' success in reaching was not impaired. The following study also provides similar conclusions, as animals that have area V1 removed can successfully reach for and obtain food pellets. Secondly, previous unpublished data from this lab, and from collaborations with others, have also shown that skilled reaching has an enhancing effect on OKT behaviour, similar to changes seen in developmental visually enhanced rats. Normal adult rats raised in standard shoebox cages until adulthood, which are then exposed to single pellet reach training show a significant 11% enhancement of OKT full field SF thresholds (Alam et al., 2008). Thirdly, skilled reaching has been shown to be significantly affected by MCA occlusion, the most common form of stroke (Gharbawie, Auer, & Whishaw, 2006; Gharbawie, Gonzalez, & Whishaw, 2005a; Gharbawie, Gonzalez, Williams, Kleim, & Whishaw, 2005b). In the following experiment, using skilled reach training in adult rats, changes in OKT thresholds following this experience will be characterized, and the effects of stroke on OKT thresholds will be examined.

The enhancing effect that a motor task, such as skilled reaching, has on OKT behaviour leads to the prediction that the brain may be drawing from plasticity in areas outside of the visual cortex. These tasks are thought to involve mainly motor areas, and are commonly used as measures of motor function. The

enhancement caused by these tasks, therefore, can be expected to take advantage of motor-visual or visual-motor connections. Because damage that results in decreased success in reaching tasks is located in motor and association areas (Gharbawie et al, 2005a; Gharbawie et al, 2005b) these cortical areas may be involved in the enhancement of OKT caused by skilled reaching. As was mentioned in Chapter 2, OKT has both a visual and motor aspect, and so motor association areas of the cortex may also be involved in this form of enhancement. Nevertheless, the previous experiment revealed no effect of damage to motor and association areas disturbed by MCA occlusion, so an investigation of both motor and association cortex damage and visual cortex damage will again be completed.

### 3.2 Effects of Two Models of Stroke on OKT Thresholds

This experiment tests the hypothesis that reach training experience has distinct effects on visuomotor outcome following stroke. In order to test this, rats were be subjected to reach training in adulthood, and OKT thresholds were obtained following experience, and following stroke. This experiment revealed that visoumotor behaviour is differentially affected by adulthood motor experience.

*Methods*

Animals were housed and handled according to Canadian Council on Animal Care (CCAC) standards and under the supervision of the University of Lethbridge Animal Care Committee.

Eleven Long Evans (LE) hooded rats were used in this experiment, 8 males and 3 females, plus 12 age-matched male controls. All animals were bred

in the Canadian Centre for Behavioural Neurosciences (CCBN) vivarium, with original stock obtained from Charles River. The animals were housed in polycarbonate cages (45.5cm L x 25.5cm W x 20cm H) in the colony room, which was maintained at an ambient temperature of 21 degrees Celsius and 35% relative humidity, with a light/dark cycle of 12/12. The animals had access to food and water *ad libitum* until the beginning of reach training.

*Experience/Single Pellet Reach Training*

*Apparatus*

Single pellet reaching boxes were made of clear Plexiglas (40cm x 45cm x 13.1cm). Animals are trained to reach through a small vertical opening 1.3cm wide x 15cm high. A 4cm wide platform to place the food pellets on is attached to the outside of the opening, 4cm above the floor. Two small indentations, 5mm in diameter and 1.5mm deep are evenly spaced in line with the edges of the vertical opening. This placement encourages the animals to use only their preferred paw, since they will have to reach across their midline to obtain the pellet. They will be much less successful at obtaining a pellet if it is placed on the same side as their preferred paw.

*Food Deprivation*

Two days before the commencement of single pellet reach training, food hoppers were removed from the animals' cages, and from this point on they were given a pre-determined amount of food each day. Food deprivation started with animals receiving 30g/d for males, and 25g/d for females (slightly above the average daily intake for Long Evans rats). However, for most animals, this

amount was too much to consume in a day (there was still food remaining in their cages at testing time the following day), and their ability to learn the task was influenced. Therefore, after 3 days of reach training at 30g/d or 25g/d, animals were permitted only 25g/d for males and 20g/day for females. This amount of food kept their body weight at 95%-100% of free-feeding weight or greater for younger animals.

*Training*

For the first 2 days of training, animals were placed in the reaching apparatus with pellets scattered both inside the reaching box and on the reaching platform. On day 3, pellets were placed only on the platform, and the paw which the animal chose to reach with more often (the preferred paw) was observed and recorded. If the preferred paw was still unclear at day 3, an additional day was used to determine the preferred paw with confidence. Once the preferred paw had been determined, pellets were placed only on the side of the platform opposite that paw. By the $5^{th}$ or $6^{th}$ day of reaching, only a single pellet was placed on the platform, opposite the animal's preferred paw. A pellet was also occasionally placed at the back of the reaching box, so that animals would travel to the back of the box to reset after each reach attempt. By day 7 of training, animals were familiar with the task, and scoring of the reach attempts began. Scoring consisted of noting the number of "hits", that is, successful reach attempts where the animal reached for a pellet, grasped it, and immediately ate it. Each animal was given 20 attempts per session, and percent success was determined. Once animals reached 50% success or greater (they successfully retrieved a pellet 50 percent of the time

or more) they were considered fully trained in the task. Reach training was continued throughout the experiments, with animals getting one to two days rest without reaching per week. Reaching experience was continued throughout the study, as it is not yet known if the OKT changes caused by reach training are permanent. If it is not, changes in OKT may have been due to the cessation of reaching.

*OKT Testing*

Animals were placed in the VOS system for approximately 30-45 minutes for each testing session in adulthood. Full visual field SF and contrast sensitivity thresholds and monocular visual fields' SF and contrast sensitivity thresholds were obtained. Testing took place the day prior to stroke induction and again at three time points after each stroke induction; two days post-stroke, one week post stroke, and two weeks post stroke. SF threshold measurements were randomly double-checked by an outside observer.

*Stroke Models*

*MCAo*

A permanent occlusion model was used for MCA stroke in this study, as described earlier. Briefly, animals were anesthetized with 2% isofuorane evaporated in 1.5L/min oxygen. An incision was made from the chest bone rostrally approximately 1.5cm, and the underlying was gently separated using a saline dampened swab and the carotid artery was isolated. The animal was then moved to the stereotaxic apparatus, where a midline incision was made, approximately 1.5 cm in length. A #11 scalpel was used to detach the muscle

from the sagittal ridge and an opening was drilled approximately 1mm anterior to bregma to approximately 5mm posterior to bregma, and extended from the mandible, to 4 mm dorsal to the mandible. The dura was gently removed, and current conducting tweezers were then used to coagulate the MCA. Both carotids were clamped with micro-aneurysm clamps (ROBOZ) for 30 minutes after which the clamps were removed and both incisions were sutured with standard suture material. Polysporin was applied to the incisions to aid healing, and 1ml Saline and 0.03ml buprinex (torbusec) were administered subcutaneously. Animals were given food and water ad libitum for while their recovery was being monitored and were returned to their home cages in the colony room after 24 hours.

*V1 Pial Strip*

V1 devascularization was completed as described earlier. An opening was made in the skull from 6mm to 12mm posterior to bregma, and from 1mm lateral to bregma to the sagittal ridge (Paxinos and Watson, 1998). The underlying dura was carefully peeled off and a cotton swab dampened with saline was used to wipe the vasculature off of the exposed brain surface. The incision was then sutured closed and animals were given 0.03ml of buprenorphine (torbusec) and a 1ml injection of saline subcutaneously. Food and water were available to the animals *ad libitum* while their recovery was being monitored and were returned to their home cages after 24 hours.

*Histology*

As described in the previous experiment, animals were sacrificed to verify lesion location and size by an intraperitoneal injection of 0.7ml Euthansyl.

Animals were then perfused with 1% buffered saline and 4% paraformaldehyde, and brains were kept in 4% paraformaldehyde for 48 hours, and then in 30% sucrose solution until slicing. Digital pictures were taken before slicing for quantification of the surface features and lesion boundaries. After slicing, the brains were stained using Cresyl Violet to view the depth of the damage.

*Statistical Analyses*

Repeated measures analysis of variance (ANOVA) was used to analyze the SF thresholds at each testing time (Statview, Version 5.0.1.0, SAS Institute Inc.). Follow up paired t-tests were used to analyze between testing sessions data and unpaired t-tests were used to compare within testing session effects (comparisons between contralateral and ipsilateral eye). The alpha level for this experiment was set at 0.05.

*Results*

There was no effect of MCA occlusion on OKT thresholds. Statistical analyses also revealed that V1 devascularization significantly reduced full visual field OKT thresholds in all groups.

*Experienced Rats - MCAo and V1 lesions - Full Field SF Thresholds*

By means of repeated measures ANOVA, a significant main effect of testing time was evident ($F(1, 10) = 405.024$, $p < 0.0001$) as well as a significant interaction between testing time and side (contralateral/ipsilateral) ($F(1, 10) = 125.936$, $p < 0.0001$) for single pellet reach trained rats with both MCAo and sequential V1 devascularization (see Figure 17 for reaching success data). Follow-up paired t-tests showed that single pellet reach training significantly

enhanced OKT thresholds ($t(11) = -110.689$, $p < 0.0001$). There was no effect of MCA occlusion at any time; therefore, measures taken after reach training are essentially the same as after MCA occlusion, and the MCA occlusion testing will be omitted from the remainder of this analysis. Further significant differences were apparent between post-reach training measures and all post V1 lesion measures (2D post unilateral V1: $t(11) = 3.613$, $p = 0.0041$; 1W post unilateral V1: $t(11) = 3.692$, $p = 0.0036$; 2W post unilateral V1: $t(11) = 3.628$, $p = 0.0040$; 2D post bilateral V1: $t(11) = 17.768$, $p < 0.0001$; 1W post bilateral V1: $t(11) = 27.421$, $p < 0.0001$; 2W post bilateral V1: $t(11) = 27.004$, $p < 0.0001$), evidence that OKT thresholds did not recover to post-reaching values, and that V1 stroke significantly decreased thresholds from post-reach training levels. OKT thresholds observed 2D after the unilateral V1 lesion were significantly different from those 2D after the bilateral lesion ($t(11) = 2.698$, $p = 0.0207$), 1W after the bilateral lesion ($t(11) = 2.408$, $p = 0.0347$), and 2W after the bilateral lesion ($t(11) = 2.356$, $p = 0.0381$). Thresholds recorded 1W after the unilateral lesion were significantly different from all three post-bilateral lesion testing sessions (2D post bilateral V1: $t(11) = 2.651$, $p = 0.0224$; 1W post bilateral V1: $t(11) = 2.370$, $p = 0.0371$; 2W post bilateral V1: $t(11) = 2.317$, $p = 0.0408$) as well as from 2W after the unilateral lesion ($t(11) = -2.259$, $p = 0.0452$). Two weeks subsequent to the unilateral lesion, OKT thresholds remained significantly different from those obtained after the bilateral lesion as well (2D post bilateral V1: $t(11) = 2.990$, $p = 0.0123$; 1W post bilateral V1: $t(11) = 2.697$, $p = 0.0208$; 2W post bilateral V1: $t(11) = 2.647$, $p = 0.0227$). These data showed that SF thresholds were

significantly reduced contralateral to the each V1 lesion. Lesion boundaries are presented in Figure 16.

Similar to previous data, significant differences between the contralateral and ipsilateral visual fields were only apparent, using unpaired t-test follow-ups, at 2D ($t(10) = 17.107$, $p < 0.0001$), 1W ($t(10) = 18.237$, $p < 0.0001$), and 2W subsequent to the unilateral V1 lesion ($t(10) = 16.537$, $p < 0.0001$). These data are represented in Figure 18, and show that thresholds were significantly reduced contralateral to the unilateral V1 lesion, and that following the bilateral lesion, the opposite visual field's thresholds were reduced to similar levels as the initially affected side.

*Experienced Rats - MCAo and V1 lesions- Monocular Field SF Thresholds*

Data collected for monocular visual field SF thresholds did not show a significant interaction when analyzed with repeated measures ANOVA ($F(1, 10) = 0.952$, $p = 0.4902$). Once again, MCA occlusion had no effect on OKT thresholds at any time point (as presented in Figure 19).

*Normal vs. Experienced Rats - MCAo and V1 lesions - Full Field SF Thresholds*

Comparisons of normal and experienced animals, using repeated measures ANOVA, revealed a significant interaction between side, group, and SF threshold measures ($F(1, 10) = 139.448$, $p < 0.0001$). Follow up unpaired t-tests showed that prior to experience or stroke, the experimental and control groups' full visual field OKT thresholds were statistically the same ($t(18) = -0.091$, $p = 0.9287$). Differences between SF thresholds of the two groups after reach training, however, were significant which suggested that reach training significantly

enhanced OKT thresholds above control thresholds ($t(18) = 78.330$, $p < 0.0001$). This difference was apparent at all testing times after MCA occlusion as well ($t(18) = 91.111$, $p < 0.0001$), as this procedure had no effect on OKT thresholds. At each testing session after unilateral V1 stroke, experimental and control groups remained significantly different from each other (2D post unilateral V1: t(26) = 3.619, p = 0.0013; 1W post unilateral V1: t(26) = 3.576, p = 0.0014; 2W post unilateral V1: t(26) = 3.999, p = 0.0005), due to the much increased thresholds of the non-affected ipsilateral visual field. Following the bilateral lesion, significant differences between the groups disappeared, as their thresholds were now very similar. These data showed that the overall pattern of OKT disruption was similar between control groups and experimental groups however, the magnitude of the loss was much greater for the enhanced animals, as can be seen in Figure 20.

*Experienced Rats - V1 Lesions - Full Field SF thresholds*

Repeated measures ANOVA demonstrated a significant main effect of testing time ($F(1, 7) = 125.823$, $p < 0.0001$) and a significant interaction between testing time and contralateral or ipsilateral side ($F(1, 7) = 108.523$, $p < 0.0001$) in animals with only sequential V1 lesions. Follow-up paired t-tests show that reach training significantly enhanced OKT from pre-experience levels ($t(15) = -36.729$, $p < 0.0001$), and that all post-lesion testing times showed thresholds that were significantly reduced from those obtained after reach training (2D post unilateral V1: $t(15) = 3.664$, $p = 0.0023$; 1W post unilateral V1: $t(15) = 3.590$, $p = 0.0027$; 2W post unilateral V1: $t(15) = 3.572$, $p = 0.0028$; 2D post bilateral V1: $t(15) = 16.954$, $p < 0.0001$; 1W post bilateral V1: $t(15) = 18.649$, $p < 0.0001$; 2W post

bilateral V1: $t(15) = 19.302$, $p < 0.0001$). Animals' thresholds did not return to reach training enhanced levels. Thresholds did, however, remain close to pre-reaching values after the V1 lesions, as only marginally significant differences were apparent between pre-reaching thresholds and post lesion thresholds. This suggested that the reach training enhancement was removed with V1, and although the animals' thresholds were slightly lower than at initial baseline testing before experience, they were much closer to naive values than to post-reach training values. Following the induction of the bilateral V1 lesion, significant differences existed between 2D post unilateral V1 lesion and 2D post bilateral lesion ($t(15) = 2.590$, $p = 0.0205$), 2D post unilateral V1 lesion and 1W post bilateral lesion ($t(15) = 2.515$, $p = 0.0283$), as well as between 2D post unilateral V1 lesion and 2W post bilateral lesion ($t(15) = 2.422$, $p = 0.0286$). The testing session at 1W after the unilateral V1 lesion was also significantly different from the post bilateral times (2D post bilateral V1: $t(15) = 2.565$, $p = 0.0215$; 1W post bilateral V1: $t(15) = 2.490$, $p = 0.0250$; 2W post bilateral V1: $t(15) = 2.396$, $p = 0.0300$), as was the testing session at 2W post unilateral lesion (2D post bilateral V1: $t(15) = 2.783$, $p = 0.0139$; 1W post bilateral V1: $t(15) = 2.713$, $p = 0.0160$; 2W post bilateral V1: $t(15) = 2.621$, $p = 0.0193$). These data showed that thresholds obtained after the V1 lesions were significantly reduced from pre-lesion thresholds

Unpaired t-test follow ups also revealed significant differences between contralateral and ipsilateral visual fields at all three testing sessions subsequent to the unilateral V1 lesion but prior to the bilateral lesion (2D post unilateral V1:

$t(14) = -18.483$, $p < 0.0001$; 1W post unilateral V1: $t(14) = -22.795$, $p < 0.0001$; 2W post unilateral V1: $t(14) = -16.136$, $p < 0.0001$). These differences show that the OKT thresholds of the visual feild contralateral to the unilateral V1 lesion were significantly reduced. Following the bilateral lesion, the thresholds measured through the other eye were then reduced to similar levels, and thresholds had evened out. The above data are represented in Figure 22, along with lesion boundaries for this group in Figure 21.

*Experienced Rats - V1 lesions - Monocular Field SF Thresholds*

As can be seen in Figure 23, data for the monocular visual fields did not show any significant differences, as repeated measures ANOVA revealed no significant main effect ($F(1, 7) = 2.074$, $p = 0.0534$), or interaction ($F(1, 7) = 0.176$, $p = 0.9895$).

*Normal vs. Experienced Rats V1 lesions – Full Field SF Thresholds*

Data comparing normal rats with reach trained rats are represented in Figure 24. These data showed that the overall pattern of OKT disruption was similar between control groups and experimental groups. However, the magnitude of the loss was much greater for the enhanced animals. Using repeated measures ANOVA a significant interaction was apparent between side (contralateral: ipsilateral), group (experimental: controls) and full field OKT thresholds at each testing time ($F(1, 7) = 53.704$, $p < 0.0001$). Unpaired t-test follow-up analyses showed that experimental and control thresholds were not different before experience or lesions ($t(30) = -0.504$, $p = 0.6178$). After reach training, however, the groups' OKT thresholds did become significantly different

($t(30) = 38.857$, $p < 0.0001$), with the reach trained animals now showing much higher thresholds than controls. After unilateral V1 lesions, the groups' thresholds remained statistically different from each other at all three testing times (2D post unilateral V1: $t(30) = 2.798$, $p = 0.0089$; 1W post unilateral V1: $t(30) = 2.776$, $p = 0.0094$; 2W post unilateral V1: $t(30) = 3.001$, $p = 0.0054$) due to the unaffected ipsilateral side. This trend was abolished after the bilateral lesion, when both groups' thresholds were reduced to the same level, and no longer differed significantly (2D post bilateral V1: $t(30) = 1.075$, $p = 0.2907$; 1W post bilateral V1: $t(30) = 1.387$, $p = 0.1756$; 2W post bilateral V1: $t(30) = 1.737$, $p = 0.0927$), which suggested that the experimental groups' thresholds were decreased much more than controls, as they were significantly higher to begin with. Overall, animals from the experimental group suffered a much greater reduction of OKT thresholds than the control group.

*Summary*

The above data shows how full field SF thresholds and monocular field SF thresholds are affected by MCA occlusion and sequential V1 lesions in rats with adulthood motor task experience. Animals' full field SF thresholds are significantly increased by 11% after single pellet reach training, further evidence that this experience enhances OKT. Following MCA occlusion, no changes were seen in OKT thresholds, suggesting that the cortical areas damaged by MCA stroke are not involved in OKT or its enhancement with skilled reaching. MCAo did reduce reaching success (Figure 17), however, showing that MCAo does interfere with circuitry involved in skilled reaching. After visual cortex lesions,

full field SF thresholds dropped 12%, to levels similar to those of normal animals following V1 stroke. V1 lesions, therefore, appear to abolish the experience-induced enhancement related to reach training. Due to these findings, the involvement of visual cortex in skilled reaching may be greater than previously assumed, and the visual cortex may be involved in sensory integration necessary for successful reaching.

With respect to the effect that experience has on behavioural function after stroke, the above study provides further evidence to support the hypothesis that experience affects the behavioural outcome observed after a stroke. It appears that experience in adulthood results in plastic changes in the brain which change baseline function. This change in baseline function subsequently results in differential behavioural outcomes after stroke.

In experiment 1 developmental visual experience resulted in a 60% reduction in OKT thresholds, yet in this experiment, only a 12% decrease was noted following adulthood motor experience. This model, therefore, is sensitive not only to developmental VOS experience, but can be used to detect plasticity in the visual cortex that is linked to experience in a seemingly non-visual task, in this case, skilled reaching.

# Chapter 4 – Effects of Stroke in Rats With Developmental Visual Experience and Adulthood Motor Experience

## 4.1 Combining Developmental Visual and Adulthood Motor Experience

The above experiments showed the effects of developmental visual experience and adulthood motor experience on stroke outcome as measured by OKT. Developmental visual experience has been shown to enhance OKT thresholds by approximately 59%, and following V1 stroke, results in a differential loss of function than in normal animals. Adulthood motor experience, on the other hand, has a similar enhancing effect on OKT which is manifested as an 11% increase in OKT thresholds, and which is also affected differently than normal or developmentally experienced animals after stroke. These two experiments then, have shown differential effects of each specific experience on stroke outcome. However, in the natural world, humans and animals are exposed to many different experiences throughout life, both during development and in adulthood. Therefore, it is necessary to test the effects of both forms of experience combined, on stroke outcome.

The following experiment aims to combine the above two studies, by using both developmental visual experience and adulthood motor experience to enhance OKT. The two previous studies have shown that both forms of enhancement are modulated by the visual cortex, as its removal or damage to it eliminates enhancement.

OKT is enhanced by both visual and motor experience, and so there may be two pools of plasticity within the visual cortex that can be drawn from, a visual

related plasticity, and a motor related plasticity. If the enhancement that occurs with developmental visual experience is essentially the same as that which occurs with skilled reaching, then we should see an additive effect of combining experiences, or possibly a ceiling effect where the greater of the two enhancements cancels out the other. Previous experiments have shown that when animals enhanced through developmental visual experience are subsequently monocularly deprived, their SF thresholds can be increased far beyond levels after the enhancing experience alone (Prusky, Alam, & Douglas, 2006). This implies that the latter suggestion of a ceiling effect is unlikely at levels following the visual experience, since enhancement above developmentally experienced thresholds have been shown in monocularly deprived animals. However, deprivation may draw on different mechanisms within the brain than those used with experience alone, and therefore results from deprivation studies may not apply to experience based studies.

On the other hand, these two methods of enhancing OKT may be drawing from separate areas of plasticity in the brain. Results may show the same behavioural manifestation in this case, either an additive effect of the enhancement or again, a cancellation of one of the enhancements. The results of combining both types of enhancement may not reveal the location in the cortex of the enhancement, but when experience is combined with the effects of stroke, a clearer picture may emerge.

If there are two plasticity pools being drawn from, different results than the previous two experiments should surface following V1 stroke. There may be

a different degree of decrease in OKT thresholds after V1 lesions. One possibility is that there could be an elimination of only one of the enhancements, that is, OKT thresholds may reduce to reach training enhanced levels, or if there is an additive effect, to developmentally enhanced levels. This would imply that the remaining enhancement is located outside of V1 when experiences are combined. However, if the plasticity is truly only present in the visual cortex, as the previous studies suggest, we should see a total elimination of all enhancements after V1 lesion.

The previous two studies have provided significant evidence to show that cortex damaged by MCA occlusion is not involved in OKT production, and that V1 removal can eliminate both visual and motor enhancement, so association areas will not be damaged in this final study.

### 4.2 Effects of Visual Cortex Stroke on OKT Thresholds

This experiment aims to provide further evidence about the cortical location of visual experience enhancement, reach training enhancement, and the effect of these experiences combined. Furthermore, the hypothesis that different experience leads to differential behavioural losses after stroke will also be tested. Animals will be subject to both developmental and adulthood experiences, and the effects of this combination will be investigated following each experience and following stroke. It has been suggested, in these results, that combining different experiences in the rat results in differential visuomotor behavioural outcomes after stroke.

*Method*

Animals were housed and handled according to Canadian Council on Animal Care (CCAC) standards and under the supervision of the University of Lethbridge Animal Care Committee.

Two male and three female LE hooded rats were used in this experiment ($N$ = 5), as well as with 8 age matched male control LE rats. All animals were bred in the Canadian Centre for Behavioural Neurosciences (CCBN) vivarium, with original stock obtained from Charles River. The animals were housed in groups of two or three in polycarbonate cages (45.5cm L x 25.5cm W x 20cm H) in the University of Lethbridge colony room. This room was maintained at an ambient temperature of 21 degrees Celsius and 35% relative humidity, with a light/dark cycle of 12/12. The animals had access to food and water *ad libitum* throughout the study, until the beginning of food deprivation described shortly. Animals were weaned at approximately 22 postnatal days, at which time males and females were separated and housed in identical conditions in the colony room.

*Experience/P15 enhancing*

Animals' thresholds were permanently enhanced as described previously, in Chapter 2.

*OKT Testing*

Animals were placed in the VOS system for approximately 30-45 minutes for each testing session in adulthood. Full visual field SF and contrast sensitivity thresholds, and monocular visual field SF and contrast sensitivity thresholds for

both sides were obtained in this time. SF threshold measurements were randomly double-checked by an outside blind observer.

*Experience/Single Pellet Reach Training*

*Apparatus*

The same single pellet reaching boxes used in the previous experiment were used here as well. Reaching boxes were made of clear Plexiglas (40cm x 45cm x 13.1cm) with a vertical opening 1.3 cm wide x 15 cm high. A 4 cm wide platform is attached to the outside of the opening, 4 cm above the floor, with two small indentations, 5 mm in diameter and 1.5 mm deep, evenly spaced in line with the edges of the vertical opening.

*Food Deprivation*

Food deprivation commenced two days before training, and as was described previously, animals were permitted only 25g/d for males and 20g/day for females, keeping their body weight at 95%-100% or greater for younger animals.

*Training*

As was described in Chapter 3, the first 4-5 days of training were used to determine paw preference. By day 7 of training, animals were familiar with the task, and scoring of the reach attempts began.

*Stroke Model*

*V1 Pial Strip*

V1 pial strip lesions were induced as described earlier, in Chapter 2.

*Histology*

Histological procedures were carried out as described in previous chapters. Digital photos were used to determine lesion boundaries, and Cresyl Violet staining was used to determine lesion depth.

*Statistical Analyses*

Repeated measures analysis of variance (ANOVA) was used to analyze the SF thresholds at each testing time (Statview, Version 5.0.1.0, SAS Institute Inc.). Follow up unpaired t-tests were used to compare within testing session effects (comparisons between contralateral and ipsilateral eye), and paired t-tests were used to analyze between testing sessions data. The alpha level for this experiment was set at 0.05.

*Results*

The following data showed that V1 devascularization significantly reduced full and monocular visual field OKT thresholds.

*Experienced Rats - V1 Lesions - Full Field SF Thresholds*

Repeated measures ANOVA revealed a significant main effect of testing time ($F(1, 7) = 23118.746$, $p < 0.0001$), and a significant interaction between testing time and contralateral/ipsilateral side ($F(1, 7) = 9247.804$, $p < 0.0001$) for rats with both developmental and adulthood experience. Follow-up paired and unpaired t- tests were used to further analyze the source of the interaction. Paired comparisons showed that reach training did not enhance OKT thresholds beyond visual experience enhancing ($t(9) = -1.5$, $p = 0.1679$). This suggests that visual

enhancing is masking the effects of the reach training enhancement, and that the reach training enhancement does not occur when visual experience has already increased thresholds in the full field. All post lesion OKT thresholds were significantly lower than both pre-reach training and post-reach training thresholds, which were equal as was previously mentioned ($M$ = 0.839 ± 0.004 and $M$ = 0.839 ± 0.004). When comparing thresholds from 2D after the unilateral V1 lesion, tests showed that thresholds were significantly higher than 2D and 1W post bilateral V1 lesion ($t(9) = 2.901$, $p = 0.0176$), as well as 2W post bilateral lesion ($t(9) = 2.842$, $p = 0.0193$). OKT thresholds recorded 1W after the unilateral lesion were also significantly higher than those obtained 2D ($t(9) = 2.929$, $p = 0.0168$), 1W ($t(9) = 2.929$, $p = 0.0168$), and 2W after the bilateral lesion ($t(9) = 2.869$, $p = 0.0185$). Similarly, measures obtained 2W after unilateral lesion were significantly different also from all three post bilateral lesion testing times (2D post bilateral V1: $t(9) = 2.978$, $p = 0.0155$; 1W post bilateral V1: $t(9) = 2.978$, $p = 0.0155$; 2W post bilateral V1: $t(9) = 2.918$, $p = 0.0171$). These data suggested that although thresholds were reduced after the unilateral V1 lesion, they were still higher following the unilateral lesion than after the bilateral lesion. This is due to the fact that the ipsilateral side was unaffected until after the induction of the bilateral lesion. OKT thresholds did not revert back to post-experience levels, and after both V1 lesions these thresholds were comparable only to those of inexperienced animals.

Unpaired t-tests showed that differences between the contralateral and ipsilateral visual fields were significant at all post unilateral lesion time points, 2D

post unilateral lesion ($t(8) = -108.757$, $p > 0.0001$), 1W post unilateral lesion ($t(8) = -102.538$, $p < 0.0001$), and 2W post unilateral lesion ($t(8) = -96.503$, $p < 0.0001$), suggesting again a significant reduction of thresholds after a unilateral lesion, and an evening out of both visual field's thresholds after a bilateral lesion. Lesion boundaries and data for this group have been presented in Figure 25 and Figure 26 respectively.

*Experienced Rats - V1 lesions - Monocular Field SF Thresholds*

Monocular visual field SF threshold data, available in Figure 27, was also analyzed using repeated measures ANOVA. A significant main effect of testing time was apparent ($F(1, 7) = 10.308$, $p < 0.0001$), as well as a significant interaction between testing time and side ($F(1, 7) = 9.736$, $p < 0.0001$). Follow-up paired t-tests showed that reach training did significantly increase OKT thresholds from values obtained before reach training ($t(9) = -4.247$, $p = 0.0022$), a trend that has only been seen with tray reach enhanced animals. Other significant differences arose after reach training, where the testing session immediately following training was found to be significantly higher than all post-lesion testing times (2D post unilateral V1: $t(9) = 2.675$, $p = 0.0254$; 1W post unilateral V1: $t(9) = 3.537$, $p = 0.0063$; 2W post unilateral V1: $t(9) = 3.924$, $p = 0.0035$; 2D post bilateral V1: $t(9) = 4.758$, $p = 0.0010$; 1W post bilateral V1: $t(9) = 4.821$, $p = 0.0009$; 2W post bilateral V1: $t(9) = 4.609$, $p = 0.0013$). These data suggested that the animals' acuities remained the same after visual experience, were increased significantly after reach training, and were then significantly reduced following damage to V1.

Using unpaired follow-up t-tests, differences between the contralateral and ipsilateral sides were apparent at 2D post unilateral lesion ($t(9) = -4.394$, $p = 0.0023$), 1W post unilateral V1 lesion ($t(9) = -2.926$, $p = 0.0191$), and 2W post unilateral lesion ($t(9) = -2.807$, $p = 0.0230$). This demonstrated that contralateral to first V1 lesion, visual field OKT thresholds reduced significantly compared to the ipsilateral (unaffected) side and that after the bilateral lesion the unaffected side reduced to thresholds similar to the initially affected side.

*Normal vs. Experienced Rats - V1 lesions - Full Field SF Thresholds*

Repeated measures ANOVA was used to check for significance between experimental and control groups. A significant interaction was found through this analysis ($F(1, 7) = 4593.928$, $p < 0.0001$). Unpaired follow-up t-tests were used to compare thresholds between experimental and control groups. A significant difference existed between P15-P30 experienced animals and controls prior to reach training, which showed again that this experience did significantly enhance OKT thresholds above inexperienced animals' thresholds ($t(24) = 259.296$, $p < 0.0001$). After reach training, this difference remained significant as thresholds were unchanged in the previously enhanced group ($t(24) = 270.009$, $p < 0.0001$). Unilateral V1 stroke reduced OKT thresholds significantly contralateral to the lesion, as mentioned earlier, but the difference between experimental and controls remained significant at all three post-unilateral lesion ($t(24) = 3.647$, $p = 0.0013$; $t(24) = 3.684$, $p = 0.0012$; $t(24) = 3.739$, $p = 0.0010$) because these data are collapsed across sides. Similar to previous experiments, the significant difference between the two groups was eliminated after bilateral V1 damage ($t(24) = -0.321$,

$p = 0.7511$; $t(24) = -0.935$, $p = 0.3592$; $t(24) = 0.124$, $p = 0.9023$), and thresholds of experimental animals and controls became statistically the same. The above data showed that experienced animals' thresholds were significantly higher than controls prior to reach training, they remained much higher than controls after reach training, were significantly reduced after unilateral V1 lesion, and were reduced further to the same level as controls after bilateral V1 lesion. These last data are represented in Figure 28.

*Summary*

This experiment combined both developmental visual and adulthood motor experiences to assess the effects of visual cortex stroke on visuomotor behaviour. The first objective was to determine the effect of combining two different and specific experiences. The second objective was to investigate the effects of additive experience on stroke outcome.

From the above experiment, the first conclusion may be that combining developmental visual experience with adulthood motor experience does not increase full field OKT thresholds above developmental visual enhancing levels. The developmental enhancement seems to cancel out, or override, the adulthood motor enhancement in the full field.

However, an unexpected finding was made, in that the monocular acuities of these animals were significantly enhanced after reach training. The reason for this is unknown, since prior studies have failed to show significant effects in the monocular visual field. It is possible that there are effects for all animals in the monocular field, but because it is such a minuscule change, this was the only

single pellet study to find it with significance. It could also be the case that combining both types of experience somehow caused the monocular changes. For example, one possibility is that developmental visual experience occupies the plasticity available in the region of V1 dedicated to the full visual field or binocular visual field, and that the motor experience enhancement was forced to move out to monocular visual field representation areas of the cortex. However, the increase in monocular fields was not equivalent to the 11% increase seen in the full field in animals with only motor experience, and was in fact much lower, at only 1%. It does not seem that the full extent of motor enhancement was relocated to monocular visual field areas. Damage to V1 eliminated the monocular visual field enhancement, and therefore it may be that relocating the enhancement to monocular areas of V1 changed the manifestation of the enhancement, and therefore the effects of combining experience may also have changed the manifestation of plastic changes in the brain. If developmental visual experience uses up the majority of the plasticity in V1, this region may no longer have the plastic capacity to allow for further experience to change the brain. That is, plasticity may be limited, and developmental experience may have used the majority of the plasticity available.

Further conclusions that can be drawn from this study include the finding that V1 lesions again eliminate enhancements of OKT caused by experience. Animals' thresholds were reduced to levels similar to normal animals with damage to V1, once again. This provides further evidence to suggest that enhancement in OKT thresholds takes place in the primary visual cortex. Though

this experiment did not include MCA occlusion, the finding that enhancement is reduced to similar levels as in the two previous studies which did include MCA occlusion, make it unlikely that this damage would cause an effect. There is still a possibility that some previously undamaged motor areas may be involved, if connections between the visual and motor cortex have been spared with MCA occlusion.

Results also imply that the plasticity pools, discussed in the introduction to this chapter, are combined in the visual cortex. Because motor experience does not increase full field OKT thresholds after visual experience enhancement, it seems that the most logical answer is that there is only one plasticity pool located in the visual cortex. The entire full field and monocular field enhancements are both removed with the visual cortex, and therefore this must be where the plasticity that allows for increased OKT thresholds is located. If there were another, undamaged plasticity pool, one would predict that OKT thresholds would not have reduced completely, or at least not to the level of other singly enhanced and normal animals, or the monocular enhancement may not have been eliminated with V1 damage.

With regard to the possibility of a ceiling effect, data is somewhat inconclusive. An additive effect was not noticed in the full visual field, and therefore it is possible that a ceiling effect has occurred. As was mentioned earlier, monocular deprivation enhances OKT thresholds beyond that of visual experience, and so it was predicted that a ceiling effect would not occur. However, as was also mentioned earlier, monocular deprivation and the resulting

increase in OKT thresholds may use different mechanisms to cause the enhancement. This is most likely the case, as the studies that have shown increases in OKT with monocular deprivation also show that once the deprivation is removed, acuities in the two eyes even out to pre-deprivation levels (Prusky et al., 2006), and therefore enhancement caused by monocular deprivation is temporary in rats, and is likely to rely on different mechanisms than our permanent enhancement models.

Because of the unexpected finding that enhancement moved to the monocular visual fields when visual and motor experience were combined, further investigation of more focal V1 lesions may reveal differential effects on the two enhancements. Since both enhancements are again removed with V1, a smaller lesion which does not abolish the entirety of V1 may reveal an area for visual enhancement, and an area for motor enhancement within V1. Smaller V1 lesions may also differentiate binocular regions of V1 from monocular regions. Further investigation of regions within V1 and their role in OKT enhancement is warranted.

The final conclusion that can be drawn from this study is that experience, motor, visual, or both, causes differential effects on behaviour after stroke. Both visual experience and motor experience cause changes in the visual cortex that effect normal function and the functional losses after stroke. It is clear that changes occur in the brain in response to experience, and these changes result in a differential loss of function after stroke, ie. baseline function changes following experience, and subsequent to these changes, the manifestation of stroke becomes

different than it would have been without the experience. This shows that variability in stroke outcome is affected by individual differences in experience in rats, though the mechanisms involved have not been elucidated here.

# Chapter 5 - Discussion

## 5.1 Summary

One of the most enigmatic features of stroke is variability. Variability in the location and size of a stroke, a patient's impairments following stroke, "spontaneous" recovery, response to treatment, and many other related factors all pose problems for researchers and clinicians in finding viable treatments for stroke. Until such variability is better understood, stroke treatments will undoubtedly remain unsatisfactory. This thesis proposes that one of the major factors contributing to variability in stroke may be individual differences in life experience. Through the use of a rat model which evaluates evolutionarily stable, reflexive visuomotor behaviours (OKT), the effects of specific developmental or adulthood experience have been investigated, as well as cortical regions which may be involved in the production of enhanced OKT.

The first discovery made in these experiments was that normal animals' full visual field OKT thresholds are significantly affected by V1 cortical lesions. Visual cortex stroke in inexperienced cage raised laboratory rats caused a small, but significant 3% decrease in OKT thresholds. Since Silver (2003) found that visual cortex aspiration does not affect normal OKT, similar to results of cortical inactivation studies (Prusky and Ramoa, 1999), these data suggest that different stroke models have differential effects on OKT.

MCA stroke, however, did not have an effect on OKT thresholds of inexperienced animals. This suggests that structures in the MCA territory are not involved in the normal functioning of OKT.

A second major finding was that full visual field OKT thresholds were significantly increased by experience, and the increase in thresholds consistently varied depending on the experience. This effect has been shown previously for developmental (Silver, 2003; Prusky et al., in press) and adulthood experience (Alam et al., 2008). For developmental visual experience, a 58-59% increase in OKT thresholds was apparent in this study. OKT thresholds were increased by 11% for animals who were exposed to adulthood reach training experience. This implies that OKT thresholds are differentially affected by different experiences. Previous data has suggested that the visual cortex is highly susceptible to change in response to experience (Hofer et al., 2006: Karmarkar & Dan, 2006), and if OKT function is dependent on the visual cortex alone, this may help to explain these findings.

The degree of functional loss following stroke was also distinct, depending on the task that animals had experienced. Developmental visual experience in the form of OKT testing resulted in an initial 58-59% increase, followed by a 60% decrease in OKT thresholds after V1 stroke. On the other hand, adulthood motor experience in the form of reach training resulted in an 11% increase in thresholds, and a 12% decrease after stroke. These data imply that experience changes baseline function, which results in a significant effect on stroke outcome. The size of the effect of stroke apparently differs depending on the experience of the animal. Though the change in baseline function was necessary to allow for the subsequent decrease in function following stroke, the experience of a laboratory rat is limited, and the increased baseline measure is

likely more indicative of "normal" function in wild animals. Therefore, reductions in OKT thresholds following stroke will likely differ from these results depending on combinations of prior experience in wild animals, or humans. This model, therefore, is sensitive to the differential effects of specific experiences on outcome following stroke. The effects of experience, however, were apparent only in the full visual field: the monocular visual field was not affected by each experience separately.

Monocular visual field OKT thresholds were only significantly affected when both developmental visual experience and adulthood motor experience were combined. These data suggest that developmental visual experience may have occupied the available full visual field enhancing plasticity in the visual cortex, and that experience later in life must then be relocated to monocular field representation areas (Schwartz, 1999). In addition, these data suggest that specific experiences alone, or combined, can alter the behavioural manifestation of experienced-induced plasticity, and that these changes have a significant effect on outcome following stroke.

### 5.2 Rat Model of Experience-dependent Variability in Stroke Outcome

Using OKT as a model for testing functional ability of motion vision after experience, and functional loss after stroke has many benefits. First, OKT is a universal response seen in all vertebrate species, and therefore, the probability that the effects of stroke on this function will be similar in rodents and humans is high. Since many treatment trials have proven successful in rodent models, yet remain unsuccessful in humans, the use of a universal response for testing

function holds promise for discovering treatment results that will transfer from one species to the next. Secondly, OKT is applicable to everyday life. OKT is essentially a response to visual input, and because humans primarily use vision to respond to their environment, this test applies to a response which humans make everyday, almost unknowingly. The third reason that this model is beneficial is that obtaining OKT thresholds is relatively quick and easy. Other methods of testing vision, such as visual discrimination and the VWT, take weeks to train animals and obtain reliable measurements, and infant animals often do not have the capacity to learn the task (Prusky et al., 2002; Douglas et, al., 2005). The VOS test of OKT takes less than 45 minutes to obtain both SF and contrast sensitivity thresholds, and no training is necessary since it relies on a reflexive response. Another benefit is that this test is sensitive to the effects of specific developmental and adulthood experience. Each new experience that animals were exposed to resulted in different changes in OKT. Therefore, this test can differentiate the effects of specific experience, a valuable characteristic for investigating the effects of experience on normal function. Also, this model has shown differential effects of stroke depending on prior experience, and so is not only sensitive to the effects of experience, but also to the effects that these experiences can have on stroke outcome. This model also allows for experience to be controlled, as laboratory rats have limited life experience and can easily be subject to one or only a few novel situations.

One of the greatest benefits of this model, however, is the promise of creating a similar test for humans. Many virtual reality therapies have been

developed, and most could easily be used as a test of OKT (Rose et al., 2005; Ansuini et al., 2006). Since the stimuli used in the VOS test of OKT is simple sinusoidal gratings, implementing this stimuli into an already much more complex apparatus should be relatively easy.

There are many directions that this research can follow within the realm of animal studies, however, 1) What are the effects of other types of experience? 2) Are other motor areas involved, which were not damaged by MCAo? 3) Are there specific areas within V1 that are responsible for each enhancement separately?

First, it has yet to be shown how experiences other than those used in the above studies affect OKT, and investigation into other types of experience and how they affect OKT is intriguing. Testing the effects of many different experiences will provide greater information about changes in the brain in response to experience. The experiences used here were purposely very specific, so that their effects could be directly attributed to visual development, or motor learning. However, investigation of the effects of different experiences may be warranted. Recent research has shown that less specific experiences, such as enriched housing and open field testing, do not effect OKT thresholds (Prusky et al., in press), and are therefore unlikely to show similar effects to those found in the above studies following stroke. Enriched housing and the open field task may have different effects on stroke outcome because they involve input to all the senses. The fact that it may be only specific experiences that affect OKT will make characterization of the effects of experience much more feasible and useful. Also, characterization of the effects of many different specific experiences and

tests which are commonly used in animal models may provide a guideline for the amount of plastic change occurring in the brain, and exactly where these change are occurring.

In these experiments, all animals' OKT thresholds reduced to very similar levels, between 0.50 and 0.52 c/d. This finding may be evidence of a "floor effect", or the possibility of such an effect may be an overgeneralization, since variability in OKT in rodents is extremely low. Examining other forms of experience may continue this pattern, or may also show that this effect is apparent only for the experiences chosen here. The possibility of a floor effect, and the reasons for such an effect, should be considered in future research within this area.

An attempt to find motor areas, not affected by MCAo damage, which may be associated with the OKT and reach training enhancement is also necessary. The above studies used MCAo to induce damage, and may have spared some or all of the dorsal pathway to area MT, a region known to be involved in motion perception (Kandel, Schwartz, & Jessel, 2000). Should the reach training enhancement remain untouched after specific motor cortex damage, the assumption can be made with certainty that motor areas of the cortex are not involved in the enhancement, directly or indirectly, and that the enhancement may be related only to the visual cortex. One could also induce this damage prior to experience to note whether motor areas are necessary for he initial induction of enhancement. However, before completely disregarding the inclusion of motor areas, subcortical motor damage must also be investigated. Because OKT is a

universal response in vertebrates, it may involve brain centers much lower than the cortex. A pilot study was conducted which induced striatal damage via endothelin-1 injection or electrolytic lesion, and found no effect on OKT in normal or experienced rats. This further complicates the issue, but may be indicative of a very specific region of involvement in subcortical structures. Also, the striatal damage study was only a pilot study, and a greater number of subjects would be necessary to make definitive conclusions. A final region of the brain that may be involved in OKT behaviour is the thalamus. Visual information travels from the retina to the thalamus, before heading on to the visual cortex (Kandel, Schwartz, & Jessel, 2000). Therefore, it is likely that thalamic damage would interrupt OKT. Investigation of this hypothesis could reveal the beginning of the pathway involved in OKT. That is, if thalamic damage does not affect OKT, we can conclude that other pathways which stem directly from the retina to other areas are the source of the production of OKT, as has been proposed previously (Douglas et al., 2005).

Finally, results presented in Chapter 4 suggest that within V1, there may be specific regions associated with certain portions of the visual field. Animals with combined developmental and adulthood experience showed an enhancement of OKT thresholds in the monocular visual field, which was also removed with V1 cortical damage. Therefore, there may be separate regions within V1 responsible for the full visual field effects, and another for monocular field effects. Smaller, more focal lesions in area V1 of animals with combined experience, may allow for the 1% monocular, adult experience-induced

enhancement to be eliminated, while sparing the 59% developmental full filed enhancement, or vice versa. Further examination of focal V1 lesions hold promise for finding separate and specific plasticity pools within this region.

### 5.3 Implications for Human Stroke

The experiments in this thesis are exciting in that they show that experience can affect both normal function and the outcome following stroke. However, there are many questions that must be answered for this information to be utilized in a clinical setting. First, do these results transfer to humans? Secondly, can human experience be analyzed in a similar way? That is, because humans have a much greater repertoire of life experiences, is it possible to break these experiences down to examine the effects of each specifically? If this is not possible, can the effects of combinations of specific experiences be determined? Thirdly, can research in this area be used to aid in the application of treatment or therapy for stroke patients?

As has been mentioned earlier, the test of OKT can be altered for use with humans. It is therefore relatively easy to test for similar effects of experience in humans. Though human experience is far more advanced than laboratory rodents, researchers could, for example, test humans with similar specific experiences to check for similarities in OKT. Another possibility would be to test OKT, then train humans on a new task and look at OKT again to examine the effects of these new experiences on original function. Similar to the experiments completed here, combinations of experience can also be investigated. Determining the effects of both specific and general experiences in humans may be useful in limiting

variability in treatment trials as well, as prior experience may be another factor which can be controlled for in participant inclusion criteria.

The information gained from these experiments may be useful in analyzing human stroke. Results showed that plasticity in response to experience may be limited. In Chapter 4 developmental experience caused a significant increase in full field OKT thresholds, yet additional experience in adulthood was unable to enhance full field OKT further. The enhancement caused by adulthood experience was relocated, and its manifestation changed from a full field enhancement of 11%, to a 1% monocular field enhancement. This suggests that there is a limited amount of plasticity available in full field representations within V1, and that further experience needs to relocate and change itself in order to affect the brain. Though the plastic capabilities of the human brain are huge (just think about all the experiences that a human is involved in throughout their lifetime), these data suggest that there may actually be limitations in the amount of plasticity that can occur in certain brain regions, or that can be utilized by certain therapies.

If plasticity in humans can be tested, using OKT or any other method, recovery may be able to take advantage of brain regions which have greater amounts of available plasticity. That is, for recovery from brain damage, researchers and clinicians may be able to draw on areas thought to have a greater capacity to accept plastic changes. This is exciting, as treatment may be directed to regions of the brain that will more readily accept plastic changes, as opposed to trying to force plasticity in areas that may have little opportunity for change.

Overall, this thesis provides evidence to show that variability in stroke outcome is linked to the experiences an individual has throughout life, and that different experiences lead to differences in normal function and in functional outcomes after stroke. Also, the VOS test of OKT has proven to be a good animal model for testing the effects of experience on the brain. It is also sensitive to the effects of experience on, at the very least, visual cortex stroke outcome. This model also holds promise as a test that can be used in human stroke patients, as not only is the function being tested the same in all vertebrate species, but technology is now available which will allow for this test to be converted into a test for humans.

# Figures

Figure 1. Virtual Optokinetic System (VOS) as shown in Douglas et al., 2005. **A.** A 3D side view cutaway of the VOS. The center platform, where the untrained animal is placed for testing, is surrounded by four LCD computer monitors which project the stimulus (sine wave grating). **B.** A virtual representation of a rotating cylinder centered on the animal. (permission obtained from Dr. Glen Prusky).

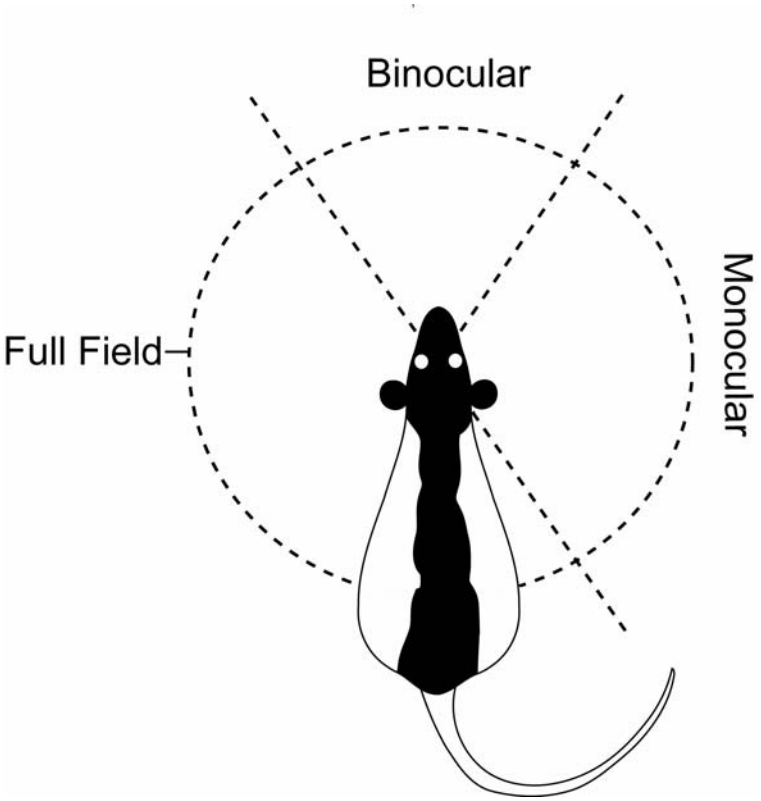


Figure 2. Representation of full, monocular, and binocular visual fields from the animal's point of view.

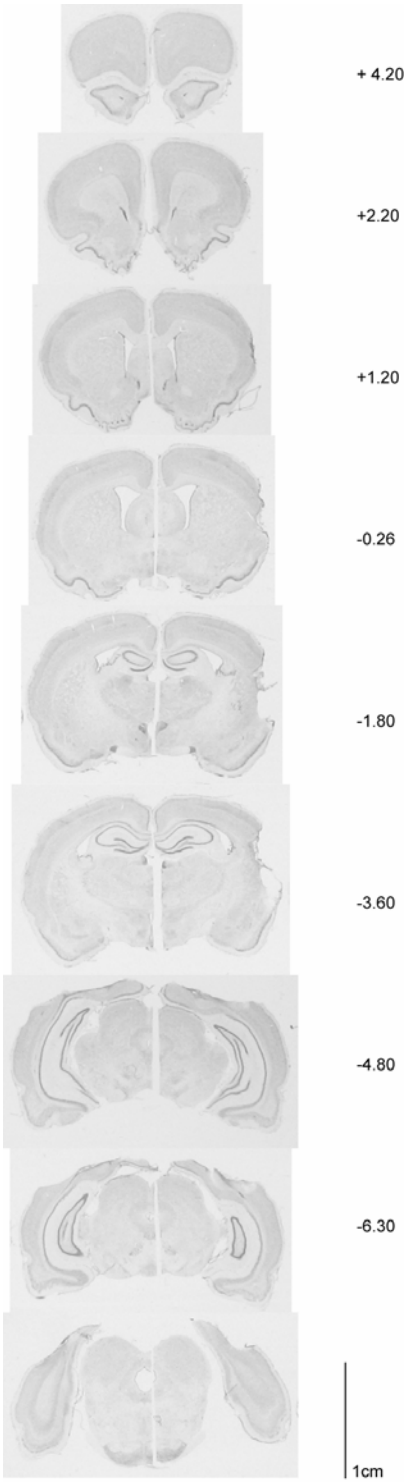


Figure 3. Typical cortical damage induced by MCAo and V1 devascularization.

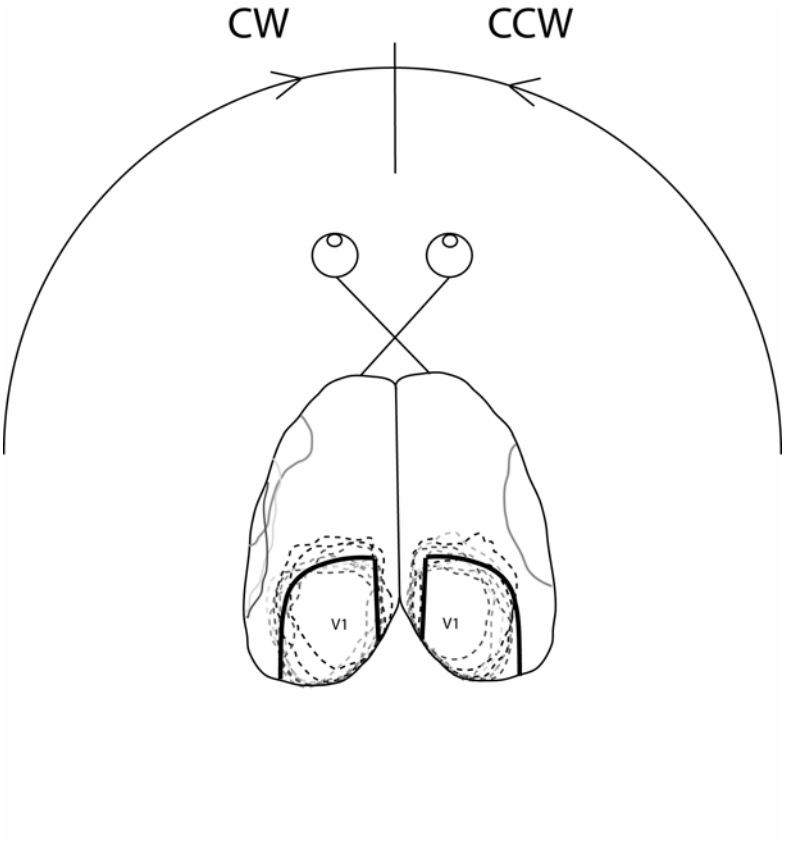


Figure 4. Representation of lesion boundaries in normal animals with typical laboratory experience; MCAo andV1 devascularization. Traces of lesions superimposed on a brain outline averaged from this group. Solid lines represent MCAo, dashed lines represent V1 damage. Arrows show direction of rotation; tracking occurs only in the naso-temporal direction, and therefore clockwise rotation (CW) tests the right hemisphere through the left eye, and counterclockwise (CCW) rotation tests the left hemisphere through the right eye. Thick black lines delineate V1 (Paxinos & Watson, 1998). Contralateral and ipsilateral, from here on, are tested through the eye opposite the first unilateral V1 lesion.

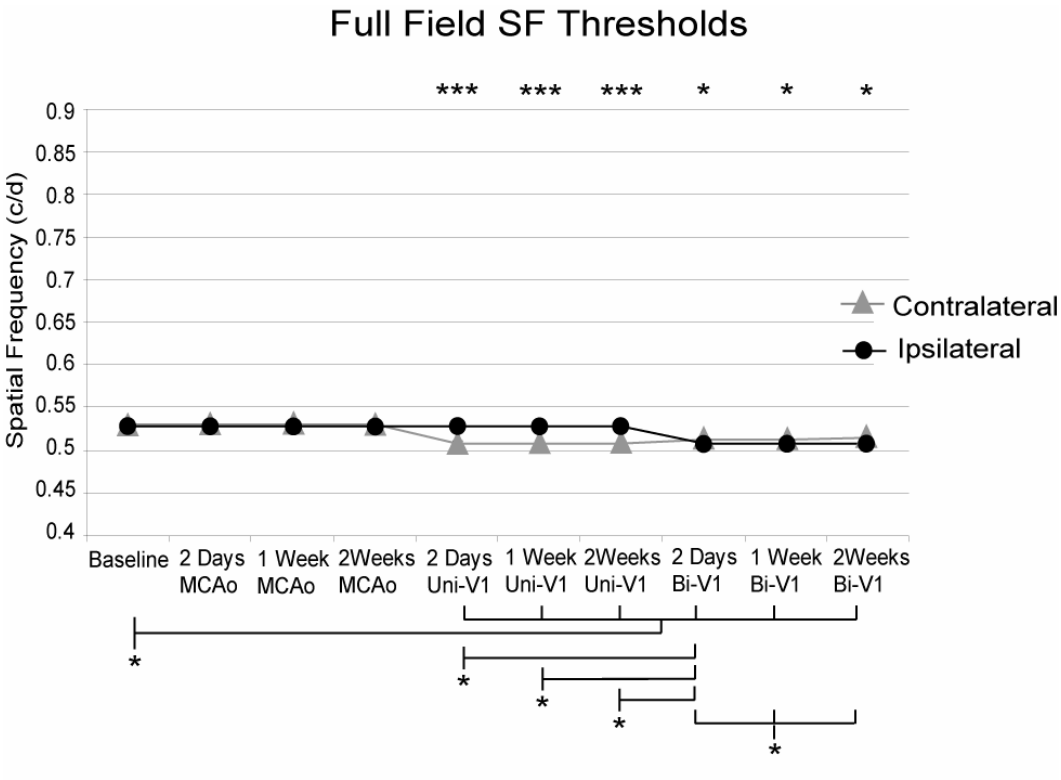


Figure 5. Full visual field (360°) SF thresholds obtained through each eye in normal animals with typical laboratory experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

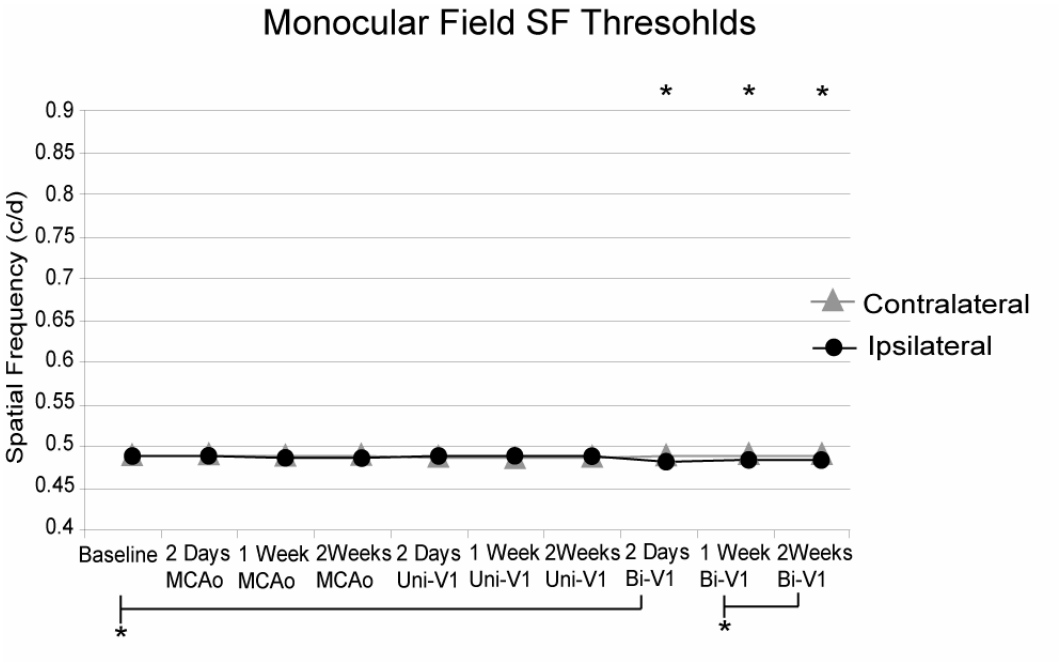


Figure 6. Monocular visual field (115º) spatial frequency (SF) thresholds obtained through each eye in normal animals with typical laboratory experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

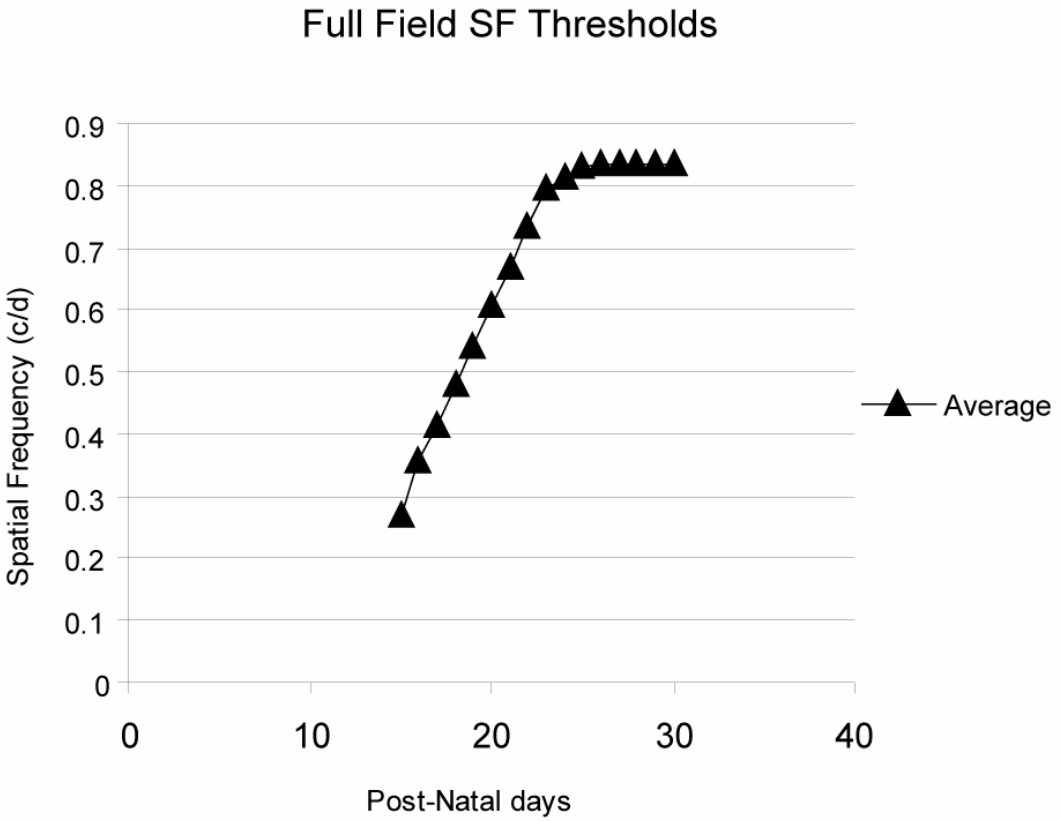


Figure 7. Average spatial frequency thresholds obtained through both eyes each day during P15-P30 developmental visual experience.

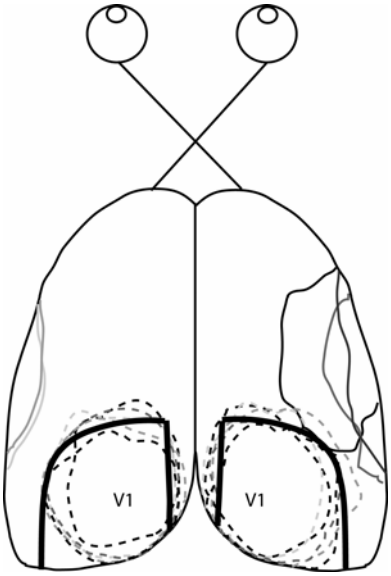


Figure 8. Representation of lesion boundaries in animals with developmental visual experience; MCAo followed by sequential V1 devascularizations. Traces of lesions superimposed on a brain outline averaged from this group. Individual animals' lesions match in colour. Thick black lines delineate V1 (Paxinos & Watson, 1998). Solid lines represent MCAo lesion boundaries, dashed lines represent V1 lesion boundaries.

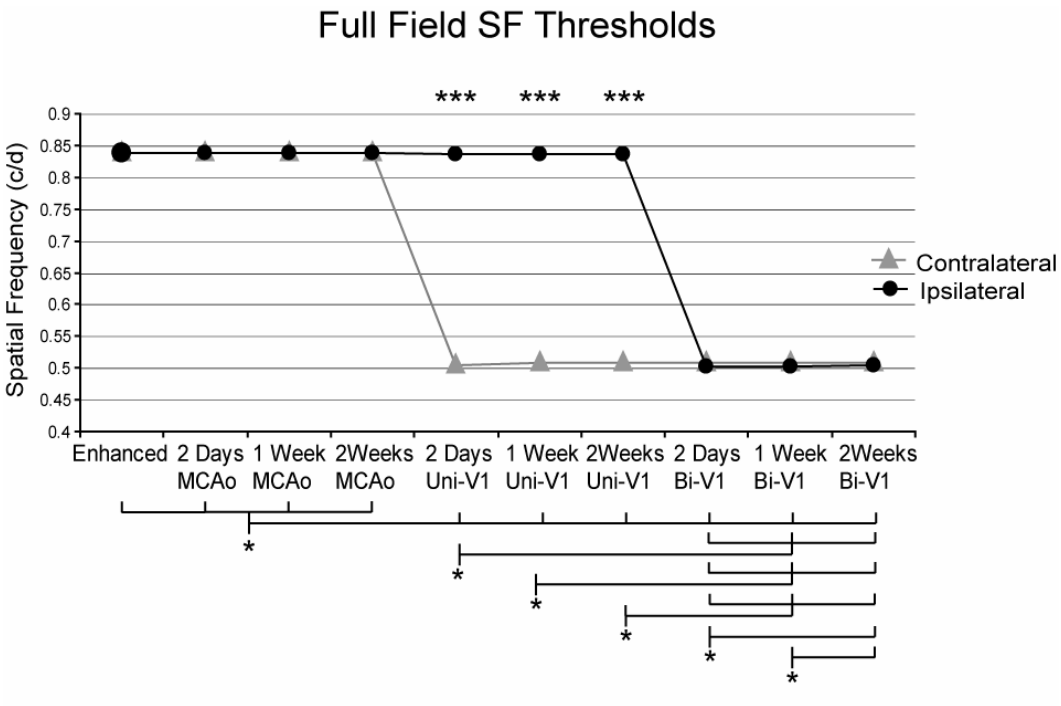


Figure 9. Full visual field (360º) spatial frequency (SF) thresholds obtained through each eye in animals with developmental visual experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

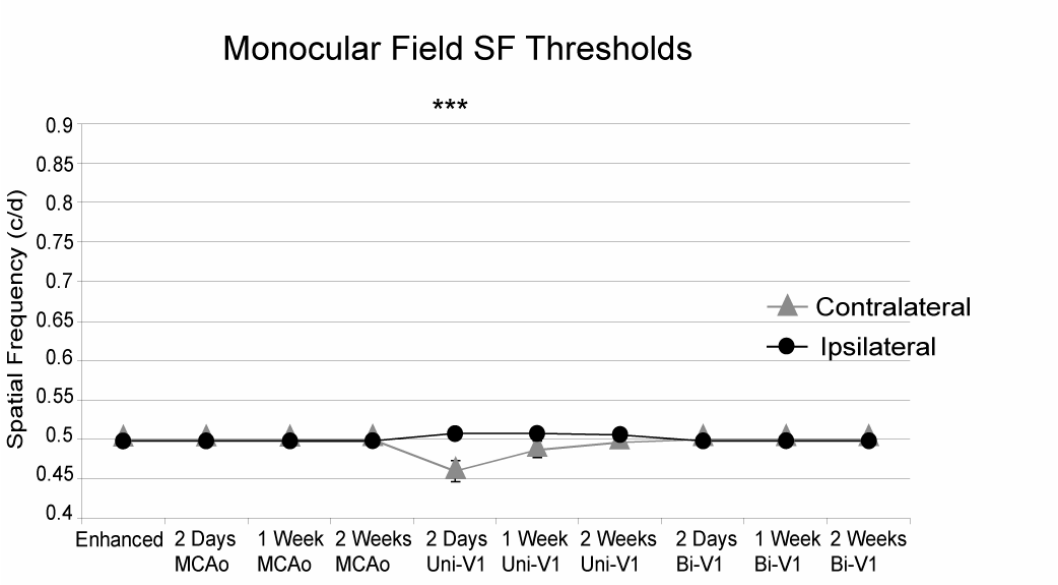


Figure 10. Monocular visual field (115º) spatial frequency (SF) thresholds obtained through each eye in animals with animals with developmental visual experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

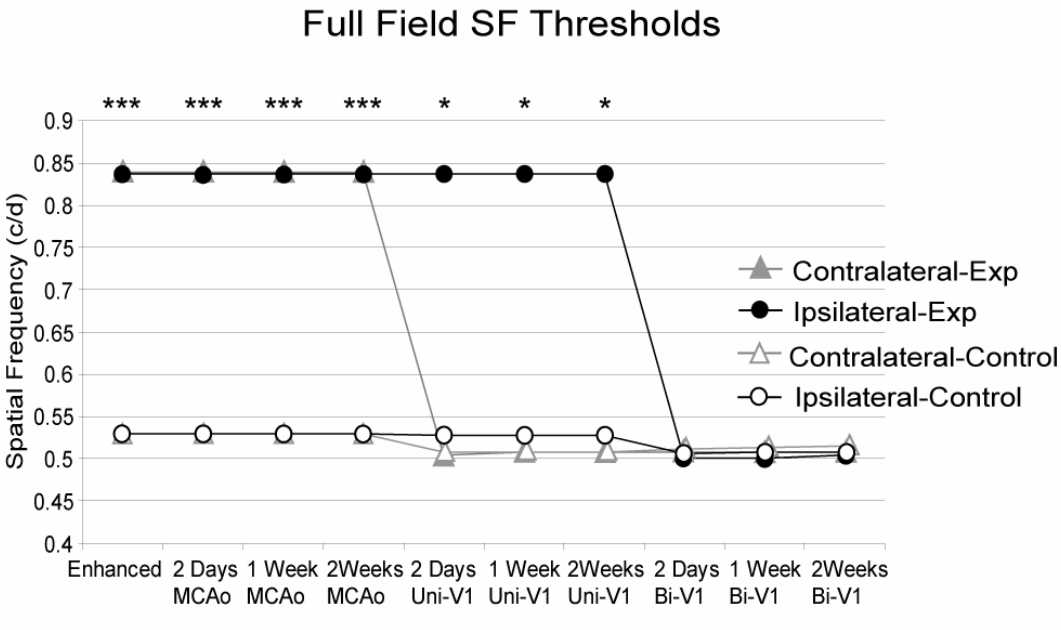


Figure 11. Full visual field (360°) spatial frequency thresholds obtained through each eye in animals with developmental visual experience and control animals without experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

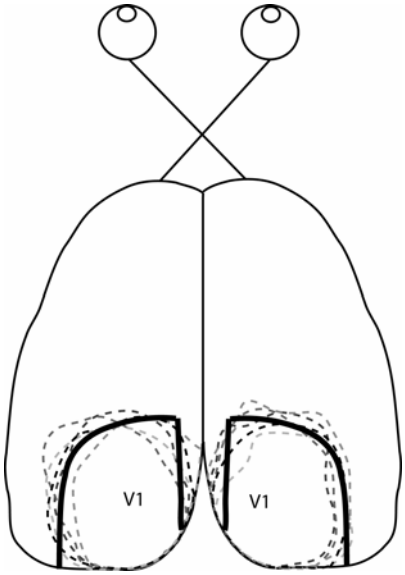


Figure 12. Representation of lesion boundaries in animals with developmental visual experience; sequential V1 devascularizations. Traces of lesions superimposed on a brain outline averaged from this group. Thick black lines delineate V1 (Paxinos & Watson, 1998). Individual animals' lesions match in colour.

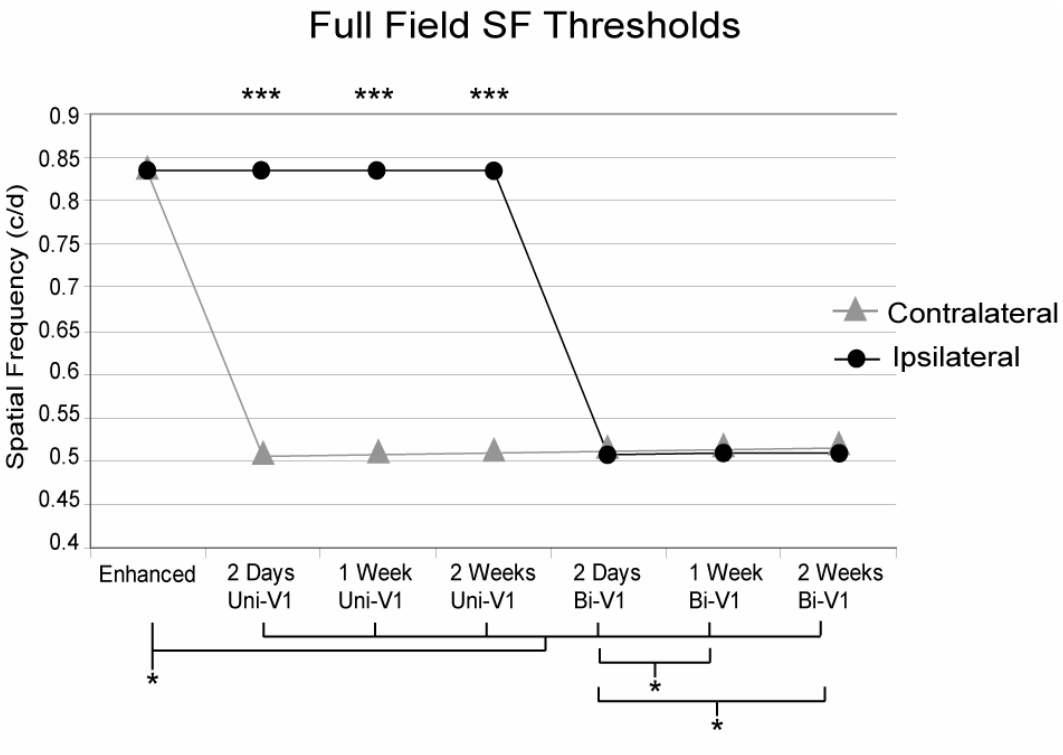


Figure 13. Full visual field (360º) spatial frequency thresholds obtained through each eye in animals with developmental visual experience; sequential V1 devasularizations. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

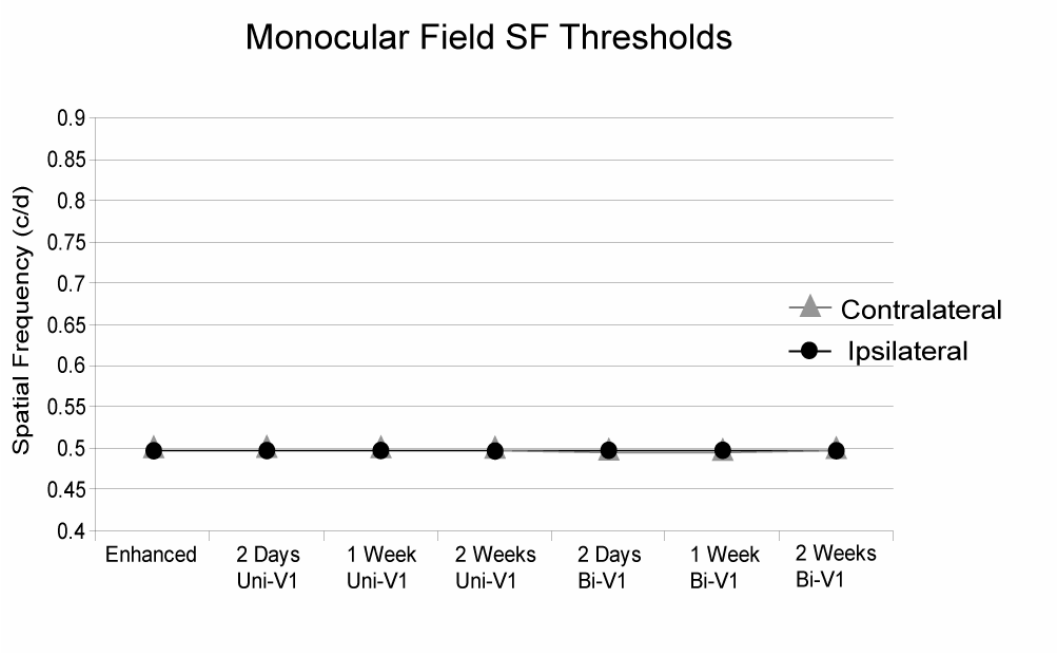


Figure 14. Monocular visual field (115º) spatial frequency thresholds obtained through each eye in animals with developmental visual experience; sequential V1 devascularization. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

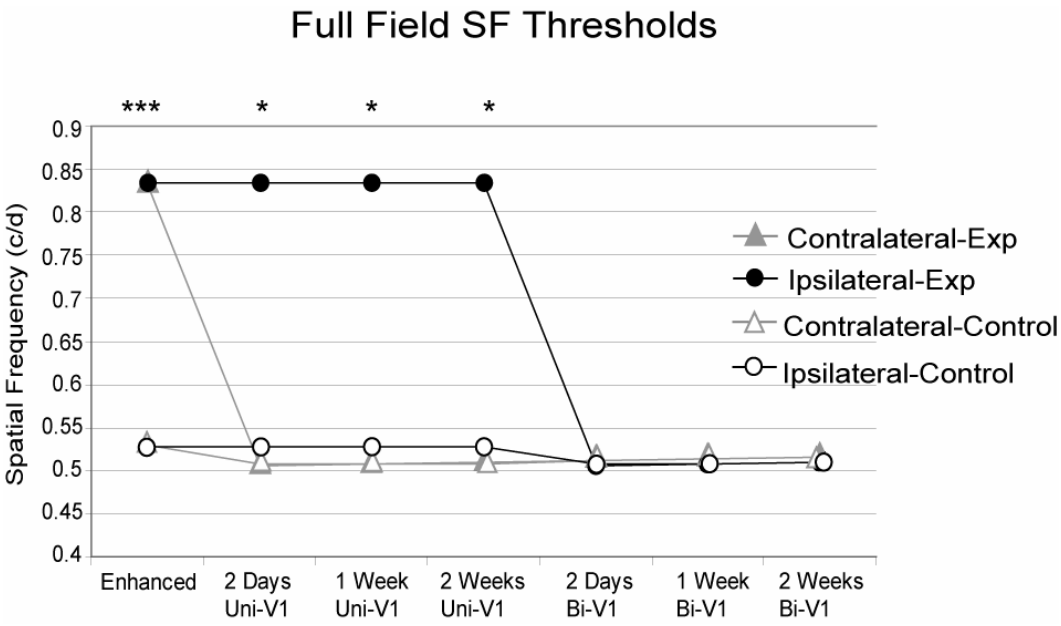


Figure 15. Full visual field (360°) spatial frequency thresholds obtained through each eye in animals with developmental visual experience and control animals without experience; sequential V1 devascularizations. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

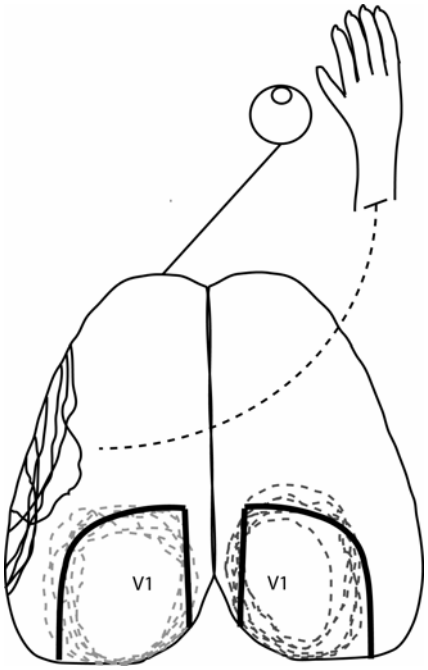


Figure 16. Representation of lesion boundaries in animals exposed to adulthood motor experience in the form of single pellet reach training; MCAo followed by sequential V1 devascularizations. Traces of lesions superimposed on a brain outline averaged from this group. Individual animals' lesions match in colour. Thick black lines delineate V1 (Paxinos & Watson, 1998). Solid lines represent MCAo lesion boundaries, dashed lines represent V1 lesion boundaries. Order of lesion induction shown by colour. Dashed line from paw shows crossed nature of motor control, ie. left hemisphere controls right side of body and vice versa. Note that not all MCAo lesions were left hemisphere, lesions were grouped on one side here to show contralateral to reaching paw.

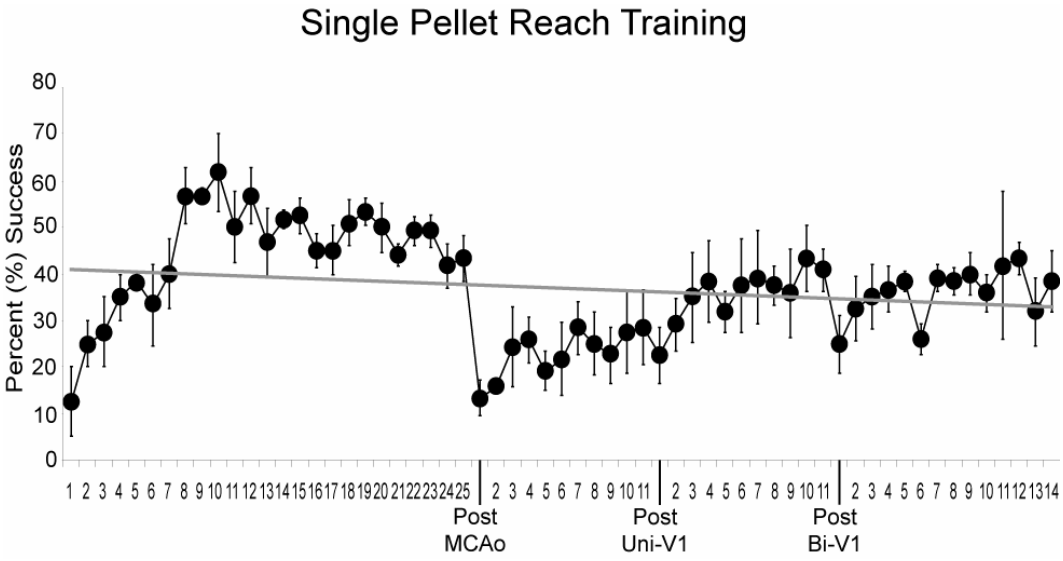


Figure 17. Success in Reaching. Percent success is represented on y-axis, days of reaching on x-axis. Note that reaching success dropped following MCAo, and was only briefly affected (approximately one day) by V1 lesions.

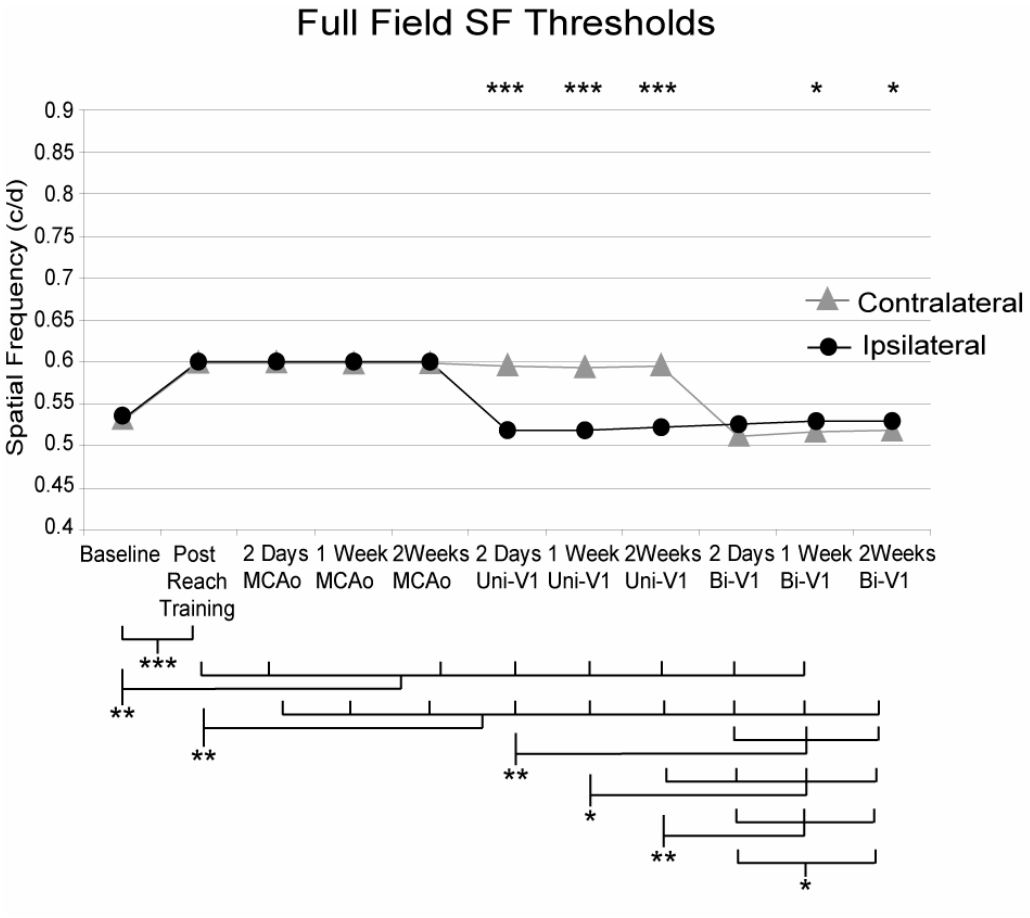


Figure 18. Full visual field (360º) spatial frequency thresholds obtained through each eye in animals with adulthood single pellet reach training experience; MCAo and sequential V1 devascularizations. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance between groups, (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

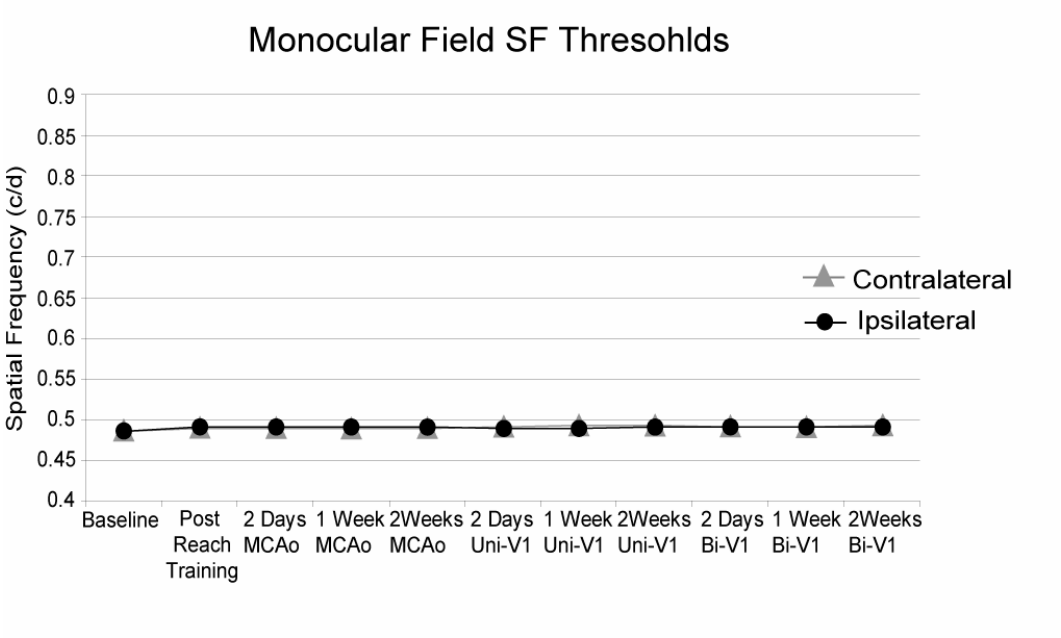


Figure 19. Monocular visual field (115º) spatial frequency (SF) thresholds obtained through each eye in animals with adulthood single pellet reach training experience; MCAo and sequential V1 devascularizations. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance between groups, (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

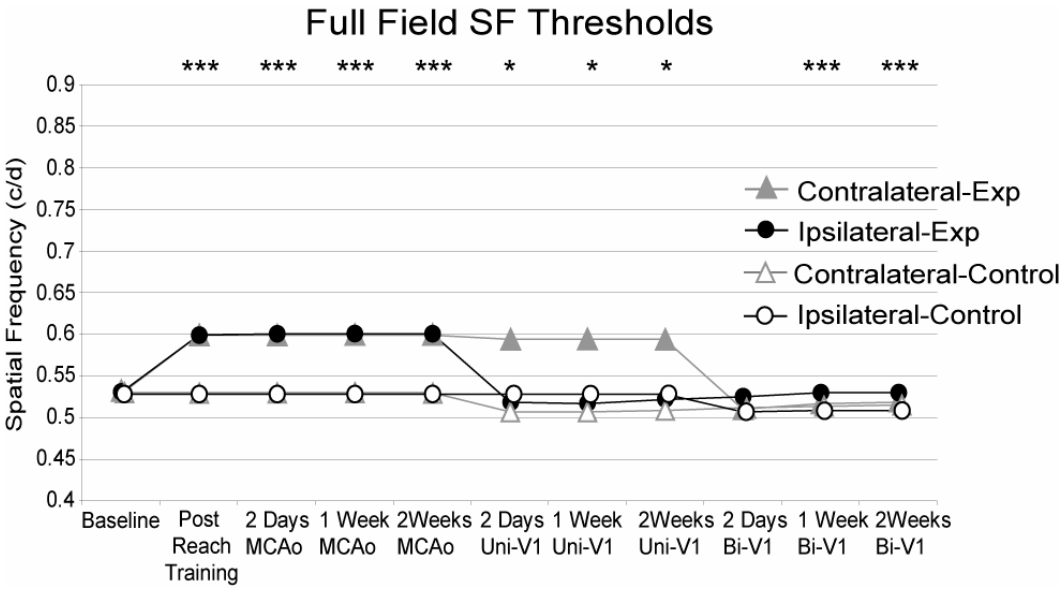


Figure 20. Full visual field (360°) spatial frequency thresholds obtained through each eye for experimental animals with adulthood single pellet reach training experience and control animals without experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance between groups, (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

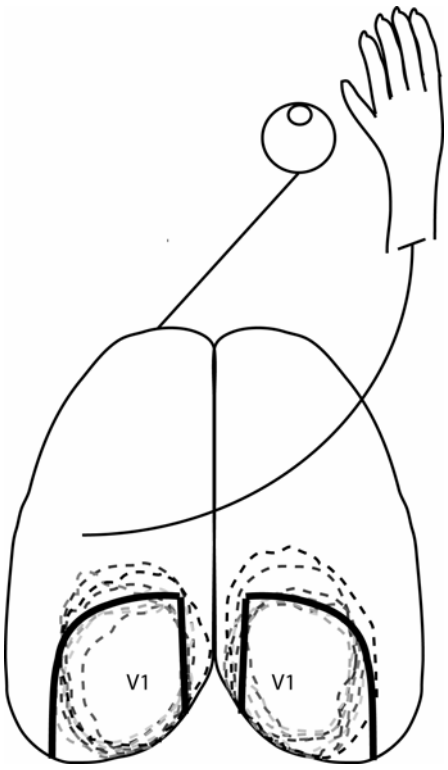


Figure 21. Representation of lesion boundaries in animals exposed to adulthood motor experience in the form of single pellet each training, with sequential V1 devascularizations. Traces of lesions superimposed on a brain outline averaged from this group. Individual animals' lesions match in colour. Thick black lines delineate V1 (Paxinos & Watson, 1998). Individual animals' lesions match in colour. Solid line from paw shows crossed nature of motor control, ie. left hemisphere controls right side of body and vice versa.

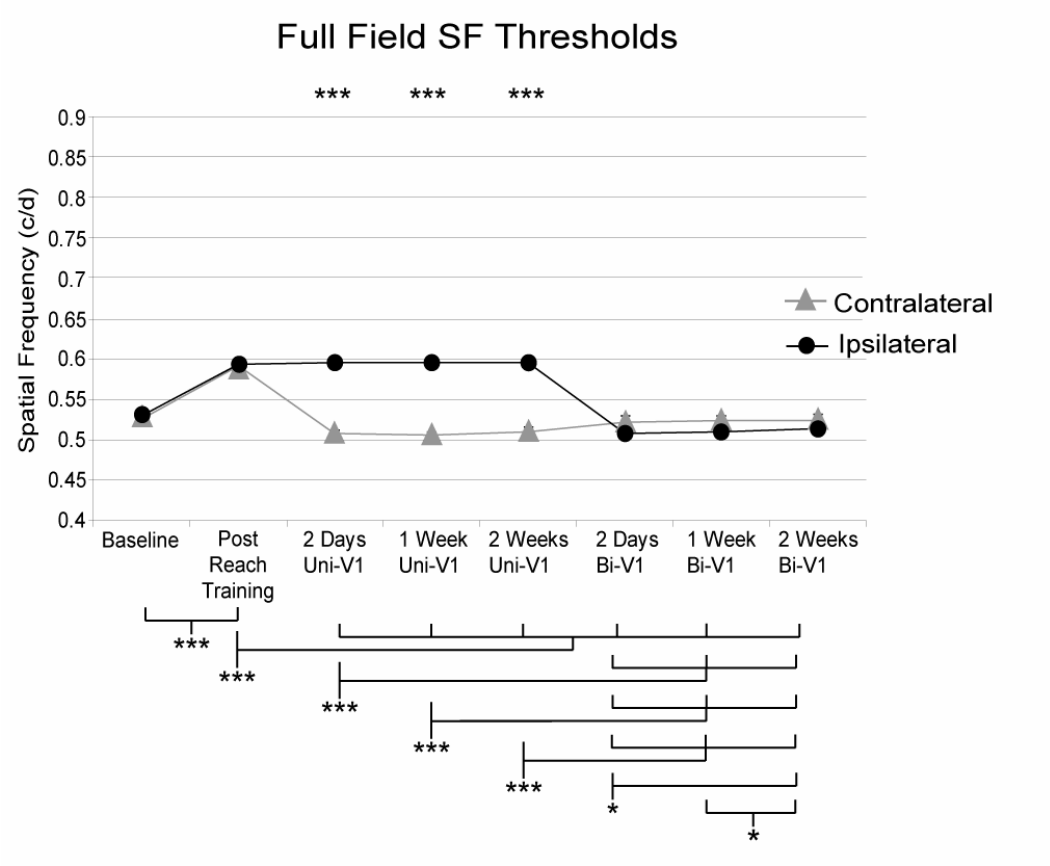


Figure 22. Full visual field (360º) spatial frequency thresholds obtained through each eye in animals with adulthood single pellet reach training experience; sequential V1 devascularizations. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance between groups, (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

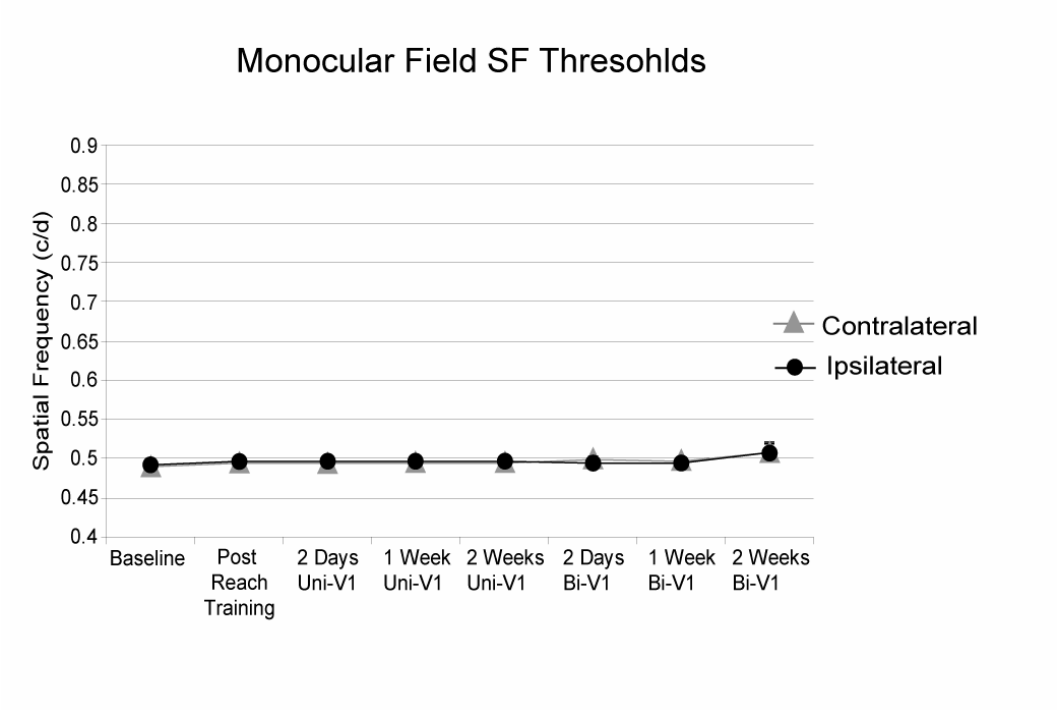


Figure 23. Monocular visual field (145º) spatial frequency thresholds obtained through each eye for single pellet reach training enhanced animals. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance between groups, (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

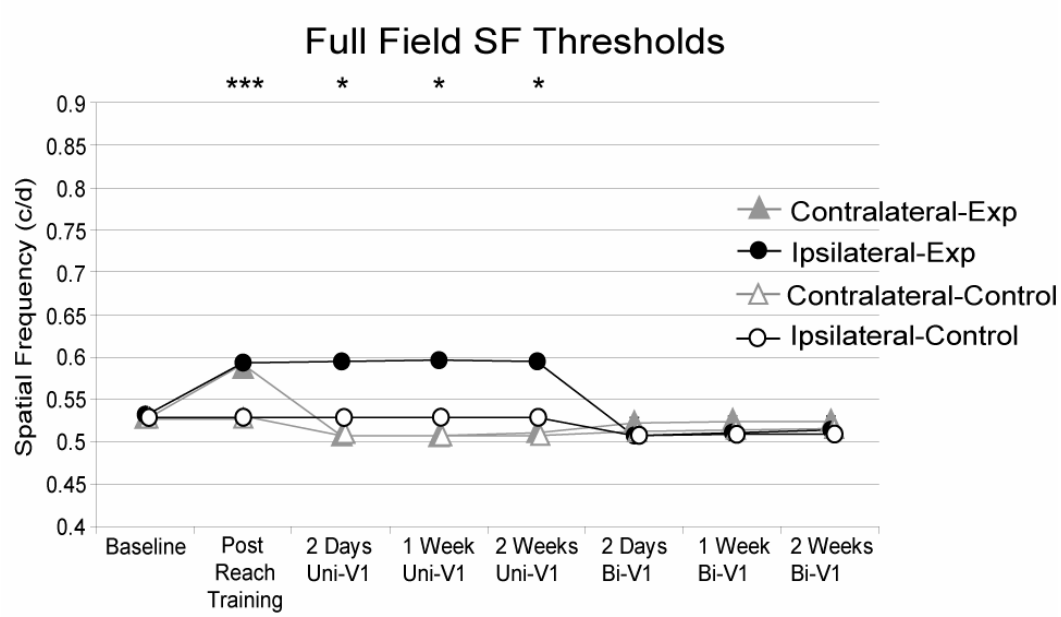


Figure 24. Full visual field (360°) spatial frequency thresholds obtained through each eye in animals with adulthood single pellet reach training experience and control animals without experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance between groups, (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

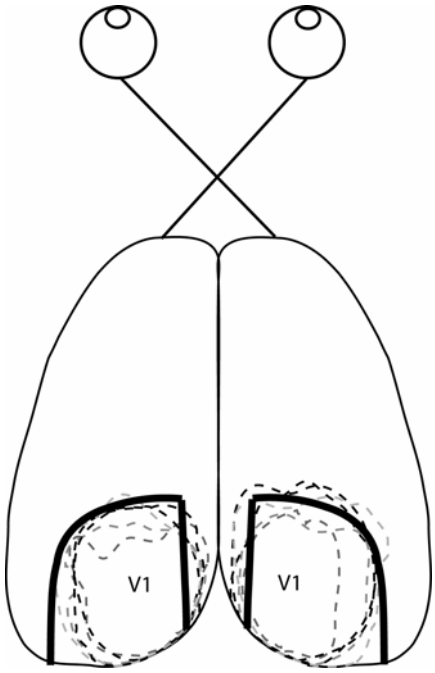


Figure 25. Representation of lesion boundaries in animals exposed to both developmental visual experience in the form of OKT testing and adulthood motor experience in the form of single pellet each training; sequential V1 devascularizations. Traces of lesions superimposed on a brain outline averaged from this group. Individual animals' lesions match in colour. Individual animals' lesions match in colour. Thick black lines delineate V1 (Paxinos & Watson, 1998). Individual animals' lesions match in colour.

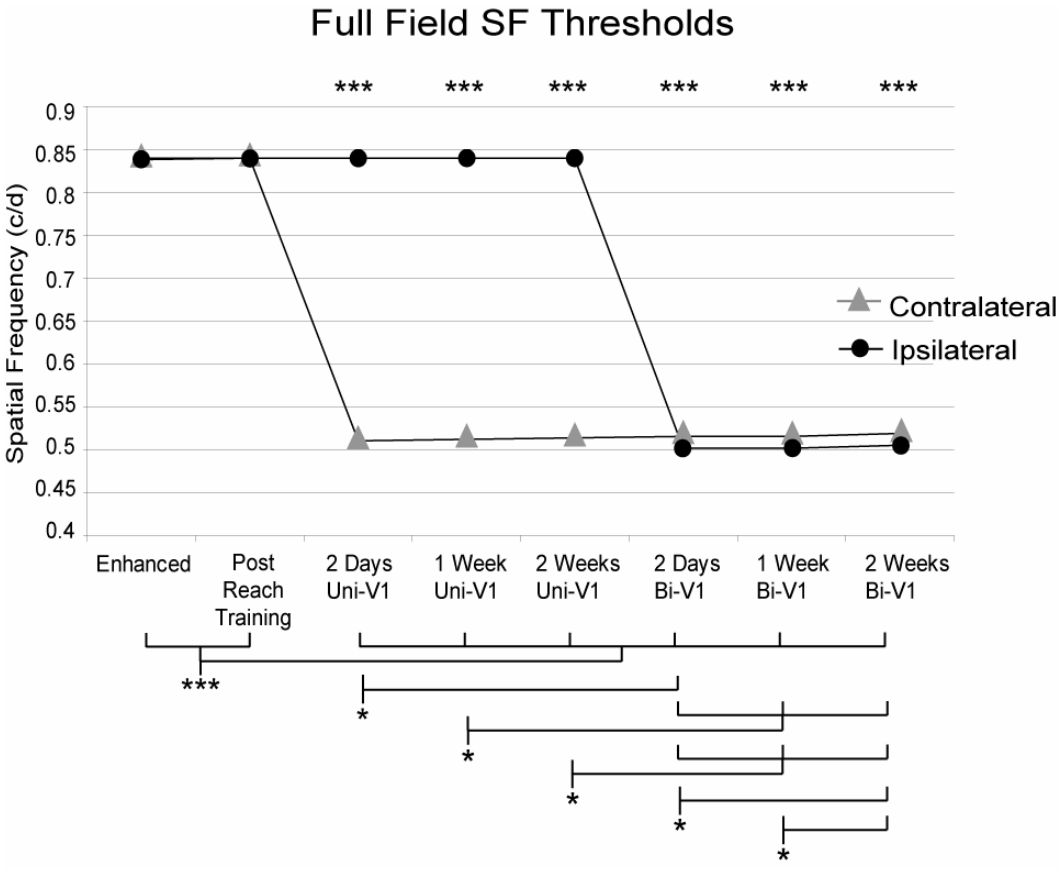


Figure 26. Full visual field spatial frequency thresholds obtained through each eye in animals with both developmental and adulthood experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

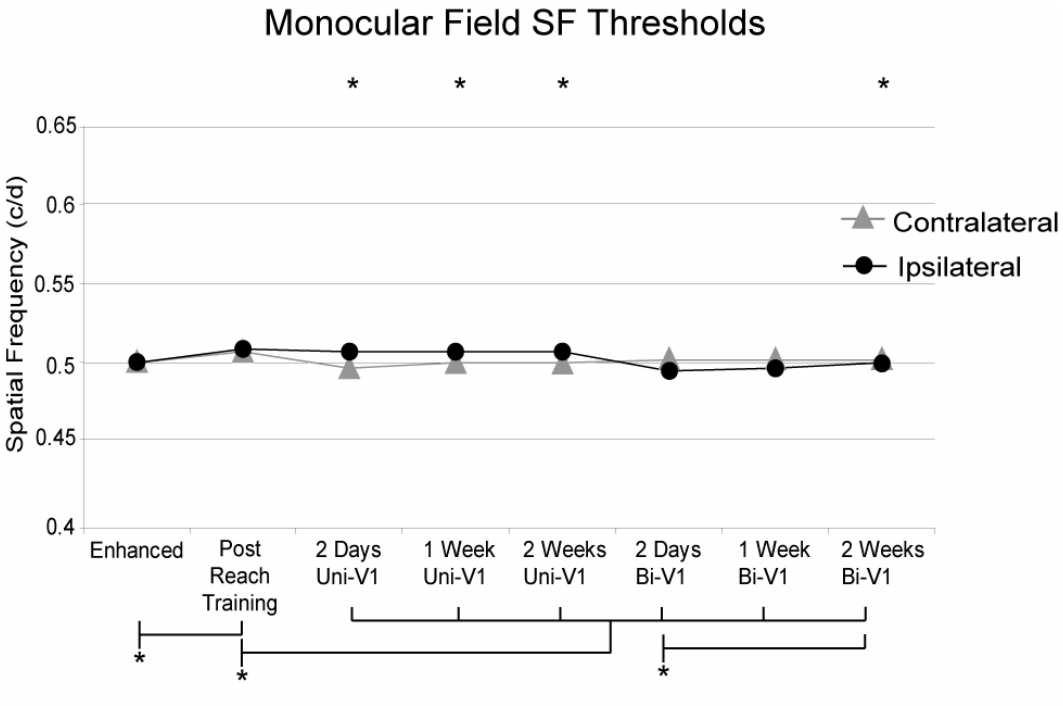


Figure 27. Monocular visual field (115º) spatial frequency thresholds obtained through each eye in animals with both developmental and adulthood experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

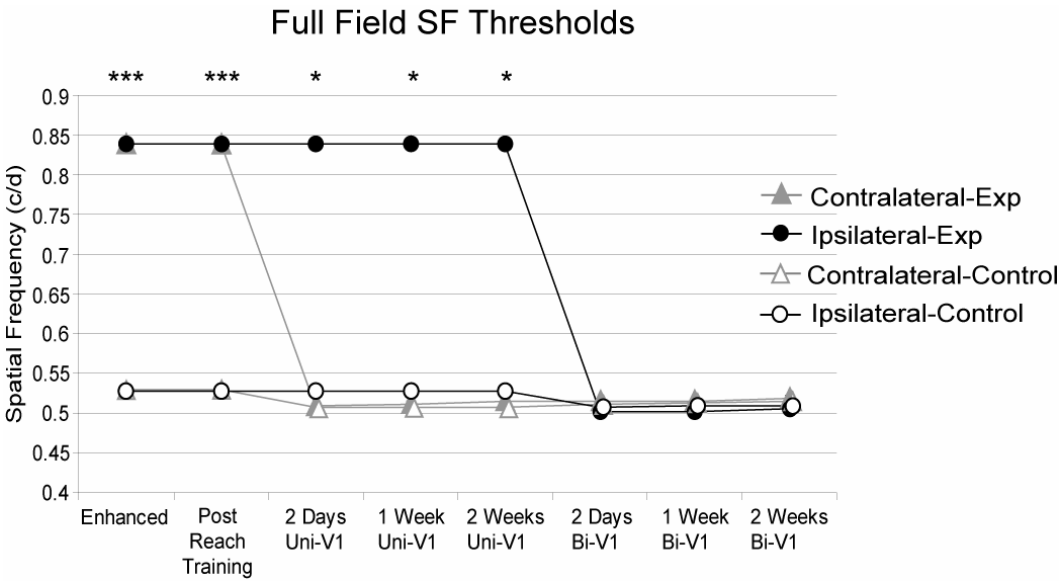


Figure 28. Full visual field spatial frequency thresholds obtained through each eye in animals with developmental and adulthood experience, and control animals without experience. Contralateral and ipsilateral represent thresholds obtained through the eye contralateral or ipsilateral to unilateral V1 lesion, respectively. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$.

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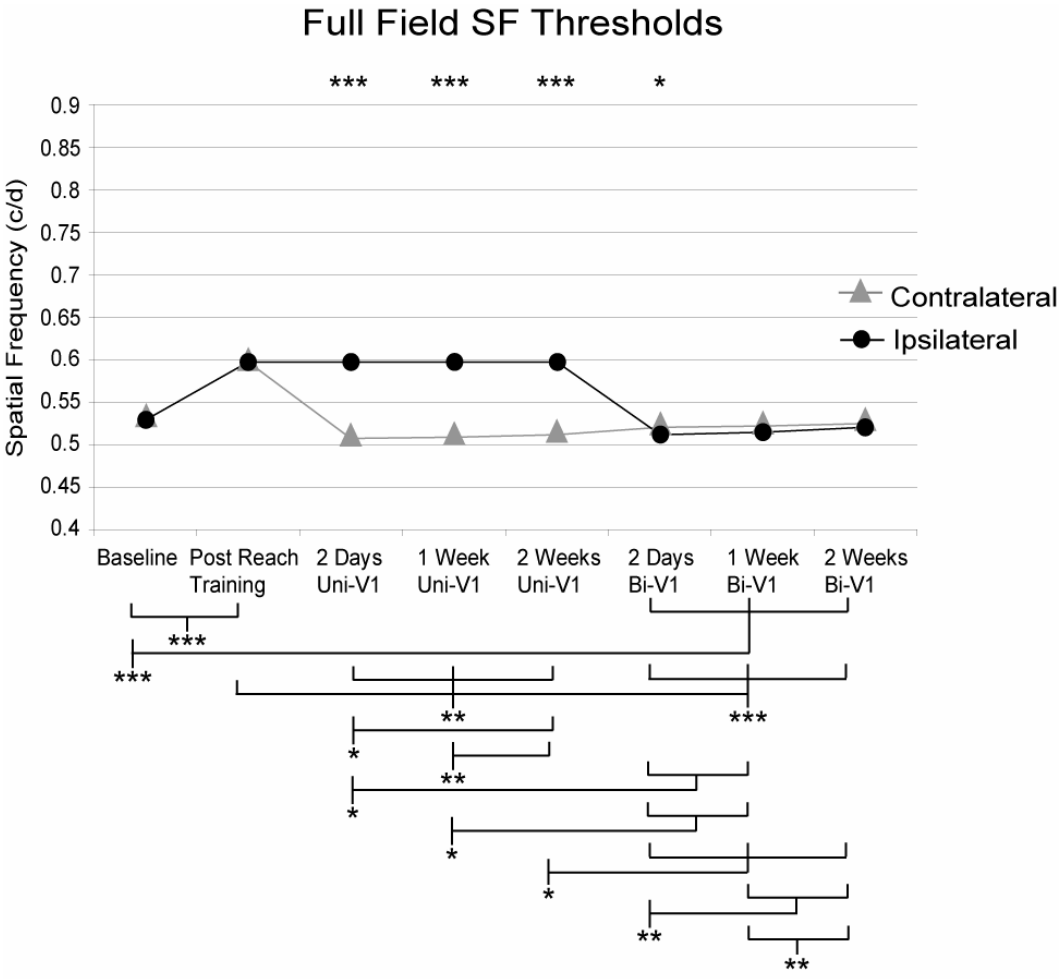
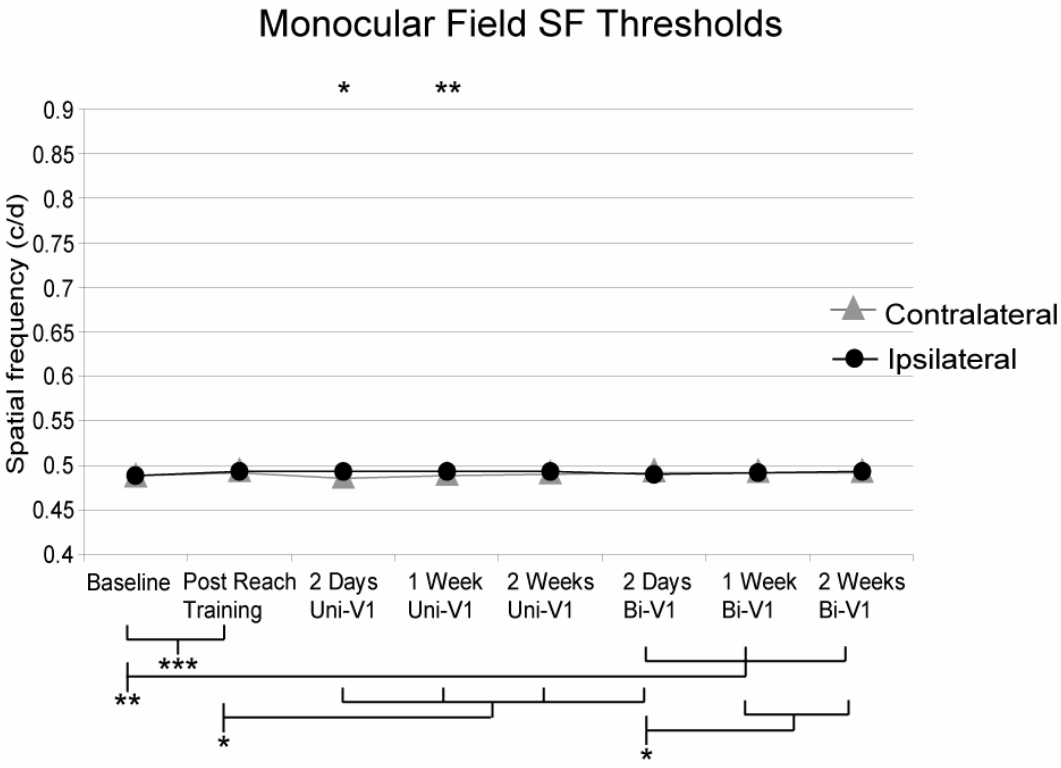
## Appendix A

Tray Reach Experience




Full visual field (360˚) spatial frequency thresholds for each eye in adult animals with tray reach experience. Contrlateral and ipsilateral to unilateral V1 lesion. (c/d) = Cycles per degree. Astericks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$. Tray reach experience enhanced OKT thresholds by 12%. Overall reduction in OKT thresholds following V1 lesions was 14%, similar to single pellet reach trained animals.

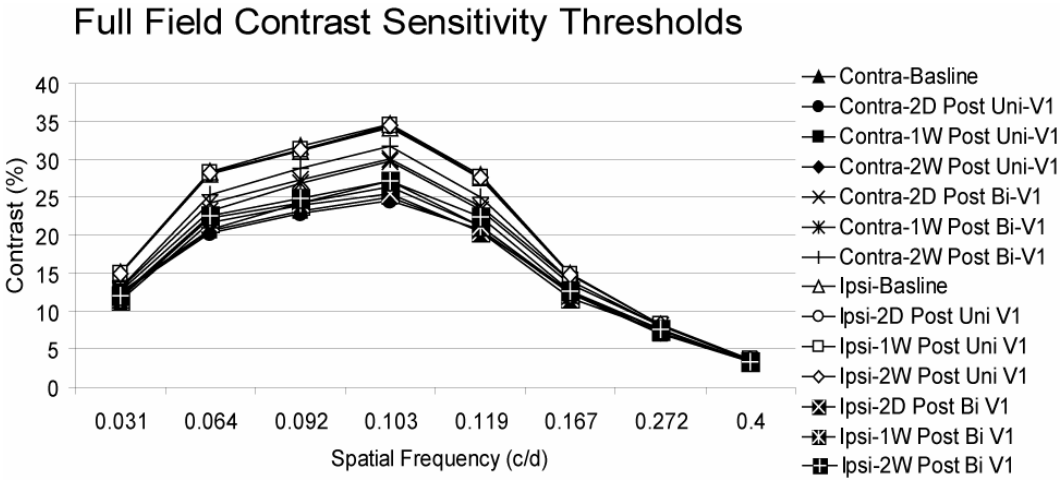



Monocular visual field (145º) spatial frequency thresholds for each eye of animals with tray reaching experience. Contralateral and ipsilateral to unilateral V1 lesion. Asterisks represent significance (*) = $p < 0.05$, (**) = $p < 0.001$, (***) = $p < 0.0001$. Tray reach experience enhanced monocular OKT thresholds significantly by 1%. Overall reduction in OKT thresholds following experience and stroke were not different.

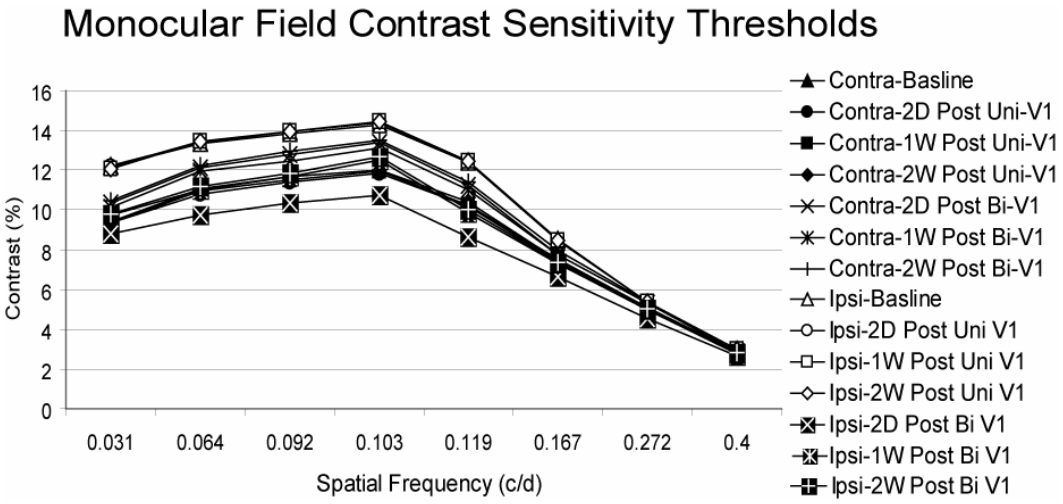
## Appendix B

Contrast Sensitivity Data

**A.**

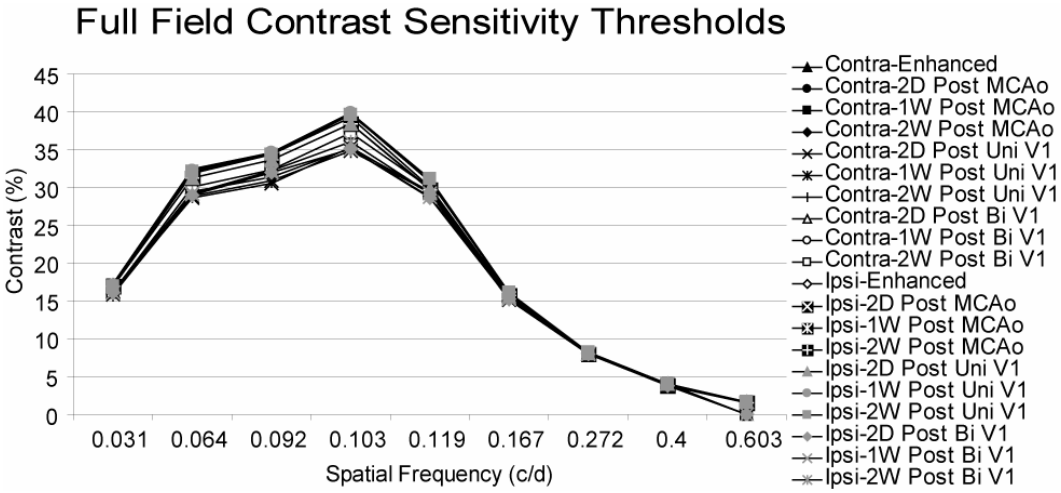



**B.**

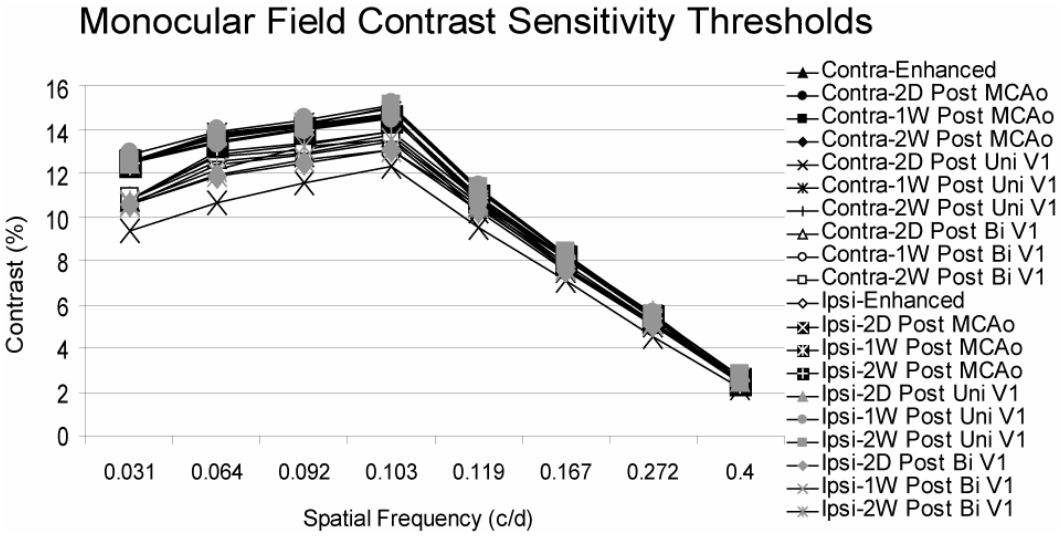



**A.** Full visual field contrast sensitivity curve for normal animals. Note there were slight differences in contrast curve, which did not match SF threshold data (see text for SF thresholds). **B.** Monocular visual field contrast curve.

**A.**

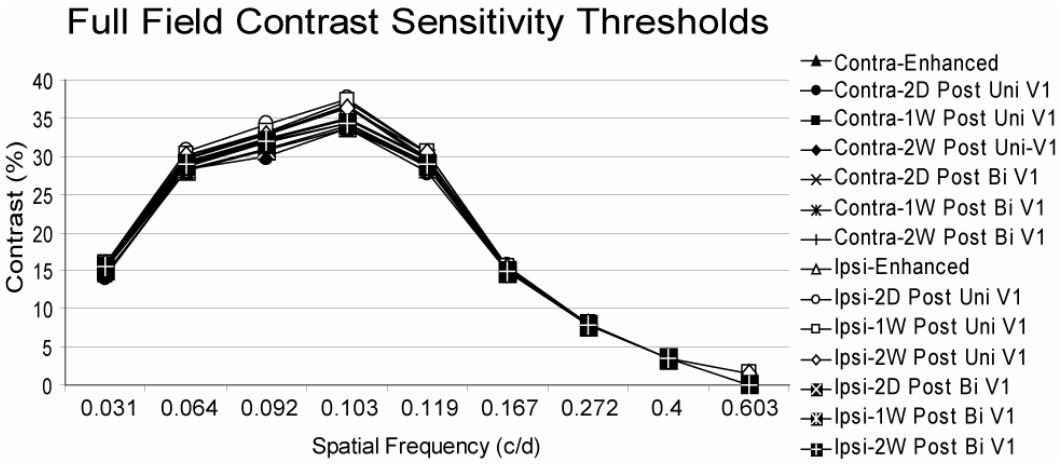



**B.**

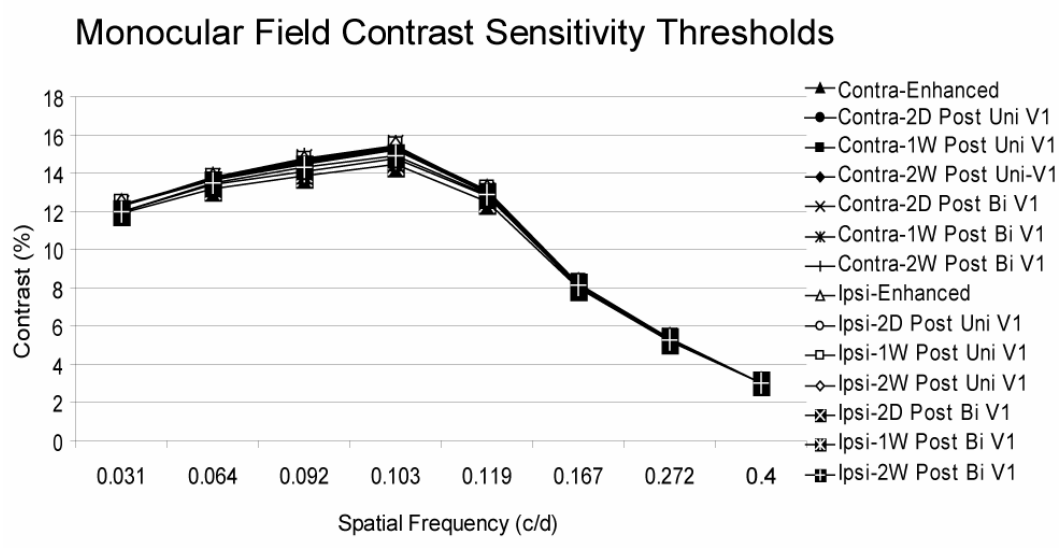



**A.** Full visual field contrast sensitivity curve of animals with developmental visual experience; both MCAo and sequential V1 lesions. **B.** Monocular visual field contrast sensitivity curve.

**A.**

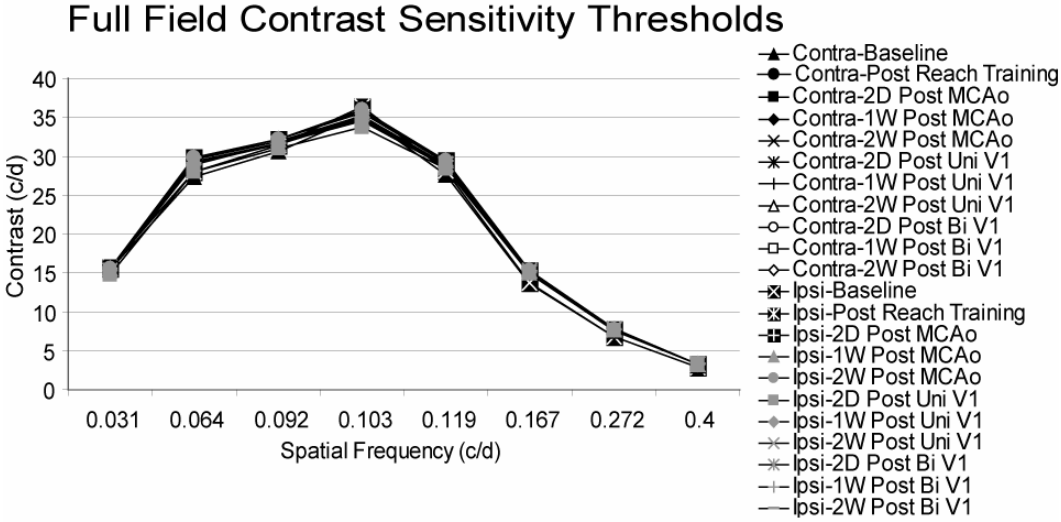



**B.**

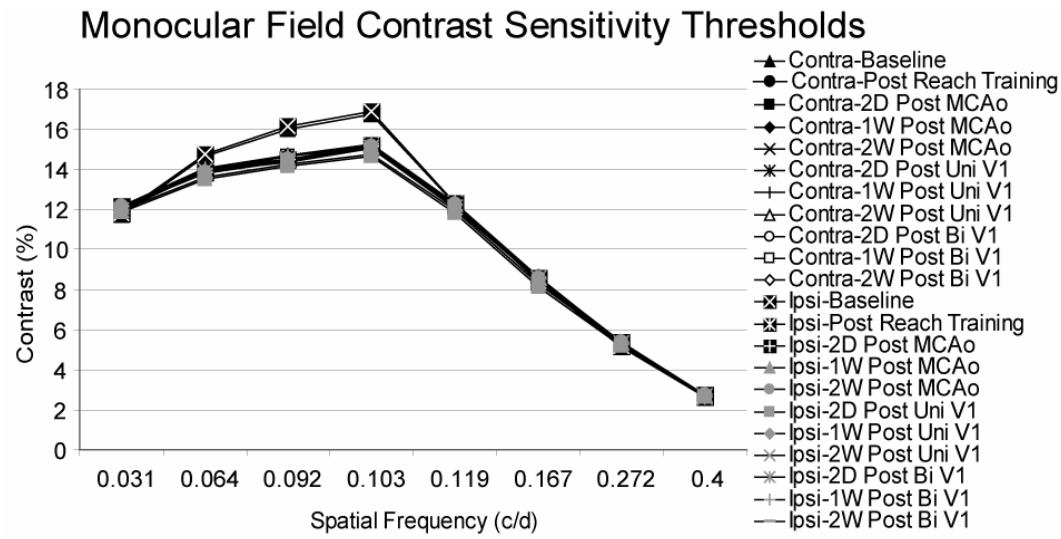



**A.** Full visual field contrast sensitivity curve of animals with developmental visual experience; sequential V1 lesions. **B.** Monocular visual field contrast sensitivity curve.

**A.**

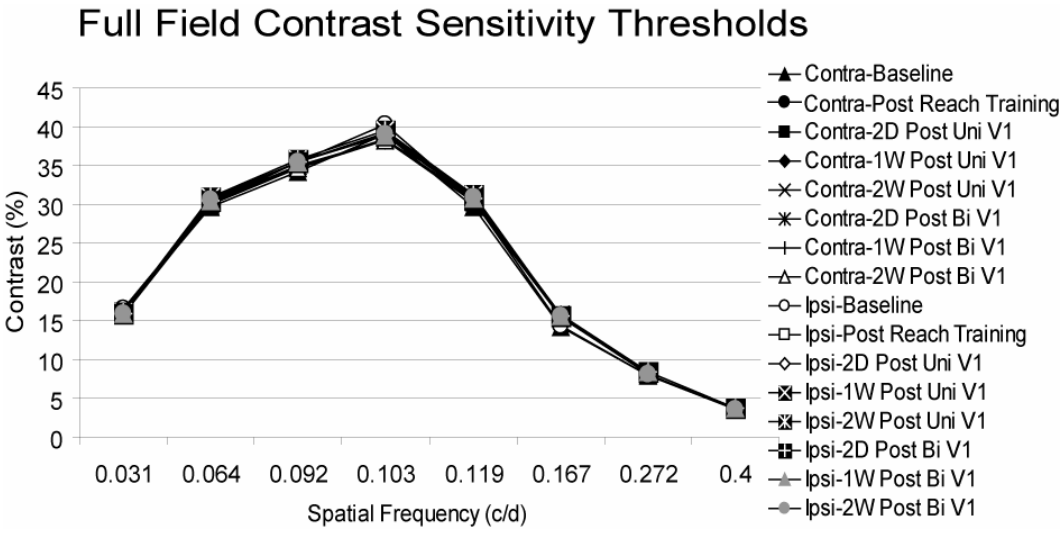



**B.**

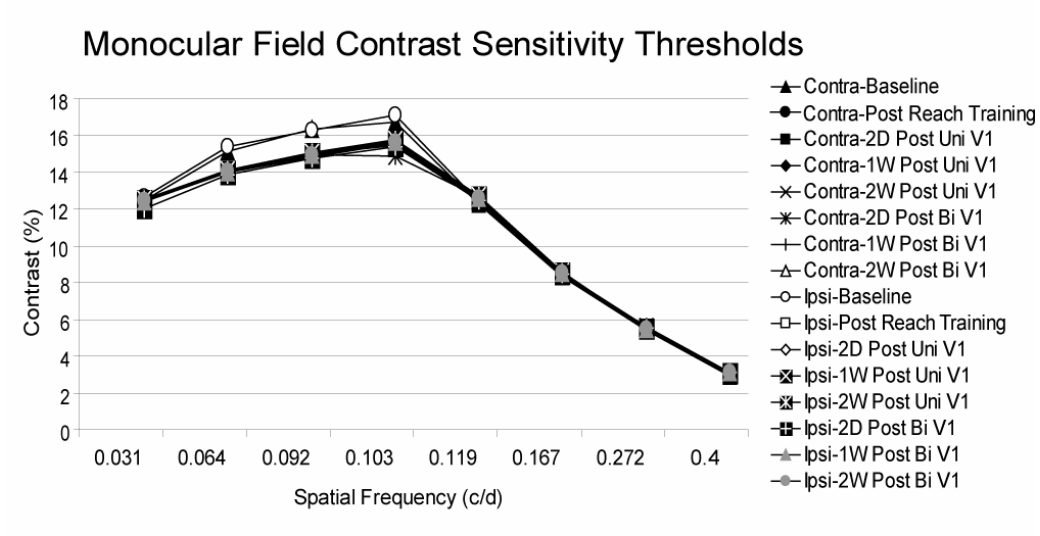



**A.** Full visual field contrast sensitivity curve of animals with adulthood single pellet reach training experience; both MCAo and sequential V1 lesions. **B.** Monocular visual field contrast sensitivity curve.

**A.**

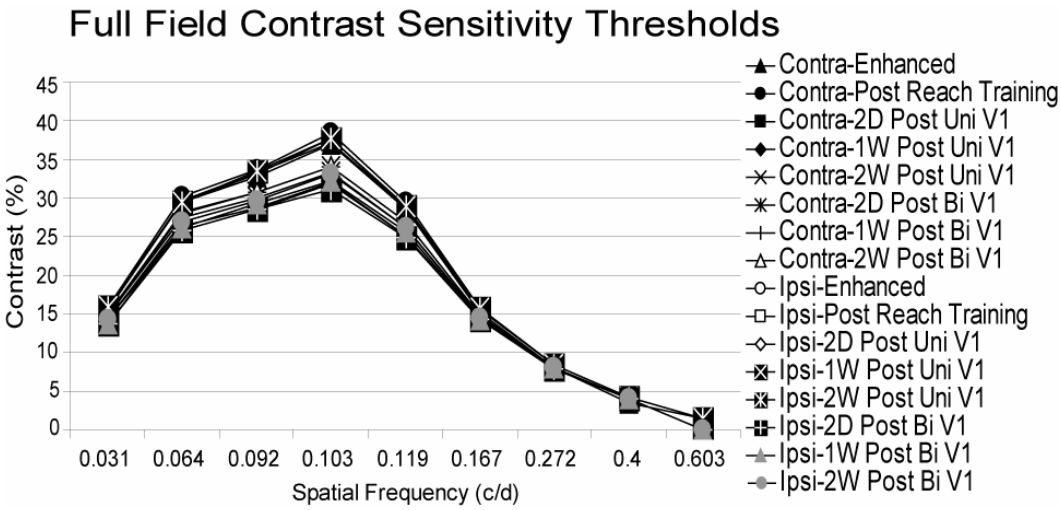



**B.**

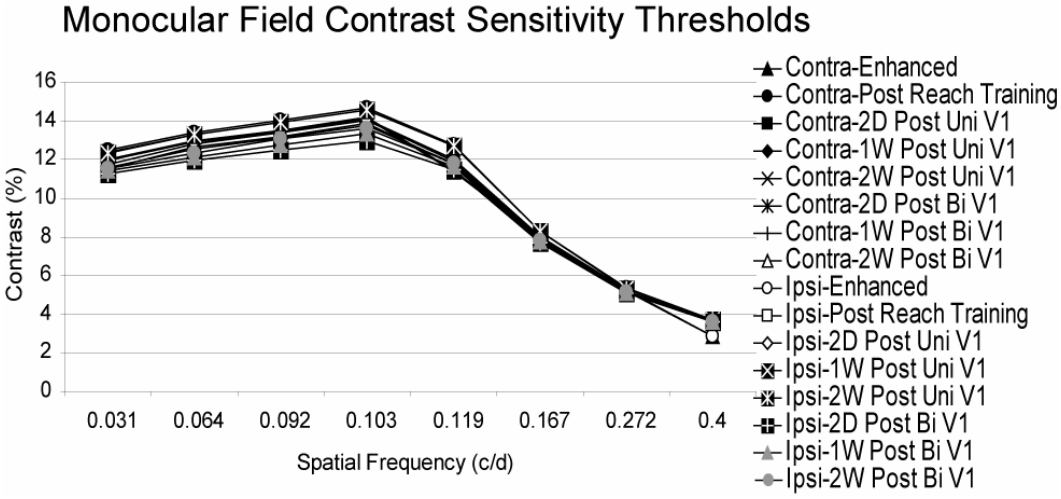



**A.** Full visual field contrast sensitivity curve of animals with adulthood single pellet reach training experience; sequential V1 lesions. **B.** Monocular visual field contrast sensitivity curve.

**A.**




**B.**




**A.** Full visual field contrast sensitivity curve of animals with both developmental visual experience and adulthood single pellet reach training experience; sequential V1 lesions. **B.** Monocular visual field contrast sensitivity curve.