# Latent Impact of Abnormal Visual Experience: Early Abnormal Vision Influences Impact of Subsequent Monocular Deprivation

by

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Dalhousie University is located in Mi'kma'ki, the
ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

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## ABSTRACT

In vision research, monocular deprivation (MD) achieved through eyelid closure is used as a method to replicate the impact of amblyopia in humans and is commonly employed to explore visual system plasticity. Following a period of MD early in development, cats later manifest discernible alterations in the dorsal lateral geniculate nucleus (dLGN) and the primary visual cortex (V1). These alterations are characterized by a reduction of soma size and loss of neurofilament in the layer of the dLGN connected to the deprived eye. Additionally, there is an absence of activity in binocular cells of when the deprived eye is stimulated. Importantly, these changes can only be induced during a critical period occurring early in development, marked by heightened plasticity peaking before the first two postnatal months in cats. This plasticity gradually diminishes into early adulthood, reaching a point where the substantial alterations caused by a period of MD can no longer be induced. The focus of the current investigations explores modulations of the profile of the classical critical period through multiple instances of abnormal visual experiences. This was achieved through investigations of the influence of an early and transient MD or monocular inactivation on the alterations in soma size and reduction of neurofilament in the dLGN resulting from a subsequent period of MD near the end of the critical period. The findings reveal an influence of early monocular vision on the impact of later MD when the opposite eye is initially deprived or inactive. Moreover, there is a subtle influence when the same eye is subjected to both instances of monocular vision. These findings suggest the potential for a latent influence of early monocular vision on the capacity for plasticity later in life.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AMPA | α-amino-3-hydroxy-5-methyl-4isoxazolepropionic acid |
| ANOVA | Analysis of Variance |
| BDNF | Brain-derived Neurotrophic Factor |
| BV | Binocular Vision |
| dLGN | Dorsal Lateral Geniculate Nucleus |
| LTD | Long-term Depression |
| LTP | Long-term Potentiation |
| MD | Monocular Deprivation |
| MI | Monocular Inactivation |
| NMDA | N-methyl-D-aspartate |
| OD | Ocular Dominance |
| ODC | Ocular Dominance Columns |
| ODI | Ocular Dominance Index |
| PBS | Phosphate Buffered Saline |
| RO | Reverse Occlusion |
| SD | Standard Deviation |
| TTX | Tetrodotoxin |
| V1 | Striate Cortex / Primary Visual Cortex |

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# CHAPTER 1:

# INTRODUCTION TO THE EFFECTS OF ABNORMAL VISUAL EXPERIENCE

## 1.1 Introduction

The period of time when most species undergo the majority of cortical development and synaptic organisation is called the critical period, during which developing neural circuitry is most adaptable to the environmental surroundings and conditions of the organism (Hensch, 2005). A phenomenon called plasticity, that refers to the ability of neural networks to change through growth and reorganization, results in modification to the structure and function of cortical cells (Bear et al., 2016). After birth, cats, among other species undergo sensory stimulation from their surrounding environment, a process that, in turn, shapes the development of the cortical regions activated during these experiences (Hubel & Wiesel, 1970). This document will primarily showcase and allude to the intricate aspects of the developing visual system. We review manipulations of visual development, recovery from different types of abnormal vision, sensitive developmental periods, and investigate the interplay of experience and age in the visual system of cats. Through a comprehensive review, our novel findings of this interplay will be introduced, supported by two empirical studies on monocular visual experiences.

The region of interest of our two empirical studies within the visual pathway was the dorsal lateral geniculate nucleus (dLGN), because of the distinctively segregated structure between the two eyes, as illustrated in Figure 1. Upon retinal stimulation by

light, the resulting signals project through the dLGN before progressing to the primary visual cortex (V1), constituting the fundamental route for visual processing (Daw, 2006). In cats, the dLGN exhibits projections to approximately 13 cortical areas associated with vision (Kaufman & Rosenquist, 1985). Notably, V1 serves as the convergence point for signals originating from both eyes, posing challenges in isolating cortical regions representing input from each eye separately (Daw, 2006). Given the segregated nature of the dLGN, our investigative focus centers on this region, rather than the integrated binocular characteristics of V1.

### 1.2 A Brief History of Monocular Deprivation

In the 1960s, Wiesel and Hubel conducted their initial studies exploring the impact of monocular deprivation (MD) on vision in cats (Wiesel & Hubel, 1963a; Wiesel & Hubel, 1963b). To induce MD, they either sutured one eyelid shut during early development or, in a few animals, placed a translucent contact occluder over one eye. This obstruction of patterned visual input to one eye persisted from birth to 8-10 weeks, whereby analysis of single-unit recordings via microelectrodes revealed differences in cells within the dLGN and V1. As measured by single-unit recordings, the overall activity in layers of the dLGN serving the deprived eye seemed less than the non-deprived layers, but was considered relatively normal (Wiesel & Hubel, 1963b). Furthermore, most cells in the dLGN of the MD animals exhibited normal on-center and off-periphery (or vice versa) receptive fields, suggesting no gross physiological change (Wiesel & Hubel, 1963b). As for the striate cortex (V1), nearly every cell they recorded from was driven by the non-deprived eye, while only 1 cell (of 84) was influenced by the deprived eye (Wiesel & Hubel, 1963b). Therefore, though changes in the dLGN were negligible,

propagation of visually evoked activity along the visual pathway resulted in a substantial reduction of activity in V1, reflecting the impact of visual deprivation from the affected eye.

Despite the segregation of visual input between the two eyes in the dorsal lateral geniculate nucleus (dLGN) through layer A and A1 in the dLGN of cats (Figure 1), V1 does not exhibit such strict segregation of the two eyes (Hubel & Wiesel, 1962). In normally reared kittens either eye is able to stimulate the vast majority of binocular cortical cells (Freeman & Olson, 1982). However, a period of MD can cause a shift in cell function where binocular cells favor input from only the non-deprived eye (Wiesel & Hubel 1963a). The degree to which a cell in V1 is stimulated by either eye is referred to as its ocular dominance (OD).

The physiological shift in OD observed in V1 was concomitant with anatomical distinctions in the dLGN. The soma size of neurons in the dLGN connected to the deprived eye were significantly smaller than those connected to the non-deprived eye in cats that had been monocularly deprived, a difference that was not found in cats that had normal binocular vision (BV; Wiesel & Hubel, 1963b). This anatomical effect of MD manifested itself by a roughly 40% decrease in the cross-sectional area of neuron somata within the deprived eye layers. Notably, the anatomical difference in cell area within the dLGN after MD was also partially attributed to hypertrophy of cells in the layers connected to the non-deprived eye. (Hickey et al., 1977). Not all cells in the dLGN were affected by MD as the changes in cell area only occurred for cells within the binocular segment of the dLGN that receive input from the non-deprived eye (Guillery & Stelzner, 1970). This is consistent with the prediction by Hubel and Wiesel (1970) who suggested

that the effects of MD are a consequence of connections from the open eye competing with connections from the closed eye. Furthermore, cats subjected to MD at 2 months of age exhibit a significantly greater reduction in soma size within the deprived eye layers of the dLGN compared to cats that undergo MD as adults (26 weeks of age or more), where no reduction in soma size is observed (Wiesel & Hubel, 1963b). Therefore, with increasing age, animals become less vulnerable to the impact of MD and undergo a decrease in the potential for dynamic changes in the visual system.

### 1.3 The Critical Period Hypothesis

The reduction in both physiological and anatomical effects of MD exhibited by the older cats used by Hubel and Wiesel is reminiscent of the critical period hypothesis proposed by the neurologist and neurosurgeon Wilder Penfield in 1959. The hypothesis claims that there is an ideal or critical period for the development of language in linguistically rich environments, after which, further language acquisition is significantly more difficult and arduous. Years later, evidence for the critical period hypothesis became impossible to ignore and is now widely accepted as a principle throughout the brain during development (Blakemore & Van Sluyters, 1974; Olson and Freeman, 1980). For cats, the physiological effect of MD within V1 peaks between 28 and 38 days which declines in magnitude from 48 to 109 days where only a marginal effect can be observed (Olson & Freeman, 1980; Jones, Spear, & Tong, 1984). The critical period in general varies between cats, rats, monkeys, and mice. Mice have the shortest and earliest critical periods, roughly 18 to 35 days of age (Gordon & Stryker, 1996), rats have the second shortest and earliest at roughly 3 to 7 weeks of age (Fagiolini et al.,1994; Guire, Lickey, & Gordon, 1999), and monkeys have the longest and latest critical period of any animal

model which lasts from birth (because they are born with theirs eyes open) to roughly 1 year of age (Daw, 2006). Research involving unilateral cataract suggests that the critical period in humans lasts from 6 weeks to nearly 10 years of age (Vaegan & Taylor, 1979; Birch et al., 1998).

The critical period of visual system plasticity varies across species in terms of duration, onset, and its conclusion. However, commonalities exist; notably, there is a peak of plasticity that gradually decreases until the critical period concludes for different species and regions of the brain. This peak signifies the period of highest plasticity, wherein MD yields the most significant effect. Therefore, between-species results can be roughly generalized. Aside from species, the duration of deprivation is another variable that influences the size of an effect. Single-unit recording of V1 in cats reveal that a short 1-day MD has a very small effect, a 2.5-to-3.5-day MD has a large effect, and a 6-to-10-day MD causes an effect that results in nearly complete domination of cortical circuitry by the non-deprived eye (Hubel & Wiesel, 1970; Olson & Freeman, 1975). A phenomenon, that is also present in higher mammals such as monkeys, wherein long lengths of MD have produced significantly larger consequences on the visual pathway (LeVay et al., 1980).

Beyond the influence of deprivation duration on the magnitude and extent of effects from MD, is another layer of complexity. Higher-level brain regions, such as the cortex, have later and usually longer critical periods compared to lower-level more primitive structures. For instance, V1 exhibits a more substantial physiological impact following MD than the dLGN, and can occur at slightly later ages (Wiesel & Hubel, 1963a). Whereas, retinal ganglion cells from kittens that experienced a period of MD

throughout the critical period, from 8 days after birth until one or more years old, show no functional abnormalities (Sherman & Stone, 1973; Cleland et al., 1980). Additionally, the effect size may vary depending on the measured dependent variable. For example, single-cell recordings within the dLGN show a slight effect from MD, while the anatomical effect on soma size may be twice as large (Wiesel & Hubel, 1963a; Wiesel & Hubel, 1963b). Furthermore, single-cell measurements of the effect in V1 are more significant and better represent the larger effect seen in anatomical measurements of the LGN (Shapley & So, 1980).

Taken together, these studies show that a multitude of variables contribute to the intricate development of the visual system, and the early decades of research on the critical period have revealed a few common properties. First, the age of a developing organism plays a crucial role, as developmental plasticity is drastically reduced following the critical period (Olson & Freeman, 1980). Second, the nature of its experience or timing of the manipulation used to evoke plasticity influences the outcome; longer periods of MD have a more significant impact on the visual system than shorter periods (Hubel & Wiesel, 1970; Olson & Freeman, 1975). Third, the type of manipulation used to induce plasticity matters; while MD has a substantial effect throughout the visual system, other forms of visual deprivation, such as enucleation, have a larger impact later in development (Cragg et al., 1976; Kratz & Spear, 1976). Last, though not exhaustive by any means, there is an interaction between age and experience; the interplay among all the contributing variables of the critical period means that a brief MD at the peak of the critical period could elicit a similar impact as a prolonged MD near the end of the critical period.

### 1.4 Other Plasticity-Inducing Manipulations to The Visual System

MD has served as the primary example of plasticity-inducing manipulation in classic visual system research because of its comparability across species and its ability to unveil the extent of plasticity capacity during postnatal development. However, there exists a similar manipulation that utilizes different properties and techniques capable of eliciting a shift in ocular dominance similar to MD when administered for a duration of more than 2 days (Rittenhouse et al., 1999). Monocular inactivation (MI) is achieved via microinjections of tetrodotoxin (TTX) into the vitreous humor of one eye. When applied, this technique produces an effect similar to MD primarily driven through potentiation of the non-deprived eye connections (Chapman et al., 1986; Frenkel & Bear, 2004).

TTX is a potent neurotoxin that inhibits neural activity through selective sodium channel blockade (Narahashi et al., 1964). Around the same time that Hubel and Wiesel conducted their first MD experiments, TTX was being investigated as a potential research tool to block the conduction of nerves and muscles. Using lobster axons, observations of the conductance were measured and compared with or without injections of TTX. It was found that the sodium increase that occurs during an action potential was absent upon application of TTX and therefore no neuronal activity was recorded (Narashashi et al., 1964). Since this discovery, numerous researchers have used TTX to selectively inhibit sodium channels, while leaving potassium channels unaffected. This has facilitated the investigation of a diverse range of topics, spanning from the mechanisms of visual system development to potential treatments for pain relief associated with various diseases or disorders. (Stryker & Harris, 1986; Goldlust et al., 2021).

The use of TTX in visual system research has aided in the identification of potential mechanisms underlying visual system plasticity, similar to the observations seen after a period of MD. An early and prominent hypothesis considered patterned electrical activity as the primary influence on the shift in neuronal connections within the cortex (Blakemore, 1976). This hypothesis posited that the development and arrangement of regions throughout the visual pathway are strongly influenced by the patterned electrical activity that occurs during development. The underlying theory suggests that if the activity patterns elicited through ganglion cells from the retina are sufficiently similar, binocularity can be sustained. Conversely, if both retinae are not stimulated simultaneously or if one retina generates a weaker response than the other, binocularity is diminished. Consequently, in the absence of patterned activity from the retinae, a downstream effect ensues from this imbalance, cascading through the cortex and causing cells within V1 to favor one eye over the other. This theory is reminiscent of the suggestion of Hubel and Wiesel that the effects of MD are a consequence of competitive interactions between both eyes (Hubel & Wiesel, 1970).

Results from anatomical investigations on the dLGN indicate that the changes in geniculate cell area after a period of MD can only occur when there is competition between input from the two eyes (Guillery, 1972; Guillery 1973), and is considered to be a retrograde interaction from V1 (Bear & Colman, 1990). Furthermore, the shift in ocular dominance within the cortex and anatomical changes within the dLGN after a period of MI can occur solely from an imbalance of retinal activity (Chapman et al., 1986). However, an investigation into whether asymmetries in activity alone could induce the effects of MD/MI, subjected kittens to injections of TTX in one eye while at the same

time the other eye was sutured shut revealed no discernible shift in ocular dominance, indicating a potential threshold for activity-dependent changes in ocular dominance (Greuel et al., 1987). Humans that experienced an imbalance in patterned activity from each eye as a result of cataracts, strabismus, or unequal refractive power can develop a visual impairment called amblyopia. The conventional treatment, referred to as patching, involves covering the dominant eye with a patch and leveraging activity-dependent plasticity—the mechanism that initially led to the impairment—as a means to recover connections of the deprived eye (Wallace, 2007).

In vision research, patching is simulated through a process known as reverse occlusion (RO). RO involves removing the lid suture from the previously deprived eye and suturing the fellow (non-deprived) eye, which induces greater recovery of visual acuity and a more pronounced correction in ocular dominance compared to a straightforward period of binocular vision (Mitchell et al., 1977). Chronic single-site recordings reveal deprivation of the newly deprived eye during a period of RO, succeeded by a slightly slower recovery of the formerly deprived eye (Mioche & Singer, 1989). Similar to MD, older cats exhibit limited recovery outcomes, a phenomenon that is attributed to the attenuated capacity for plasticity associated with the classic critical period (Olson & Freeman, 1980). Various studies of critical periods from cats and monkeys imply, without explicitly reporting, that the critical period of RO is shorter in duration compared to MD (Olson and Freeman, 1980; LeVay, Wiesel, & Hubel, 1980; Blakemore and Van Sluyters, 1974). Additionally, findings from a study that systematically investigated whether MI could be used as a potential treatment for the effects of a long-term MD indicate that MI of the fellow eye leads to greater recovery

compared to binocular vision, RO, or binocular inactivation (Duffy et al., 2018). Moreover, the impact of MI and MD in normal animals were compared in order to investigate differences between the hypertrophy and atrophy of soma size in the non-deprived and deprived layers of the dLGN. First, it was found that MI produced a significantly larger effect than MD throughout the entire critical period by about a factor of two, and that MI produced an effect within the dLGN past the traditional critical period (Duffy et al., 2023). Furthermore, MI produced a change in soma size within both the binocular and monocular regions of the dLGN, whereas MD only affects the binocular region of the dLGN, suggesting that the effects of MI derive from a loss of activity from the retinae as well as from imbalanced competition within V1 (Duffy et al., 2023).

These investigations, have revealed that MI for more than 2 days yields comparable effects to MD, including a shift in ocular dominance and difference in soma area of the dLGN. However, MI seems to rely upon different mechanisms than MD and can elicit a more substantial impact that operates outside the confines of the same critical period as MD. Moreover, the heightened influence of MI appears to correlate with increased recovery when employed within a RO-type paradigm, suggestive of an influence or interaction between the two interventions.

### 1.5 Interactions of Multiple Manipulations

As stated in the sections above, MD causes many effects throughout the visual pathway. These include a reduction of visual acuity in the deprived eye (Mitchell, 1988; Murphy & Mitchell 1991), characterised by loss of soma size in the dLNG and imbalance of visually evoked potentials in V1 which leads to a loss of binocularity (Dews and

Wiesel, 1970; Giffin and Mitchell, 1978; Timney, 1983; Vorobyov et al., 2007). Furthermore, these effects are constrained to a critical period outside of which these interventions do not produce much if any effects (Olson & Freeman, 1980). Therefore, it is understood that the mammalian brain is most plastic, or most susceptible to developmental change, only for a certain period of early life. However, an early transient MD imposed on mice pups can impact neural connections within the visual cortex and the way they respond to future environmental manipulation well after the MD is relieved and the mice recover from the effects (Hofer et al., 2009).

A latent effect of an early and transient period of experience influencing future development is also found from engaging in repeated sensorimotor tasks, or encountering foreign languages early in development which has been found to enhance the edification of similar tasks or information later in life (Mcgonigle & Flook, 1978; Kuhl, 2004). This phenomenon was evident in the visual system of the mice pups mentioned above; after a brief 5-day period of MD early in the critical period produced a shift in cortical ocular dominance of the mice with recorded cells showing reduced responses to stimulation of the deprived eye, though reintroducing binocular vision eventually restored a normal response. Imposing a second MD to the same eye in adulthood led to a quicker and more robust shift in ocular dominance compared to mice without the prior MD early in the critical period (Hofer et al. 2006). Thus, despite the animals being the same age and ostensibly in the same stage of the critical period, the experience of an early period of MD that had fully recovered amplified the effects of a second MD applied to the same eye later in life.

In a parallel manner, but with a result in the opposite direction to that of the study mentioned above, cats exposed to an early period of ocular misalignment (strabismus) demonstrated a diminished impact of a period of MD later in life compared to cats reared with normal vision until the same period of MD imposed at the same age (Mustari and Cynader, 1981; Faulkner et al., 2005). The attenuation of MD effects observed in these animals suggests that, like the facilitation of the effect from a subsequent same eye MD, early visual experiences influence the adaption and development resulting from future manipulation of visual experience. These results suggest the potential of an interaction between early and subsequent periods of monocular visual experiences that impact plasticity in a manner that results in a more rapid and robust or diminished MD effect compared to age-matched animals reared with normal visual experience. This theoretical framework provides an explanation for the results mentioned above indicating that the critical period for the effects of RO in monkeys and cats ended earlier than the critical period for the effects of MD; the initial MD appears to influence the impact of the subsequent reversed MD (Olson and Freeman, 1980; LeVay et al., 1980; Blakemore and Van Sluyters, 1974).

Studies involving juvenile barn owls exposed to repeated associations between auditory cues and vision through the use of prismatic spectacles have also revealed a latent and enduring effect of early abnormal visual experiences (Linkenhoker et al., 2005). Experiencing early abnormal visual input via prismatic spectacles led to the development of aberrant axonal projections to the external nucleus of the inferior colliculus (Linkenhoker et al., 2005). Remarkably, these connections endured even after removal of the prisms, and when exposed to the same sensory conditions in adulthood the

owls exhibit a readaptation to the association between auditory cues and abnormal visual experiences (Knudsen, 1998). Notably, this adaptation does not occur in adult owls exposed to identical sensory conditions without the prior history of such exposure (Brainard and Knudsen, 1998).

A principle that encompasses these interactions is metaplasticity, a form of synaptic plasticity that accounts for changes in the ability to induce synaptic plasticity throughout life (Abraham & Bear, 1996). Metaplasticity refers to the ability to change the capacity for plasticity within a synapse given the history of activation. Again, the exact mechanisms of metaplasticity are not fully known but alteration of post-synaptic receptor subunits after plasticity-evoking events seem to be highly involved in modulating the ability to induce synaptic plasticity (Cho et al., 2009). Furthermore, there are many factors (experiences) that can contribute to the overwhelming range of influence and interactions of metaplasticity (Kolb et al., 2017), therefore accounting for the lack of precise empirical investigation.

Results from studies investigating an early abnormal visual experience that is repeated in adulthood in mice, owls, and cats have provided a theoretical framework that underscores an aspect of developmental plasticity within the critical period, namely an interaction of plasticity-inducing experiences. The commonly held belief is that the critical period opens and closes at a specific age, and the potential for plasticity is presumed to exhibit a similar trajectory in relation to age. However, if the outcome resulting from the interplay of early and late experiences during the critical period modulate the capacity for plasticity, then predicting plasticity becomes contingent on both the age of the animal and its experiential history. A hypothesis of considerable

importance for therapeutic interventions that relies on a higher capacity of plasticity for effective treatment outcomes, such as patching to treat amblyopia (Nelson, 2009), that constitutes the focus of inquiry for the experiments detailed in chapters 2 and 3 of this document.

### 1.6 Introduction to Investigation of An Interaction Effects

Exploration of the impact of early abnormal visual experience will be addressed by the results from two experiments that follow the same design. The first experiment (Chapter 2) will present anatomical results from cats subjected to early MD at the peak of the critical period followed by a period of binocular vision before undergoing a later period of MD at the end of the critical period. Likewise, in the second experiment (Chapter 3), findings will be presented from cats that underwent early MI at the peak of the critical period followed by a period of binocular vision before being subject to MD at the end of the critical period. In both experiments, the probe MDs at the end of the critical period and the initial manipulations of visual experience took place at the same ages for each animal. The initial manipulation was conducted on either the same or opposite eye as the probe MD. The effects from the probe MD from all these animals will be compared to results from animals from a control condition that were raised with normal vision until the end of the critical period at which point, they received the probe MD. This comparison willow assessment of the influence of earlier manipulations.

For decades, the cat visual system has served as a foundation for exploring the repercussions of abnormal visual experiences and the critical period of plasticity during the development of the visual pathway (Wiesel and Hubel, 1963a; 1963b; Giffin and Mitchell, 1978; Rittenhouse et al., 1999). The following chapters, describe experiments

that examine the ability of a period of abnormal visual experiences to influence neural adaptation in the visual system of cats to a later period of deprivation. Measurements from two anatomical hallmarks of deprivation, the reduction of soma size and loss of neurofilament protein within the deprived-eye layers of the LGN (Wiesel and Hubel, 1963; Guillery and Stelzner, 1970; Bickford et al., 1998; Kutcher and Duffy, 2007; Duffy et al., 2018), will be presented as a means to assess the impact of a probe period of MD. These indicators of visual system plasticity will serve as dependent variables in testing the hypothesis that early monocular visual experience influences neural adaptation to future manipulations of visual experience later in the critical period.

# CHAPTER 2:

# EFFECTS OF MONOCULAR DEPRIVATION FOLLOWING PRECURSORY MONOCULAR DEPRIVATION

## 2.1 Effect of Prior Monocular Deprivation

Disruption of normal binocular vision early in life produces neural changes that cascade throughout the primary visual pathway, and that can impair vision for a lifetime. Amblyopia is a vision disorder that is characterised by poor or blunted sight usually from one eye and, as mentioned in chapter 1, is most commonly treated by patching the dominant eye. This is an ancient treatment that was suggested by Buffon in 1743 (Daw, 2006). Roughly 200 years later, it is understood that amblyopia is the result of a developmental defect that obstructs normal vision, such as a cataract, strabismus, or anisometropia (Von Noorden, 1990). Removal of the obstruction along with patching of the fellow (opposite eye) has been found to be effective in reversing the effects from the amblyogenic cause, provided it is implemented early in life (Loudon et al., 2002). As suggested by the critical period hypothesis, patching was found to be successful only in young children and was not an effective treatment for older children (Von Noorden, 1990). Furthermore, children with amblyopia who have undergone ineffective patching treatment demonstrate less recovery with a second round of patching compared to children of a similar age that have not undergone an initial intervention (Scheiman et al., 2005; Scheiman et al., 2008; Holmes et al., 2011).

If the original amblyogenic event is akin to a period of MD, and patching treatment is akin to RO, the poor recovery of children with a prior history of patching

echoes the findings from animals that the critical period for RO is shorter than the critical period for MD (Olson and Freeman, 1980; LeVay, Wiesel, & Hubel, 1980; Blakemore and Van Sluyters, 1974). Both sets of results support the hypothesis that early abnormal visual experience influences the impact from another later period of abnormal visual experience. In Chapter 1 it was mentioned that sequential periods of monocular deprivation to the same eye in mice produced a *prior MD effect* that facilitated the adaptation of the period of MD after the critical period (Hofer et al., 2006). It is important to note that these mice recovered from the effects of the initial MD before the second MD was administered in adulthood. This implies a latent effect of the first period of MD on the extent to which the subsequent MD influenced the visual system. Furthermore, in cats that were rendered strabismic by myotomy early in the critical period, an attenuated effect of MD near the end of the critical period was observed when compared to controls of the same age (Mustari and Cynader, 1981; Faulkner et al., 2005). The juxtaposition of these two findings, a facilitation and an attenuation of MD effects following prior abnormal visual experience, suggests the possibility that prior experience can bidirectionally influence the capacity for developmental plasticity.

The marked reduction of soma size in deprived-eye recipient layers of the dLGN, are concomitant with a loss of the cytoskeletal protein neurofilament in the same layers of dLGN (Bickford et al., 1998; Duffy & Livingstone, 2005). Neurofilaments are a class of structural proteins found in the cytoplasm of neurons which provide structural support and play a crucial role in maintenance of cell integrity (Morris & Lasek, 1982; Hoffman et al., 1987). Rigid stability is acquired by neurofilament through phosphorylation which subsequently diminishes vulnerability to fragmentation by proteolysis (Pant, 1988). This

process occurs during later stages of the critical period and extends the stability into adulthood when there is negligible plasticity potential (Liu et al., 1994; Song et al., 2015). As stable scaffolding of neurons, neurofilament is thought to limit plasticity and favor the conservation of cytoskeletal organisations within the cells (Morris & Lasek, 1982). Neurofilament is mostly found in projection cells rather than inhibitory interneurons (Duffy et al., 2012). Therefore, the concurrent decrease in neurofilament and soma size following a period of MD indicates diminished stability in the cells responsible for the propagation of input from the retina to V1.

As an attempt to create similar sensory history to the cats that experienced MD after being rendered strabismic, and the mice that experienced two period of MD to the same eye, the current investigation uses multiple bouts of MD in cats to reveal any influence of prior monocular visual experience on plasticity potential. In this experiment, the expectation was that a precursory period of MD would influence the reduction of soma size and loss of neurofilament produced by a subsequent period of MD later in the critical period. Specifically, we predicted that a precursory period of MD to the same eye would facilitate the effects of the probe MD while a precursory MD to the opposite eye would attenuate the effects of the probe MD, thereby demonstrating a bidirectional influence contingent on which eye undergoes the preceding monocular vision.

## 2.2 Methods

The rearing histories of the animals described below are detailed in Table 1 and the rearing manipulations for each group in the experiment are depicted in Figure 2A.

### 2.21 Animals

Anatomical studies were conducted on 19 cats born and raised in a closed breeding colony at Dalhousie University. 12 of the cats used in this experiment were part of previous studies (Kutcher and Duffy, 2007; O'Leary et al, 2012), and their tissue was reanalyzed with those reared for the current study. All rearing and experimental procedures were conducted in accordance with protocols that were approved by the University Committee on Laboratory Animals at Dalhousie, and that conformed to guidelines from the Canadian Council on Animal Care. The current study investigated the effect of 10 days of right-eye MD imposed at 8 weeks of age (Probe MD) compared between 3 groups. The first group received a left eye MD for 7 days at postnatal day 30 before 3 weeks of binocular recovery and administration of the right eye probe MD (Incongruent MD; n = 3). The second group received a right eye MD for 7 days at postnatal day 30 before 3 weeks of binocular recovery and administration of the right eye probe MD (Congruent MD; n = 3). The third group only received the right eye probe MD after 8 weeks of normal binocular vision (Control MD; n = 4). To ensure recovery of the Incongurent and Congruent MD groups after the precursory MD, 3 recovery controls were also examined. The recovery control groups consisted of a group that only received a 7-day MD at postnatal day 30 (MD; n = 4), a group that received a 7-day MD at postnatal day 30 followed by a week of binocular vision (MD + BV; n = 2), and a normal group at postnatal day 30 (Normal; n = 2). Data from the Congruent MD and Control MD groups have been used in a previous study (Henneberry et al., 2023), but were reanalysed for the current investigation.

### 2.22 Monocular Deprivation

The periods of monocular deprivation were administered while the cats were under general gaseous anesthesia (3-4% isoflurane in oxygen) and involved suture of the upper and lower palpebral conjunctivae with sterile 5-0 vicryl followed by closure of the eyelids with 5-0 silk sutures. After the sutures were administered, animals were given oral Metacam (meloxicam; 0.05 mg / kg) for postprocedural analgesia, a local anesthesia of Alcaine sterile ophthalmic solution (1% proparacaine hydrochloride; CDMV, Canada), and broad-spectrum topical antibiotic (1% chloromycetin; CDMV) to prevent infection. The eyes were monitored daily to mitigate risk of infection and ensure the eye was fully closed. Animals in the Congruent and Incongruent MD groups had their deprived eye open under gaseous anesthesia (3-4% isoflurane in oxygen) by removing the sutures. After the sutures were removed, animals were given Metacam and a broad-spectrum topical antibiotic once again.

**2.23 Tissue Preparation**

Animals were euthanized by administration of a lethal dose of sodium pentobarbital (Pentobarbital Sodium; 150 mg/kg) and were immediately exsanguinated by transcardial perfusion with 150 ml of phosphate buffered saline (PBS) followed by 4% paraformaldehyde dissolved in PBS. The brain was extracted from the skull and the thalamus was dissected to prepare tissue samples for sectioning and histological processing of the LGN. The dissected thalamus was cryoprotected and manually cut coronally into 25- μm thick sections using a sliding microtome. The sections that were used for neurofilament labeling were cut at a thickness of 50- μm. The tissue slices were then immersed in an antigen preservative solution (Burke et al., 2009) until being used

for the study. Extra tissue from these animals were cryoprotected and stored for use in other studies.

**2.24 Nissl**

Sections containing the left and right LGN were mounted onto glass slides and stained with a 1% Nissl solution (ab246817; Abcam, USA) before being differentiated in 70% ethanol and dehydrated in a graded series of ethanol and cleared with Histo-Clear (National Diagnostics, Atlanta, GA). The slides were then coverslipped with Permount mounting medium (Fisher Scientific, Waltham, MA) and left to dry for roughly 2-4 weeks before microscopic evaluation.

**2.25 Neurofilament**

Sections containing the left and right LGN were placed in a PBS solution containing mouse monoclonal antibody (1:1000) targeted against neurofilament protein-H (SMI-32, RRID:AB_509998; Biolegend, San Diego, CA) and left free-floating for 12 hours. Afterward, the sections were washed 3 times with PBS before being immersed in biotinylated goat anti-mouse antibody (1:500) (115-065-003; Jackson ImmunoResearch, West Grove, PA) for 1 hour. After being washed a second time, sections were immersed in a PBS solution containing avidin and peroxidase-conjugated biotin (PK6100; Vector Laboratories, Burlingame, CA) for 1 hour. Immunolabeling was performed by immersing these sections to a PBS solution containing hydrogen peroxide (1:1000) and the chromogen 3,3'-diaminobenzidine (0.5mg / ml) until sections became tan, then were washed with PBS and mounted onto glass slides. Once sections were dry on the slides, they were dehydrated using a graded series of ethanol and cleared with Histo-Clear. The

slides were then coverslipped with Permount mounting medium and left to dry for roughly 2-4 weeks before microscopic evaluation.

**2.26 Quantification**

Quantification of soma size and counts of neurofilament-positive cell density were performed blind to the rearing condition of each animal. Measurements were made from sections that spanned coronal planes 6-7 (Sanderson, 1971), roughly midway along the anterior-posterior axis of the LGN. The cross-sectional area of neuron somata within the A and A1 layers of the left and right LGN were measured from at least 3 Nissl-stained sections per animal using the nucleator probe from a computerized stereology system (newCAST; VisioPharm, Denmark). All area measurements were performed using a BX-51 compound microscope via 4X objective and 60X oil-immersed objective (Olympus; Markham, Ottawa, Canada). Criteria for neurons consisted of cells with dark cytoplasmic and nucleolar staining, and those with light nuclear staining (Duffy et al., 2012) and were distinguished from glial cells using established criteria (Wiesel and Hubel, 1963a; Guillery and Stelzner, 1970). Adherence to these criteria allowed avoidance of cell caps and ensured that each measurement was taken from neurons cut through an approximate somal midline. Roughly 2000-4000 neurons were measured from each animal.

Quantification of neurofilament-positive cell density were performed on sections labeled for neurofilament and imaged via an Olympus VS200 slide scanner using a 20X objective. Density was calculated for each layer by dividing cell counts, taken using bioimaging analysis software, QuPath (Bankhead et al., 2017), by the area of each region of interest specified within the A and A1 layers of the LGN. Criteria of neurofilament-positive neurons consisted of distinct cytoplasmic labelling with weak or absent labeling

in the nucleus. Adherence to these criteria ensured that neurons positive for neurofilament and not labeling of cell caps were counted towards the density.

### 2.27 Statistical Analysis

The effect of the probe MD was assessed using an ocular dominance index (ODI) used in similar investigation (Duffy et al., 2023). The ODI revealed the percentage difference between effects to the eye-specific dLGN layers for each animal separately, and was calculated with measurements from each layer as follows:

$$ODI = (((\text{Non-Deprived Layer A} + \text{Non-Deprived Layer A1}) - (\text{Deprived Layer A} + \text{Deprived Layer A1})) / (\text{Non-Deprived Layer A} + \text{Non-Deprived Layer A1})) * 100$$

Since the ODI is a within-animal metric it enabled assessment of the effects of MD for each individual animal by employing the non-deprived eye as a reference. Analysis of such a metric helps mitigate the effect of having low number of animals per group by reducing the chance of Type I statistical error. To investigate whether there was cause for further analysis of each group a Kruskal-Wallis one-way analysis of variance was performed on the experimental groups. Statistical comparisons between ODI from each animal were achieved using one-tailed Mann-Whitney tests in the direction of the predictions made above. Significance for all statistical tests was set at 0.05.

### 2.3 Results

To ensure appropriate comparisons between the incongruent MD, congruent MD, and control MD (experimental) groups, it is essential for the effects of the MD + BV group to recuperate to a balanced ODI that is not significantly different from the Normal

group. Without recovery from the initial MD the results from the experimental groups can not be meaningfully interpreted because of carry-over effects from the first episode of deprivation.

### 2.31 Recovery from Early and Brief MD

*Nissl:*

The normal group of animals at postnatal day 30 (P30) produced an average ODI of 0.3% (SD = 4.302; 95% CI [-10.34, 11.03]), meaning that the cross-sectional soma size of neurons connected to the left and right eye was about the same. Following 7 days of MD at P30, the soma area of neurons within the deprived-eye recipient layers of the LGN was reduced compared to the non-deprived layers of the LGN. Quantification of soma area for this MD group revealed that deprived eye neurons (mean = 134$\mu m^2$; SD = 15$\mu m^2$) were smaller than non-deprived eye neurons (mean = 169$\mu m^2$; SD = 18$\mu m^2$) which resulted in an average ODI of 19% (SD = 3.208; 95% CI [13.94, 24.15]). Quantification of soma area following 8 days of BV after 7 days of MD at P30 in the MD + BV group revealed that neurons within previously deprived eye layers (mean = 168$\mu m^2$; SD = 10$\mu m^2$) were comparable in size to neurons in the non-deprived eye layers (mean = 167$\mu m^2$; SD = 7$\mu m^2$) which produced an average ODI of -0.8% (SD = 5.128; 95% CI [-46.89, 45.26]). An unpaired t-test between the normal and MD groups revealed a significant difference ($t(5) = 6.646$, $p = 0.001$); however, no difference was found between the normal and MD + BV groups ($t(3) = 0.2765$, $p = 0.8001$), meaning an MD at P30 produced a significant effect compared to normal controls but 8 days of BV was sufficient to recover soma area to normal.

*Neurofilament:*

Similar to quantification of soma area, from the same animals presented above, neurofilament labeling of normal animals produced an ODI of 0.6% (SD = 4.615, 95% CI [-40.87, 42.05]). Quantification of neurofilament-positive cell density of the animals in the MD group revealed an average ODI of 48% (SD = 5.238; 95% CI [39.18, 55.85]) with a reduction in neurofilament-positive neurons in deprived eye layers (mean = 44 neurons/$\mu m^2$; SD = 13 neurons/$\mu m^2$) compared to the non-deprived eye layers (mean = 90 neurons/$\mu m^2$; SD = 11 neurons/$\mu m^2$) of the LGN. Quantification of neurofilament-positive cell density of the animals in the MD + BV group revealed an average ODI of -0.4% (SD = 2.5; 95% CI [-22.88, 22.05]) with a similar count of neurofilament-positive neurons in previously deprived eye layers (mean = 87 neurons/$\mu m^2$; SD = 7 neurons/$\mu m^2$) and non-deprived eye layers (mean = 86 neurons/$\mu m^2$; SD = 11 neurons/$\mu m^2$) of the LGN. An unpaired t-test between normal and MD groups revealed a significant difference ($t(4) = 10.65$, $p < 0.001$), however no difference was found between the normal and MD + BV groups ($t(2) = 0.2698$, $p = 0.8126$). Therefore, similar to measurements of soma size, the reduction of neurofilament labeling produced by early and brief MD recovered after 8 days of binocular vision.

**2.32 Impact of Congruent and Incongruent MD on Soma Size**

The immediate impact of the early and transient MD on soma size and neurofilament was recovered after just 8 days of binocular vision. Consequently, as animals from the three experimental groups each received 3 weeks of binocular vision following the initial period of MD, they had normal and balanced ODIs at the time they

received the period of MD 3 weeks later. Quantification of soma area for the *control* MD group that received only the 10-day probe MD at 8 weeks of age and experienced binocular vision up until that point revealed smaller neurons in the dLGN layer serving the deprived eye (mean = 155 $\mu m^2$; SD = 24 $\mu m^2$) compared to neurons serving the non-deprived eye (mean = 183 $\mu m^2$; SD = 24 $\mu m^2$). This resulted in an average ODI of 15% (SD = 1.119; 95% CI [13.22, 16.78]) which served as the baseline effect size for the probe MD and the ODI to which the other two experimental conditions were compared. Quantification of the *incongruent* MD group revealed slightly smaller neurons in deprived eye layers of the LGN (mean = 166 $\mu m^2$; SD = 13 $\mu m^2$) compared to non-deprived eye layers (mean = 179 $\mu m^2$; SD = 24 $\mu m^2$), which produced an average ODI of 7% (SD = 3.881; 95% CI [-3.119, 16.16]). This was a difference in soma size between layers of the LGN that was visibly smaller than what was observed in both the *control* and *congruent* MD groups when inspected at high and low magnification (Figure 3). Quantification of the *congruent* MD group revealed much smaller neurons in the layer of the LGN serving the deprived eye (mean = 134 $\mu m^2$; SD = 58 $\mu m^2$) compared to neurons serving the non-deprived eye (mean = 167 $\mu m^2$; SD = 79 $\mu m^2$), which produced an average ODI of 20 % (SD = 7.294; 95% CI [2.006, 38.24]). Furthermore, the *incongruent* group exhibited a noticeable disparity in cell size between the layers of the dLGN that was visibly larger than the *control* and *incongruent* MD groups upon examination at both low and high magnifications (Figure 3).

A Kruskal-Wallis one-way analysis of variance revealed a significant difference between the three experimental groups ($H(3) = 5.982$, $p = 0.034$) which can be seen in the graph from Figure 2B. Further analysis using Mann-Whitney tests (one-tailed) performed

on the *congruent* MD group indicated ODIs were not significantly larger than the ODIs of the *control* MD group (U (14,14) = 4, $p = 0.3143$) or the *incongruent* MD group (U (6,15) = 0, $p = 0.05$). Whereas, a Mann-Whitney test (one-tailed) performed on the *incongruent* MD group revealed significantly smaller ODIs from ODIs of the *control* MD group (U (22,6) = 0, $p = 0.0286$). Results from these statistical analyses are presented in Table 2.

### 2.33 Impact of Congruent and Incongruent MD on Neurofilament

Similar to the results for soma size, quantification of neurofilament-positive cell density for the *control* MD group revealed smaller densities in layers of the LGN serving the deprived eye (mean = 39 neurons/$\mu m^2$; SD = 9 neurons/$\mu m^2$) compared to layers serving the non-deprived eye (mean = 87 neurons/$\mu m^2$; SD = 7 neurons/$\mu m^2$), which resulted in an average ODI of 43% (SD = 10.15; 95% CI [26.73, 59.02]) and served as a baseline effect of the probe MD. Quantification of the *incongruent* MD group revealed slightly smaller densities in layers of the LGN serving the deprived eye (mean = 59 neurons/$\mu m^2$; SD = 7 neurons/$\mu m^2$) compared to layers serving the non-deprived eye (mean = 80 neurons/$\mu m^2$; SD = 8 neurons/$\mu m^2$), which produced an average ODI of 20% (SD = 8.395; 95% CI [-0.52, 41.19]). The difference in cell density between the layers of the LGN was clearly different at low magnification (Figure 2B). Lastly, quantification of the *congruent* MD group revealed much smaller densities in layers of the LGN serving the deprived eye (mean = 28 neurons/$\mu m^2$; SD = 7 neurons/$\mu m^2$) compared to layers serving the non-deprived eye (mean = 51 neurons/$\mu m^2$; SD = 12 neurons/$\mu m^2$), which produced an average ODI of 45% (SD = 4.01; 95% CI [34.94, 55.29]).

A Kruskal-Wallis one-way analysis of variance revealed that there was no significant difference between the three experimental groups ($H(3) = 5.727$, $p = 0.05$) for neurofilament-positive cell density, but a difference can be seen in the graph and images from Figure 2C. Further analysis using Mann-Whitney tests (one-tailed) performed on the *congruent* MD group indicated ODIs were not significantly larger than ODIs of the *control* MD groups ($U (16,12) = 6$, $p = 0.5$) or the *incongruent* MD group ($U (6,15) = 0$, $p = 0.05$). Whereas a Mann-Whitney test (one-tailed) performed on the *incongruent* MD group revealed significantly smaller ODIs from the *control* MD group ($U (22,6) = 0$, $p = 0.0286$). Results from these statistical analyses are presented in Table 3.

**2.4 Interpretation of Results**

With respect to both measures of anatomical change, soma size and neurofilament-positive cell density, animals recovered from a 7-day period of MD initiated at P30 with subsequent binocular vision. Furthermore, the effect size from a 10-day MD at 8 weeks of age is reduced in animals that received a precursory MD to the opposite eye (*incongruent* MD group) compared to animals with no precursory MD (*control* MD group). In contrast, animals that received a precursory period of MD to the same eye (*congruent* MD group) as the probe MD exhibited a similar effect as animals with no precursory MD. These results suggest that a precursory MD can influence the degree to which a later MD to the opposite eye affects the visual system, a hypothesis that has been alluded to in past research.

A protective effect of strabismus on the effects of a later MD has been postulated from studies of cats that were rendered strabismic early in the critical period and importantly a few weeks before a period of MD was imposed (Mustari & Cynader, 1981;

Faulkner et al., 2005). This effect is thought to be produced by a breakdown of binocularity that occurs from abnormal patterned activity during strabismic rearing (Wiesel & Hubel, 1965; Trachtenberg and Stryker, 2001). Such disruption could introduce irregularities in the pruning of cortical connections during binocular competition, resulting in a deficient or abnormal competition. For the recovery of the visual system, it is crucial to sustain a competitive interaction between connections from both eyes, maintaining such a balance is necessary for binocular recovery from the impact of MD (Kind et al., 2002). An example of this can be produced by rearing kittens with an alternating MD using an opaque occluder placed over one eye each day. Doing so during the critical periods leads to nearly complete loss of binocular inputs within V1 (Wiesel & Hubel, 1965; Blasdel and Pettigrew, 1979) which also influences the effects of a period of MD induced later in life (Blasdel & Pettigrew, 1978). In fact, the early visual experience of kittens is crucial even if no normal vision is allowed until the peak of the critical period. In kittens that were reared in the dark until the peak of the critical period, those that received an MD immediately after darkness showed less recovery from an RO imposed later in life than kittens that were allowed binocular vision after darkness (Blasdel & Pettigrew, 1978).

The influence of a smaller loss of neurofilament in the LGN for animals that received the periods of MD in the opposite eyes highlights the possibility that neurofilament plays a crucial role in the influence of prior abnormal visual experience. As a cytoskeletal protein important for stability and intracellular scaffolding, neurofilament is responsible for maintaining the structural integrity of neurons (Morris and Lasek, 1982; Yuan et al., 2006). It accumulates postnatally in V1 following a time

curve that is inverse to the critical period (Song et al., 2015) and remains low in cats that are exposed to complete darkness who exhibit an enhanced capacity for plasticity (O'Leary et al., 2012; Duffy and Mitchell, 2013). This inverse correlation with the critical period has led to the suggestion that the role of neurofilament in the visual system, and perhaps in the nervous system more generally, is to act as a plasticity inhibitor by increasing stability and maintenance of neurons (Duffy and Mitchell, 2013; Hensch and Quinlan, 2018). Therefore, the attenuated loss of neurofilament may be implicated in the reduced effect of MD on soma size in the *incongruent* MD animals, as the precursory MD may have produced a lasting stabilization of neurons serving the originally non-deprived eye. In terms of the contrasting effects from a precursory MD to the same eye, a similar but opposite lasting destabilization of the deprived layer of the LGN would produce an equivalent or larger effect compared to naïve animals. In other words, the stabilization of the non-deprived layer of the dLGN as evidenced by high levels of neurofilament, results in either an attenuated impact of a future MD to the opposite eye or a facilitated impact from an MD to the same eye.

The results of the current study are clear in showing that a precursory MD to the opposite eye attenuates the effects from a later MD, however the influence of the initial MD to the same eye are not as clear. In mice that receive two periods of MD to the same eye, separated by a period of binocular vision, the effect of the second MD was brought about quicker and was more robust than mice that did not receive the precursory MD (Hofer et al., 2006; Hofer et al., 2009). Therefore, it was expected that the probe MD from the current experiment would elicit a more robust effect than exhibited by age-matched controls. However, the results observed in the current experiment indicate only a

small and non-significant difference in ODI between the *control* MD and *congruent* MD groups, with a slightly larger effect for the *congruent* MD group. Together, the influence of a precursory MD to the opposite eye and the slight influence when applied to the same eye suggest that the period of monocular visual experience early in the critical period affected the manner in which the visual system could adapt later in life. This is consistent with evidence suggesting that reverse occlusion in cats and mice promotes a maladaptive plasticity phenotype (Balsor et al., 2019) that yields little recovery from the effects of the primary MD (Kaneko & Stryker, 2023).

### 2.5 Conclusion

There seems to be a lasting and underlying neural adaptation elicited by an initial period of visual deprivation to one eye that influences effects from subsequent periods of abnormal monocular vision, aside from the impact on soma size and neurofilament. Whether the influence is bidirectional, meaning that it either attenuates or facilitates plasticity, based on the form, time, and to which eye the deprivation is imposed, remains unclear. However, statistical analysis of these experimental groups paired with visual observation of the tissue reveals an effect from the prior MD in the *incongruent* MD group, whereas only visual inspection of the tissue from the *congruent* MD group revealed a prior MD effect as compared to the *control* MD group.

# CHAPTER 3:

# EFFECTS OF MONOCULAR DEPRIVATION FOLLOWING PRECURSORY MONOCULAR INACTIVATION

## 3.1 Effect of Prior Monocular Inactivation

One corollary to the evidence supporting the influence that monocular lid closure has on later adaptations of the visual system presented in the preceding chapter is whether the form, duration, or extent of the prior abnormal visual experience modulates the effect. Similar in effect to a period of MD is the effects of monocular retinal inactivation, though the latter can produce an effect that is as much as twice the magnitude of the former (Duffy et al., 2023). In fact, the heightened effectiveness of MI also extends to the recovery capability of reversing the effects of MD. Immediate MI to the non-deprived eye of cats that received a period of MD during the critical period has promoted recovery surpassing what is achieved by a period of RO (Fong et al., 2016). Furthermore, the recovery that is promoted by a period of MI imposed on the fellow eye has been shown to occur outside the classical and well-defined critical period of recovery, and also from the effects of a long-term deprivation (Duffy et al., 2018; Fong et al., 2021). In other words, a period of MI may promote a similar but more extreme adaptation of the visual system compared to MD.

With a more potent manipulation comes the possibility of a more potent and potentially irreversible effect that is not observed following MD. For instance, it is conceivable that retinal inactivation could permanently alter retinal function, which would complicate assessment of deprivation-induced adaptations within the primary

visual pathway. Examination of the retinae and optic nerves following intraocular administration of TTX for various abnormalities in cats and monkeys has so far produced no evidence of damage or degeneration (Foeller and Tychsen, 2019; DiCostanzo et al., 2020; Duffy et al., 2023). Furthermore, examination of ocular axial length and refractive error of kittens that received 10 days of MI induced to the dominant eye after a period of MD were unchanged as compared to measurements before inactivation (Hogan et al., 2023). It has been suggested that though the effects of MI overlap with those of MD, the mechanisms that produce the effects are different, as MI elicits significantly less long-term depression in visual cortex than MD (Rittenhouse et al., 1999). Taken together, though the effects of MI seem to be more potent and superior to the effects induced by MD, possibility of injury to the visual system is unlikely and therefore suit the purposes of the current investigation.

Following the design of the experiment presented in chapter 2, the current investigation uses animals that received a brief period of right or left eye MI early in the critical period followed by a period of binocular recovery before receiving a probe MD of the right eye to measure their capacity for plasticity. In parallel to the investigation in Chapter 2, the probe MD was administered to either the same eye or opposite eye as the MI, and to ensure recovery from the first episode of MI, a group of animals was examined before receiving the probe MD. Since the probe MD served the same purpose as the previous investigation, the current investigation will use the *control* MD group from Chapter 2 to compare the influence of the prior MI. The same hypothesis remains for this investigation: the precursory period of MI will influence the effects of the probe

MD in a bidirectional manner depending on whether the manipulations are performed to the same eye or not.

### 3.2 Methods

The rearing histories of the animals described below are detailed in Table 3, and the rearing manipulations for each group in the experiment are depicted in Figure 4A.

### 3.21 Animals

Anatomical studies were conducted on 9 cats born and raised in a closed breeding colony at Dalhousie University. All rearing and experimental procedures were conducted in accordance with protocols that were approved by the University Committee on Laboratory Animals at Dalhousie, and that conformed to guidelines from the Canadian Council on Animal Care. The current study investigated the effect of 10 days of right-eye MD imposed at 8 weeks of age (Probe MD) compared between 3 groups. The first group received 6 days of MI imposed to the left eye at postnatal day 30 before 3 weeks of binocular recovery and administration of the right eye probe MD (Incongruent TTX-MD; n = 4). The second group received 6 days of MI imposed to the right eye at postnatal day 30 before 3 weeks of binocular recovery and administration of the right eye probe MD (Congruent TTX-MD; n = 3). The third group only received 6 days of MI imposed to the right eye at postnatal day 30 before 3 weeks of binocular vision (TTX + BV; n = 2) to ensure recovery occurred from MI in the Incongruent and Congruent TTX-MD groups. Retinal inactivation was induced for 6 days via 2 injections of TTX to closely mimic the 7-day period of MD without exceeding the specified duration.

### 3.22 Monocular Inactivation

Animals that received a period of MI were first anesthetized with 3-4% isoflurane in oxygen then the eye was administered intravitreal injection of TTX (ab120055; abcam, USA) solubilized in citrate buffer at 3 mM. Animals that later received an MD had their eye lids sutured according to the procedure described in chapter 2.22. TTX dosage was scaled for each animal according to individual eye size while simultaneously considering age (Thorn et al.,1976); 0.5 µl of TTX per mm of vitreous chamber length. A dosage that blocks action potentials of affected cells while mitigating fast axoplasmic transport (Ochs & Hollingsworth, 1971). A small puncture was made in the sclera at the pars plana using a sterile 30-gauge needle to create an injection administration location. While using a surgical microscope, the TTX solution was injected into the vitreous chamber via sterilized Hamilton syringe (Hamilton Company, USA) with a 30-gague needle (point style 4) positioned through the injection administration location and roughly 5-10 mm into the chamber angled away from the lens. After the full measured volume of TTX was dispensed slowly into the chamber, the needle was held in place for roughly one minute before it was retracted to ensure full injections. Following the procedure, a topical antibiotic (1% chloromycetin) and a local anesthetic (1% proparacaine hydrochloride) were administered to mitigate risk of infection and prevent complications alongside Metacam (0.05 mg/kg) to provide analgesia. To accomplish 6 days of inactivation, a second injection was administered halfway through the 6 days of inactivation, for a total of 2 injections per animal. A single dose of TTX, administered according to the specifications listed above, eliminates visual responses for roughly 3 days (Wong-Riley and Riley, 1983; Stryker and Harris, 1986; Linden et al., 2009; Fong et al., 2016). Basic assessments of visual behavior while the non-inactivated eye was occluded with an

opaque contact lens were performed to ensure vision was inactivated. The absence of pupillary light reflex, lack of visuomotor behaviors including visual placing, visual startle, and tracking of a laser was confirmed during the period of inactivation.

### 3.23 Procedure

Lid closure, tissue preparation, Nissl and neurofilament staining, quantification, and statistical analysis was performed in accordance with the methods described in Chapter 2 (Chapter 2.22 – 2.27).

## 3.3 Results

Results from the current study on the effects of MI require that there is recovery from the MI following provision of binocular vision. To ensure that animals in the *incongruent* and *congruent* TTX-MD groups entered the probe MD with a normal ODI measurements, the *TTX + BV* group must show recovery from the effect of the MI.

### 3.31 Effects of Brief and Transient Monocular Inactivation

*Nissl:*

After 3 weeks of binocular vision following the 6-day period of MI, neurons within the deprived eye layer (mean = 193 $\mu m^2$; SD = 89 $\mu m^2$) were similar in size to those of the non-deprived layer (mean = 207 $\mu m^2$; SD = 101 $\mu m^2$) of the LGN, which produced an average ODI of 6.77% (SD = 4.058; 95% CI [-29.69, 43.23]). Suggesting that the effects of the brief period of MI have mostly recovered and returned the dLGN to a state only slightly deviating from normal.

*Neurofilament:*

Similarly, the neurofilament-positive cell density within the deprived eye layer (mean = 34 neurons/$\mu m^2$; SD = 10 neurons/$\mu m^2$) of the *TTX + BV* group was comparable to that of the non-deprived layer (mean = 42 neurons/$\mu m^2$; SD = 10 neurons/$\mu m^2$) of the LGN, which produced an average ODI of 19.2% (SD = 8.837; 95% CI [-60.20, 98.58]). Indicative of a partial recovery that returned the dLGN to a state that deviates slightly from normal.

### 3.32 Impact of Congruent and Incongruent MI on Soma Size

The *control* MD group that received only the right-eye MD after 8 weeks of normal binocular vision from birth produced an average ODI of 15% (SD = 1.119; 95% CI [13.22, 16.78]) for soma area, which again served as the baseline effect size for the probe MD and the ODI in which the following experimental conditions are compared. Quantification of soma area for the *Incongruent* TTX-MD group that received 6 days of MI to the left eye followed by 3 weeks of binocular recovery before receiving the right eye probe MD revealed obviously smaller neurons in the layer of the LGN serving the deprived eye (mean = 178 $\mu m^2$; SD = 81 $\mu m^2$) compared to neurons serving the non-deprived eye (mean = 196 $\mu m^2$; SD = 86 $\mu m^2$). This resulted in an average ODI of 9% (SD = 4.051; 95% CI [2.617, 15.51]), an effect smaller than the *control* MD group. Quantification of soma area for the *Congruent* TTX-MD group that received 6 days of MI to the right eye followed by 3 weeks of binocular recovery before receiving the right eye probe MD revealed much smaller neurons in the layer of the LGN serving the deprived eye (mean = 123 $\mu m^2$; SD = 52 $\mu m^2$) compared to neurons serving the non-deprived eye (mean = 164 $\mu m^2$; SD = 75 $\mu m^2$). This produced an average ODI of 25% (SD = 7.966; 95% CI [5.365, 44.94]), an effect larger than the *control* MD group.

A Kruskal-Wallis one-way analysis of variance on soma size revealed a significant difference between the three TTX experimental groups and the *control* MD group ($H(3) = 8.909$, $p = 0.001$) which can be seen from the first graph in Figure 4B. Further analysis using Mann-Whitney tests (one-tailed) performed on the *control* MD indicated ODIs were significantly smaller than the *congruent* TTX-MD group ODIs ($U(18,10) = 0$, $p = 0.0286$), and were significantly larger than the *incongruent* TTX-MD group ODIs ($U(10,26) = 0$, $p = 0.0143$). Similarly, the *congruent* TTX-MD group ODIs were significantly larger than the *incongruent* TTX-MD group ODIs ($U(10,18) = 0$, $p = 0.0286$). Results from these statistical analyses are presented in Table 4.

### 3.33 Impact of Congruent and Incongruent MI on Neurofilament

The *control* MD group (see chapter 2) that received only right-eye MD after 8 weeks of normal binocular vision from birth produced an average ODI of 43% (SD = 10.15; 95% CI [13.22, 16.78]) for neurofilament-positive cell density, which again served as the baseline effect size for the probe MD and the ODI in which the following experimental conditions are compared. Quantification of the *Incongruent* TTX-MD group that received 6 days of MI to the left eye followed by 3 weeks of binocular recovery before receiving the right eye probe MD revealed a smaller density of neurofilament positive cells in the layer of the dLGN serving the deprived eye (mean = 33 neurons/$\mu m^2$; SD = 9 neurons/$\mu m^2$) compared to the density of the non-deprived layer (mean = 43 neurons/$\mu m^2$; SD = 13 neurons/$\mu m^2$), which produced an average ODI of 23% (SD = 5.36; 95% CI [14.26, 31.32]). Quantification of neurofilament positive cell density for the *Congruent* TTX-MD group that received 6 days of MI to the right eye followed by 3 weeks of binocular recovery before receiving the right eye probe MD revealed an even

smaller density in the layer of the dLGN serving the deprived eye (mean = 28 neurons/$\mu m^2$; SD = 7 neurons/$\mu m^2$) compared to the density of the non-deprived layer (mean = 51 neurons/$\mu m^2$; SD = 12 neurons/$\mu m^2$), which produced an average ODI of 35% (SD = 9.36; 95% CI [12.24, 58.74]).

Another Kruskal-Wallis one-way analysis of variance revealed a significant differences of the impact on neurofilament between the TTX experimental groups and the *control* MD group ($H(3) = 5.667$, $p = 0.0469$) which can be seen from the second graph in Figure 4C. Further analysis using Mann-Whitney tests (one-tailed) performed on the *control* MD indicated ODIs were not significantly smaller than the *congruent* TTX-MD group ($U(10,18) = 4$, $p = 0.3143$) and ODIs for the *congruent* TTX-MD were not significantly larger than the *incongruent* TTX-MD group ($U(12,16) = 2$, $p = 0.1143$). Whereas, a Mann-Whitney test performed between the and the *control* MD and *incongruent* TTX-MD groups revealed significant larger ODIs for the *control* MD group ($U(10,26) = 0$, $p = 0.0143$). Unlike the results from soma size, only the *incongruent* TTX-MD group revealed a significant difference from the *control* MD group, suggesting a period of MI to the same eye does not influence the effect of the probe MD on neurofilament as it does on soma size. Results from these statistical analyses are presented in Table 6.

### 3.4 Interpretation of Prior MI Results

Kittens that received only the early and transient period of MI appeared to have somewhat recovered from the effects of MI within the 3 weeks of binocular vision (3.31). When kittens experienced this same period of MI first to the left eye and later received

the right eye probe MD (*Incongruent* TTX-MD) the loss of neurofilament and difference in soma size within the dLGN were diminished compared to kittens that only received the probe MD. In contrast, kittens that experienced a period of MI to the right eye and later received the right eye probe MD (*Congruent* TTX-MD) exhibited a larger reduction in soma size and an equivalent loss of neurofilament compared to kittens that only received the probe MD.

Results from the current study are consistent with the hypothesis that early monocular visual experience influences adaptation to further monocular vision and corroborate the findings presented in Chapter 2. In both experiments, when kittens were exposed to an early period of monocular vision in the left eye and later exposed to the right eye probe MD, the impact on neurofilament and soma size within the dLGN was diminished. This result suggests that the impact of an MD late in the critical period can be attenuated by an earlier period of monocular vision. However, results were not as clear for the influence of an early period of monocular vision to the right eye before the right eye probe MD. Only the *congruent* TTX-MD group showed a significantly larger effect than the *control* MD group, and therefor the facilitation of the impact of a late MD is still unclear.

# CHAPTER 4:

# CONCLUSION AND SYNTHESIS OF EFFECTS FROM MULTIPLE MONOCULAR VISUAL EXPERIENCES

## 4.1 Recapitulation of Effects from Early Monocular Vision

Over a half century ago, the gross structural modifications of neurons within the LGN resulting from a period of monocular deprivation were discovered (Wiesel & Hubel, 1963b). Since then, further exploration has delved into the degree and age at which these changes can occur (Olson & Freeman, 1980; Mitchell & Maurer, 2022). In an effort to contribute to these explorations, the experiments presented above assessed how a period of monocular vision can affect the visual system's response to subsequent manipulation. Importantly, following an initial period of MD or MI, animals recover from the impact of these manipulations when simply provided binocular vision. Subsequent MD, following this recovery, led to variations in the impact on soma size and neurofilament loss within the dLGN, contingent on the initial experience of monocular vision. When the animal underwent the initial inactivation or deprivation of the left eye, followed by subsequent deprivation of the right eye, then the effect size of the probe MD was significantly smaller than normal animals. In contrast, when a period of deprivation or inactivation is initially administered to the right eye, followed by subsequent deprivation of the right eye, the observed effect size was slightly larger than normal animals upon microscopic examination. However, this increased effect size was statistically significant only when measured by changes in soma size within the animals that initially received inactivation.

Though, given that a similar trend was observed following an initial period of MD or MI, the influence of the prior monocular visual experience is difficult to rule out.

The impact of a prior monocular visual experience suggests the possibility that the depth and duration of an initial amblyogenic event could play a role in the degree to which the dominant eye yields input to the weaker eye during treatment for amblyopia. Similar to the influence of an initial period of MD or MI that resulted in a smaller or larger impact on a subsequent MD, the original amblyogenic event may influence treatment outcomes from patching the fellow eye. If true, this emphasizes the advantages of providing the most potent treatment available to optimize potential plasticity resources.

### 4.2 Impact of Early Monocular Vision

The influence of an early period of monocular vision has been observed in children with amblyopia undergoing retreatment with patching therapy, showing less recovery compared to similarly aged children undergoing treatment for the first time (Scheiman et al., 2005; Scheiman et al., 2008; Holmes et al., 2011). Analogous to this is the diminished effectiveness of reverse suturing as compared to the efficacy of an initial MD in animal models (Olson and Freeman, 1980; LeVay et al., 1980; Blakemore and Van Sluyters, 1974). Both situations involve abnormal visual experience of just one eye that is later followed by a period of monocular vision of the other eye and demonstrate a reduction in the ability to produce adaptation of neural circuitry. In contrast, when two periods of monocular vision are experienced through manipulations performed to the same eye, an increase in the ability to produce change occurs (Hofer et al., 2006; Hofer et al., 2009). Therefore, the impact of preceding monocular vision can be bidirectional, contingent on which eye is later subjected to monocular vision.

The findings from the experiments detailed in chapters 2 and 3 support the effects noted in multiple episodes of monocular vision affecting opposite eyes. However, the quantitative results from the same eye did not precisely align with previous research, though this may be due to small sample sizes as the qualitive differences are clear. Nevertheless, the influence of early monocular vision appears to affect the potential for plasticity induced by subsequent episodes of monocular vision. Consequently, the effects of early monocular vision are not only an immediate impact on soma size and neurofilament within the dLGN but also a latent impact that is only observed after subsequent monocular visual experience.

### 4.21 Impact of Early and Later Monocular Vision to the Same Eye

Ocular dominance indices (ODIs) from soma size and neurofilament-positive cell density quantification were in agreement that the subsequent MD produced roughly equal effects for the *control* MD and *congruent* MD groups. However, results from previous research (Hofer et al., 2006; Hofer et al., 2009) would suggest that the *congruent* MD group exhibit higher ODIs than the *control* MD group. The variation in these results may indicate a species dependent influence of prior monocular vision. Rodents, such as the mice used by Hofer (2006), have a visual system that differs significantly from that of higher mammals such as cats and primates. In contrast to cats, rodents lack the segregation of ocular dominance columns in V1 but instead posses a distribution of cells exhibiting ocular dominance for either eye in a “salt and pepper” pattern (Ohki & Reid, 2007). Given the absence of ocular dominance columns in rodents, it is conceivable that the competitive interaction arising from input received by both retinas throughout the visual cortex in higher mammals is more specialized in nature and necessitates more

robust manipulations to produce a comparable impact from prior monocular vision. Therefore, while mice demonstrate a quantitative enhancement in the impact from multiple periods of MD to the same eye (Hofer et al., 2006), cats may only manifest the qualitative differences observed in Chapter 2.

When comparing the impact of an early MI rather than an early period of MD, slightly different results were observed. The ODIs from soma size quantification revealed a significant difference between the *control* MD and *congruent* TTX-MD groups, a difference that was not found from ODIs of neurofilament-positive cell density. If the significant difference of soma size ODIs represents an influence of the prior manipulation, and not a type I statistical error, this suggests that the depth of the effects from the early period of monocular vision may in part determine the degree of influence to later adaptations. Since a period of MI imposed during the peak of the critical period produces effects that are twice the size of a period of MD (Kuppermann and Kasamatsu, 1983; Duffy et al., 2023) it may have been enough of an early adaptation to produce a latent influence on the probe MD, whereas the weaker manipulation of MD did not produce a strong enough effect to produce the same impact. This underscores the idea that cats require more robust manipulations to elicit a comparable influence of prior monocular vision to mice. Conversely, given that the ODIs derived from neurofilament-positive cell density revealed no significant difference from the same group of animals, this could be suggestive of a type 1 error in analysis of soma size or a saturation of the impact of MD on neurofilament that progresses more rapidly than the impacts on soma size (Lingley et al., 2019). Either way, the significant difference of impact on soma size following a period of MD in cats that underwent a prior MI compared to normal cats,

along with the qualitative differences from the other animals, imply an influence of multiple episodes of monocular vision to the same eye, akin to the findings of Hofer and colleagues (2006).

### 4.22 Impact of Early and Later Monocular Vision to the Opposite Eye

ODIs from soma size and neurofilament-positive cell density quantification from the *incongruent* MD group revealed a significant difference from both the *control* and *congruent* MD groups. These results were clear in showing that 7 days of early MD reduced the effect of a subsequent probe MD, but did not completely block the effect from the second MD. The effect sizes from both soma size and neurofilament-positive cell density were reduced by roughly 50% in the *incongruent* MD group as compared to the *control* MD group. Similarly, ODIs from soma size quantification from the *incongruent* TTX-MD group revealed a significant difference from the *control* and *congruent* MD groups, and a significant difference from the *control* MD group was revealed from neurofilament-positive cell density quantification. Again, the effect sizes for both soma size and neurofilament difference were reduced by about 50% in the *incongruent* TTX-MD group as compared to the *control* MD group, and a difference in soma size that was reduced as much as 65% compared to the *congruent* TTX-MD group.

Together, these results suggest that disruption to normal vision during the critical period can reduce the impact of a subsequent episode of monocular vision later in life, even when the monocular vision is caused by two different types of deprivation. For instance, in kittens rendered strabismic by myotomy during the critical period that later experienced a period of MD, there was a reduction in the ocular dominance shift induce by the period of MD as compared to normally reared kittens (Mustari & Cynader, 1981;

Faulkner et al., 2005). Further, kittens reared strabismic exhibited a slower rate of recovery from the effects of MD irrespective of the eye that received the MD but were limited to the contralateral hemisphere (Faulkner et al., 2005). However, the physiological effect, as measured by VEPs, in the strabismic animals was not significantly different than VEPs of normal animals. This finding contrasts the results of the current investigations, though it could be attributed to the more substantial impact of MD on the visual system in comparison to strabismus (Mower & Duffy, 1983). To replicate results resembling those outlined in Chapters 2 and 3, an influence greater than that of strabismus might be necessary. This may also allude to the distinctions between the effects of prior MI compared to MD, as the more potent manipulation (MI) appears to influence an equal or slightly larger impact on subsequent monocular visual experience. Taken together, this reinforces the likelihood that the depth and duration of preceding periods of monocular visual experience contribute to shaping the impact of subsequent adaptations to monocular vision.

### 4.3 Development of Abnormal Visual Framework

It is well documented that when kittens have their eyelids sutured to prevent vision in just one eye, anatomical and physiological effects occur throughout the visual pathway contingent on the duration of the suture (Wiesel & Hubel, 1963a; Wiesel & Hubel, 1963b), but no observable effects can be elicited after 8 months to one year of age (Olson & Freeman, 1980; Daw et al., 1992). Before this age in cats, MD influences the number of neurons that will be responsive to stimulation of the non-deprived eye (Wiesel & Hubel, 1963a). In mice, this results from experience-dependent alignment of neurons with similar tuning properties, including binocularity, orientation selectivity and spatial

frequency, throughout V1 (Brown & McGee, 2022). Following a period of MD, mice exhibit mechanisms that alter the composition of binocular and monocular neurons in V1, resulting in a reduced ratio of monocular neurons responsive to the contralateral versus the ipsilateral eye (Brown & McGee, 2022). Thus, this rearrangement gives rise to an abnormal framework of neural circuitry, potentially contributing to the influence of prior monocular vision revealed by subsequent periods of monocular deprivation. This suggests that the underlying visual framework of an animal that has recovered from the impacts of MD may also be abnormal compared to animals without prior history of monocular vision.

Another anomaly of the visual framework of animals exposed to MD may occur within the retinogeniculate pathway which normally segregates into dLGN layers serving each eye based on afferent action potential activity from the two eyes (Linden et al., 1981; Cucchiaro & Guillery 1984). Simply silencing the experience-independent spontaneous prenatal activity of the retinae responsible for normal retinogeniculate development (Penn et al., 1998) using TTX early in embryonic development prevents segregation of the layers in the dLGN, though the neuronal connections are otherwise normal (Shatz, 1990). Similarly, the geniculocortical afferents that drive normal development of V1, including segregation into ocular dominance columns (ODCs), rely on spontaneous experience-independent activity during embryonic development (Horton & Hocking, 1996). Inhibiting the spontaneous activity of both eyes via TTX injection during the normal period of ocular dominance segregation in V1 prevents the development of ODCs (Stryker & Harris, 1986). These findings demonstrate that disrupting neural activity during early development interferes with the organized

arrangement of the visual pathway, leading to significant abnormalities in the underlying framework.

Interestingly, even when the normal segregation of the retinogeniculate pathway is disrupted, normal single-cell responses and topographic representation persist in the dLGN (Huberman et al., 2002). This suggests that the significant abnormalities of the underlying visual system framework escape detection through finer independent measures. Consequently, it is plausible that, without overt consequences to cell integrity and physiology, a notable impact of the visual framework endures following a period of monocular vision. An adaptation of the sort could contribute to the impact of subsequent adaptations. Furthermore, this contribution may not necessarily occur on a circuit-wide scale but could manifest at a synaptic level, as the potential to induce synaptic plasticity shifts based on an animal's visual history (Abraham & Bear, 1996), a phenomenon that is commonly referred to as metaplasticity.

### 4.4 Metaplastic Development and BCM Theory

The theory of competitive binocularity proposes that neural activity stimulated independently from each eye compete for representation within V1 (Levelt, 1965). This concept is rooted in principles of Hebbian synaptic modification, where connections are strengthened the more they fire in unison (Hebb, 1949). In essence, the connections representing each eye engage in continual competition within V1, with those more correlated with postsynaptic activity gaining increasing favor throughout the visual pathway.

The fundamental concept that patterned post-synaptic activity dictates the preferred connections within V1 constitutes the core theory underlying metaplasticity, known as BCM theory (Bienenstock et al., 1982). According to BCM theory, the modification threshold to evoke synaptic plasticity is dynamic and depends on the correlation of average post-synaptic activity. Changes in glutamate-gated-ion channels, particularly N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4isoxazolepropionic acid (AMPA) receptors, mediate the modification threshold (Bear et al., 2016). Repeated stimulation of the postsynaptic neuron activates NMDA receptors, enhances synaptic efficacy in part by increasing the amount of AMPA receptors on the post-synaptic terminal, thereby strengthening synaptic transmission (Bear et al., 2016); a phenomenon known as long term potentiation (LTP). Conversely, when presynaptic neuron activity is uncorrelated with its postsynaptic target, the connection weakens, and the efficiency of the cellular mechanisms that enable neuron firing decrease (Lüscher and Malenka, 2012; Bear et al., 2016); a phenomenon known as long term depression (LTD). Moreover, prolonged LTD can lead to the elimination and retraction of the presynaptic neuron (Coleman et al., 2010; Piochon et al., 2016). Together, the synaptic weakening (LTD) and synaptic strengthening (LTP) modify the modification threshold for inducing further plasticity based on the history of post-synaptic activation (Kirkwood et al., 1996).

It is proposed that the shift in modification threshold is driven by an adaptation in the composition of NMDA receptors, comprising an obligatory GluN1 subunit and a mix of GluN2A-D subunits (McBain & Mayer, 1994). Throughout normal development, there is a regular adaptation in the mixture of GluN2 subunits, with downregulation of GluN2B and upregulation of GluN2A during the critical period, leading to alterations in receptor

function (Flint et al., 1997; Nase et al., 1999; Roberts & Ramoa, 1999). Crucially, this adaptation is influenced by the history of visual experience and produces an increased GluN2A/GluN2B ratio coinciding with the end of the critical period (Quinlan, Olstein, & Bear, 1999; Quinlan et al., 1999; Philpot et al., 2001). Consequently, a lower ratio reduces the threshold for induction of LTP (Philpot et al., 2007), whereas a higher ratio increases the threshold for LTP associated with the more mature stable cells corresponding with post-critical period ages (Tovar & Westbrook, 1999).

Animals exposed to a period of MD exhibit a reduction in GluN2A/GluN2B ratio, a change reminiscent of the GluN2A/GluN2B ratio reduction in dark reared animals that only occurs within cells representing the deprived eye (Chen & Bear, 2007). Therefore, following MD, connections that receive input from the non-deprived eye would be strengthened through consistent LTP while connections that receive input from the deprived eye are weakened. After being re-exposed to light, the GluN2A/GluN2B ratio in dark reared animals rapidly increases and LTD is instead promoted for a short time (Chen & Bear, 2007). Animals initially subjected to a preceding MD followed by a period of binocular vision, would also exhibit promotion of LTD for connections associated with the deprived eye along with an increase in the GluN2A/GluN2B ratio. The promotion of LTD in the connections representing the deprived eye may persist until the subsequent MD in the cats presented in Chapters 2 and 3. Establishing a scenario in which a subsequent MD to the same eye would yield an enhanced effect, attributed to the promotion of LTD in the previously deprived synapses. Conversely, a subsequent MD to the opposite eye would lead to a diminished effect, given the challenge in inducing LTP of the deprived eye synapses.

Therefore, BCM theory, and metaplasticity more generally, offers a potential theoretical foundation for understanding the influence of an initial MD on subsequent adaptions of the visual system. The chemical and cellular alterations in synapses associated with metaplasticity create an avenue to further investigate the unique effects of prior monocular vision. Utilizing the ratio of GluN2 subunits of the NDMA receptor as an indicator for plasticity in the visual system could potentially yield evidence for the effects of prior abnormal visual experience in future investigations.

### 4.5 Abnormal Spine Density

While BCM theory and metaplasticity align well with the results presented above, it may not comprehensively explain all the factors contributing to the observed prior experience effect. The functional recovery of the previously deprived eye after a period of binocular vision appears to be accompanied by a full anatomical recovery, however an accumulation of small changes may not recover. In mice subjected to two periods of MD to the same eye, the elevation in dendritic spine density associated with an initial period of MD, which persists thereafter, does not manifest following a second period of MD imposed during adulthood (Hofer et al., 2009). This difference can not be attributed to age alone, as the age-matched control mice that received only a single period of MD in adulthood exhibited the typical alterations to spine density characteristic of an initial period of MD (Hofer et al., 2009). The newly formed dendritic spines, arising after a period of MD likely strengthen the synaptic connections linked to the non-deprived eye (Knott et al., 2006; Nägerl et al., 2007), and therefore act as a safeguard against the effects of a subsequent MD. Interestingly, the increased spine density is also associated with an enhanced ability to induce LTP that occurs prior to the post-synaptic increase of

AMPA receptors (Kopec et al., 2006). Implying that the density of dendritic spines may contribute to the influence of prior monocular visual history through alterations in the ability to induce LTP.

### 4.6 Atypical Expression of Brain-Derived Neurotrophic Factor

The impact of MD on spine density as a contributor to the prior experience effect once again points to LTP as a primary factor in the underlying mechanism. An alternative factor contributing to this could come from an alteration in the levels of brain-derived neurotrophic factor (BDNF) following a period of MD. BDNF is a protein thought to play a key role in the maturation of cortical connections and a large contributor to the closure of the critical period (Huang et al., 1999). Indeed, after a period of MD, there is a reduction in both BDNF and the expression of messenger RNA for BDNF in the visual cortex of rats contralateral to the deprived eye (Bozzi et al., 1995; Rossi et al., 1999). Furthermore, the impact of neurotrophic administration during a period of MD prevents the typical dystrophic effects of MD (Riddle et al., 1995; Gillespie et al., 2000). Moreover, after a short period of MI, BDNF levels within layers of the dLGN and in V1 ODCs associated with the inactivated eye were significantly lower than the regions associated with the non-inactivated eye, a difference that was not present in control animals (Lein & Shatz, 2000). Consequently, during these periods of monocular visual experience, an atypical expression of BDNF is elicited that potentially disrupts conventional maturation of the affected neurons. However, the expression of BDNF within the non-deprived/inactivated layers of the dLGN has yet to be established. If the non-deprived layers of the dLGN exhibit an elevated expression of BDNF, it might lead to a premature maturation of neurons representing the non-deprived eye. In such a

scenario, a decrease of BDNF in neurons connected to the deprived eye and an increase within neurons connected to the non-deprived eye could occur following an initial period of MD. This, akin to alterations in spine density or the modification threshold for synaptic plasticity, could establish an underlying neuronal framework facilitating the effects of a subsequent MD to the same eye while mitigating the effects of MD when imposed on the opposite eye.

### 4.7 Implications to Understanding of Critical Period

Modifications to the critical periods of the visual system in cats with a prior abnormal visual history were demonstrated in a study involving dark-rearing. After several months of dark-rearing, cats subjected to a period of MD at the end of the critical period exhibited a shift in ocular dominance while normal cats exhibited little to no effect of an MD imposed at the same age (Cynader & Mitchell 1980). Additionally, dark-rearing was found to delay the offset of the critical period, as MD imposed early in the critical period has a more significant effect on dark-reared compared to light-reared animals (Mower, 1991; Beaver et al., 2001).

The conventional understanding of the critical period for visual system plasticity suggests that it occurs within a specific age range, with the exception of animals subjected to dark-rearing. However, insights from the current investigations, along with previous studies alluding to the influence of prior abnormal visual experiences (Knudsen, 1998; Linkenhoker et al., 2005; Hofer et al., 2006; Faulkner et al., 2005), introduce additional considerations regarding the history of visual experience within the critical period. These findings provide evidence that monocular visual experience to the same eye facilitates the effects of MD and enhances the capacity for plasticity in V1 and the LGN,

whereas monocular visual experience to the opposite eye diminish the effects of MD. Consequently, the critical period for MD in these cats was demonstrated to both extend and shorten based on the animals' relative history of visual experience.

### 4.8 Conclusion

As empirical evidence highlighting the role of visual history in influencing the start, end, and duration of the critical period accumulates, there is a need to minimize the emphasis on strictly defining the critical period within a specific age range. This shift in perspective holds significant implications for clinical treatment, emphasizing the importance of considering the patient's visual experience history for their optimal recovery.

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# APENDIX A: Tables

| | Rearing Manipulations | | | | |
|---|---|---|---|---|---|
| | **Normal** | **LE MD** | **RE MD** | **BV** | **Probe MD (RE)** |
| **Normal** | | | | | |
| N = 2 | P0 – P30 | x | x | x | x |
| **MD** | | | | | |
| N = 3 | P0 – P30 | P30 – P37 | x | x | x |
| **MD + BV** | | | | | |
| N = 2 | P0 – P30 | P30 – P37 | x | P37 – P45 | x |
| **Congruent MD** | | | | | |
| N = 3 | P0 – P30 | x | P30 – P37 | P37 – P56 | P56 – P66 |
| **Incongruent MD** | | | | | |
| N = 3 | P0 – P30 | P30 – P37 | x | P37 – P56 | P56 – P66 |
| **Control MD** | | | | | |
| N = 4 | P0 – P56 | x | x | x | P56 – P66 |

Table 1. Animal rearing conditions for Normal, MD, MD + BV, Congruent MD, Incongruent MD, and Control MD groups.

Animals in the *Normal* group (N = 2) were exposed to normal binocular vision until postnatal day (P) 30. Animals in the *MD* group (N = 3) were exposed to normal binocular vision until P30 then subjected to left-eye MD until P37. Animals in the *MD + BV* group (N = 2) were exposed to normal binocular vision until P30 then subjected to left-eye MD until P37 followed by binocular vision until P45. Animals in the *Congruent MD* group (N = 3) were exposed to normal binocular vision until P30 then subjected to a right-eye MD until P37 followed by binocular vision until P56 and anther right-eye MD from P56 until P66. Animals in the *Incongruent MD* group (N = 3) were exposed to normal binocular vision until P30 then subjected to a left-eye MD until P37 followed by binocular vision until P56 then a right-eye MD from P56 until P66. Animals in the *Control MD* group (N = 4) were exposed to normal binocular vision until P56 then subjected to a right-eye MD until P66.

| MD – MD NF ODI | | | | |
|---|---|---|---|---|
| *Condition* | *U* | *df* | *p* | *95% CI* |
| *Mann-Whitney Test:* | | | | |
| Cong. Vs. Control | 4 | 14,14 | 0.3143 | (-4.04 – 13.09) |
| Incong. Vs. Control | 0 | 22,6 | 0.0286 | (-14.15 – -3.845) |
| Cong. Vs. Incong. | 0 | 6,15 | 0.05 | (2.376 – 24.67) |

Table 2. Statistical analysis of ODIs produced from Nissl data of the *Congruent* MD (Cong.), *Incongruent* MD (Incong.), and control MD groups.

The percent difference (ODI) between soma size within layers A and A1 of the dLGN for the *Congruent* MD (Cong.), *Incongruent* MD (Incong.), and control MD groups were compared using Mann-Whitney tests.

| MD – MD NF ODI | | | | |
|---|---|---|---|---|
| *Condition* | *U* | *df* | *p* | *95% CI* |
| *Mann-Whitney Test:* | | | | |
| Cong. Vs. Control | 6 | 16,12 | 0.5 | (-11.98 – 15.33) |
| Incong. Vs. Control | 0 | 22,6 | 0.0286 | (-41.33 – -5.48) |
| Cong. Vs. Incong. | 0 | 6,15 | 0.05 | (12.63 – 37.54) |

Table 3. Statistical analysis of ODIs produced from Neurofilament data of the *Congruent* MD (Cong.), *Incongruent* MD (Incong.), and control MD groups.

The percent difference (ODI) between neurofilament-positive cell density within layers A and A1 of the dLGN for the *Congruent* MD (Cong.), *Incongruent* MD (Incong.), and control MD groups were compared using Mann-Whitney tests.

| | Rearing Manipulations | | | | |
|---|---|---|---|---|---|
| | **Normal** | **LE MI** | **RE MI** | **BV** | **Probe MD (RE)** |
| **TTX + BV** | | | | | |
| N = 2 | P0 – P30 | x | P30 – P36 | P36 – P56 | x |
| **Congruent TTX-MD** | | | | | |
| N = 3 | P0 – P30 | x | P30 – P36 | P36 – P56 | P56 - P66 |
| **Incongruent TTX-MD** | | | | | |
| N = 4 | P0 – P30 | P30 – P36 | x | P36 – P56 | P56 - P66 |

Table 4. Animal rearing conditions for TTX + BV, Congruent TTX-MD, and Incongruent TTX-MD groups.

Animals in the *TTX + BV* (N = 2) were exposed to normal binocular vision until postnatal day (P) 30 then subjected to monocular inactivation (MI) of the right eye via microinjection of tetrodotoxin (TTX) until P36 followed by binocular vision until P56. Animals in the *Congruent TTX-MD* (N = 3) were exposed to normal binocular vision until P30 then subjected to MI of the right eye via TTX until P36 followed by binocular vision until P56 whereupon they were subjected to a right-eye MD until P66. Animals in the *Incongruent TTX-MD* (N = 4) were exposed to normal binocular vision until P30 then subjected to MI of the left eye via TTX until P36 followed by binocular vision until P56 whereupon they were subjected to a right-eye MD until P66.

| TTX – MD NISSL ODI | | | | |
|---|---|---|---|---|
| *Condition* | *U* | *df* | *p* | *95% CI* |
| *Mann-Whitney Test:* | | | | |
| Control Vs. Cong. | 0 | 18,10 | 0.3142 | (-8.756 – 28.01) |
| Control Vs. Incong. | 0 | 10,26 | 0.0128 | (4.958 – 36.53) |
| Cong. Vs. Incong. | 0 | 10,18 | 0.0698 | (-3.926 - 26.15) |

Table 5. Statistical analysis of ODIs produced from Nissl data of the *Congruent* TTX - MD (Cong.), *Incongruent* TTX-MD (Incong.), and Control MD groups.

The percent difference (ODI) between soma size within layers A and A1 of the dLGN for the *Congruent* TTX-MD (Cong.), *Incongruent* TTX-MD (Incong.), and control MD groups were compared using Mann-Whitney tests.

| TTX – MD NF ODI | | | | |
|---|---|---|---|---|
| *Condition* | *U* | *df* | *p* | *95% CI* |
| *Mann-Whitney Test:* | | | | |
| Control Vs. Cong. | 4 | 18,10 | 0.3142 | (-8.756 – 28.01) |
| Control Vs. Incong. | 0 | 10,26 | 0.0128 | (4.958 – 36.53) |
| Cong. Vs. Incong. | 2 | 12,16 | 0.0698 | (-3.926 - 26.15) |

Table 6. Statistical analysis of ODIs produced from Neurofilament data of the *Congruent* TTX -MD (Cong.), *Incongruent* TTX-MD (Incong.), and Control MD groups.

The percent difference (ODI) between neurofilament-positive cell density within layers A and A1 of the dLGN for the *Congruent* TTX-MD (Cong.), *Incongruent* TTX-MD (Incong.), and control MD groups were compared using Mann-Whitney tests.

## APENDIX B: Figures

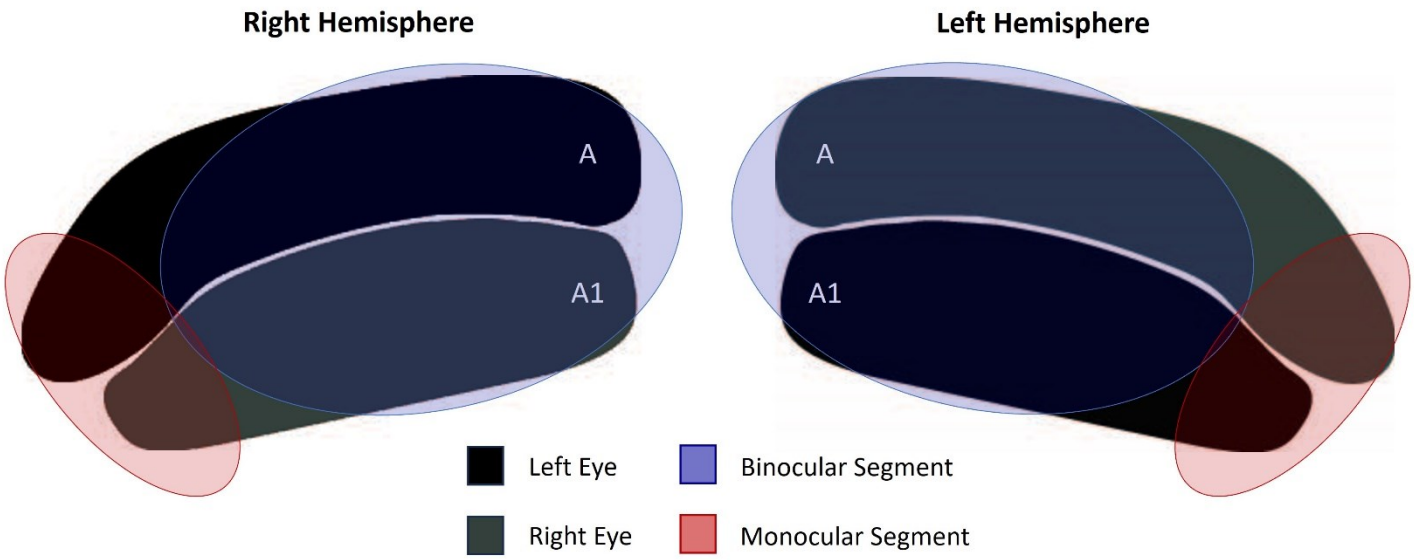


Figure 1. Dorsal Lateral Geniculate Nucleus of the Cat.

Depiction of the dorsal lateral geniculate nucleus from the right and left hemisphere of a cat. Input from the left (black) and right (grey) eyes are segregated between layers A and A1. Binocular (blue) and monocular (red) segments post-synaptic of both eyes and the contralateral eye respectively.

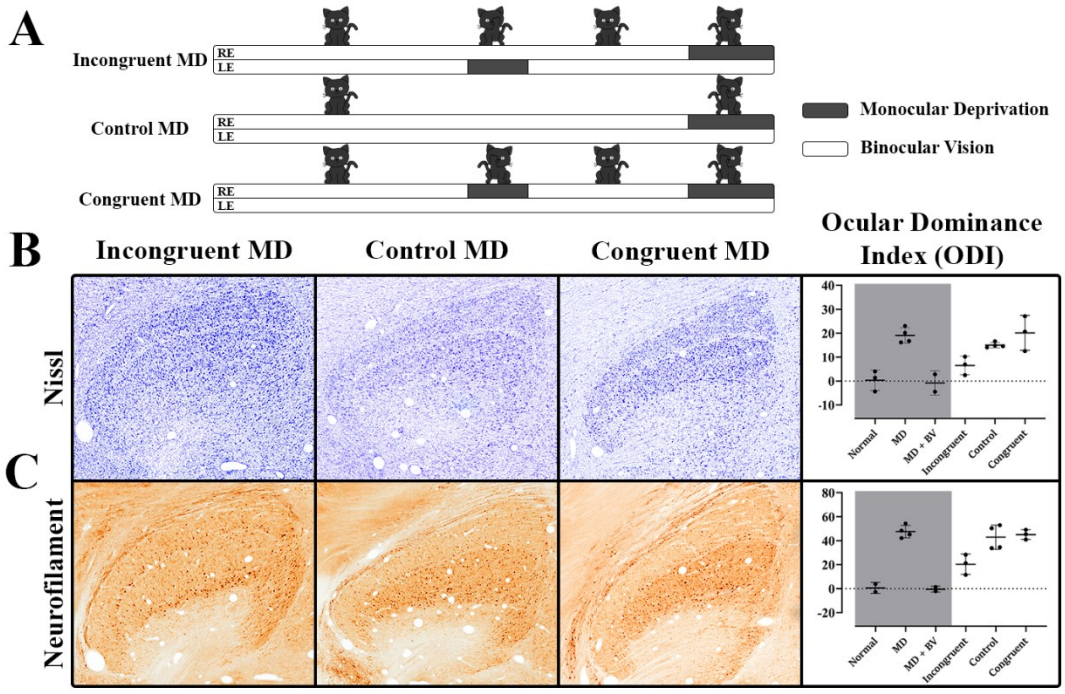


Figure 2. Nissl and Neurofilament results from the *Congruent* MD, *Incongruent* MD, and Control MD groups.

Depiction of animal rearing timelines for the *Congruent* MD, *Incongruent* MD, and Control MD groups (A). Images of Nissl stained dLGN sections from *Incongruent* (left), *Control* (middle), and *Congruent* (right) MD groups followed by a scatterplot of the percent difference between layers A and A1 of the dLGN (ODI) for the recovery groups (*Normal, MD, MD + BV*) in grey, then the *Incongruent* MD, *Control* MD, and *Congruent* MD groups thereafter (B). The soma size ODIs from the *Incongruent* MD, *Control* MD, and *Congruent* MD groups were found to be significantly different ($F(2, 7) = 7.1$, $p = 0.021$). Images of SMI-32 stained dLGN sections for neurofilament from *Incongruent* (left), *Control* (middle), and *Congruent* (right) MD groups followed by a scatterplot of neurofilament-positive cell density ODIs from the recovery groups in grey, and the *Incongruent* MD, *Control* MD, and *Congruent* MD groups thereafter (C). The neurofilament-positive cell density ODIs from the *Incongruent* MD, *Control* MD, and

*Congruent* MD groups were found to be significantly different ($F(2, 7) = 8.46$, $p = 0.014$). Graphs from B and C were adapted from Henneberry and colleagues (2023).

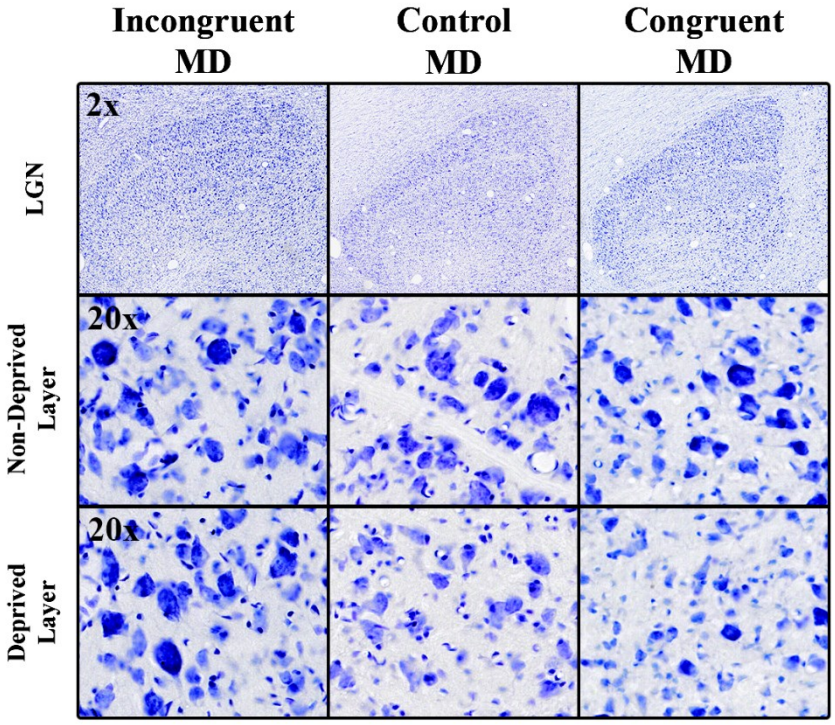


Figure 3. Nissl stained sections of the dLGN from the Incongruent, Control, and Congruent MD groups.

Low magnification (2x objective) images of Nissl stain from the dLGN from the *Incongruent, Control*, and *Congruent* MD groups reveal a difference in soma size between layers A and A1 (top). High magnification (20x) images of Nissl stained dLGN layer connected to the non-deprived eye of the *Incongruent, Control*, and *Congruent* MD groups reveal normal soma size (middle). High magnification (20x) images of Nissl stained dLGN layer connected to the deprived eye of the *Incongruent, Control*, and *Congruent* MD groups reveal smaller soma size in *Control*, and *Congruent* MD groups than the *Incongruent* MD group (bottom).

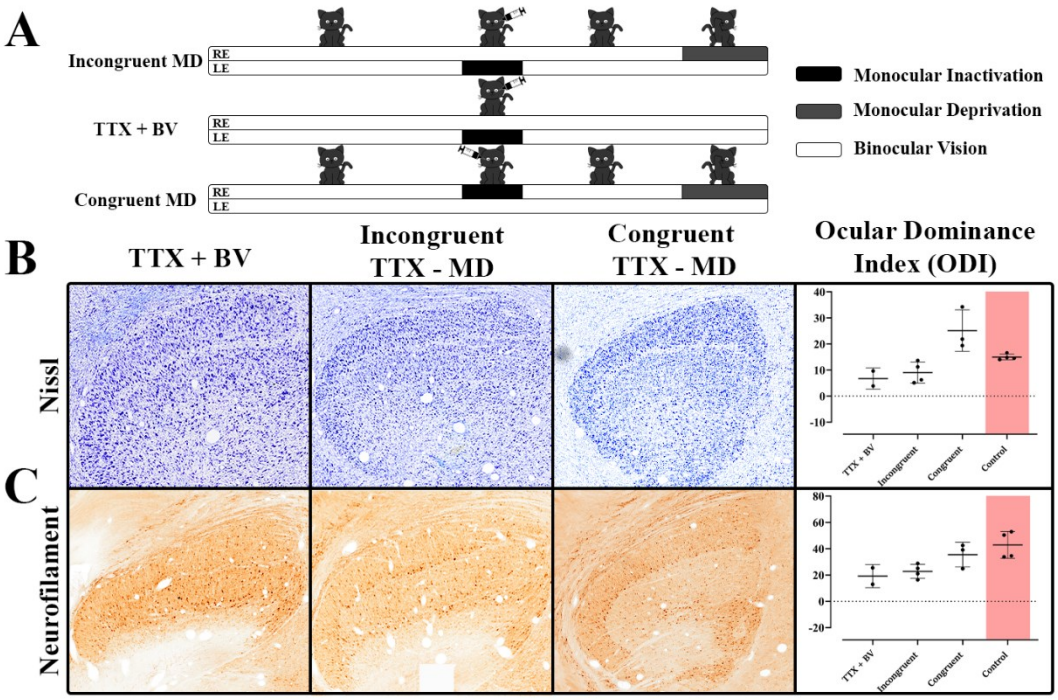


Figure 4. Nissl and Neurofilament results from the TTX + BV, Incongruent TTX-MD, and Congruent TTX-MD groups.

Depiction of animal rearing timelines for the *Congruent* MD, *Incongruent* MD, and Control MD groups (A). Images of Nissl stained dLGN sections from *Incongruent* (left), *Control* (middle), and *Congruent* (right) MD groups followed by a scatterplot of the percent difference between layers A and A1 of the dLGN (ODI) for the recovery control groups (*Normal, MD, MD + BV*) in grey, and the *Incongruent* MD, *Control* MD, and *Congruent* MD groups thereafter (B). The soma size ODIs from these groups were found to be significantly different ($F(3, 9) = 8.837$, $p = 0.005$). Images of SMI-3s stained dLGN sections for neurofilament from *Incongruent* (left), *Control* (middle), and *Congruent* (right) MD groups followed by a scatterplot of ODI for the recovery control groups (*Normal, MD, MD + BV*) in grey, and the *Incongruent* MD, *Control* MD, and *Congruent* MD groups thereafter (C). The neurofilament-positive cell density ODIs from these groups were found to be significantly different ($F(3, 9) = 5.42$, $p = 0.021$).