

**Neuromodulatory and metaplastic regulation of long-term synaptic plasticity in the  
primary visual cortex of adult rats**

by

Peter J. Gagolewicz

A thesis submitted to the Centre for Neuroscience Studies  
In conformity with the requirements for  
the degree of Doctor of Philosophy

Queen's University  
Kingston, Ontario, Canada  
(September, 2018)

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## Abstract

Synaptic plasticity in the neocortex of mammals is influenced by various neuromodulators and metaplasticity mechanisms. These regulatory processes allow synaptic networks to operate and adjust to various behavioral states and the constantly changing inputs from the sensory environment. The neurochemical milieu created by neuromodulators is an important regulator of synaptic plasticity at the time of induction, while metaplastic-processes regulate neuronal networks based on their history of activity. This thesis investigated the effects of the neurochemical milieu and past experiences (learning) on long-term potentiation (LTP) of synapses *in vivo* in the primary visual cortex (V1) of adult rats. Initial experiments revealed that the application of the neuromodulator serotonin (5-HT) in V1 was ineffective at altering LTP. However, LTP was facilitated in the presence of a 5-HT<sub>1A</sub>-receptor antagonist. Interestingly, in juvenile rats, the same antagonist exerted the opposite effect (i.e., inhibited LTP), indicating an age-specific role of 5-HT<sub>1A</sub> receptors in the gating of V1 synaptic plasticity. Next we examined the role of visual experience (visual discrimination training) on LTP in the adult V1. Rats trained to discriminate visual cues in a Y-shaped water maze apparatus subsequently exhibited enhanced LTP compared to naive or control (visually untrained) rats. The facilitation of LTP in visually trained rats was reversed by an antagonist of GluN2B subunits of the N-methyl-D-aspartate receptor (NMDAR) in V1. Consequently, we examined potential changes in NMDAR functioning in rats trained in the visual discrimination task. Patch clamp recordings of principal neurons in V1 revealed that behavioral training enhanced GluN2B-mediated NMDAR conductance. Finally, we examined the behavioral consequences of blocking GluN2B receptors during visual discrimination training, using a sequential task that involved two distinct rounds of training (involving different sets of visual cues). Results of these experiments showed that systemic GluN2B antagonism during the second round of visual training impaired task acquisition relative to controls. Together, the experiments in this thesis highlight the importance

of neuromodulation and metaplasticity in V1 for the gating of LTP, one of the principal mechanisms of learning and memory storage in the cortex of mammalian species.

## Co-Authorship

In all cases, I (Peter Gagolewicz) participated in the study design, data acquisition, data analysis, and manuscript preparation. Hans C. Dringenberg contributed to the study design, data analysis, and manuscript preparation.

**Chapter 2** has been published in its entirety, and can be cited as:

**Gagolewicz, P. J., & Dringenberg, H. C. (2016).** Age-Dependent Switch of the Role of Serotonergic 5-HT<sub>1A</sub> Receptors in Gating Long-Term Potentiation in Rat Visual Cortex In Vivo. *Neural Plasticity*, 2016, 1-11.

**Chapter 3** has been published in its entirety, and can be cited as:

**Gagolewicz, P. J., & Dringenberg, H. C. (2011).** NR2B-subunit dependent facilitation of long-term potentiation in primary visual cortex following visual discrimination training of adult rats. *European Journal of Neuroscience*, 34, 1222-1229.

**Chapter 4** has been published in part. Experiments describing the temporal summation of NMDA EPSCs have not been published previously. The remaining experiments of this chapter have been published and can be cited as:

Hager, A. M., **Gagolewicz, P. J.**, Rodier, S., & Dringenberg, H. C. (2015). Metaplastic up-regulation of LTP in the rat visual cortex by monocular visual training: requirement of task mastery, hemispheric specificity, and NMDA-GluN2B involvement. *Neuroscience*, 293, 171-186.

**Chapter 5** has not been published at this time.

## Acknowledgements

This work has been a humbling experience. The final product was the combination of knowledge, wisdom, time, money, effort (physical and emotional), sacrifice, endurance, failure, success, and endless support – for which I am grateful.

Dr. Hans Dringenberg, thank you for taking me on as your student, an experience that shattered my views of the world and rearranged everything to where it fits today, hopefully a positive outcome. Hans, you have been a phenomenal mentor and a mountain of support, endless support; thank you.

Dr. Eric Dumont, thank you for being part of the thesis committee and for training me how to patch clamp neurons. Dr. Richard Beninger, thank you for joining the thesis committee, for words of wisdom, and for coming out of retirement to help defend me. Also, to numerous faculty and staff members at the CNS and Psychology, your various forms of help are appreciated. Dr. Barrie Frost, thank you for the informal discussions regarding learning, memory and the visual system while relaxing in your comfortable office chair. A mention to Dr. David Andrew and Dylan Petrin, thank you immensely for helping me navigate through some rough waters near the end. It is also vital to acknowledge the help of various members of the Neuroplasticity Lab. Audrey, wish that you did not leave so early. Chloe, helpful, professional, dependable, thank you. And to the others at this lab (Diala, Min-Ching, Jenny, Matthew, Claudia, Laura, and Carolyn), appreciate all of your help in forming the memories.

At this point I must also thank Nathalie, for handling the glory of living with a graduate student.

Your patience, positivity and reminders to focus on what is important helped me to find the end.

Lastly I thank my parents for their enduring care, love and support. Miro, we know you wanted to be a doctor but you had no patience.

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## List of Abbreviations

5-HT	serotonin (5-hydroxytryptamine)
5-HT <sub>1A</sub>	serotonin 1A receptor subtype
ACh	acetylcholine
aCSF	artificial cerebrospinal fluid
ANOVA	analysis of variance
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AP	anterior-posterior
BDNF	brain-derived neurotrophic factor
Ca <sup>2+</sup>	calcium
CNS	central nervous system
ECoG	electrocorticogram
EPSC	excitatory postsynaptic current
fPSPs	field postsynaptic potentials
HFS	high frequency stimulation
GluN2B	glutamate [NMDA] receptor subunit epsilon-2
i.p.	intraperitoneal
LGN	lateral geniculate nucleus of the thalamus
LTD	long-term depression
LTP	long-term potentiation
LY 341495	(2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid
mGluRs	metabotropic glutamate receptors
NMDA	N-methyl-D-aspartate
P+	hidden escape platform

P-	absence of escape platform
Ro 25-6981	R- (R*,S*)]- - (4-hydroxyphenyl) - -methyl-4- (phenylmethyl) -1- piperidinepropanol hydrochloride
s.c.	subcutaneous
S.E.M.	standard error of the mean
SSRI	selective serotonin reuptake inhibitor
TBS	theta-burst stimulation
V1	primary visual cortex

# Chapter 1

## General introduction

### 1.1 The role of LTP in learning and memory

The 20<sup>th</sup> century was a period of rapidly advancing knowledge, bridging the gaps in our understanding between the processes occurring inside of the brain and how these relate to observable behavior. Science has come a long way in describing the physiological changes occurring within the brain following various types of experience. These experiences can affect/alter activity at neuronal synapses and result in alterations in the biochemical environment surrounding the cells. Of the many discoveries in the field of brain electrophysiology, one of the most significant was the initial report of a form of plasticity termed long-term potentiation (LTP) in the hippocampal dentate gyrus of anesthetized rabbits (Bliss & Lomo, 1973). This discovery revealed that, following high frequency patterned electrical stimulation, neurons tended to respond more strongly to subsequent input signals. Eventually, additional classic experiments revealed that interfering with the mechanisms of hippocampal LTP could disrupt spatial learning in rats (Morris et al., 1986), as measured in a newly designed apparatus known as the Morris Water Maze. Since these pioneering experiments, further evidence has accumulated, and it now is widely accepted that LTP is one of the core, cellular-level physiological mechanisms of learning and memory (Morris et al., 1990; Eichenbaum & Otto, 1993). One particularly illuminating aspect of these early experiments on synaptic plasticity was that LTP could not only be generated electrically by directly stimulating specific interconnected neuronal pathways, but that it could also occur naturally following various types of experience. Specific patterns of behavior and/or learning experiences could elicit LTP at a selective subset of synapses within the brain (Whitlock

et. al., 2006), a phenomenon that appeared to be very similar to LTP occurring following electrical stimulation of neurons. Further work in the field of synaptic plasticity has provided a detailed description of the mechanisms of, and factors affecting LTP in the nervous systems of animals and throughout different stages of development and aging.

## **1.2 The mechanism of LTP**

Although the process of LTP was discovered in Oslo, Norway in the laboratory of Per Andersen, the actual term "LTP" was first used in the literature by Douglas and Goddard (1975). Just as the name implies, the phenomenon of LTP can be characterized as follows: after the establishment of a stable baseline of field excitatory postsynaptic potentials (fEPSPs), a brief, high frequency stimulation will result in subsequently potentiated fEPSPs, an effect that can last for hours or days, depending on the specific methodology employed. This effect was initially described in the dentate gyrus of the hippocampus by monitoring fEPSPs in the dentate gyrus that were generated by brief, single pulses of electrical current applied to the perforant path (Bliss & Lomo, 1973). While the name has become a buzzword in the recent neuroscientific literature, the true beauty of LTP is in its function as a coincidence detector at synapses between two neurons (Bliss & Collingridge, 1993). At the core of the coincidence detection mechanism is the N-methyl-D-aspartate receptor (NMDAR). The unique properties of NMDARs permit them to be classed as ionotropic cation channels, which exhibit a particularly high permeability to calcium ( $\text{Ca}^{2+}$ ) ions. However, NMDARs can also function much like typical metabotropic receptors, which influence intracellular signaling cascades and even gene transcription, by altering local  $\text{Ca}^{2+}$  levels within their host neuron. At resting membrane potentials of neurons, the central channels of NMDARs are typically blocked by a magnesium ion ( $\text{Mg}^{2+}$ ), and hence cannot conduct charge, even if the receptors are in the open configuration (Ascher & Novak, 1998). This

Mg<sup>2+</sup> obstruction is relieved when the membrane surrounding the NMDAR becomes sufficiently depolarized. If there is concurrent (or close to concurrent) glutamate release into the vicinity of the NMDAR and a relief of the Mg<sup>2+</sup> pore plug, glutamate binding causes a conformational change in the receptor, the central channel opens, and cations, particularly Ca<sup>2+</sup>, can flood into the neuron across the membrane. In this way, the NMDARs function as detectors of concurrent activity between two connected neurons; activity of the first (presynaptic) neuron is expressed by the release of glutamate, while activity of the second (postsynaptic) neuron is indexed by the depolarization of the membrane surrounding the glutamate-binding NMDARs (Malenka & Bear, 2004). The latter effect usually results from the binding of glutamate to a second class of glutamatergic receptor, the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA; Collingridge & Bliss, 1987). This process of LTP induction, which results from concurrent pre- and post-synaptic activity, has been simplified by a popular phrase of Carla Shatz (1992); “cells that fire together, wire together”.

Once the NMDARs are activated and opened, Ca<sup>2+</sup> can flood the intracellular compartment of the host neuron, and if concentrations rise high enough following repeated channel activations, Ca<sup>2+</sup>-sensitive kinases will begin to activate intracellular signaling cascades. This can lead to a number of changes, including the transcription of genes, together resulting in stronger neuronal responses to subsequent synaptic inputs (Malenka & Nicholl, 1999). LTP that is sensitive to drugs that block NMDAR function is known as NMDA-dependent LTP. It is important to note that various other (NMDAR-independent) forms of LTP have also been described throughout the brain (for reviews, see Nicoll & Schmitz, 2005; Chevaleyre et al., 2006; Citri & Malenka, 2008), however, for the purpose of this thesis, I will focus on the classical, NMDAR-dependent type of LTP.

### 1.3 LTP at sensory cortical synapses including the primary visual cortex

The phenomenon of NMDA-dependent LTP is not constrained to hippocampal synapses; in fact, it appears to occur throughout the brain and has been suggested to be a basic property of most excitatory synapses throughout the mammalian nervous system (Malenka & Nicoll, 1999). Although the initial experiments describing LTP in the dentate gyrus were conducted in the whole brain (*in vivo*) preparation, the utilization of brain slices (*in vitro* studies) increased in popularity, as this method permitted advantages for precisely delineating the mechanisms of LTP at microcircuits containing the synapses of interest. In slices of rat neocortex, NMDA-dependent LTP can be induced in layers II/III of the primary somatosensory cortex by stimulating underlying cortical layers using brief, high-frequency pulses (Castro-Alamancos et al., 1995); interestingly, this effect was not present in the primary motor area (Castro-Alamancos et al., 1995). Similarly, LTP of layer IV synapses elicited by white matter stimulation was described in slices of the primary auditory cortex (Zhang et al., 2013). Further, LTP can be readily induced in the primary visual cortex (V1) of adult rats when stimulation is delivered in layer IV and recordings occur in the more superficial layer III (Kirkwood et al., 1993). On the other hand, in adult rats, *in vitro* LTP is more difficult to achieve when the stimulations occur in the deep white matter and responses are recorded in layer III (Kirkwood et al., 1995). The rationale for these differences is that NMDA-dependent LTP in the deeper layers of V1 is under a strict, developmental regulation, with synaptic plasticity progressively declining with age (Kato et al., 1991). The experimental descriptions of sensory cortex LTP *in vivo* are less numerous than in those in slices, although they are not necessarily less informative. LTP was reported in the primary somatosensory cortex of adult rats *in vivo* (Lee & Ebner, 1992). Reports from intact brain experiments also exist for the primary auditory cortex of adult rats (Speechly et al., 2007), which exhibits clear, NMDAR-dependent LTP. Lastly, *in vivo* LTP has also been studied at rat

thalamocortical visual synapses (Heynen and Bear, 2001). Here NMDA-dependent LTP can be achieved by stimulating the lateral geniculate nucleus (LGN) of the thalamus, while recording with electrodes in V1. Importantly, thalamocortical visual fibers readily exhibit LTP even in adult animals, a finding that contrasts with many *in vitro* studies demonstrating a loss of LTP with postnatal maturation (Komatsu et al., 1998; Yoshimura et al., 2003).

#### **1.4 The functions of LTP in the primary visual cortex**

The evidence provided above demonstrates that LTP can be readily induced at V1 synapses. However, at present, there is only limited information regarding the functional roles of LTP in V1 in various types of learning and memory storage occurring at synapses throughout this part of the rodent neocortex. Fortunately, the visual system of the developing and adult rat has been thoroughly investigated, providing some clues to the possible functionality of LTP in V1.

In slices of brains perfused with aldehyde, the V1 of rats is about 1490  $\mu\text{m}$  in thickness and contains a prominent layer IV (granular), with lower-density layers II/III and V above and below (supragranular and infragranular), respectively. The lateral part of V1 contains neurons that respond to binocular vision, while the medial V1 processes monocular vision (Adams and Forrester, 1968; Montero, 1973; Thurlow and Cooper, 1988). Receptive field properties of neurons from the rat V1 (Montero, 1981; Parnavelas et al., 1981) indicate that over 80% of the cells are orientation selective. Neurons here can be classified as simple, complex, or hypercomplex, the latter properties only appearing in pyramidal cells. Orientation-selective neurons are intermingled throughout V1 (Ohki et al. 2005), as opposed to following the pinwheel patterns observed in higher mammals. This arrangement makes it less complicated to record visually evoked potentials (VEP) in response to visual stimuli. Ablation of the V1 in rats results in the modest deterioration of spatial acuity when measured behaviorally (Dean, 1981), an

observation suggesting that the contribution of V1 information processing to certain aspects of vision could be considered minor. The properties of neurons in V1 also suggest that this area of the neocortex seems to be mainly involved in acuity mechanisms, rather than primary pattern and form analyses.

With these V1 functional properties in mind, there exists evidence of LTP-like changes at synapses between the LGN and V1 following perceptual learning, a form of learning that improves the perception of basic visual features such as orientation, motion, and contrast. When rodents are presented with high-contrast visual stimuli depicting gratings of a specific orientation, increases in VEPs recorded in V1 can be detected following several days of repeated stimulus presentation. The enhancement in cortical VEP response is orientation (i.e., input) specific and has been termed "stimulus-selective response potentiation" (SRP). This plasticity phenomenon in V1 has also been shown to depend on mechanisms shared with classical NMDAR-dependent LTP (Frenkel et al., 2006; Cooke and Bear, 2013). SRP is sensitive to NMDA receptor antagonists or the presence of a peptide that prevents AMPA receptor insertion into the neuronal membrane (Frenkel et al., 2006). Remarkably, visual SRP can occlude subsequent electrically induced thalamocortical LTP, while the induction of LTP using TBS can also occlude SRP (Cooke and Bear, 2010). Similarly, Sale et al. (2011) demonstrated that rats trained to visually discriminate between specific visual stimuli containing gratings of different spatial frequency results in the enhancement of VEPs at V1 synapses in layer II/III, making these connections resistant to further strengthening by subsequent TBS.

Although the evidence for LTP occurring at V1 synapses during perceptual learning is correlational, there are good arguments for the striking similarity of cellular mechanisms recruited during various types of learning and following TBS-generated LTP. For brain areas outside of V1, there exists additional, compelling evidence of LTP-like changes following

behavioral training, such as motor learning and inhibitory avoidance training (Rioult-Pedotti et al., 2000; Whitlock et al., 2006). Overall, the establishment of a direct overlap between synaptic strengthening that occurs during LTP induction and during learning/memory has been difficult to delineate, partly because various learning processes can be encoded by widely distributed synaptic alterations throughout the brain (Cooke and Bear, 2010). However, as summarized above, there is now strong evidence of mechanistic parallels between the synaptic modifications that mediate learning and LTP.

### **1.5 Neuromodulation and metaplasticity of LTP at the visual cortex**

The neurochemical milieu, which is related to behavioral state, can influence the magnitude of LTP at cortical synapses. The presence of certain neurotransmitters/neuromodulators at the time of plasticity induction may enhance or impair LTP; this is commonly referred to as “neuromodulation”. The same synapses can also be influenced by particular experiences, which occurred minutes or even days prior to the induction of LTP. Importantly, these experiences may prime synapses for subsequent plasticity induction, without themselves eliciting detectable changes of synaptic strength (at the time of the priming event). This concept is referred to as metaplasticity, or the “plasticity of synaptic plasticity” (see Figure 1.1; Abraham & Bear, 1996).

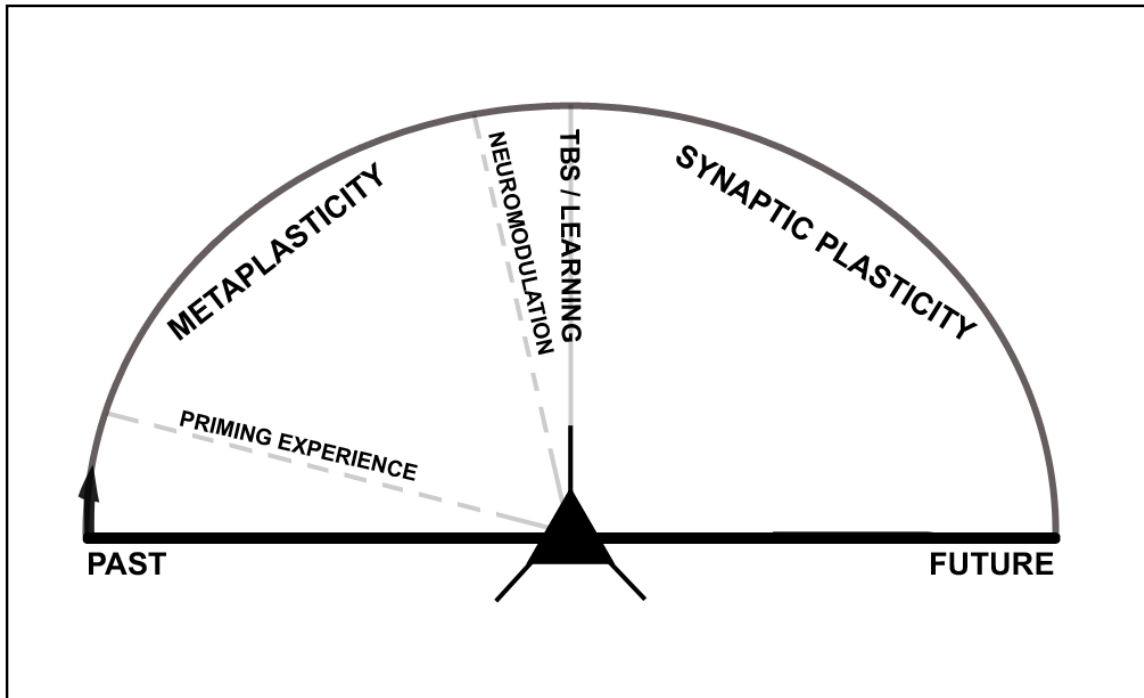


Figure 1.1. A space-time schematic of neuromodulation and metaplasticity at neuronal synapses. Neuromodulation by various factors present around the time of plasticity induction (TBS; theta-burst stimulation) can influence the amount of LTP (synaptic plasticity) expressed at surrounding pyramidal cell (black triangle) synapses. Alternately, previous experiences can prime synapses (metaplasticity) for subsequent LTP induction (by TBS or learning) without detectably altering synaptic strength at the time of priming. Time is represented as past, present, future (left, middle, right; respectively). Adapted from Abraham, 2008.

The cells in V1 are innervated by various neuromodulatory transmitters (e.g., acetylcholine, serotonin, noradrenaline, histamine), which are released by the axon terminals of neurons whose cell bodies are located outside of the cortical visual areas. For example, the cell bodies of neurons that innervate V1 with acetylcholine are located in the nucleus basalis magnocellularis or the diagonal band of Broca of the basal forebrain (Mesulam et al., 1983; Carey & Rieck, 1987; Laplante et al., 2005). The induction threshold and magnitude of LTP in V1 is sensitive to neurochemical changes occurring at, or in the vicinity of the synapse. Various factors, including neurotransmitters present around the time of induction (e.g., acetylcholine, histamine;

Dringenberg et al., 2007; Kuo & Dringenberg, 2008) can interact with parts of the cellular machinery that regulates the induction, expression, and maintenance of LTP. Previous work *in vivo* has shown that the release of endogenous acetylcholine through stimulation of the basal forebrain facilitates LTP in V1 (Dringenberg et al., 2007; Gagolewicz & Dringenberg, 2009). This observation compliments earlier studies *in vitro*, where acetylcholine enhanced LTP in slices of V1 (Bröcher et al., 1992; Gu, 2003). The visual cortex also receives neuromodulatory innervation from the serotonergic system (Smith & Sweet, 1978). Neurons containing the neurotransmitter 5-hydroxytryptamine (5-HT), or serotonin, have been shown to project throughout various brain areas, including V1 (Papadopoulos et al., 1987; Bennett-Clarke et al., 1991; Koh et al., 1991). The serotonergic fiber inputs to V1 are heavily branched and can diffusely release 5-HT in all cortical layers to affect receptors on pyramidal cells and interneurons (Gu & Singer, 1995; Bunin & Wightman, 1998). Consequently, 5-HT receptors are present in the visual cortex, even though they are not as abundant as in other brain areas, for example the prefrontal cortex or the hippocampus (Wright et al., 1995; Barnes & Sharp, 1999). The 5-HT receptors are classified into seven families (i.e., 5-HT<sub>1</sub>-5-HT<sub>7</sub>), and these comprise a total of fourteen receptor subtypes (Humphrey et al., 1993). The intricacies of serotonergic innervation and receptor diversity have made it difficult to agree on the functions of 5-HT and different 5-HT receptors in the neuromodulation of LTP in the visual cortex. Evidence from *in vitro* work suggests that 5-HT can facilitate or inhibit LTP in the V1 of mature rats (Park et al., 2012; Edagawa et al., 1998a). The inhibition of LTP was suggested to be a developmental phenomenon, which is supported by evidence of increasing 5-HT levels in visual cortex of rats as they mature towards adulthood (Edagawa et al., 2001). Overall, the role of 5-HT in the neuromodulation of LTP at V1 synapses *in vivo* is currently not well understood and needs to be assessed in more detail.

NMDAR-containing synapses in V1 are not only sensitive to the effects of neuromodulators at the time of plasticity induction, but also keep a molecular signature of levels of prior activity occurring at these synapses. Until recently, these changes have been difficult to detect, especially since they are not easy to notice until subsequent plasticity induction (for example LTP). One form of metaplasticity that exists in the visual cortex depends on the functioning of the NMDA receptor and, more specifically, the combination of different receptor subunits that comprise functional NMDARs. The NMDAR consists of four subunits: there are two compulsory GluN1 subunits, and two variable GluN2 subunits that are regulated by various factors, including age. The regulated GluN2 subunits can be a mixture of GluN2A, GluN2B, GluN2C and (to a lesser extent) GluN2D, thus allowing for a combination of different types of NMDARs to appear at the synapse (Seeburg, 1993; Mori & Mishina, 1995). The precise grouping of GluN1 and GluN2 subtypes within the heterotetrameric NMDAR determines the functional characteristics of the complete complex. Interestingly, receptors containing GluN2B subunits conduct more  $\text{Ca}^{2+}$  upon opening, due to longer durations of channel opening upon NMDAR activation by glutamate (Monyer et al., 1994). The proportion of GluN2B subunits that make up complete NMDARs increases throughout early development, and subsequently remains relatively stable. GluN2A subunits follow a profound developmental increase into adulthood (Yoshimura et al., 2003). In the developed, adult brain, experience-dependent modifications of NMDAR subunits appear to be driven by alterations to GluN2B levels, as opposed to GluN2A (He et al., 2006). So, how do NMDAR subunits contribute to metaplasticity in V1? Synapses containing a higher ratio of GluN2B-containing NMDARs are capable of conducting more  $\text{Ca}^{2+}$ . Therefore, a higher proportion of GluN2B subunits due to previous priming should facilitate subsequent LTP induction. Indeed, dark rearing of postnatal rats facilitates subsequent LTP obtained in slices of V1, an effect that is mediated by GluN2B subunits (Kirkwood et al., 1995, 1996). Similarly in

adult rats *in vivo*, induction thresholds for LTP in V1 can be reduced by as little as 12 hours of dark exposure (Manahan-Vaughan, 2007). Further, Kuo & Dringenberg (2009) reported that 5 hours of dark exposure was sufficient to achieve thalamocortical LTP in V1 using weak induction protocols that would otherwise be ineffective. These data, together with evidence demonstrating increases in GluN2B subunits following light deprivation (Chen & Bear, 2006), are suggestive of a role of this NMDA subunit in metaplastic regulation of synaptic plasticity in V1. In contrast, experimental evidence utilizing transgenic mice suggests that this may not be the case by highlighting that GluN2A subunits may be important for experience-dependent modifications of LTP induction thresholds. Mice lacking the GluN2A subunit show disruptions in experience-dependent metaplasticity (Philpot et al., 2007). Similarly, when the GluN2A subunit is deleted and metaplasticity is disrupted, visual stimulus selectivity is attenuated in the visual cortex (Fagiolini et al., 2003). Under certain conditions, dark rearing rodents can also result in elevated levels of both GluN2A and GluN2B subunits (Tropea et al., 2006). Clearly, the description of NMDAR-experience-dependent metaplasticity is incomplete.

One suggested function of NMDAR-mediated metaplasticity is to maintain synapses within a proper operating range by preventing their extinction or oversaturation in response to insufficient or excessive prior network activity, respectively. The most current theory of experience-dependent metaplasticity is based on the Bienenstock, Cooper, and Munro (BCM) theory (Bienenstock et al., 1982). It suggests that the thresholds for the induction of LTP (or the opposing process of long-term depression, LTD), are controlled by the history of neuronal activity at those synapses. Accordingly, periods of low network activity shift the threshold of plasticity induction in favor of strengthening or LTP. Conversely, when previous network activity is high, subsequent strengthening (LTP) is more difficult to achieve. This model of metaplasticity incorporates changes at NMDARs, suggesting that deprivation leads to increased levels/function

of GluN2B subunits, resulting in the facilitation of LTP (Kind, 1999). On the other hand, increases in network activity result in increased GluN2A subunits, leading to impairments of LTP. One possible issue with this theory is that observations exist supporting metaplastic-facilitation of LTP in V1 following certain types of sensory experience (Sale et al., 2007; Mainardi et al., 2010; Hager & Dringenberg, 2010). However, it is currently unknown if these types of experience-related changes in plasticity properties can be attributed to NMDAR-mediated metaplasticity.

Overall, there is clear evidence to suggest that visual experiences can act to modify the composition of NMDARs in V1, even though the details and specific contributions of various NMDAR subunits to metaplasticity are not fully understood and require further investigation.

## **1.6 Objectives**

The goal of the following chapters was to examine mechanisms of neuromodulation and metaplasticity of LTP in the V1 of adult rats. I will describe the role of 5-HT in the modulation of thalamocortical LTP *in vivo* through pharmacological and electrophysiological characterization. The effects of 5-HT on synaptic plasticity in V1 have been well documented *in vitro*, although the overall interpretation of the results is complex, and the experimental evidence *in vivo* is sparse. Importantly, this thesis also examined the role of experience-dependent metaplasticity following visual discrimination training using behavioral, electrophysiological (*in vivo* and *in vitro*) and pharmacological characterization. The possible link between V1 metaplasticity and sequential learning, within the context of a visual discrimination task, were also explored.

Chapter 2 examines the role of the neuromodulator 5-HT in gating synaptic plasticity (LTP) in the thalamocortical fiber pathways projecting to V1. The third chapter explores the roles of learning a visual discrimination task on subsequent synaptic plasticity (LTP) induction *in vivo*, focusing on the metaplasticity operating at thalamocortical fibers. The fourth chapter expands on the findings from chapter three, further characterizing the metaplastic-changes *in vitro* in V1 pyramidal neurons of adult rats. The fifth chapter evaluates the behavioral roles of metaplastic-changes at NMDA receptors (previously described in chapter 3 and chapter 4), in the sequential learning of visual discrimination tasks. Together, the evidence presented here advances our understanding of the regulation of cortical synaptic plasticity by the neurochemical milieu and prior experience, acting via metaplastic mechanisms, which may also serve to adjust behavior and learning within a dynamic, constantly changing sensory environment.

## Chapter 2

### **Age-dependent switch of the role of serotonergic 5-HT<sub>1A</sub> receptors in gating long-term potentiation in the rat visual cortex in vivo**

*Published in its entirety in “Age-Dependent Switch of the Role of Serotonergic 5-HT<sub>1A</sub> Receptors in Gating Long-Term Potentiation in Rat Visual Cortex In Vivo” by Gagolewicz, P. J., & Dringenberg, H. C. (2016). Neural Plasticity, 2016, 1-11.*

#### **2.1 Abstract**

The rodent primary visual cortex (V1) is densely innervated by serotonergic axons and previous in vitro work has shown that serotonin (5-HT) can modulate plasticity (e.g., long-term potentiation (LTP)) at V1 synapses. However, little work has examined the effects of 5-HT on LTP under in vivo conditions. We examined the role of 5-HT on LTP in V1 elicited by theta-burst stimulation (TBS) of the lateral geniculate nucleus in urethane-anesthetized (adult and juvenile) rats. Thalamic TBS consistently induced potentiation of field postsynaptic potentials (fPSPs) recorded in V1. While 5-HT application (0.1-10mM) itself did not alter LTP levels, the broad-acting 5-HT receptor antagonists methiothepin (1mM) resulted in a clear facilitation of LTP in adult animals, an effect that was mimicked by the selective 5-HT<sub>1A</sub> receptor antagonist WAY 100635 (1mM). Interestingly, in juvenile rats, WAY 100635 application inhibited LTP, indicative of an age-dependent switch in the role of 5-HT<sub>1A</sub> receptors in gating V1 plasticity. Analyses of spontaneous electrocorticographic (ECoG) activity in V1 indicated that the antagonist-induced LTP enhancement was not related to systematic changes in oscillatory activity in V1. Together, these data suggest a facilitating role of 5-HT<sub>1A</sub> receptor activation on LTP in the juvenile V1, which switches to a tonic, inhibitory influence in adulthood.

## 2.2 Introduction

Long-term potentiation (LTP), a form of brain plasticity characterized by a long-lasting increase in synaptic coupling of neurons, has been suggested as a candidate mechanism mediating processes of learning and memory in the nervous system (Bliss & Collingridge, 1993; Martin & Morris, 2002). First characterized in the hippocampal formation (Bliss & Lomo, 1973), LTP has now been shown to occur at synapses throughout the nervous system, including cortical sensory areas such as the primary visual (V1), auditory, and somatosensory cortices (Tsumoto, 1992; Bennett, 2000; Malenka & Bear, 2004). In V1, LTP has been successfully induced under both *in vitro* and *in vivo* conditions, with work in slice preparations indicating that LTP is limited to a well-defined time window during early postnatal life, after which V1 synapses become resistant to LTP induction (Komatsu et al., 1988; Kojic et al., 1997; Kojic et al., 2000; Kim et al., 2006; Vetencourt et al., 2008; Park et al., 2012). Interestingly, under *in vivo* conditions, LTP is readily induced in the fully matured V1 of adult rodents (Heynen & Bear, 2001; Dringenberg et al., 2007; Gagolewicz & Dringenberg, 2011), indicative of some fundamental differences in the induction of LTP in V1 between *in vivo* and *in vitro* conditions.

An important aspect of LTP regulation lies in the role of various neuromodulators present in the extracellular environment. For example, acetylcholine (ACh) exerts a powerful, modulatory effect by enhancing LTP in V1, an effect that is apparent under both *in vitro* and *in vivo* conditions and for a number of different (e.g., weak and strong) LTP induction protocols (Bröcher et al., 1992; Kojic et al., 2001; Dringenberg et al., 2007). A similar, facilitating effect on LTP is also seen with histamine application directly in V1 of rats *in vivo* (Dringenberg & Kuo, 2008), highlighting the importance of a variety of neuromodulators as gating mechanism for the induction of plasticity at cortical synapses (Gu, 2002; Gu, 2003; Dringenberg & Kuo, 2008).

The central serotonergic (5-hydroxytryptamine, 5-HT) system has also been implicated in the modulation of cortical synaptic plasticity. Serotonergic fibers originating in the dorsal and median raphe nuclei provide a dense innervation of the entire forebrain, including V1 (Papadopoulos et al., 1987; Bennett-Clarke et al., 1991; Koh et al., 1991; Jacobs and Azmitia, 1992). The presence of 5-HT receptors in V1 has been confirmed by radioligand-binding experiments (Dyck & Cyander, 1993; Rakic & Lidow, 1995) and in situ hybridization techniques (Wright et al., 1995), implying functionality of the 5-HT input to V1.

Previous work on the role of 5-HT in the regulation of LTP in V1 has yielded inconsistent results. In V1 slices obtained from kittens (40-80 days old), 5-HT application facilitated LTP induction in layer 4 neurons, effects that were absent in older (more than 120 days) animals (Kojic et al., 1997; Kojic et al., 2000). Similarly, in the immature rat V1 *in vitro*, LTP of layer 2/3 neurons elicited by layer 4 stimulation was impaired by 5-HT depletion or bath application of antagonists of 5-HT<sub>1A</sub> or 5-HT<sub>2</sub> receptors (Inaba et al., 2009). Together, these observations suggest a facilitating role for 5-HT in LTP induction in the immature V1, findings that have recently been extended to more mature (8-10 week old) rats (Park et al., 2012).

Surprisingly, others have reported an inhibition of LTP in layers 2/3 of V1 slices obtained from juvenile rats following bath application of 5-HT (Edagawa et al., 1998a; Park et al., 2012). These results have led to the suggestion that the developmentally increasing serotonergic tone in V1 is responsible for the loss of LTP throughout cortical maturation (Edagawa et al., 2001; Kim et al., 2006), at least under *in vitro* conditions (see above). However, it is unclear how to reconcile this assumption with data demonstrating serotonergic facilitation of LTP in the more mature V1 (Park et al., 2012), as well as observations that both LTP and ocular dominance plasticity in V1 can be reinstated in adult rodents following treatment with the selective serotonin reuptake

inhibitor (SSRI) fluoxetine (Vetencourt et al., 2008).

The above summary of prior work suggests that the role of 5-HT in the regulation of LTP in V1 is not fully understood, and may also depend on the age and developmental status of the animal. Further, there is a clear lack of information regarding serotonergic effects on LTP assessed in the intact brain *in vivo*, which appears important, given the differences in LTP induction in the mature V1 between *in vivo* and *in vitro* preparations (see above). To clarify some of these unresolved issues, we examined the role of 5-HT and some 5-HT receptors in the induction of LTP in the V1 of juvenile and adult rats using intact, urethane-anesthetized animals, an experimental preparation that continues to express LTP well into adulthood (Heynen & Bear, 2001; Dringenberg et al., 2007; Gagolewicz & Dringenberg, 2011).

## **2.3 Materials and methods**

### **2.3.1 Subjects**

All procedures adhered to the guidelines of the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee. Experiments were conducted on adult (300-550 g; > 70 days old) or juvenile (190-240 g; mean age: 45 days, age range: 42-48 days) male Long-Evans rats (Charles River Laboratories, Saint-Constant, QC, Canada). The animals were housed in a colony room under a reversed 12:12-h light cycle (lights on at 19:00 h), with water and food access ad libitum. Each animal was used for only one experiment. All efforts were made in order to minimize animal suffering and the number of animals employed for these experiments.

### **2.3.2 Surgery**

Each animal was deeply anesthetized with 2.0 g/kg urethane (Sigma-Aldrich, Oakville, ON, Canada), administered intraperitoneally (i.p.) as four doses of 0.5 g/kg each, given every 20 min. Additional 0.5 g/kg supplements of urethane were administered when necessary. Fifteen minutes prior to the start of surgery, the local analgesic bupivacaine (Marcaine; Hospira Healthcare Corporation, Montreal, QC, Canada) was administered subcutaneously to the skin and tissue along the incision line over the skull (two or three injections; total of 5 mg/kg). Throughout the surgical procedure and experiment, body temperature was monitored with a rectal thermometer and maintained at  $37 \pm 1^\circ\text{C}$  by means of an electrical heating pad and fleece insulating blankets surrounding the body. After anesthesia induction, a rat was placed in a stereotaxic apparatus, the skull bone was exposed, and small holes were drilled overlying the following areas (all measurements taken from bregma and the horizontally level skull surface): lateral geniculate nucleus (LGN), anterior-posterior  $-4.1$  mm, lateral  $+4.1$  mm, and ventral  $-4.8$  to  $-5.1$  mm; V1, anterior-posterior  $-7.6$  mm, lateral  $+3.6$  mm, and ventral  $-0.8$  to  $-1.2$  mm. Two additional holes were drilled in the bone overlying the left and right cerebellum to secure jewelry screws, which served as ground and reference connections. A concentric, bipolar stimulation electrode (SNE-100; Rhodes Medical Instruments, David Kopf Instruments, Tujunga, CA) was lowered into the LGN, while a monopolar recording electrode (125  $\mu\text{m}$  diameter Teflon-insulated stainless steel wire) was placed in the superficial layers of V1. The final, ventral depth of both electrodes was adjusted to yield maximal amplitude field postsynaptic potentials (fPSPs) recorded in V1 in response to single-pulse LGN stimulation.

### **2.3.3 Electrophysiology**

Stimulation of the LGN (single 0.2 ms pulses) was achieved by connecting the stimulation electrode to a stimulus isolation unit (ML 180 Stimulus Isolator; AD Instruments, Toronto, ON, Canada) providing a constant current output. The fPSPs in V1 were recorded

differentially, with the recording electrode referenced against a screw in the bone overlying the cerebellum. The V1 signal was amplified (half-amplitude filters at 0.3 Hz to 1 kHz), digitized (10 kHz) by an A/D converter (PowerLab 4/s system running Scope software v. 3.6.5; AD Instruments), and stored for offline analysis.

For each rat, an input-output curve was established by stimulating the LGN at increasing intensities (0.1-1.0 mA in 0.1 mA increments) and the intensity yielding approximately 50-60% of the maximal fPSP amplitude was then used for the remainder of the experiment (see Fig. 2.1).

Cortical fPSPs were recorded every 30 s until 30 min of stable baseline recordings were achieved ( $\leq 5\%$  difference between successive data points for fPSPs averaged over 10 min epochs). Subsequently, theta burst stimulation (TBS) was delivered to the LGN, consisting of five single pulses (at 100 Hz) per burst, with bursts repeated at 5 Hz for a total of 10 bursts (pulse intensity and duration were the same as stated above). Recordings of fPSPs (every 30 sec) continued for 2 h following TBS delivery.

In all experiments, spontaneous electrocorticographic (ECoG) activity was also recorded through the same V1 electrode used for fPSP recordings. For the ECoG, the cortical signal was digitized (200 Hz), band-pass filtered (0.3-50 Hz), and analyzed offline for peak power in the main frequency bands (low delta, 0.5-1 Hz; delta, 1-4 Hz; theta, 4-8 Hz; alpha, 8-12 Hz; beta, 12-20 Hz; and gamma, 20-40 Hz). The ECoG was sampled (5 sec epochs) prior to the onset of fPSP baseline recordings and at the end of experiment, i.e., 120 min after TBS).

#### **2.3.4 Pharmacology**

To investigate the roles of 5-HT and different 5-HT receptors, independent groups of animals received one of the following drug treatments: 5-hydroxytryptamine hydrochloride (5-HT; 0.1 or 10 mM; Sigma- Aldrich); *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-

pyridinylcyclohexanecarboxamide maleate (WAY 100635; 1 mM; Tocris Bioscience, Ellisville, MO, USA); 1-[10,11-Dihydro-8- (methylthio)dibenzo(Z)[b,f]thiepin-10-yl]-4-methylpiperazine maleate (methiothepin; 1 mM; Tocris Bioscience); 8-Hydroxy-2-(di-n-propylamino)-tetralin hydrobromide (8-OH-DPAT; 1 mM; Tocris Bioscience). WAY 100635 and 8-OH- DPAT act as potent 5-HT<sub>1A</sub> receptor antagonist and agonist, respectively (Middlemiss & Fozard, 1983; Forster et al., 1995; Fletcher et al., 1996), and 8-OH-DPAT also exhibits an affinity for 5-HT<sub>7</sub> receptors (see Nikiforuk, 2015). Methiothepin is a potent 5-HT<sub>2</sub> receptor antagonist, but also acts as an antagonist at 5-HT<sub>1</sub>, 5-HT<sub>5A</sub>, 5-HT<sub>5B</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors (Hoyer et al., 1994; Bard et al., 1996). All compounds were dissolved in artificial cerebrospinal fluid (aCSF), consisting of: 118.3 mM NaCl, 4.4 mM KCl, 1.2 mM MgSO<sub>4</sub>, 1.0 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub>, 22.1 mM NaHCO<sub>3</sub>, and 10.0 mM glucose, with the exception of methiothepin, which was dissolved in either a mixture of aCSF and dimethyl sulfoxide (25 % DMSO; n = 5), saline (n = 4), or aCSF and distilled water (n = 4; there were no significant differences in LTP among these different vehicle solutions and rats were combined into a single methiothepin group).

Drugs were applied locally in V1 by means of reverse microdialysis. The dialysis probe (Mab 6.14.2, 15,000-Da cut-off polyether sulfone membrane, outer diameter 0.6 mm; S.P.E. Limited, North York, Ontario, Canada) was mounted immediately adjacent to the V1 recording electrode, with the probe tip extending approximately 1 mm past the electrode tip. Drug concentrations reaching the brain are estimated to be ~10% of the aCSF content within the vicinity of the probe membrane (about 1 mm; Benveniste & Huttemeier, 1990; Oldford & Castro-Alamancos, 2003). The dialysis probe was connected to a 2.5 mL Hamilton syringe using FEP microtubing (S.P.E. Limited). The syringe was driven by a microdialysis pump (CMA 402; CMA Microdialysis, Solna, Sweden) at a flow rate of 1 µL/min, with perfusion beginning 20 min prior to the acquisition of baseline fPSP recordings and continuing throughout the entire experiment.

### **2.3.5 Histology**

At the conclusion of electrophysiological data acquisition, all animals received a supplementary dose (1.0 mL) of urethane and, after 5-10 min, were perfused through the heart with 0.9% saline (~50 mL) followed by 10% formalin (~100 mL). The brains were removed and stored in 10% formalin for a minimum of 24 h before sectioning (40  $\mu$ m slices) with a cryostat. Slices were then mounted onto microscope slides and inspected with a digital microscope to verify electrode placements. Histological inspections and decisions on the accuracy of electrode placements were made by an experimenter who was blind to the results of individual animals. Data from inaccurate placements were omitted from this study (77 and 39 rats included and rejected due to missed placements, respectively).

### **2.3.6 Data analysis**

Cortical fPSPs were analyzed with Scope software (v. 3.6.5; AD Instruments, Colorado Springs, CO). With the electrode configuration employed in the present study, fPSPs elicited in V1 consisted of a predominant, large-amplitude, negative-going component. The amplitude of this component was automatically detected and computed with Scope software (using the Data Pad function) by measuring the voltage difference between the maximal fPSP negativity and the baseline voltage sampled immediately prior to the stimulation artifact. Once individual fPSPs were analyzed in this manner, they were averaged over successive 10-min intervals (i.e., 20 fPSPs/interval) and then normalized by dividing them by the average baseline (pre-TBS) amplitude of each animal. Amplitude data are presented as mean  $\pm$  standard error of the mean (S.E.M.) and were analyzed with a repeated-measures analysis of variance (ANOVA) and followed up, where statistically appropriate, with Bonferroni post-hoc tests or unpaired Student's t-tests using SPSS software (v. 15.0; SPSS, Colorado Springs, CO, USA). The analysis was conducted with time (10-min averaging epochs) as the repeated/within-subjects factor and drug

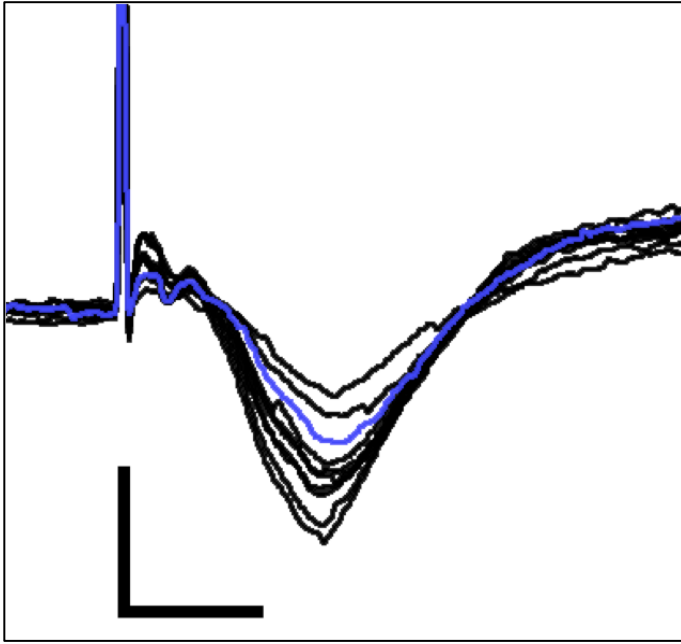
group as the between- subjects factor. The Greenhouse-Geisser correction was applied in all cases when the assumption of sphericity was violated, as tested by the Mauchly's test of sphericity (using SPSS software).

Five-second epochs of ECoG activity were analyzed using Chart software (v. 5. 5. 6; AD Instruments). For each epoch, spectral analysis was used to determine the % of total power contained in each of the following frequency bands: 0.5-1 Hz, 1-4 Hz, 4-8 Hz, 8-12 Hz, 12-20 Hz, and 20-40 Hz. Data from individual experiments were averaged across treatment groups and statistically analyzed by ANOVA using SPSS software.

## **2.4 Results**

### **2.4.1 Characteristics of fPSPs in V1 elicited by LGN stimulation**

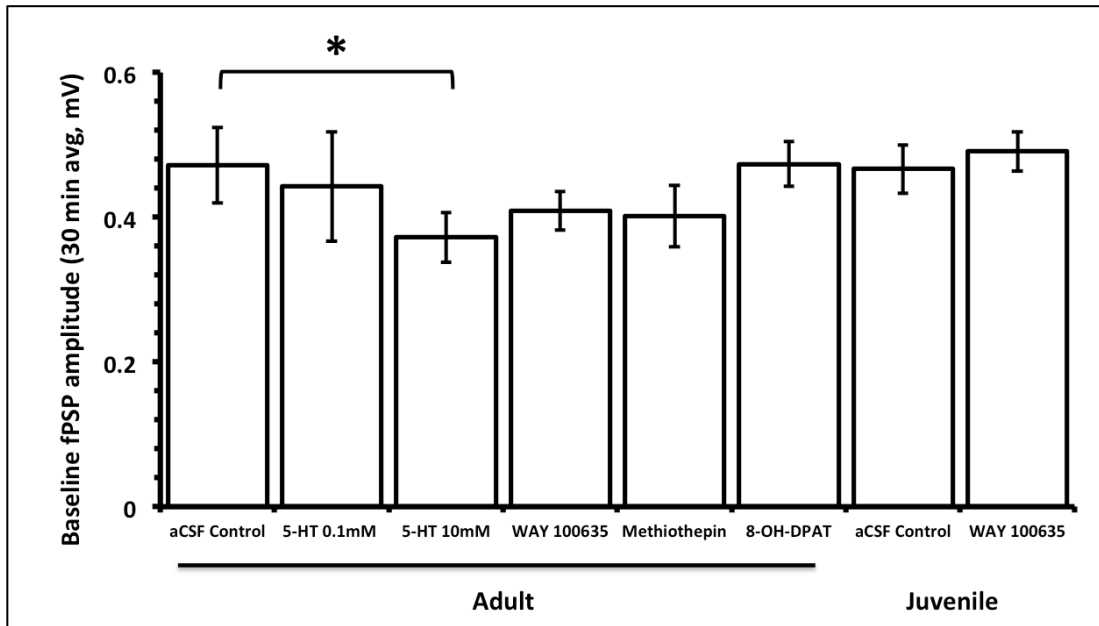
In urethane-anesthetized animals, single-pulse stimulation of the LGN reliably elicited fPSPs in the ipsilateral V1 (Figure 2.1). In agreement with previous work using this experimental preparation (Dringenberg et al., 2007; Dringenberg & Kuo, 2008; Gagolewicz & Dringenberg, 2011), fPSPs were composed mainly of a large amplitude (up to 0.5 mV), negative component, with a latency to peak of 16-18 ms following LGN stimulation (Figure 2.1). These fPSPs appear to reflect, for the most part, excitatory current sinks originating in layers 2/3 of V1 (Heynen & Bear, 2001).



**Figure 2.1.** Typical fPSPs recorded in V1 in response to single-pulse stimulation of the ipsilateral LGN in a urethane-anesthetized rat. fPSPs were recorded during input-output stimulation (0.1 to 1.0 mA stimulation current); note the increase in fPSP amplitude with increasing stimulation intensities. The blue trace (elicited by 0.3 mA) was the intensity used for the subsequent data collection (bottom; calibration bars indicate 0.5 mV vertical and 10 ms horizontal).

#### **2.4.2 Effects of pharmacological treatments on baseline (pre-TBS) fPSP amplitude**

Levels of LTP may be influenced by differences in baseline (i.e., prior to LTP induction) synaptic strength. Thus, we initially computed and compared the baseline amplitude of fPSPs in all experimental groups prior to the delivery of TBS to induce LTP. As shown in Fig. 2.2, for all groups, baseline fPSP amplitudes were in the range of 0.37 to 0.47 mV. Application of the high concentration of 5-HT (10 mM) resulted in a modest, but significant ( $P < 0.05$ , t-test) suppression of fPSP amplitude relative to rats receiving aCSF application in V1. None of the other pharmacological treatments produced a significant change in fPSP amplitude relative to the aCSF condition (Fig. 2.2).

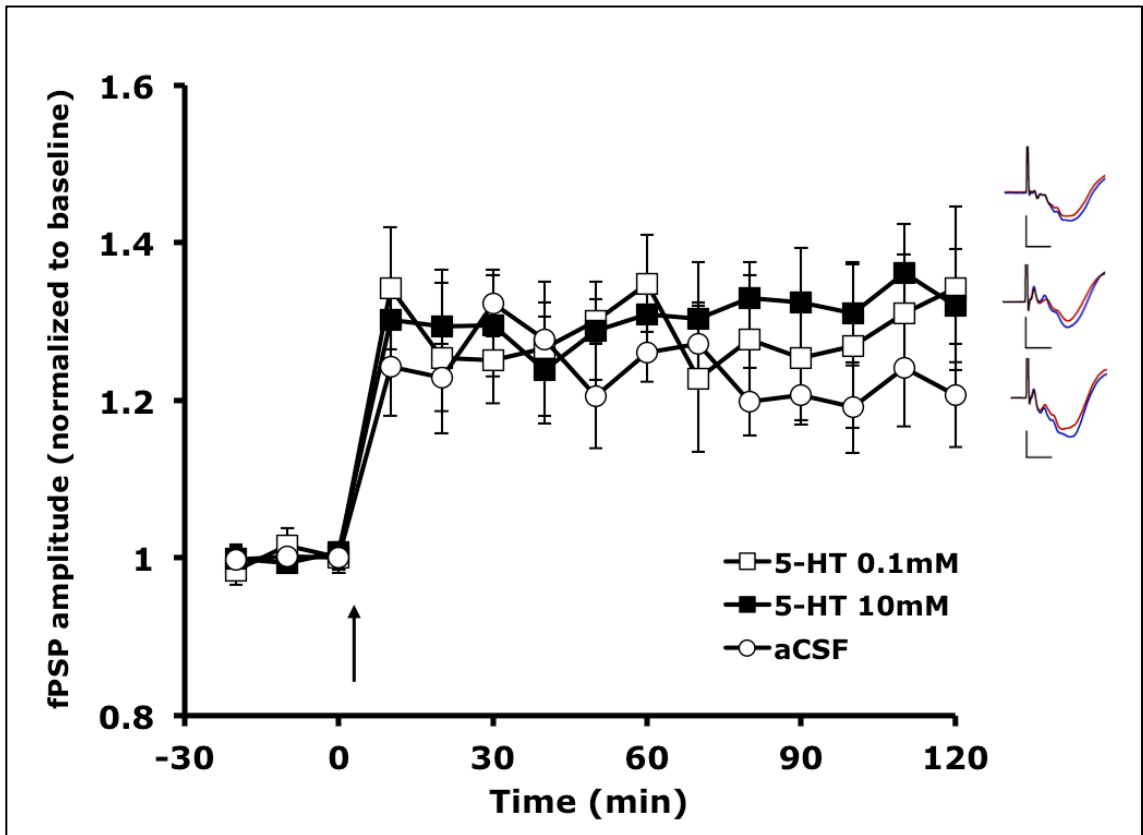


**Figure 2.2.** The effect of application (in V1 by means of reverse microdialysis; application started 20 min prior to the onset of recordings) of either aCSF (adult and immature animals), 5-HT (0.1 and 10 mM; adults), WAY 100635 (1 mM; adults and immature), methiothepin (1 mM; adults), or 8-OH-DPAT (1 mM; adults) on baseline (pre-TBS delivery) amplitude of fPSP recorded in V1. Each bar represents averaged fPSP amplitudes during 30 min (i.e., 60 fPSPs) of baseline recordings. The high concentration of 5-HT (10 mM) resulted in a significant ( $P < 0.05$ ; t-test) depression of fPSP amplitude compared to aCSF application. None of the other pharmacological treatments resulted in a significant change of fPSP amplitudes relative to aCSF application.

#### 2.4.3 Effect of 5-HT on LTP in adult rats

To determine the effects of 5-HT application on LTP, separate groups of adult animals received application of either aCSF, or 5-HT at 0.1 mM or 10 mM. Application occurred locally in V1 by means of reverse microdialysis, with the dialysis probe situated immediately adjacent to the cortical recording electrode. During aCSF application ( $n = 8$ ), TBS of the LGN reliably induced LTP, with fPSP amplitude increasing to  $121 \pm 4\%$  of baseline (Fig. 2.3; all values reported here are averages of the last 30 min of the experiment, i.e., from 91 to 120 min after TBS delivery). During application of 0.1 mM ( $n = 5$ ) or 10 mM of 5-HT ( $n = 7$ ), fPSP amplitude reached  $131 \pm 5\%$  and  $133 \pm 4\%$  of the baseline, respectively (Fig. 2.3). These values appeared

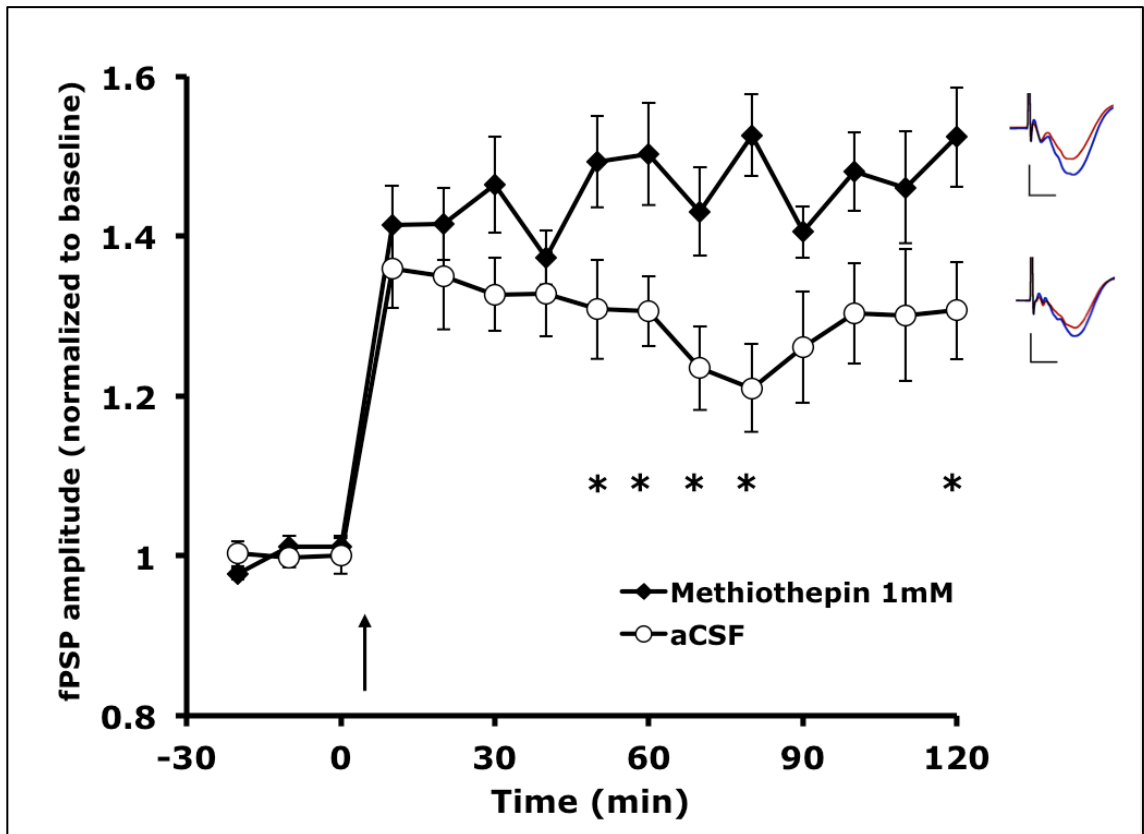
higher than those observed during aCSF application, but this difference did not reach statistical significance, as revealed by the ANOVA showing a significant main effect of time, but not of group ( $F(2, 17) = 0.5, P > 0.6$ ) or a time x group interaction ( $F(10, 88) = 0.91, P > 0.5$ ). Thus, under the present, experimental conditions, 5-HT did not exert a significant influence on LTP induction in the thalamocortical visual system of adult rats.



**Figure 2.3.** The effect of application (in V1 by means of reverse microdialysis) of either aCSF ( $n = 8$ ) or 5-HT (0.1 or 10 mM;  $n = 5$  and 7, respectively) on LTP following TBS (at arrow) of the LGN in urethane-anesthetized rats. Application of 5-HT did not result in any significant changes in LTP relative to rats receiving aCSF. Inserts depict typical fPSPs before (red) and after (blue) LTP induction for animals in the presence of 0.1 mM 5-HT (top), 10 mM 5-HT (middle), or aCSF (bottom; calibration bars indicate 0.5 mV vertical and 10 ms horizontal; each fPSP trace is an average of 30 min of continuous recording).

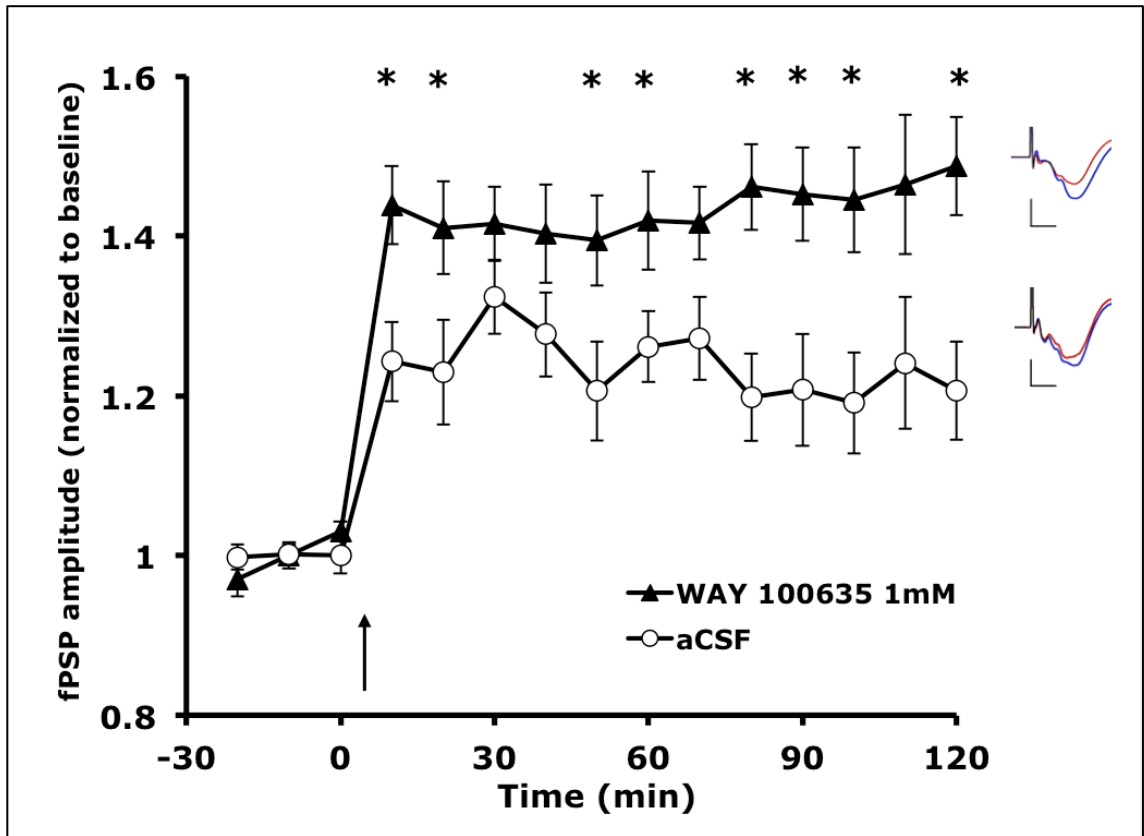
#### 2.4.4 Effect of 5-HT receptor antagonists or a 5-HT<sub>1A</sub> agonist on LTP in adult rats

To examine a possible effect of 5-HT blockade on LTP, we initially applied the broad-acting 5-HT receptor antagonist methiothepin (1.0 mM) in a separate group of adult animals (n = 13). In these rats, TBS of the LGN resulted in an increase of fPSP amplitude to  $149 \pm 3\%$  of baseline during the last 30 min of the experiment (Fig. 2.4). Given that methiothepin was dissolved in several, different vehicle solutions (see Section 2.3.4) a new group of control rats was also examined (n = 11; consisting of aCSF/DMSO, n = 4; saline, n = 3; and aCSF/H<sub>2</sub>O, n = 4). In these rats, fPSP amplitude reached  $130 \pm 4\%$  of baseline during the final 30 min of the experiment (Fig. 2.4). An ANOVA revealed that methiothepin enhanced LTP levels, as indicated by significant main effects of time ( $F(5, 116) = 38.8, P < 0.001$ ) and group ( $F(1, 22) = 6.38, P < 0.02$ ), as well as a significant time x group interaction ( $F(3, 116) = 3.47, P \leq 0.005$ ).



**Figure 2.4.** The effect of application (in V1 by means of reverse microdialysis) of either aCSF/vehicle (n = 11; see text for detail regarding this control group) or methiothepin (1 mM, n = 13) on LTP following TBS (at arrow) of the LGN in urethane-anesthetized rats. Application of methiothepin resulted in a significant increase in LTP relative to control animals. Inserts depict typical fPSPs before (red) and after (blue) LTP induction in the presence of methiothepin (top) and control animals (bottom; calibration bars indicate 0.5 mV vertical and 10 ms horizontal; each fPSP trace is an average of 30 min of continuous recording). \* indicates a significant ( $P < 0.05$ , t-test) difference between the two groups.

In order to further characterize the LTP enhancement seen with 5-HT receptor blockade, we tested the effect of V1 application of WAY 100635 (1 mM, n = 10), a highly selective antagonist at 5-HT<sub>1A</sub> receptors (Edagawa et al, 1998a; Forster et al., 1995). In this group of rats, TBS increased fPSP amplitude to  $146 \pm 4\%$  of baseline (Fig. 2.5). This level of LTP was significantly higher than that in rats receiving application of aCSF (same group as in Fig. 2.3;  $121 \pm 4\%$  of the baseline, n = 8), as indicated by the main

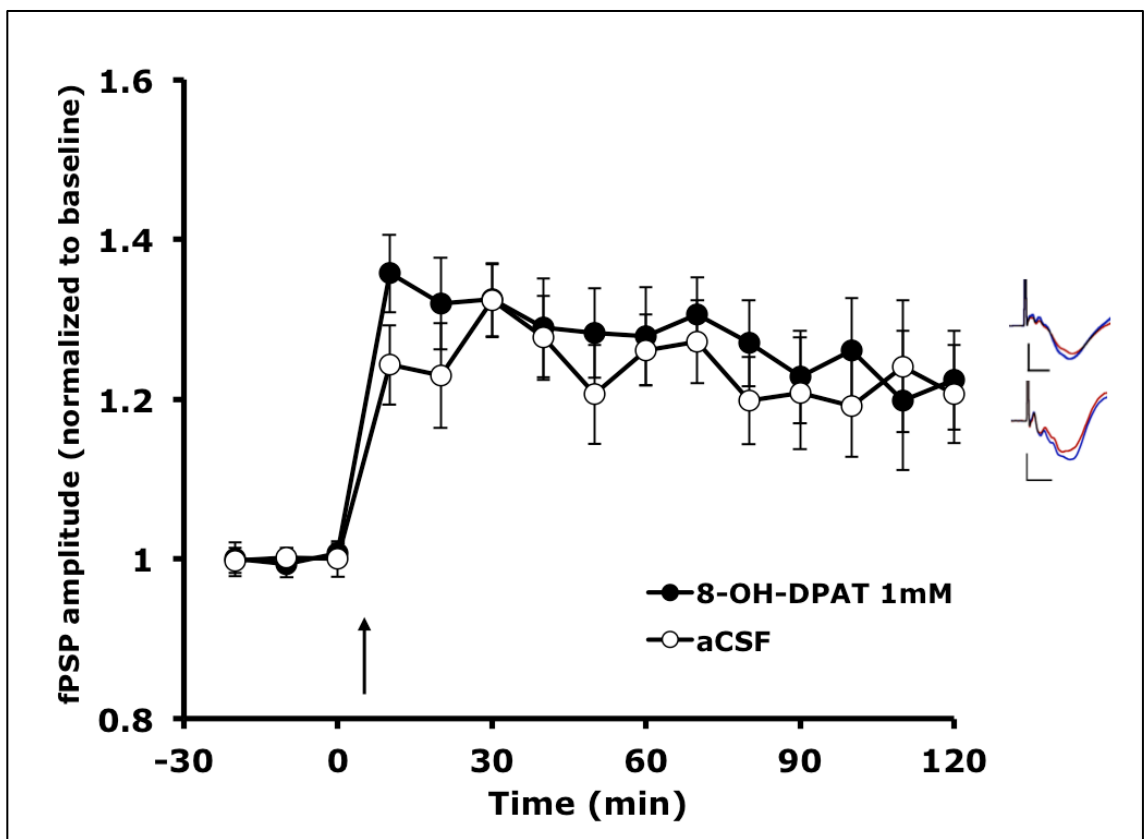


**Figure 2.5.** The effect of application (in V1 by means of reverse microdialysis) of either aCSF (n = 8; same group as in Fig. 3) or WAY 100635 (1 mM, n = 10) on LTP following TBS (at arrow) of the LGN in urethane-anesthetized rats. Application of WAY 100635 resulted in a significant increase in LTP relative to control animals. Inserts depict typical fPSPs before (red) and after (blue) LTP induction in the presence of WAY 100635 (top) and aCSF (bottom; calibration bars indicate 0.5 mV vertical and 10 ms horizontal; each fPSP trace is an average of 30 min of continuous recording). \* indicates a significant ( $P < 0.05$ , t-test) difference between the two groups.

effects of time ( $F(5, 88) = 22.9$ ,  $P < 0.001$ ) and group ( $F(1, 16) = 10.51$ ,  $P < 0.005$ ), and by a time x group interaction ( $F(5, 88) = 2.75$ ,  $P \leq 0.02$ ).

Based on the results reported above, it appears that 5-HT<sub>1A</sub> receptors exert an inhibitory effect over LTP induction in the mature V1. To further examine the role of these receptors, we applied the highly selective 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT in V1 (1 mM, n = 8). In these animals, TBS elicited LTP, with fPSP amplitude reaching  $123 \pm 3\%$  of baseline during the last 30

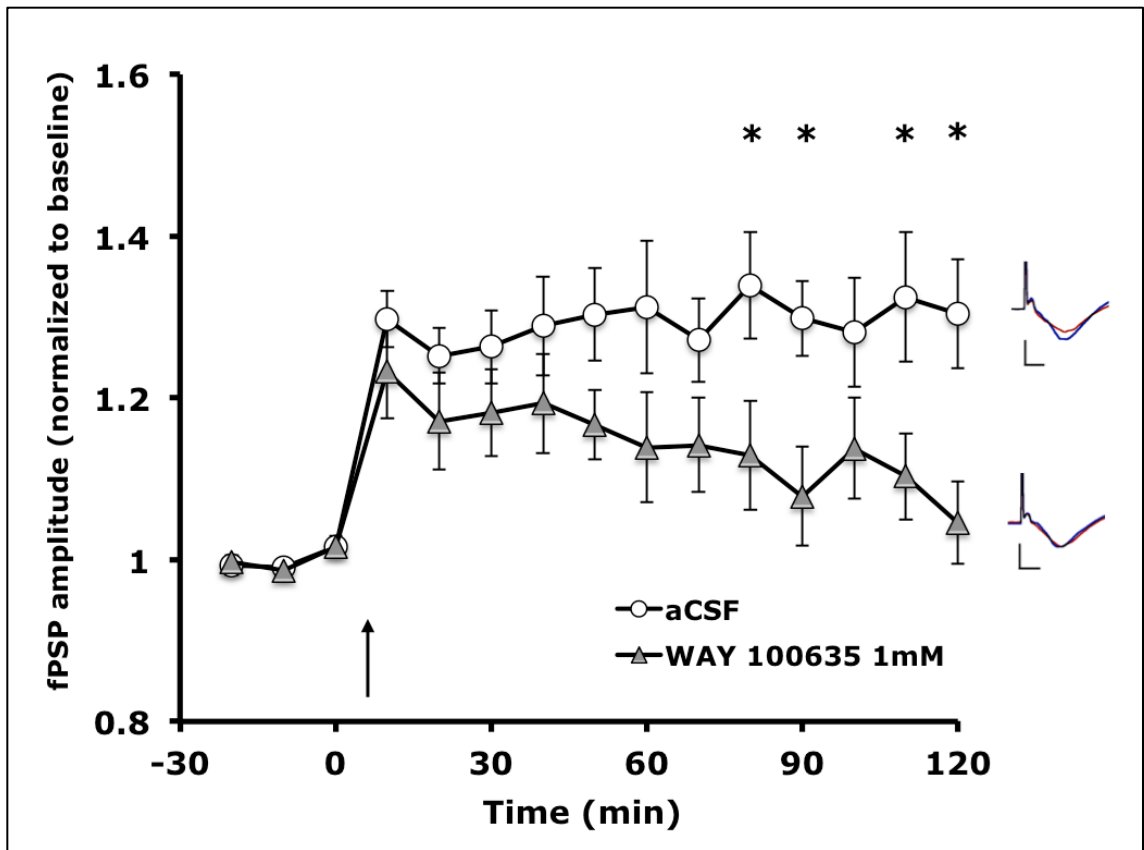
min of the experiment (Fig. 2.6). These values were not significantly different from those in rats receiving aCSF application (same group as in Fig. 2.3), as indicated by a significant main effect of time ( $F(5, 68) = 20.8, P < 0.001$ ), but not of group ( $F(1, 14) = 0.7, P = 0.4$ ), and no significant time x group interaction ( $F(5, 68) = 0.8, P = 0.6$ ). Thus, blockade of 5-HT<sub>1A</sub> receptors facilitates LTP, but 5-HT<sub>1A</sub> receptor activation does not result in further inhibition of LTP, similar to the effects noted above with direct 5-HT application in V1.



**Figure 2.6.** The effect of application (in V1 by means of reverse microdialysis) of either aCSF ( $n = 8$ ; same group as in Fig. 2.3) or 8-OH-DPAT (1 mM,  $n = 8$ ) on LTP following TBS (at arrow) of the LGN in urethane-anesthetized rats. Application of 8-OH-DPAT did not result in a significant change in LTP relative to animals receiving aCSF. Inserts depict typical fPSPs before (red) and after (blue) LTP induction in the presence of 8-OH-DPAT (top) and aCSF (bottom; calibration bars indicate 0.5 mV vertical and 10 ms horizontal; each fPSP trace is an average of 30 min of continuous recording).

#### 2.4.5 Effect of 5-HT<sub>1A</sub> blockade on LTP in juvenile rats

Considerable evidence from *in vitro* experiments suggest that 5-HT plays a role in developmental plasticity, including the timing and closure of the “critical period” for plasticity in the rodent V1 (Bennett-Clarke et al., 1991; Dyck & Cyander, 1993; Kojic et al., 1997; Kojic et al., 2000; Kojic et al., 2001; Inaba et al., 2009; Park et al., 2012). Thus, we also investigated the effects of 5-HT<sub>1A</sub> receptor blockade by WAY 100635 application (1 mM) in a group of juvenile animals (age range: 42-48 days). In juvenile control animals (n = 7; mean age 44 days) receiving aCSF application in V1, TBS resulted in LTP, with fPSP amplitude at  $130 \pm 7\%$  of the baseline during the final 30 min of the experiment (Fig. 2.7). Surprisingly, in the presence of WAY 100635, juvenile rats (n = 8; mean age 44.9 days) showed reduced LTP, with fPSP amplitude at  $110 \pm 6\%$  of baseline during the last 30 min of the experiment (Fig. 2.7). Levels of LTP in these two groups of juvenile rats were different, as highlighted by a main effect of time ( $F(4, 47) = 14.4, P < 0.001$ ) and a significant time x group interaction ( $F(4, 47) = 2.984, P < 0.03$ ), even though the main effect of group only approached statistical significance ( $F(1,13) = 4.3, P = 0.058$ ). Together, these observations suggest that there is a developmental switch in the role of 5-HT<sub>1A</sub> receptors in regulating V1 plasticity, with 5-HT<sub>1A</sub> receptor activation promoting plasticity in the juvenile V1, while assuming an inhibitory role in V1 of adult, fully matured animals.



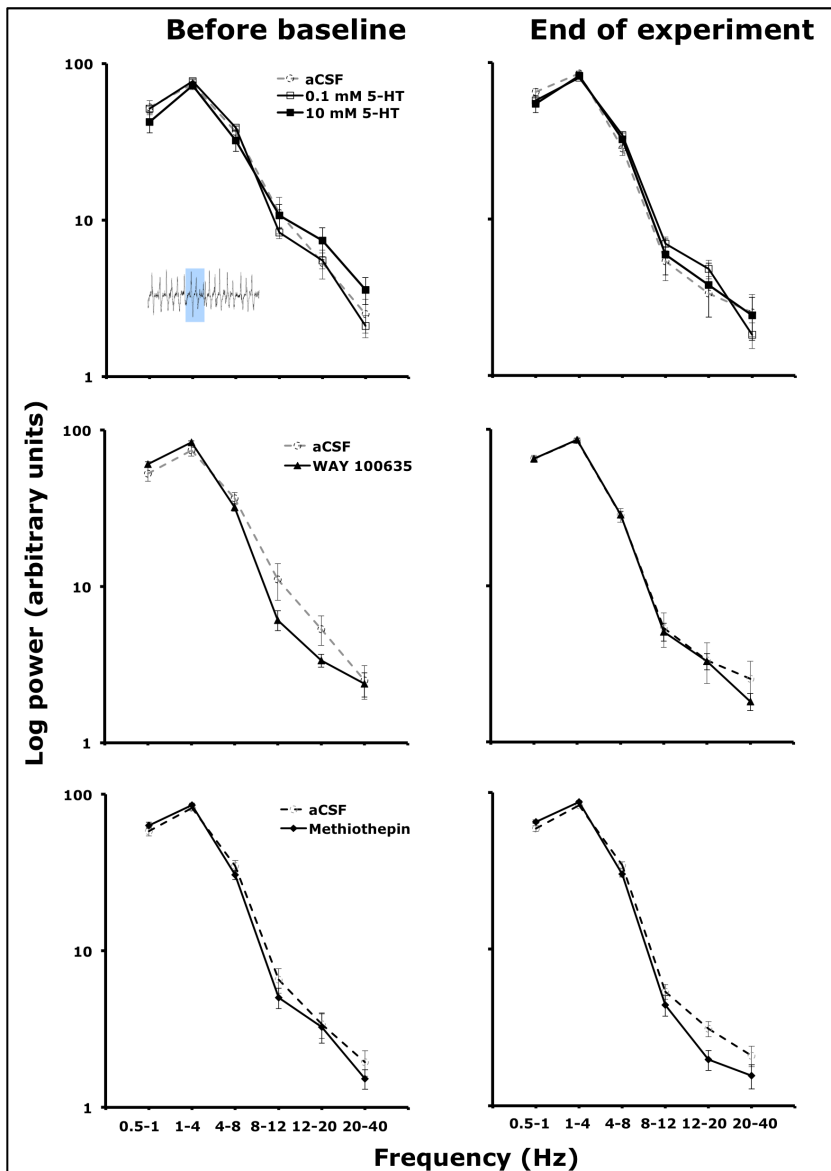
**Figure 2.7.** The effect of application (in V1 by means of reverse microdialysis) of either aCSF (n = 7) or WAY 100635 (1 mM, n = 8) on LTP following TBS (at arrow) of the LGN in juvenile (mean age 45 days, range 42-48 days), urethane- anesthetized rats. Application of WAY 100635 resulted in a suppression of LTP relative to animals receiving aCSF during four of the last five 10 min-recording epochs relative to animals receiving aCSF. Inserts depict typical fPSPs before (red) and after (blue) LTP induction in the presence of aCSF (top) and WAY 100635 (bottom; calibration bars indicate 0.5 mV vertical and 10 ms horizontal; each fPSP trace is an average of 30 min of continuous recording). \* indicates a significant ( $P < 0.05$ , t-test) difference between the two groups.

#### 2.4.6 Effects of 5-HT and 5-HT antagonists on the ECoG in V1

In addition to sampling evoked fPSPs, we also recorded ECoG activity in V1 to assess potential effect of 5-HT or drug application on spontaneous, oscillatory activity of the cortex. For all experiments, ECoG activity was assessed before the onset of baseline fPSP recordings (i.e., 20

min after the onset of drug application) and at the end of the experiment. In adult animals, the ECoG was dominated by large amplitude, slow oscillations, with peak power concentrated in the low delta (0.5-1 Hz) and delta (1-4 Hz) frequency bands, as determined by power spectral analyses (Fig. 2.8). Power in all frequency bands (0.5-1 Hz, 1-4 Hz, 4-8 Hz, 8-12 Hz, 12-20 Hz, and 20-40 Hz) remained quite stable over the course of the experiment when aCSF was applied to V1 (Fig. 2.8). Further, application of 5-HT, methiothepin, or WAY 100635 did not result in any significant changes in ECoG activity over the course of the experiment when compared with aCSF application (Fig. 2.8; all group effects and group by time effects non-significant,  $P$ 's > 0.05).

Similar observations were made for ECoG activity in juvenile rats, which also exhibited peak power in the low delta (0.5-1 Hz) and delta (1-4 Hz) frequency bands that was not significantly altered by application of WAY 100635 (data not shown). These data suggest that changes in spontaneous, oscillatory activity in V1 do not account for the effects of methiothepin and WAY 100635 to alter LTP in V1 following TBS of the LGN in urethane-anesthetized animals.



**Figure 2.8.** The effect of application (in V1 by means of reverse microdialysis; starting 20 min prior to the onset of recordings) of either aCSF, 5-HT (top; 0.1 and 10 mM), WAY 100635 (middle; 1 mM), or methiothepin (bottom; 1 mM) on electrocorticographic (ECoG) activity in V1 of adult, urethane-anesthetized rats. The ECoG was recorded and analyzed (5 sec epochs) by power spectral analysis before the onset of baseline recordings and at the end of the experiment. Throughout the experiment, ECoG activity was dominated by large-amplitude, slow activity, with most power concentrated in the low delta (0.5-1 Hz) and delta (1-4 Hz) frequency bands. None of the pharmacological treatments resulted in a significant change in ECoG activity. Insert depicts typical ECoG activity in V1, with the shaded area representing a 5 sec epoch used for the analysis (group sizes the same as in previous figures).

## 2.5 Discussion

The present set of experiments examined the role of 5-HT and 5-HT<sub>1A</sub> receptors in gating thalamocortical plasticity between LGN and V1 of juvenile and adult rats studied under urethane anesthesia. Application of 5-HT (0.1 to 10 mM) in V1 did not affect the induction or maintenance of LTP in adult animals under the present, experimental conditions. However, V1 application of the broad-acting 5-HT receptor antagonist methiothepin or the selective 5-HT<sub>1A</sub> receptor antagonist WAY 100635 resulted in a clear facilitation of LTP in adult rats, suggestive of a suppression of LTP by endogenous 5-HT release and 5-HT receptor activation. In contrast, WAY 100635 reduced LTP when tested in a group of juvenile rats (mean age 45 days). None of the pharmacological treatments that altered LTP induction exerted significant effects on baseline synaptic transmission (fPSP amplitude prior to LTP induction; note that 5-HT at 10 mM suppressed baseline fPSP amplitude, but did not affect LTP). Similarly, none of the pharmacological treatments altered spontaneous ECoG activity recorded in V1 over the course of the experiment. Together, these results indicate that 5-HT<sub>1A</sub> receptors play an important, age-dependent role in gating plasticity in the thalamocortical visual system of rats, with 5-HT<sub>1A</sub> receptor activation facilitating LTP in juveniles, but inhibiting LTP in the brains of adult animals.

We were surprised that, in our experiments, 5-HT application exerted no detectable effect on LTP induction in V1. A considerable amount of *in vitro* work has shown that 5-HT can alter LTP in V1 slice preparations, even though there are considerable inconsistencies in the specific results among various studies. In V1 slices of kittens, 5-HT has been shown to enhance LTP recorded in layer 4, effects that were absent in slices obtained from adult animals (Kojic et al., 1997; Kojic et al., 2000). Depletion of 5-HT (in combination with noradrenaline depletion) has been shown to impair LTP in layers 2/3 of the immature rat V1 *in vitro*, an effect that was mimicked by application of either 5-HT<sub>1A</sub> or 5-HT<sub>2</sub> receptor antagonists (Inaba et al., 2009).

Recently, these findings have been extended by showing that bath application of 5-HT enhances (in fact, reinstates) LTP in layers 2/3 of V1 of adult (8-10 weeks old) rats, confirming that 5-HT can exert a facilitating effect on the induction of long-lasting plasticity in V1 (Park et al., 2012).

In clear contrast, other studies have indicated that 5-HT application results in a pronounced suppression of LTP at synapses between layers 4 and 2/3 of V1 of rats (3-5 weeks old), an effect that is mediated by both 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors (Edagawa et al., 1998; Edagawa et al., 1998a; Edagawa et al., 2000; Kim et al., 2006). In fact, in V1 slices of 5 week-old rats, LTP could no longer be induced; however, acute bath application of the 5-HT receptor antagonist methysergide, acute 5-HT depletion, or neurotoxic ablation of serotonergic neurons restored LTP (Edagawa et al., 2001; Kim et al., 2006). A similar, inhibitory effect of 5-HT application has also been demonstrated for the induction of long-term depression in V1 *in vitro* (Jang et al., 2010). Interestingly, 5-HT levels in V1 show a significant increase (up to 3.5-fold from 3 to 5 weeks of age) over postnatal development (Edagawa et al., 2001; Kim et al., 2006). These observations have led to the hypothesis that 5-HT, in concert with other, neurochemical changes (particularly the maturation of GABAergic circuits (Jiang et al., 2005; Jang et al., 2009), contributes to the loss of V1 plasticity over postnatal life, resulting in the closure of the “critical or sensitive” period of V1 development (Edagawa et al., 2001; Kim et al., 2006; Jang et al., 2010).

Our data are consistent with the hypothesis that the release of endogenous 5-HT exerts a tonic, inhibitory influence on LTP induction, at least in adult rats. Cortical 5-HT is detectable in urethane-anesthetized rats, even during periods of ECoG synchronization (Dringenberg et al., 2003). We speculate that, under the present conditions, this effect of endogenous 5-HT is maximal, making the application of exogenous 5-HT ineffective in further suppressing LTP. However, future studies using 5-HT-depleted animals are necessary to examine this hypothesis.

The presumed, inhibitory effect of endogenous 5-HT is relieved by methiothepin and WAY 100635, indicative of a role of 5-HT<sub>1A</sub> receptors, even though we do not rule out the involvement of other 5-HT receptor types (e.g., 5-HT<sub>7</sub> receptors, which are blocked by methiothepin and have recently been implicated in processes of learning, memory, and synaptic plasticity (Nikiforuk, 2015)). In V1 slices of young (5-week old) rats, both 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors mediate the effect of 5-HT in the suppression of LTP induction (Kim et al., 2006; Edagawa et al., 1998a; Edagawa et al., 2000). It is noteworthy, however, that WAY 100635 and methiothepin application resulted in very similar levels of LTP enhancement, indicating that 5-HT<sub>1A</sub> receptor blockade alone is sufficient to result in a substantial disinhibition of LTP induction mechanisms under the present, experimental conditions.

In contrast to the enhancement of LTP in adult animals, application of WAY 100635 in juvenile animals (42-48 days) suppressed LTP, indicative of an age-related switch in the role of 5-HT<sub>1A</sub> receptors in gating V1 plasticity. A similar phenomenon has previously been described for direct 5-HT application, albeit in a direction opposite to that revealed by the current set of experiments. Park et al. (2012) observed that, in V1 slices obtained from juvenile rats (5 week old), 5-HT suppressed LTP, while 5-HT enhanced LTP in slices of adult animals (8-10 weeks old). At present, it is not clear why this pattern contradicts the data obtained in our experiments. However, it is noteworthy that the effects noted by Park et al. were mediated by 5-HT<sub>2</sub> receptors (2012), while our data clearly indicate a role for 5-HT<sub>1A</sub> binding sites in V1 (see above). Thus, it is possible that different receptor populations do, indeed, exert opposing effects on plasticity gating in V1, an assumption that requires a critical assessment with further investigations (also see (Jang et al., 2015) for a differential effect of 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptor activation on inhibitory transmission in V1).

To the best of our knowledge, the current experiments are the first to assess the role of 5-HT in gating LTP in the rodent V1 under *in vivo* conditions. Work conducted *in vitro* clearly offers significant advantages in terms of delineating microscopic and mechanistic properties of synaptic transmission and plasticity, such as the concurrent analysis of changes in inhibitory and excitatory synapses in V1 during LTP induction (Moreau et al., 2013). At the same time, there appear to be some important differences between LTP studied *in vivo* compared to *in vitro* conditions. Numerous studies have shown that V1 synapses studied *in vitro* become increasingly resistant to LTP induction with postnatal maturation, and slices harvested from adult animals do not show LTP with standard induction protocols (Komatsu et al., 1988; Kato et al., 1991; Kirkwood et al., 1995; Kojic et al., 1997; Edagawa et al., 2001; Kim et al., 2006; Vetencourt et al., 2008; Park et al., 2012). In sharp contrast, LTP is readily induced and maintained in V1 when studied in adult, intact-animal (anesthetized) preparations (Bröcher et al., 1992; Heynen & Bear, 2001; Dringenberg & Kuo, 2006; Dringenberg et al., 2007; Kuo & Dringenberg, 2008; Gagolewicz & Dringenberg, 2011). Also of interest, protocols that effectively elicit LTD in V1 *in vitro* - often fail to do so when tested under *in vivo* conditions (Jiang et al., 2003; Hager & Dringenberg, 2010). Thus, there clearly are some fundamental differences in the mechanisms and/or conditions that govern the induction of long-lasting plasticity at V1 synapses between *in vitro* and *in vivo* conditions, which might explain some of the apparent discrepancies among studies (see above). The use of tissue harvested from very young animals (20 days old or less), the routine use of some pharmacological agents in the bath solution (e.g., GABAergic antagonists), the removal of long-range (cortico-cortical, thalamo-cortical, subcortical) projections, and the loss of neuromodulatory inputs in slice preparations all introduce conditions that are very different from those present *in vivo* (see Steriade (2001) for a detailed discussion of the advantages and disadvantages of electrophysiological work conducted *in vivo* and *in vitro*).

These questions and discrepancies highlight the need for further work, in particular in intact animals, which preserve the anatomical connectivity and complex, physiological interactions among cortical and subcortical networks (Steriade, 2001) that regulate activity and plasticity of synapses in V1 and elsewhere. Importantly, anesthetized and non-anesthetized preparations should also be compared to assess whether the systemic effects of urethane or other anesthetics alter the plasticity properties of V1 synapses (Steriade, 2001; Hager & Dringenberg, 2010).

The results of the present experiments indicate that 5-HT<sub>1A</sub> receptors exert a tonic, inhibitory influence over LTP in adult animals, but facilitate LTP in the juvenile V1. Prior work has shown that 5-HT<sub>1A</sub> receptors are located (albeit in different densities) on both interneurons and pyramidal cells of V1 (Moreau et al., 2010). Thus, direct effects on principal neurons and/or the modulation of inhibitory tone in V1 are likely candidate mechanisms for the effects noted in our experiments. The observation that 5-HT<sub>1A</sub> receptor activation can suppress NMDA receptor functions in principal V1 cells (Edagawa et al., 1999) suggests a relatively direct action, but this does not preclude an involvement of other mechanisms (e.g., disinhibition or changes in the excitatory-inhibitory balance; Moreau et al., 2010; Moreau et al., 2013) in the effects observed here.

For the present experiments, we employed drug concentrations that are higher than those used for typical *in vitro* experiments. There are several reasons why we decided to use these higher concentrations: (a) pharmacological agents were applied by means of reverse microdialysis; it is generally assumed that only about 10% of drug molecules will diffuse across the dialysis probe membrane and into the surrounding, neural tissue (Benveniste & Huttemeier, 1990; Oldford & Castro-Alamancos, 2003); (b) drugs applied under *in vivo* conditions by reverse dialysis (but also during direct infusions) undergo extensive degradation, due to interactions with the probe membrane and lipophilic molecules, as well as diffusion and continuous enzymatic

breakdown (Adell & Artigas, 1998; Benveniste & Huttemeier, 1990). Thus, as has been pointed out by others (Adell & Artigas, 1998; Greenshaw, 1998), there is a clear discrepancy in terms of effective drug concentrations when comparing experimental *in vivo* and *in vitro* approaches. Nevertheless, we do acknowledge that it will be important for future work to establish whether the effects reported here can be elicited with drug concentrations that are lower than those employed for the present set of experiments.

There is a growing body of evidence that 5-HT plays an important role in shaping plasticity of the developing and mature nervous system, and that alterations in 5-HT transmission can result in neurodevelopmental and psychiatry disorders (Daubert & Condron, 2010; Lesch & Waider, 2012). Prior work has shown that LTP in V1, in addition to its important role in ocular dominance plasticity (Malenka & Bear, 2004; Vetencourt et al., 2008; Cooke & Bear, 2013), may also mediate processes of visual (perceptual, recognition) learning and memory storage (Cooke & Bear, 2013; Cooke et al., 2015). Based on these hypotheses and the results of the present investigation, we anticipate that altering serotonergic transmission in V1 exerts profound, age-dependent effects on visual processing and learning. For example, blockade of 5-HT<sub>1A</sub> receptors in V1 may enhance perceptual learning in adults, when 5-HT acts to stabilize synaptic connectivity; opposite behavioral effects would be expected in juvenile animals, when 5-HT<sub>1A</sub> receptors act to facilitate plasticity induction in V1. Clearly, investigations that involve a combination of behavioral, pharmacological, and electrophysiological approaches are required to characterize the role of 5-HT in visual behavior and directly test some of the hypothesis stated above.

Finally, the modulatory effects of 5-HT on activity- and experience- dependent plasticity also are of relevance to the potential treatment of various nervous system disorders. For example, chronic treatment with a selective serotonin reuptake inhibitor has been shown to reinstate ocular

dominance plasticity (and LTP) in the V1 and allow recovery from amblyopic visual deficits in adult rodents (Vetencourt et al., 2008), highlighting the therapeutic potential of serotonergic manipulations that alter the plasticity potential of cortical circuits (but see (Beshara et al., 2015)). It remains to be established whether the effects following chronic, serotonergic manipulations relate to the role of 5-HT in acute plasticity gating described with the present set of experiments.

## Chapter 3

### **NR2B-subunit dependent facilitation of long-term potentiation in primary visual cortex following visual discrimination training of adult rats**

*Published in its entirety in “NR2B-subunit dependent facilitation of long-term potentiation in primary visual cortex following visual discrimination training of adult rats” by Gagoiewicz, P. J., & Dringenberg, H. C. (2011). European Journal of Neuroscience, 34, 1222-1229.*

#### **3.1 Abstract**

Long-term potentiation (LTP) is an important mechanism thought to mediate changes in synaptic connectivity following various types of experience. We examined the effects of visual discrimination training on LTP in the mature, rodent thalamocortical visual system. Adult rats underwent visual discrimination training in a modified Morris Water Maze containing a Y-maze insert, requiring rats to associate visual cues with the location of a hidden escape platform placed in one of the two goal arms of the Y-maze insert. On the day following successful task acquisition (average of nine training days), rats were anesthetized (urethane), and LTP in the thalamocortical system was characterized. In task-naïve rats, theta-burst stimulation of the lateral geniculate nucleus resulted in modest (~40%) potentiation of field postsynaptic potentials recorded in the primary visual cortex (V1). Rats trained on the visual discrimination task showed significantly greater levels of LTP (~60%), an effect that was not seen in rats trained to swim in the maze without a predictive association between visual cues and platform location. An antagonist of the N-methyl-d-aspartate (NMDA) receptor NR2B subunit ([R- (R\*,S\*)]-a-(4-hydroxyphenyl)-b-

methyl-4-(phenylmethyl)-1-piperidinepropanol hydrochloride (Ro 25-6981); 2 mM, applied locally at the recording site in V1) reversed the training-induced LTP enhancement without affecting LTP in task-naïve rats. An antagonist of metabotropic glutamate receptors [(2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY 341495); 2 mM] was ineffective in reversing the training-induced LTP facilitation. These data suggest that behavioral (visual) training can result in changes in plasticity exhibited by the mature, thalamocortical visual system that requires activation of NMDA receptors containing the NR2B subunit.

### **3.2 Introduction**

Long-term potentiation (LTP) constitutes a form of plasticity thought to mediate changes in synaptic connectivity during brain development and various forms of learning in the adult nervous system (Martin & Morris, 2002; Malenka & Bear, 2004). Although a close similarity between learning-related plasticity and LTP has been established in several cases (e.g. Rioult-Pedotti et al., 2000; Whitlock et al., 2006), there continues to be a vigorous debate regarding the empirical evidence required to demonstrate a causal role for LTP (or other plasticity forms) in the long-lasting storage of behavioral experiences by neural networks (Martin & Morris, 2002; Neves et al., 2008).

Recent work has shown that, in the mature rodent primary visual cortex (V1) *in vivo*, LTP can readily be induced by electrical theta-burst stimulation (TBS) of the lateral geniculate nucleus (LGN) (Heynen & Bear, 2001; Dringenberg et al., 2004). Interestingly, similar effects are induced by visual stimuli (flashes and grating patterns), which elicit enhanced synaptic responses to V1 with repeated exposure (Clapp et al., 2006; Frenkel et al., 2006; Kuo & Dringenberg, 2009;

Cooke & Bear, 2010). LTP in V1 is regulated by neuromodulators (acetylcholine and histamine) present around the time of induction (Dringenberg et al., 2007; Kuo & Dringenberg, 2008), as well as priming factors occurring at time points remote from the initiation of plasticity, a phenomenon termed metaplasticity (Abraham & Bear, 1996; Bear, 2003; Abraham, 2008). In the visual system, metaplastic effects are typically studied by altering activity in visual pathways through manipulations of visual input. For example, eliminating visual input by dark exposure (for hours to days) enhances LTP in V1 (Tsanov & Manahan-Vaughan, 2007; Kuo & Dringenberg, 2009). Surprisingly, exposure to enriched environments or visual discrimination training also enhances LTP in V1 (Hager & Dringenberg, 2010a; Mainardi et al., 2010), confirming that the history of visual experience exerts profound effects on the levels of plasticity in the adult thalamocortical visual system.

Several mechanisms have been implicated in mediating metaplasticity phenomena, many of which relate to the regulation of  $\text{Ca}^{2+}$  levels in postsynaptic neurons (Abraham & Bear, 1996). For example, the specific configuration of N-methyl-d-aspartate (NMDA) receptor subunits (i.e. NR2A and NR2B) is regulated, at least in part, by visual experience, and alters the entry of  $\text{Ca}^{2+}$  and subsequent LTP induction in cortical neurons (Bear, 2003; He et al., 2006; Kopp et al., 2007). Alternatively, activation of metabotropic glutamate receptors (mGluRs) results in the release of  $\text{Ca}^{2+}$  from intracellular stores (among other effects), which also acts to regulate plasticity induction at central synapses (Nakamura et al., 1999; Topolnik et al., 2006). Whether these mechanisms mediate the changes in LTP seen in V1 after behavioral experiences has not been determined.

Here, we confirm that visual discrimination training results in enhanced LTP in the thalamocortical visual system of adult rats. Local application of antagonists of mGluR or NR2B

subunits in V1 showed that this effect depends on NR2B subunits at the time of LTP induction. Thus, behavioral training appears to facilitate plasticity induction in the mature, cerebral cortex, an effect that requires NR2B-containing NMDA receptors.

### **3.3 Materials and methods**

#### **3.3.1 Subjects**

Experiments were conducted on adult (300-600 g) male Long-Evans rats (Charles River Laboratories, Saint-Constant, Quebec, Canada). The animals were singly housed in a colony room under a reversed 12:12-h light cycle (lights off between 07:00 and 19:00 h), with food and water access ad libitum. Experiments were conducted between 08:00 and 18:00 h. All procedures were conducted in accordance with guidelines of the Canadian Council on Animal Care, and were approved by the Queen's University Animal Care Committee. Each animal was used for one experiment.

#### **3.3.2 Visual discrimination training**

Behavioral training was conducted in a modified Morris Water Maze containing a Y-maze insert. The water maze consisted of a cylindrical pool (180 cm in diameter, and 60 cm in height, filled with water to a height of 40 cm). The water was maintained at a temperature of  $22 \pm 1$  °C, and rendered opaque by the addition of white, non-toxic paint. The pool contained a clear Plexiglas Y-maze insert (height, 60 cm; length, 140 cm; width, 50 cm at the proximal release site and 80 cm at the distal goal arms), which was kept in the same position throughout the entire training period. A black Plexiglas divider (height, 50 cm; length, 60 cm) separated the two goal arms. A clear Plexiglas rectangular platform (height, 38 cm; width, 12 cm; length, 36 cm) was placed 10 cm from the end of one of the goal arms, 2 cm below the water surface. The water

opacity ensured that the platform was not visible.

Two distinct visual cues were used for discrimination training. Cues were printed on white sheets of paper (21.5 x 28 cm), and consisted of either three black, horizontal bars (width, 3 cm; length, 15 cm; spaced 3 cm apart) or three vertical bars (same dimension and spacing as above). The visual cues were mounted at the end of the two Y-maze goal arms 1 cm above the water line.

Discrimination training was divided into three phases. For all phases, animals were released into the Y-maze insert facing the pool wall and required to swim towards the goal arms in order to reach the hidden escape platform located in one of the arms.

Phase 1: The initial training phase (1 day) served to familiarize animals with swimming in the pool and finding the hidden platform without the use of visual cues in the maze. Each animal was released into the pool and given a maximum of 200 s to find the platform. If an animal failed to do so, it was manually guided to the platform by the experimenter. Animals remained on the platform for 15 s before commencement of the next trial. Trials were repeated (the platform remained in the same arm) until an animal performed five consecutive correct responses, or a maximum of 10 trials, whichever came first (this constituted a ‘trial block’). Correct trials were scored when an animal entered the correct goal arm and mounted the platform; errors were scored when an animal entered the incorrect goal arm with at least half of its body. Each trial block was followed by a rest period of ~5 min, during which animals were returned to a holding cage with holes in the bottom to allow water to drain. Following the rest period, the next trial block was administered with the same procedures as above, with the exception that the platform was moved to the opposite goal arm. A total of four trial blocks were administered, each followed by a 5 min rest period. After completion of training, animals were placed under a heat lamp for a minimum

of 15 min before being returned to their home cage.

Phase 2: The same training procedure as that outlined above was used for phase 2 (1 day), with the exception that two distinct visual cues were placed at the end of the goal arms, one indicating the presence of the platform (P+), and the other indicating its absence (P-). The assignment of cues as P+ and P- was counterbalanced across rats. Platform location and associated visual cues remained unchanged during each trial block (10 trials), but alternated between blocks (four blocks in total), with the platform-cue association being kept constant for all blocks. However, for a group of control animals, the platform location alternated between blocks, whereas the visual cues remained constant to ensure that they did not predict the platform location.

Phase 3: The final training phase commenced on day three and continued until animals reliably discriminated the two visual cues. Procedures were the same as for phase 2, with the following exceptions. On each day, animals received 10 training trials, each followed by a 30 s rest period in a holding cage. For each trial, platform location was randomly assigned to one goal arm. Again, for one group of (trained) animals, visual cues alternated together with the platform, so that P+ and P- consistently indicated the presence and absence, respectively, of the platform. For the control group, platform location was changed randomly from trial to trial, but visual cues remained stationary, and thus lacked a consistent association with the platform. For trained animals, daily training continued until an animal reached a criterion of at least 80% correct (i.e. 8 /10 daily trials) over three consecutive days. For control animals, training for phase 3 was carried out for an average of nine consecutive days (range of 7-11 days), which was the mean number of days required by trained animals to reach the performance criterion outlined above.

### **3.3.3 Surgery**

Electrophysiological procedures were carried out on the day following completion of

behavior training. Each animal was deeply anesthetized with urethane (Sigma-Aldrich, Oakville, Ontario, Canada; 2.0 g/kg, given intraperitoneally as four doses of 0.5 g/kg, one every 20 min; additional top-ups consisting of 0.5 g/kg were administered when necessary), prior to being mounted in the stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Fifteen minutes prior to the start of surgery, the local analgesic bupivocaine (Marcaine; Hospira Healthcare Corporation, Montreal, Quebec, Canada) was administered subcutaneously to the skin and tissue along the incision line over the skull (two or three injections; total of 5 mg/kg). Throughout the experiment, body temperature was monitored with a rectal thermometer and maintained between 36 and 37 °C by means of an electrical heating pad and fleece insulating blankets surrounding the body.

The skull bone was exposed, and small holes were drilled overlaying the following areas (all measurements taken from bregma and the skull surface): LGN, anterior-posterior -4.1 mm, lateral +4.1 mm, and ventral -4.8 to -5.1 mm); V1, anterior-posterior -7.6 mm, lateral +3.6 mm, and ventral -0.8 to -1.2 mm. Two additional holes were drilled in the bone overlying the left and right cerebellum to secure screws, which served as ground and reference connections. A stimulation electrode (Series 100 concentric bipolar electrode; Rhodes Medical Instruments, David Kopf Instruments) was lowered into the hole above the LGN. A monopolar recording electrode (125 µm diameter Teflon-insulated stainless steel wire) was lowered onto the surface of V1. The final, ventral depths of both electrodes were adjusted to yield maximal amplitude field postsynaptic potentials (fPSPs) in V1 in response to single-pulse LGN stimulation.

### **3.3.4 Electrophysiology**

Stimulation of the LGN (single 0.2-ms pulses) was delivered by the concentric stimulation electrode connected to a stimulus isolation unit (ML 180 Stimulus Isolator; AD

Instruments, Toronto, ON, Canada) providing a constant current output. The fPSPs in V1 were recorded with the wire electrode referenced against a screw in the bone overlying the cerebellum. The recording electrode was connected to an amplifier and A/D converter (PowerLab 4/s system running Scope software v. 3.6.5; AD Instruments), allowing the signal to be amplified, filtered (0.3 to 1 kHz), digitized (10 kHz), and stored for offline analysis.

Initially, input-output curves were established by stimulating the LGN at increasing intensities (0.1-1.0 mA in 0.1 mA increments). Based on these input-output curves, the LGN stimulation intensity yielding approximately 50-60% of the maximal fPSP amplitude in V1 was then used for the remainder of the experiment.

Cortical fPSPs (every 30 s) were recorded until 30 min of stable baseline were achieved ( $\leq 5\%$  difference between successive data points for fPSPs averaged over 10 min epochs). Subsequently, TBS was delivered to the LGN, consisting of five single pulses (at 100 Hz) per burst, with bursts repeated at 5 Hz for a total of 10 bursts (pulse intensity and duration were the same as above). Cortical fPSPs were recorded (every 30 s) for 1 h following the first TBS. Two more TBS episodes were delivered, each of which was followed by 1 h of fPSP recordings.

### **3.3.5 Pharmacology**

To investigate the roles of different receptor populations in LTP elicited by TBS, independent groups of animals received one of the following drug treatments: (2S)-2-amino-2-[[1S,2S)-2-carboxycyclo-prop-1-yl]-3-(xanth-9-yl) propanoic acid (LY 341495) (2 mM; Tocris Bioscience, Ellisville, MO, USA); and [R-(R\*,S\*)]-a- (4-hydroxyphenyl) -b-methyl-4-(phenylmethyl)-1-piperidinepropanol hydrochloride (Ro 25-6981) (2 mM; Sigma-Aldrich). LY 341495 exhibits antagonistic properties across all subtypes of mGluRs, even though it has somewhat greater affinity for mGluR2 and mGluR3 subtypes (Kingston et al., 1998; Linden et al.,

2009). Ro 25-6981 is a potent antagonist of NMDA receptors containing the NR2B subunit, with a > 5000-fold selectivity for NR2B over NR2A subunits (Fischer et al., 1997). Drug concentrations were chosen on the basis of previous work demonstrating effective antagonism of LTP or long-term depression at cortical and subcortical synapses (Fitzjohn et al., 1998; Sawtell et al., 1999; Rao & Daw, 2004; Wu et al., 2008; Hogsden & Dringenberg, 2009). Drugs were dissolved in artificial cerebrospinal fluid (ACSF), consisting of (in mM): 124.0 NaCl, 4.4 KCl, 1.2 MgSO<sub>4</sub>, 1.0 NaH<sub>2</sub>PO<sub>4</sub>, 2.5 CaCl<sub>2</sub>, 26.0 NaHCO<sub>3</sub>, and 10.0 glucose.

Drugs were applied locally in V1 by means of reverse microdialysis. The dialysis probe (Mab 6.14.2, 15 000-Da cut-off, polyether sulfone membrane, outer diameter 0.6 mm; SPE Limited, Concord, Ontario, Canada) was mounted alongside the V1 recording electrode, with the probe extending approximately 1 mm ventrally past the electrode tip. The dialysis probe was connected to a 2.5 mL Hamilton syringe, using FEP microtubing (SPE Limited). The syringe was driven by a microdialysis pump (CMA 402; CMA Microdialysis, Solna, Sweden) at a flow rate of 1 µL/min, with perfusion beginning 20 min prior to acquisition of baseline fPSP recordings.

### **3.3.6 Histology**

At the conclusion of the experiment, all animals were perfused through the heart with 0.9% saline (~50 mL) followed by 10% formalin (~100 mL). The brains were removed and stored in 10% formalin for a minimum of 24 h before sectioning (40 µm slices) with a cryostat. Slices were then mounted onto microscope slides and inspected with a digital microscope to verify electrode placements, using a rat brain atlas as a reference (Paxinos & Watson, 1998). Histological inspections and decisions on the accuracy of electrode placements were made by an experimenter who was blind to the results of individual animals. Data from inaccurate placements were omitted from this study.

### **3.3.7 Data analysis**

Behavioral data were expressed as mean  $\pm$  standard error of the mean (SEM) to reach the predetermined performance criterion (see above). For the electrophysiological experiments, fPSPs were acquired and analyzed with Scope software (v. 3.6.5; AD Instruments). With the electrode configuration employed in the present study, fPSPs elicited in V1 consist of a predominant, large-amplitude, negative-going component with a latency to peak of about 16-18 ms (see Fig. 3.3 insert), similar to that shown in previous work using equivalent stimulation and recording techniques (Heynen & Bear, 2001; Dringenberg et al., 2007; Hager & Dringenberg, 2010a). This major, negative fPSP component appears to reflect current sinks originating in layers II/III of V1 (Heynen & Bear, 2001). The amplitude of this component was automatically detected and computed with Scope software (using the Data Pad function) by measuring the difference between the maximal fPSP negativity and a baseline epoch sampled immediately prior to the stimulation artifact. Once individual fPSPs were analyzed in this manner, they were averaged over 10 min intervals (20 fPSPs in total) and then normalized by dividing them by the average baseline amplitude of each animal. Electrophysiological data are presented as mean  $\pm$  SEM, and were analyzed with mixed, two-way ANOVAs and followed up, where statistically appropriate, with Bonferroni post hoc tests and unpaired Student's t-tests with spss (v. 15.0; SPSS, Colorado Springs, CO, USA). The Greenhouse-Geisser correction was applied in all cases when the assumption of sphericity was violated, as tested by the Mauchly's test of sphericity (using SPSS software).

## **3.4 Results**

### **3.4.1 Visual discrimination training**

Animals (n = 55) were trained to associate visual cues with the location of the escape platform in the water maze. A total of 54 animals reached the predetermined acquisition criterion (8/10 correct arm entries per day for 3 consecutive days) after an average of nine training days (range, 6-13 days) (Fig. 3.1). One animal failed to reach the criterion after 13 days of training and was excluded from the study. A group of control animal (n = 11) that underwent training without pairing of visual cues with platform location performed at about chance levels (approximately 5/10 correct arm entries per day) throughout the entire training period (Fig. 3.1; average of 9 days; range, 7-11 days).

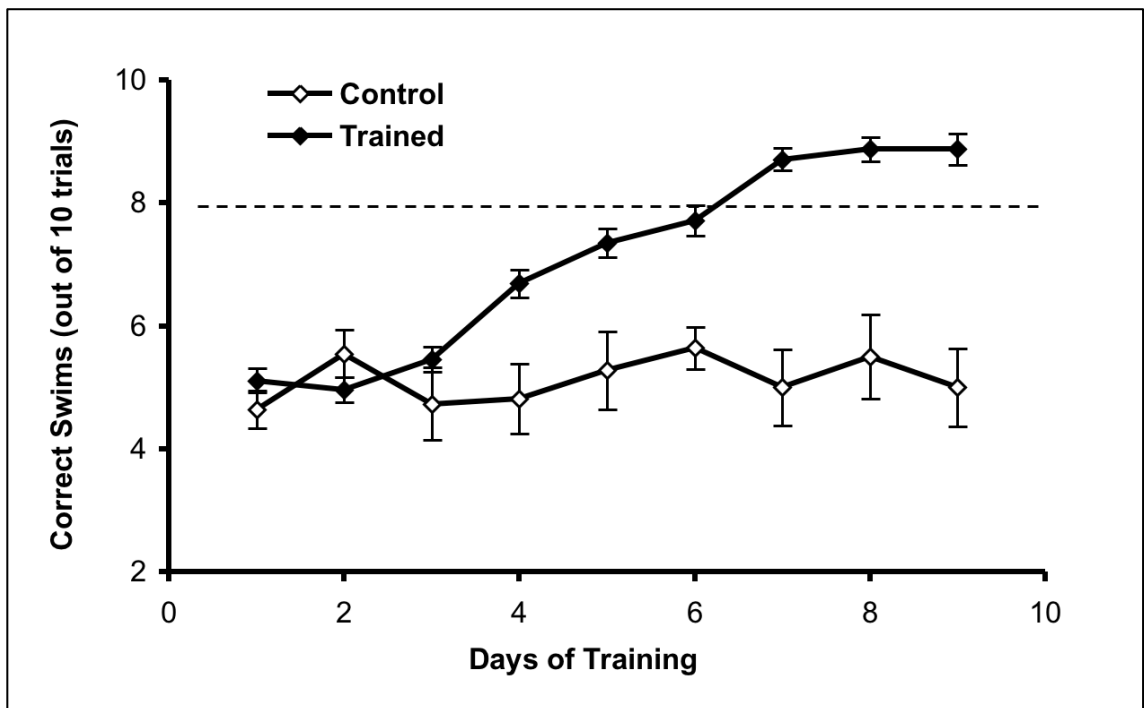
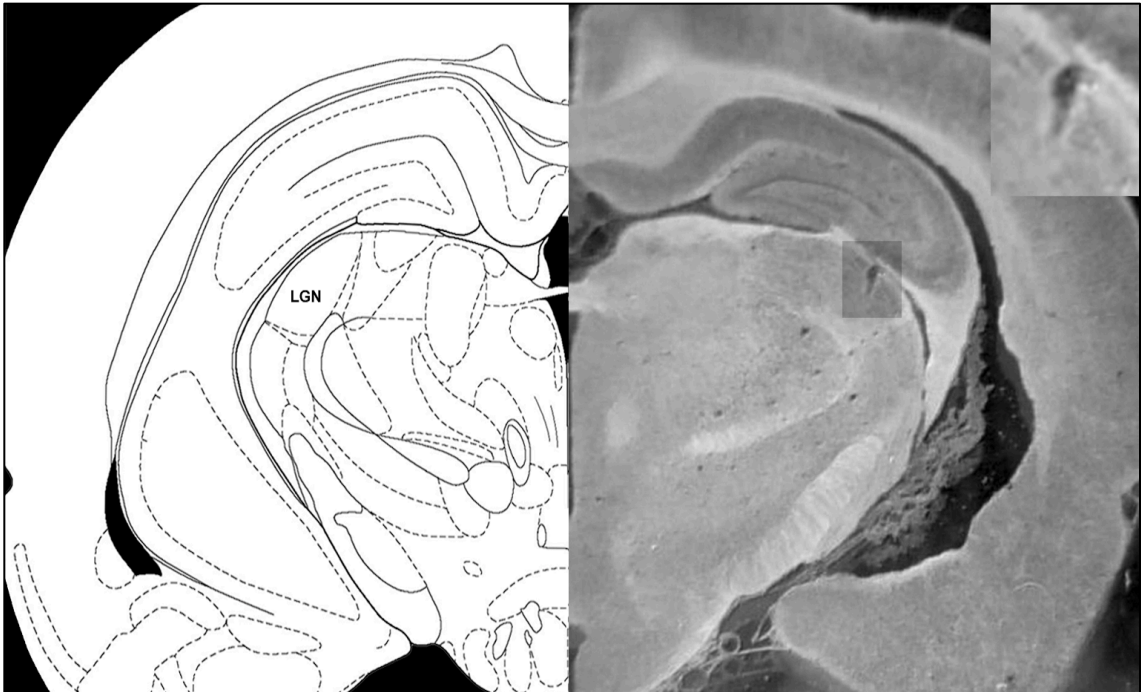


Figure 3.1. Average ( $\pm$  SEM) correct goal arm entries for trained (n = 54) and control (n = 11) animals during visual discrimination training. Trained animals took an average of 9 days to meet the training criterion ( $\geq 8/10$  correct trials for three consecutive days; see dashed line). Note that control animals that did not experience pairing of the visual cues with the platform location performed at chance levels (5/10 trials) throughout training.

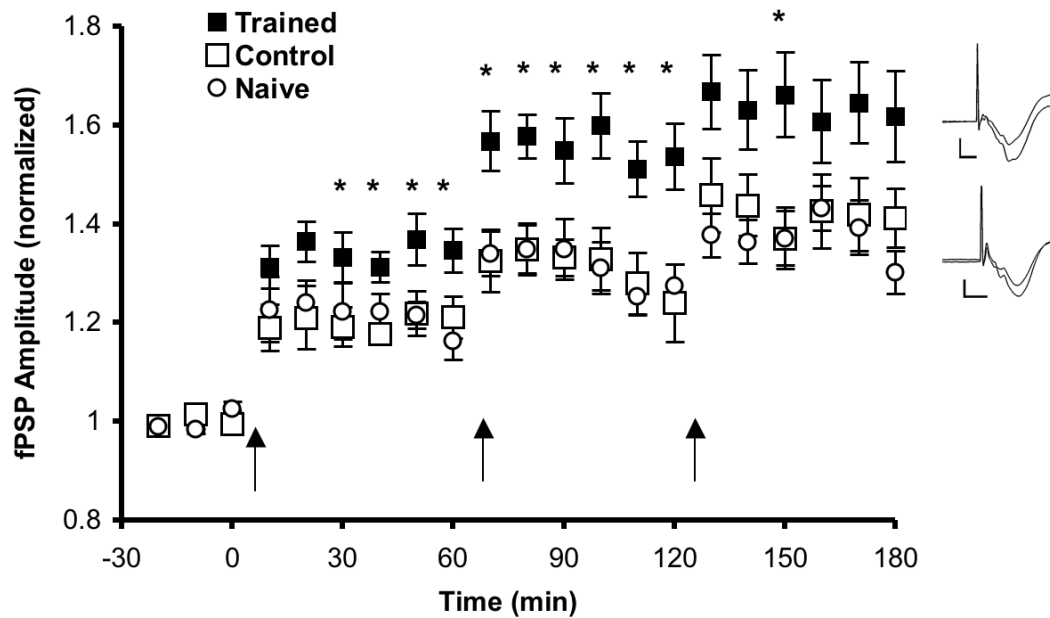
### 3.4.2 Effects of training on LTP

One day following the completion of discrimination training, all animals were deeply anesthetized with urethane, and stimulation and recording electrodes were placed in the LGN (Fig. 3.2) and the surface of V1, respectively, as described previously (Hager & Dringenberg, 2010). The fPSPs recorded in V1 elicited by single-pulse stimulation of the LGN consisted of a



**Figure 3.2.** Image depicting a typical placement of a stimulation electrode in the LGN. The right side of this figure is a digital microscope image of a brain slice (40  $\mu\text{m}$  in thickness) showing the vertical electrode track, with the ventral tip magnified (insert, top right); the left side shows a corresponding, modified section from the rat brain atlas by Paxinos and Watson (1998).

large-amplitude, negative-going component with a latency to peak of about 16-18 ms (Fig. 3.3 insert), similar to those described previously (Heynen & Bear, 2001; Dringenberg et al., 2007; Hager & Dringenberg, 2010a).



**Figure 3.3.** The effect of three episodes (every 60 min, at arrows) of TBS on the amplitude of fPSPs in V1 elicited by stimulation of the LGN. Animals ( $n = 15$ ) trained to associate visual cues with escape platform location exhibited greater LTP than both control animals ( $n = 8$ ; trained in the absence of cue-platform associations) and naïve animals ( $n = 7$ ; never trained in the maze). \*Significant ( $P < 0.05$ ) difference between trained and control animals. Inserts depict typical fPSPs before (smaller amplitude) and after LTP induction for a trained (top) and a control (bottom) animal (calibration bars indicate 0.2 mV vertical and 10 ms horizontal; each fPSP is an average of 20 min of recording).

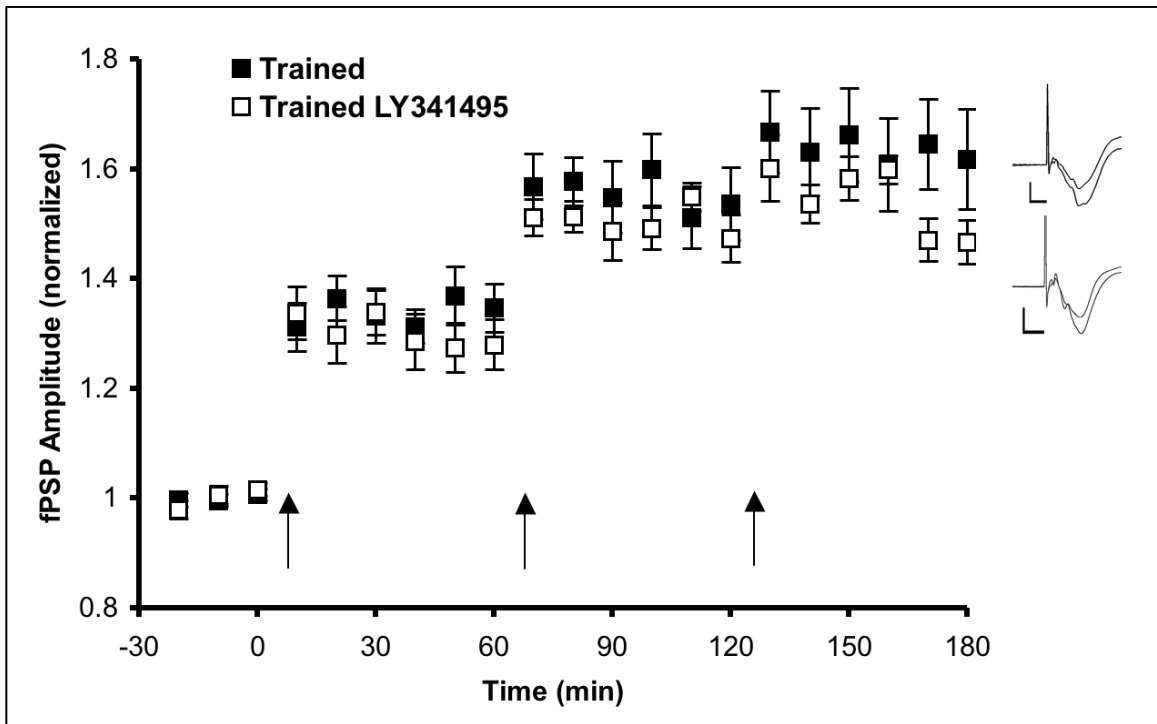
To examine the effects of visual discrimination training on LTP in the thalamocortical projection system, three separate groups of animals received TBS of the LGN. In naïve animals ( $n = 7$ ) that did not receive behavioral training, three episodes of TBS (60 min apart) elicited synaptic potentiation, with fPSP amplitude reaching  $138 \pm 5\%$  of baseline during the last 30 min of the experiment (Fig. 3.3). Control animals ( $n = 8$ ) receiving water maze training in the absence

of pairing of visual cues with the escape platform exhibited LTP of a similar magnitude, with fPSP amplitude reaching  $142 \pm 7\%$  of baseline during the final 30 min of the experiment (Fig. 3.3). In contrast, animals ( $n = 15$ ;  $n = 9$  for no drug application;  $n = 6$  for ACSF application; ANOVA showed that these two subgroups were not different) trained to associate a visual cue with the escape platform showed significantly greater synaptic enhancement, with levels of LTP at  $162 \pm 9\%$  of baseline during the final 30 min of recordings (Fig. 3.3). A mixed two-way ANOVA revealed main effects of time ( $F(20, 540) = 34.472, P < 0.001$ ) and group ( $F(2, 27) = 6.664, P < 0.01$ ), as well as a time x group interaction ( $F(20, 540) = 1.730, P < 0.01$ ). Follow-up ANOVAs comparing trained with either control or naïve animals revealed significant group and time x group effects for both analyses (trained vs. controls, group,  $F(1, 21) = 7.510, P < 0.05$ ; trained vs. controls, time x group,  $F(20, 420) = 1.676, P < 0.05$ ; trained vs. naïve, group,  $F(1, 120) = 7.913, P < 0.05$ ; trained vs. naïve, time x group,  $F(20, 400) = 2.166, P < 0.003$ ). Overall, these results indicate that visual discrimination training resulted in a facilitation of LTP in the thalamocortical visual system of rats.

### **3.4.3 Effect of mGluR blockade on training-induced LTP enhancement**

To determine the role of mGluRs in the facilitation of LTP observed in trained animals, the antagonist LY 341495 (2 mM) was applied locally in V1 by means of reverse microdialysis. In trained animals ( $n = 6$ ) given LY 341495, three episodes of TBS (60 min apart, as above) elicited synaptic potentiation, with fPSP amplitude at  $151 \pm 4\%$  of baseline during the last 30 min of the experiment (Fig. 3.4). A mixed two-way ANOVA comparing trained animals given and not given (same trained group as in Fig. 3.3) LY 341495 revealed a main effect of time, but not of group ( $F(1, 19) = 0.623, P > 0.1$ ), and time x group interaction ( $F(20, 380) = 0.568, P = 0.934$ ). Also, the apparent small decrease in LTP in rats given LY 341495 relative to untreated rats ( $151 \pm 4\%$  and  $162 \pm 9\%$ , respectively) was attributable to the fact that LTP was reduced only during

the last 20 min of the experiment, whereas other data points fell within the range of untreated animals (Fig. 3.4). Overall, these observations suggest that LY 341495, at the concentration tested, did not result in a significant reversal of the LTP facilitation seen after visual discrimination training.

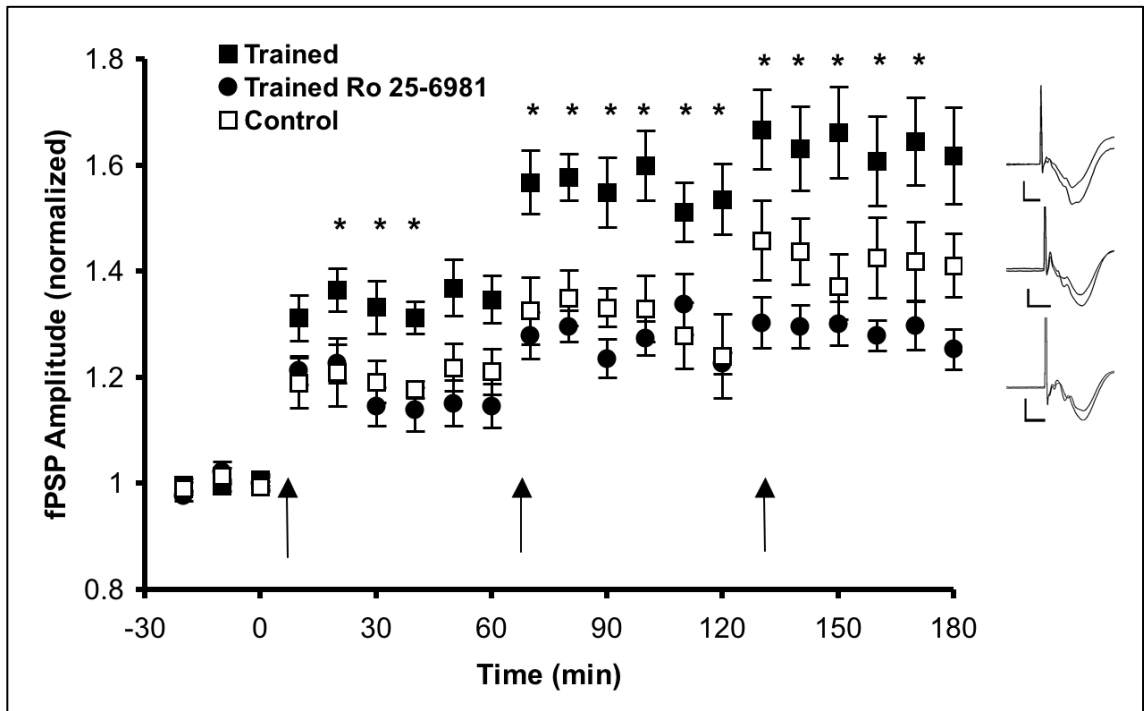


**Figure 3.4.** The effect of LY 341495 application (2 mM, reverse microdialysis) at the recording site in V1 on LTP following visual discrimination training. Application of LY 341495 in trained animals ( $n = 6$ ) did not result in a significant change in LTP relative to trained animals not given the drug (total,  $n = 15$ ;  $n = 6$  for ACSF application,  $n = 9$  for no application, same group as in Fig. 3; arrows indicate TBS). Inserts show typical fPSPs before (smaller amplitude) and after LTP induction for trained animals in the presence of ACSF (top) or LY 341495 (bottom; calibration bars indicate 0.2 mV vertical and 10 ms horizontal; each fPSP is an average of 20 min of recording).

group interaction ( $F(20, 380) = 0.568, P = 0.934$ ). Also, the apparent small decrease in LTP in rats given LY 341495 relative to untreated rats ( $151 \pm 4\%$  and  $162 \pm 9\%$ , respectively) was attributable to the fact that LTP was reduced only during the last 20 min of the experiment, whereas other data points fell within the range of untreated animals (Fig. 3.4). Overall, these observations suggest that LY 341495, at the concentration tested, did not result in a significant reversal of the LTP facilitation seen after visual discrimination training.

#### **3.4.4 Effect of NR2B blockade on training-induced LTP enhancement**

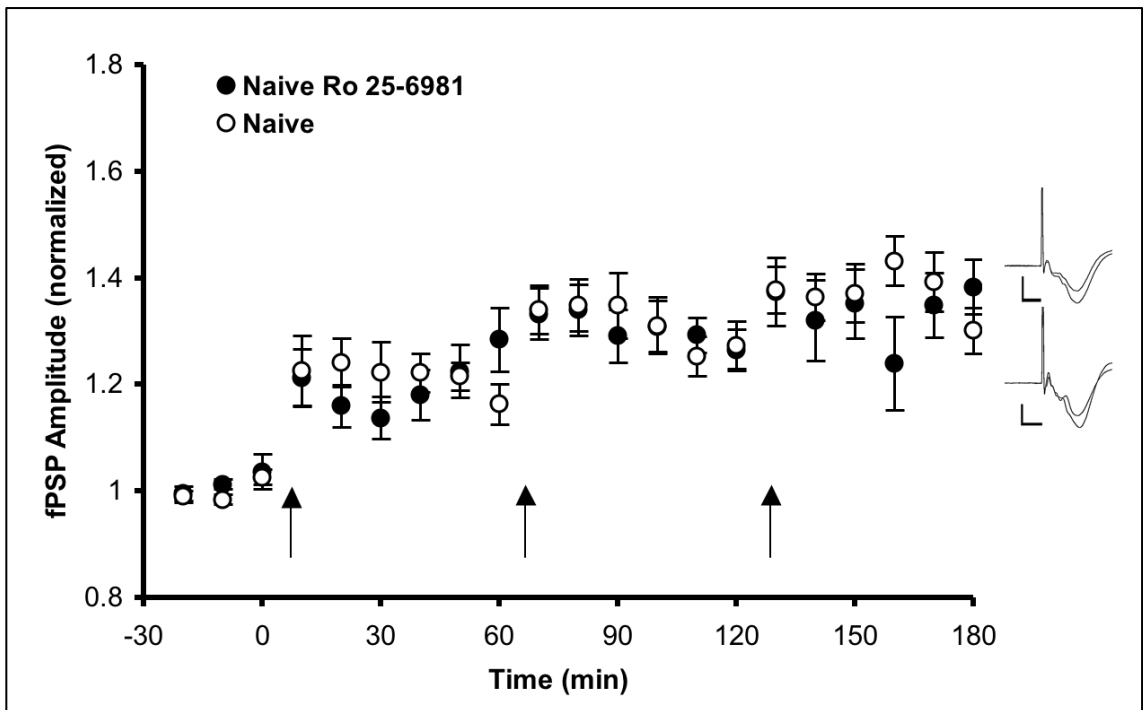
To examine a possible role of NMDA receptors containing the NR2B subunit in the facilitation of LTP observed in trained animals, Ro 25-6981 (2 mM) was applied locally in V1. In trained animals ( $n = 8$ ) given Ro 25-6981, three episodes of TBS (60 min apart) elicited synaptic potentiation, with fPSP amplitude at  $128 \pm 4\%$  of baseline during the last 30 min of the experiment (Fig. 3.5). A mixed two-way ANOVA comparing control animals (i.e. trained without cue-platform association; same control group as in Fig. 3.3), trained animals without drug application (same as in Fig. 3.3) and trained animals given Ro 25-6981 revealed main effects of time ( $F(20, 560) = 34.364, P < 0.001$ ) and group ( $F(2, 28) = 9.638, P = 0.001$ ), as well as a time x group interaction ( $F(40, 560) = 2.511, P < 0.001$ ). A follow-up ANOVA comparing trained animals given and not given Ro 25-6981 revealed a significant effect of group ( $F(1, 21) = 5.739, P < 0.001$ ) and a group x time interaction ( $F(20, 420) = 3.737, P < 0.001$ ). A further analysis comparing trained animals given Ro 25-6981 and control animals revealed a time x group interaction ( $F(20, 420) = 1.650, P < 0.05$ ), but no main effect of group ( $F(1, 14) = 1.523, P = 0.238$ ); the interaction appeared to be attributable to the fact that trained animals given Ro 25-6981 showed less LTP towards the end of the experiment than control animals (Fig. 3.5). Together, these data indicate that Ro 25-6981 blocked the LTP facilitation normally seen following visual discrimination training.



**Figure 3.5.** The effect of Ro 25-6981 application (2 mM, reverse microdialysis) at the recording site in V1 on LTP facilitation following visual discrimination training. Application of Ro 25-6981 in trained animals ( $n = 8$ ) resulted in a significant decrease in LTP relative to trained animals not given the drug (total,  $n = 15$ ;  $n = 6$  for ACSF application,  $n = 9$  for no application, same group as in Fig. 3; arrows indicate TBS). Trained animals given Ro 25-6981 also showed less LTP than control animals ( $n = 8$ , trained in the absence of cue-platform associations, same as in Fig. 3.3) during the last 1 h of the experiment. \*Significant ( $P < 0.05$ ) difference between trained animals given and not given Ro 25-6981. Inserts show typical fPSPs before (smaller amplitude) and after LTP induction for a trained (top) animal, a trained animal given Ro 25-6981 (middle), and a control animal (bottom) (calibration bars indicate 0.2 mV vertical and 10 ms horizontal; each fPSP is an average of 20 min of recording).

In order to assess whether the effect of Ro 25-6981 in reducing LTP is selective for the training-induced enhancement of LTP, a further group of naïve animals ( $n = 10$ ) given Ro 25-6981 (2 mM) was also examined. In these animals, three episodes of TBS (60 min apart) elicited synaptic potentiation, with fPSP amplitude at  $132 \pm 7\%$  of baseline during the last 30 min of the experiment (Fig. 3.6). An ANOVA comparing naïve animals given or not given (same naïve group as in Fig. 3.3) Ro 25-6981 revealed a significant effect of time ( $F(20, 280) = 15.580$ ,  $P <$

0.001), but no main effect of group ( $F(1,14) = 0.000$ ,  $P = 0.995$ ) or time x group interaction ( $F(20, 280) = 0.973$ ,  $P = 0.495$ ). Thus, Ro 25-6981 application in V1 does not result in non-selective inhibition of LTP induction mechanisms.



**Figure 3.6.** The effect of Ro 25-6981 application (2 mM, reverse microdialysis) at the recording site in V1 on LTP in task-naïve animals. Application of Ro 25-6981 in naïve animals ( $n = 10$ ) did not result in a significant change in LTP relative to naïve animals not given the drug ( $n = 7$ , same as in Fig. 3.3; arrows indicate TBS). Inserts show typical fPSPs before (smaller amplitude) and after LTP induction for a trained (top) animal and a naïve (bottom) animal (calibration bars indicate 0.2 mV vertical and 10 ms horizontal; each fPSP is an average of 20 min of recording).

### 3.5 Discussion

In the present set of experiments, visual discrimination training led to an enhancement of LTP induced in V1 by means of TBS of the LGN in vivo. This LTP facilitation was not sensitive

to application of the mGluR antagonist LY 341495, suggesting that this effect does not require mGluR activation. Surprisingly, application of Ro 25-6981, a selective antagonist of NR2B subunits (Fischer et al., 1997), completely reversed the training-induced LTP facilitation, whereas LTP in task-naïve animals was unaffected by the drug. Consequently, it appears that visual discrimination training enhances plasticity induction in the fully matured V1 of adult rats, an effect that depends on NR2B-containing NMDA receptors.

Considerable evidence has shown that the subunit composition of NMDA receptors in the rodent V1 shows a consistent, developmental trajectory. During early postnatal life, high levels of NR2B-containing NMDA receptors are apparent, and appear to be largely maintained into adulthood. In contrast, NR2A-containing NMDA receptors are sparse in the immature cortex, but show clear, experience-driven upregulation throughout postnatal life (for review, see Dumas, 2005). This change in subunit expression and the related shift in the ratio of NR2A/NR2B-containing receptors have been implicated in alterations of synaptic plasticity during cortical development. It appears that, in general, elevated plasticity (or a reduced plasticity threshold) is associated with higher relative levels of NR2B subunits at cortical synapses. NMDA receptors containing NR2B subunits display longer channel opening and current duration, resulting in an increased entry of  $Ca^{2+}$  into postsynaptic cells (Monyer et al., 1994; Sobczyk et al., 2005). Furthermore, the preferential interaction of NR2B subunits with intracellular, plasticity-related signaling proteins, especially  $Ca^{2+}$ /calmodulin-dependent protein kinase II (Strack and Colbran, 1998; Bayer et al., 2001), is also consistent with their role in promoting heightened levels of plasticity during early postnatal life (Yashiro & Philpot, 2008).

A number of studies support this idea. For instance, application of the NR2B-preferring antagonist ifenprodil reduces LTP in slices of V1 obtained from juvenile, but not adult, mice (de Marchena et al., 2008). Similarly, heightened levels of LTP seen in the juvenile primary auditory

cortex are reduced to lower, adult-like levels by cortical application of different NR2B antagonists (Hogsden & Dringenberg, 2009). Additionally, transgenic overexpression of NR2B subunits has been shown to enhance LTP in the hippocampal formation of mice (Tang et al., 1999), whereas transient overexpression of the NR2B C-terminus, which blocks the NR2B-Ca<sup>2+</sup>/calmodulin-dependent protein kinase II interaction, reduces the levels of hippocampal LTP (Zhou et al., 2007). Together, these and other studies (e.g. Lu et al., 2001; Zhao et al., 2005) have convincingly demonstrated that NR2B subunits act as a potent regulator of levels of plasticity at central synapses.

The fact that we observed an enhancement of LTP that is sensitive to NR2B subunit antagonism appears to be consistent with the experimental evidence summarized above. However, it is important to highlight that the LTP enhancement after training does not appear to fit current models of the role of LTP in information ('memory') encoding, or characterized mechanisms of experience-dependent metaplasticity in the adult visual cortex. A number of studies have now demonstrated that various types of learning experience (fear conditioning; motor, avoidance and perceptual learning) result in LTP-like changes of specific sets of synapses, limiting the amount of LTP that can be induced by electrical stimulation protocols (Rogan et al., 1997; Rioult-Pedotti et al., 2000; Monfils & Teskey, 2004; Whitlock et al., 2006; Sale et al., 2011). This 'occlusion approach' is generally taken as evidence that learning and LTP share common cellular and molecular mechanisms (Martin & Morris, 2002). The fact that LTP was not occluded and, in fact, showed facilitation in the present set of experiments might suggest that synapses in the thalamocortical visual system do not mediate the long-term storage of information acquired during the visual discrimination training (that is, they are not potentiated). Previous work has shown that lesions of the hippocampal formation impair the long-term recall of cues acquired during a training protocol very similar to that used here, indicating that the hippocampus

is part of the network involved in the long-term storage of visual information (Epp et al., 2008). Whether synapses in visual pathways play a role during the earlier stages of training and encoding of visual information (and consequently show LTP occlusion) is currently unknown.

Interestingly, control animals that underwent maze training in the absence of a predictive association of visual cues and platform location did not exhibit facilitation of LTP. In fact, LTP in these animals was not significantly different from that in task-naïve animals despite the fact that control animals repeatedly experienced the complex, visual environment of the testing room. Thus, it appears that a high level of specific, detailed visual processing and/or the behavioral significance attributed to visual cues in the trained group are required to elicit the plasticity facilitation noted in the present experiments. Control animals were subjected to the swimming procedure for the same average number of days as trained animals (mean of nine training days for both groups; see above). Thus, control animals did experience swimming in the maze and the associated motor activity and stress for an equivalent time period, even though we did not match individual trained and control animals on measures of swim time or distance. In fact, given that control animals did not ‘know’ where the escape platform was located on each trial, they actually spent more time in the maze than trained animals that took direct swim paths to the platform during the later training stages. This observation raises the concern that control animals may have experienced more prolonged or greater levels of stress during training, which could result in a suppression of LTP. However, given that potentiation in control animals was equivalent to that in completely task-naïve animals it appears that over the extended training period, stress levels were not sufficient to affect LTP induction mechanisms, at least for the experimental conditions employed in the present investigation.

Even though the facilitation of LTP following visual discrimination training does not fit with the ‘occlusion approach’ summarized above, our observation that prior experiences can alter

plasticity responses of synapses is in line with a number of previous studies. Several investigations have shown that housing adult rodents in enriched environments facilitates LTP induction in the thalamocortical visual system (Sale et al., 2007; Mainardi et al., 2010), even though it is unknown whether this effect is related to changes in NMDA receptor activity. Surprisingly, dietary factors [e.g. caloric restrictions and dietary (fruit) supplements] or hormonal manipulations (estradiol treatment) also appear to facilitate LTP in the forebrain, effects that have been linked to enhanced NR2B subunit functioning (Fontan- Lozano et al., 2007; Coutrap et al., 2008; Snyder et al., 2011). These data, together with the evidence provided here, clearly support the notion that NR2B subunits in the mature brain can contribute to enhancements in plasticity following various environmental, experience-related or chemical manipulations.

It is noteworthy that ‘priming’ factors that have been demonstrated to be effective in increasing LTP (housing, hormonal, diet, and prior training) have all been shown to enhance performance in a variety of behavioral tasks (e.g. Duffy et al., 2001; Xu & Zhang, 2006; Fontan-Lozano et al., 2007; Hager & Dringenberg, 2010a). For example, previous experience with visual discrimination training not only results in greater LTP, but also facilitates subsequent acquisition of novel visual stimuli (Hager and Dringenberg, 2010). This facilitation of discrimination performance may be attributable to the fact that, in addition to learning to distinguish specific visual cues, rats have acquired the general, procedural requirements involved in effective task performance, especially using/attending to visual information as a means to escape from the water. It is possible that the LTP enhancement noted here reflects, at least to some degree, this procedural learning and/or the accompanying visual attention and processing, which then leads to faster acquisition of visual information during new learning trials. At present, direct evidence to support this notion is lacking. However, future investigations that aim to interfere with mechanisms implicated in plasticity enhancements will be able to establish a more direct link

between the physiological and behavioral effects resulting from various types of preceding experiences.

## Chapter 4

### **Changes in NR2B-receptor subunit conductance in V1 (layer II/III) neurons following visual discrimination training of adult rats.**

*The following experiments have been published along with additional experiments in “Metaplastic up-regulation of LTP in the rat visual cortex by monocular visual training: requirement of task mastery, hemispheric specificity, and NMDA-GluN2B involvement” by Hager, A. M., Gagolewicz, P. J., Rodier, S., & Dringenberg, H. C. (2015). Neuroscience, 293, 171-186.*

#### **4.1 Abstract**

NMDA receptors (NMDARs) are key molecular devices for controlling synaptic plasticity and memory function. We examined effects of visual discrimination training on NMDAR conductance in the mature, rodent primary visual cortex by training rats in a modified Morris Water Maze containing a Y-maze insert. Rats learned to swim into the goal arms of the maze to associate visual cues with the location of an underwater hidden platform (learning rats, LR). A second group of rats was trained in the maze, but experienced a random association of visual cues with the escape platform location (no learning rats, NLR). Following successful task acquisition, brain slice electrophysiology was used to identify training-induced alterations to neurons in layers II/III of the primary visual cortex (V1). Whole-cell patch clamp recordings from pyramidal cells pharmacologically isolated with DNQX (50  $\mu$ M; applied to slice bath) did not reveal differences in AMPA/NMDA excitatory post-synaptic current (EPSC) ratios between the LR, NLR and task naïve groups. Whole-cell patch clamp recordings from cells treated with NR2B antagonist Ro 25-6981 (1  $\mu$ M; applied to slice bath) revealed significantly reduced (30%) EPSCs in LR, compared

to NLR and task naïve (18%; 19%, respectively) rats. These results suggest that behavioural (visual) training can alter NMDAR conductance in V1, an effect dependent on NR2B subunits.

## **4.2 Introduction**

Long-term potentiation (LTP) constitutes a synaptic plasticity mechanism thought to mediate the encoding and storage of new information in the adult nervous system (Martin & Morris, 2002). A number of studies have shown that various learning experiences (e.g., fear conditioning, motor, perceptual, and passive avoidance training) result in increases in synaptic strength at specific sets of synapses (Rogan et al., 1997; Rioult-Pedotti et al., 2000; Monfils & Teskey, 2004; Whitlock et al., 2006; Sale et al., 2011). Importantly, this training-induced synaptic potentiation often competes with, or occludes, the subsequent induction of LTP induced by high-frequency electrical stimulation (Rioult-Pedotti et al., 2000; Monfils & Teskey, 2004; Whitlock et al., 2006), an observation that is consistent with the notion that learning and LTP share common cellular and molecular mechanisms (Martin & Morris, 2002).

Interestingly, other investigations have shown that behavioral experiences can also facilitate subsequent LTP induction, rather than lead to the occlusion effect noted above. For example, housing adult rodents in an enriched environment enhances LTP induction in the primary visual cortex (V1; Sale et al., 2007; Mainardi et al., 2010), while sound discrimination training has been shown to result in greater LTP in the primary auditory cortex (A1) of adult rats (Zhang et al., 2013). Similar to the latter observation, visual discrimination training leads to a facilitation of LTP in the thalamocortical visual system between the lateral geniculate nucleus (LGN) and V1 of adult rats (Hager & Dringenberg, 2010a), an effect that appears to involve the NR2B subunit of N-methyl-D-aspartate (NMDA) receptors located in V1 (Gagolewicz & Dringenberg, 2011). Together, the studies summarized above clearly emphasize that behavioral

experience can exert complex, multi-directional effect on plasticity mechanisms, with occlusion or facilitation of LTP as possible outcomes.

One of the key molecules for determining the magnitude and direction of synaptic plasticity at mature V1 synapses are NMDA receptors. These membrane-bound, multi-subunit complexes exist as di-heteromers or tri-heteromers. Functional NMDA receptors are composed of two obligatory NR1 subunits that forms pairs with one or two of the NR2A-D subunits (Seeburg, 1993; Mori and Mishina, 1995). The NR2 subunits are particularly important to the regulation of synaptic plasticity, as these control the time course of NMDAR currents, set the voltage dependence of the channel  $Mg^{2+}$  block, and affect the intracellular binding elements involved in regulating the molecular composition of synapses and signal transduction (Kopp et al., 2007). The NR2A and NR2B subunits have been extensively studied in the mature rodent cortex and hippocampus (see Barria and Malinow, 2002; Dumas, 2005 for review), where they are predominant (Cull-Candy & Leszkiewicz, 2004). In V1, NR2B receptor expression levels plateau within the first two weeks of development, while NR2A subunits continue to increase gradually with maturation (Quinlan et al., 1999; Sheng et al., 1994). In addition, evidence exists supporting experience-dependent modifications to NR2 subunit composition at NMDA-containing synapses in V1 of mature rodents (Yashiro & Philpot, 2008; Smith et al., 2009). In general, sensory deprivations conducted in adults result in reduced ratios of NR2A/NR2B receptors in visual cortex neurons (He et al., 2006; Yashiro et al., 2005). However, no clear consensus exists regarding the experience-dependent regulation of NR2B receptors in the V1 of adults.

With the present experiments, we assessed the effects of visual discrimination training on NMDA receptors properties in adult rats. As mentioned, our previous work has showed that discrimination training results in a facilitation of LTP in V1, an effect dependent on the NR2B

subunit of NMDA receptors (Gagolewicz & Dringenberg, 2011). Here, by assessing electrophysiological properties of cortical layer II/III pyramidal neurons in V1, we further probe the role of NMDA receptor subunits in the plasticity modulation seen following visual discrimination training. We show that V1 neurons exhibit an enhanced response to the potent NR2B antagonist Ro 25-6981 following mastery of the visual discrimination task. These results suggest that, under certain conditions, visual experience, rather than visual deprivation, can lead to an upregulation of NR2B function in upper layer principal neurons in V1.

With the present experiments, we assessed the effects of visual discrimination training on NMDA receptors properties in adult rats. As mentioned, our previous work has showed that discrimination training results in a facilitation of LTP in V1, an effect dependent on NR2B receptors (Gagolewicz & Dringenberg, 2011). Here, by assessing electrophysiological parameters of cortical layer II/III pyramidal neurons in V1, we further probe the role of NMDA receptor subunits in the plasticity modulation seen following visual discrimination training. We show that cortical synapses show a differential response to the potent NR2B antagonist Ro 25-6981 following mastery of the visual discrimination task. These results suggest that, under certain conditions, visual experience, rather than visual deprivation, can lead to an upregulation of NR2B function in upper layer principal neurons in V1.

## **4.3 Materials and methods**

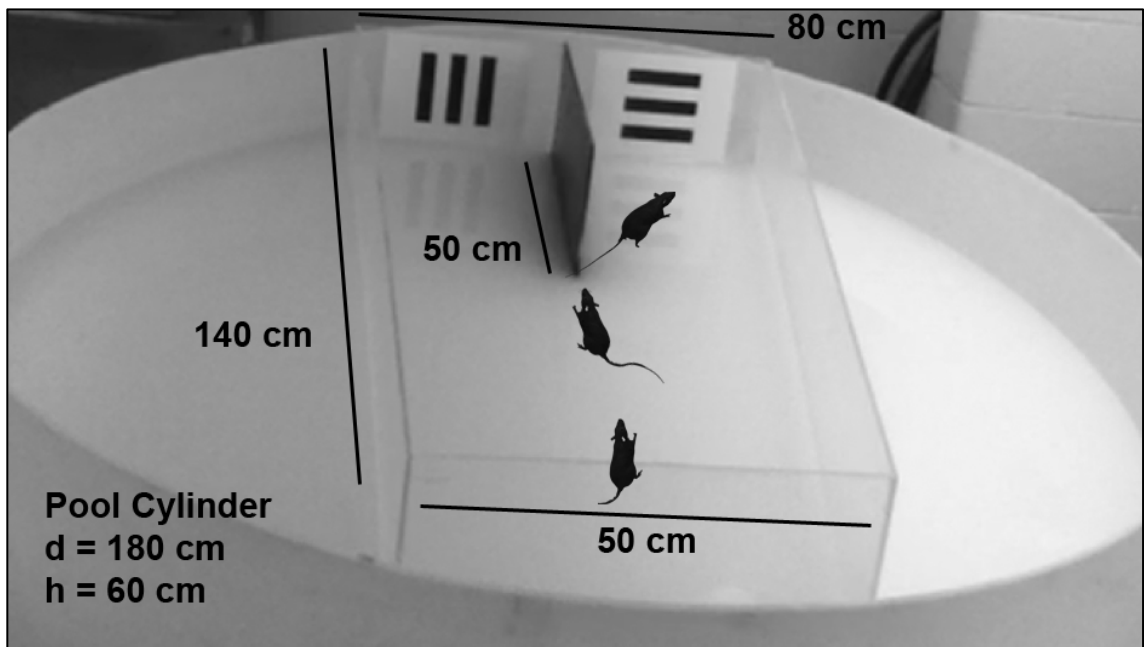
### **4.3.1 Subjects**

*Subjects.* Experiments were conducted on adult (300-600 g) male Long-Evans rats (Charles River Laboratories, Saint-Constant, Quebec, Canada). The animals were singly housed in a colony room under a reversed 12:12-h light cycle (lights off between 07:00 and 19:00 h),

with food and water access *ad libitum*. Experiments were conducted between 09:00 and 18:00 h. All procedures were conducted in accordance with guidelines of the Canadian Council on Animal Care, and were approved by the Queen's University Animal Care Committee. Each animal was used for one experiment.

#### 4.3.2 Visual discrimination training

Behavioral training was conducted in a modified Morris Water Maze containing a Y-maze insert. The water maze consisted of a cylindrical pool (Fig. 4.1; 180 cm in diameter, and 60 cm in height, filled with water to a height of 40 cm). The water was maintained at a temperature of  $22 \pm 1$  °C, and rendered opaque by the addition of white, non-toxic paint. The pool contained a clear Plexiglas Y-maze insert (height, 60 cm; length, 140 cm; width, 50 cm at the proximal



**Figure 4.1.** Photograph of the circular water pool and the Y-maze insert apparatus used for visual discrimination training. Rats were placed at the foot of the maze and swam to the goal arms,

which contained two distinct visual cues. Trained rats learned to associate one of the cues with a submerged invisible escape platform. Rat and dimensional graphics are superimposed.

release site and 80 cm at the distal goal arms), which was kept in the same position throughout the entire training period. A black Plexiglas divider (height, 50 cm; length, 60 cm) separated the two goal arms. A clear Plexiglas rectangular platform (height, 38 cm; width, 12 cm; length, 36 cm) was placed 10 cm from the end of one of the goal arms, 2 cm below the water surface. The water opacity ensured that the platform was not visible. Two distinct visual cues were used for discrimination training. Cues were printed on white sheets of paper (21.5 x 28 cm), and consisted of either three black, horizontal bars (width, 3 cm; length, 15 cm; spaced 3 cm apart) or three vertical bars (same dimension and spacing as above). The visual cues were mounted at the end of the two Y-maze goal arms 1 cm above the water line. Discrimination training was divided into three phases. For all phases, animals were released into the Y-maze insert facing the pool wall and required to swim towards the goal arms in order to reach the hidden escape platform located in one of the arms.

Phase 1: The initial training phase (1 day) served to familiarize animals with swimming in the pool and finding the hidden platform without the use of visual cues in the maze. Each animal was released into the pool, initially facing the pool wall, and given a maximum of 100 s to find the platform. If an animal failed to do so, it was manually guided to the platform by the experimenter. Animals remained on the platform for 15 s before commencement of the next trial. Trials were repeated (the platform remained in the same arm) until an animal performed five consecutive correct responses, or a maximum of 10 trials, whichever came first (this constituted a 'trial block'). Correct trials were scored when an animal entered the correct goal arm and mounted the platform; errors were scored when an animal entered the incorrect goal arm with at least half of

its body. Each trial block was followed by a rest period of ~5 min, during which animals were returned to a holding cage with holes in the bottom to allow water to drain. Following the rest period, the next trial block was administered with the same procedures as above, with the exception that the platform was moved to the opposite goal arm. A total of four trial blocks were administered, each followed by a 5 min rest period. After completion of training, animals were placed under a heat lamp for a minimum of 15 min before being returned to their home cage.

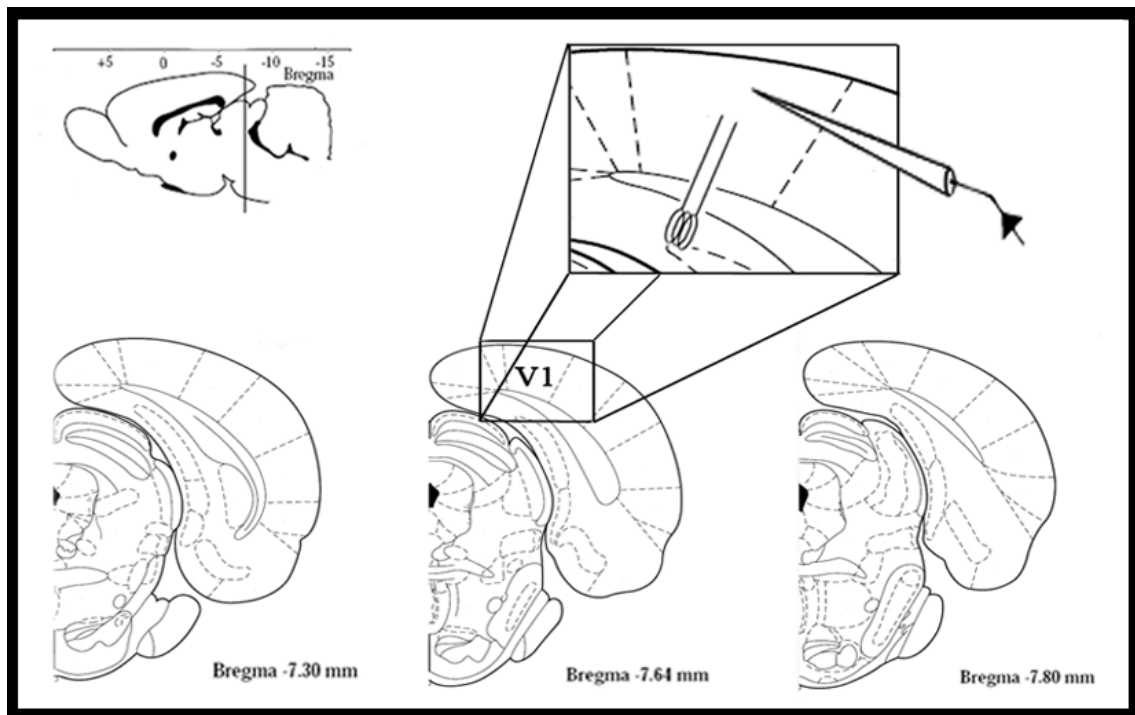
Phase 2: The same training procedure as that outlined above was used for phase 2 (1 day), with the exception that two distinct visual cues were placed at the end of the goal arms, one indicating the presence of the platform (P+), and the other indicating its absence (P-). The assignment of cues as P+ and P- was counterbalanced across rats. Platform location and associated visual cues remained unchanged during each trial block (10 trials), but alternated between blocks (four blocks in total), with the platform-cue association being kept constant for all blocks. However, for a group of control animals, the platform location alternated between blocks, whereas the visual cues remained constant to ensure that they did not predict the platform location.

Phase 3: The final training phase commenced on day three and continued until animals reliably discriminated the two visual cues. Procedures were the same as for phase 2, with the following exceptions. On each day, animals received 10 training trials, each followed by a 30 s rest period in a holding cage. For each trial, platform location was randomly assigned to one goal arm. Again, for one group of (trained) animals, visual cues alternated together with the platform, so that P+ and P- consistently indicated the presence and absence, respectively, of the platform. For the control group, platform location was changed randomly from trial to trial, but visual cues remained stationary, and thus lacked a consistent association with the platform. For trained animals, daily training continued until an animal reached a criterion of at least 80% correct (i.e. 8 / 10 daily trials) over three consecutive days. For control animals, training for phase 3 was carried

out for an average of eight consecutive days (range of 7-8 days), which was the mean number of days required by trained animals to reach the performance criterion outlined above.

#### 4.3.3 Preparation of brain slices

Electrophysiological procedures were carried out one day following completion of behavior training. Rats were anesthetized with isoflurane, euthanized, and the brains were extracted for slice preparation. Coronal sections (300  $\mu\text{m}$ ; Fig. 4.2) were prepared on a vibrating

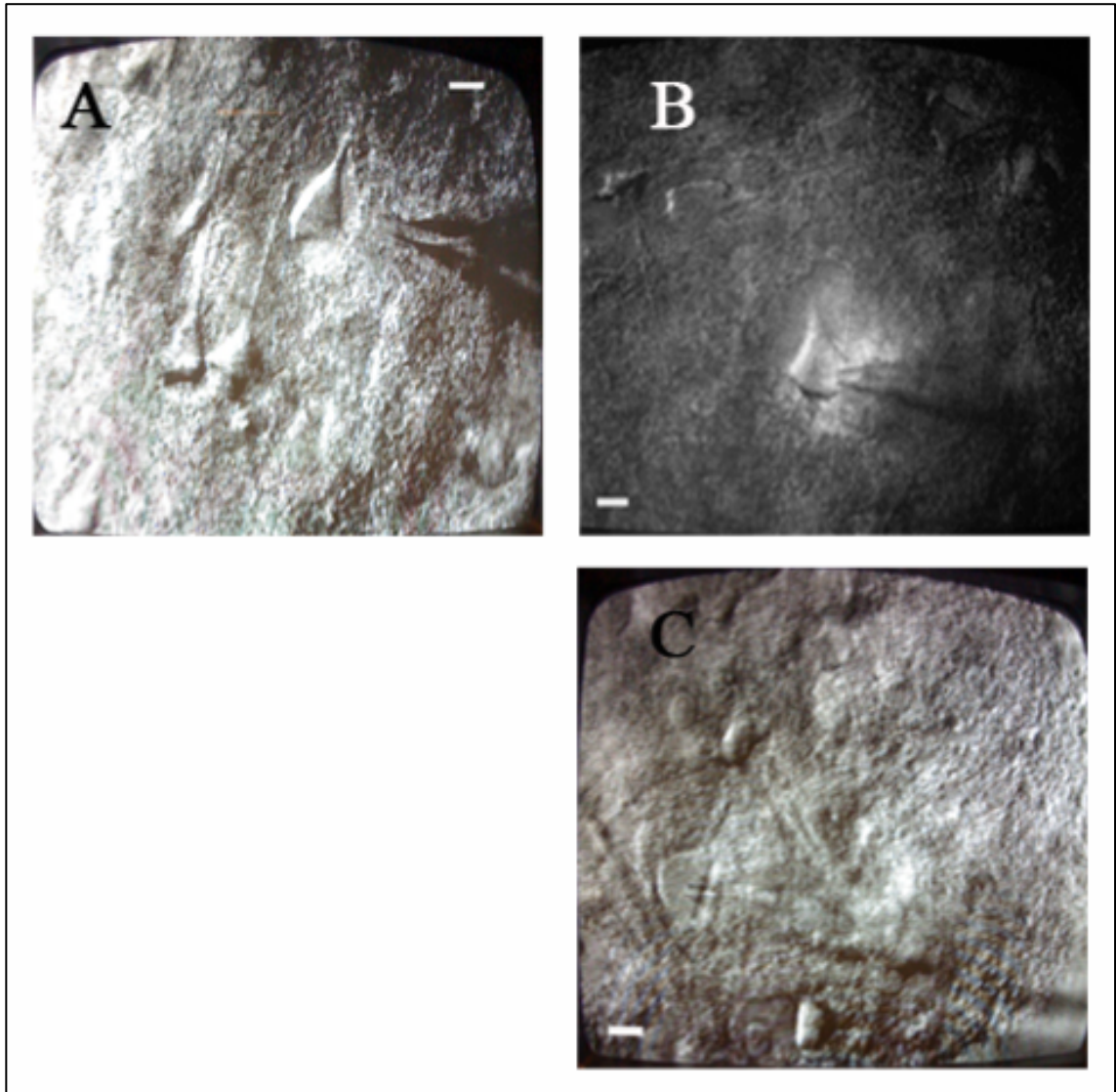


**Figure 4.2.** Schematic depicting the anatomical site of electrode placements employed for the electrophysiological experiments. Scale surrounding the lateral brain section indicates anterior/posterior or ventral coordinates (in mm) based on the skull landmark bregma. Stimulation electrodes were placed in V1, ventral to layer II/III. Recordings were typically constrained to the area shown in the magnified insert. Up to a maximum of 6 coronal brain slices (300  $\mu\text{m}$  in thickness; 3 per hemisphere) were obtained from each animal one-day following completion of the behavioral training. Brain and slice diagrams obtained from the rat brain atlas by Paxinos and Watson (1998).

blade microtome in an ice-cold, oxygenated physiological solution containing (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl<sub>2</sub>, 2.6 CaCl<sub>2</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 21.4 NaHCO<sub>3</sub> and 12.5 D-Glucose, osmolarity at ~320-290 mOsm. Throughout brain slicing sections of cortical lobes were separated from the midbrain, these were collected while the latter were discarded. Slices were incubated in oxygenated physiological solution at 34°C for at least 60 min, and next transferred to a holding bath for patch-clamp electrophysiology. During patch clamp recordings, slices were constantly perfused (2.0 ml/min) with physiological solution maintained at 34°C and equilibrated with 95% O<sub>2</sub>/ 5% CO<sub>2</sub>. To ensure accurate recording locations a maximum of three coronal sections were kept per brain. This yielded six slices or three per hemisphere.

#### **4.3.4 Electrophysiology**

The V1 was visualized and pyramidal neurons were identified by shape (Fig. 4.3), and located using landmarks such as the slice surface plus white matter. Whole-cell voltage clamp recordings were obtained from layer II/III neurons with borosilicate glass tip pipettes (1.5-2.5 MΩ tip resistance) containing (in mM) 140 CH<sub>3</sub>CsO<sub>3</sub>S, 10 HEPES, 4 NaCl, 1 MgCl<sub>2</sub>, 1 EGTA, 10 P-creatine, 4 MgATP, 0.2 GTP, with pH adjusted to 7.25-7.4 and osmolarity at ~290-270 mOsm. Recordings were obtained with a Multiclamp 700B amplifier connected to a Digidata 1440A digitizer (Molecular Devices Scientific, Sunnyvale, CA). Data were collected and



**Figure 4.3.** Photos of typical layer II/III neurons encountered throughout recordings in V1 slices under darkfield illumination. (A) Pyramidal neurons at the recording site, glass microelectrode visible on the top right. (B) The characteristic appearance of a dimple on the cell body prior to G $\Omega$  seal formation. (C) Whole-cell patch clamp configuration of a cortical neuron. White scale bar represents 10  $\mu$ m.

analyzed using Axograph X for windows (V 1.4.3, AxographX.com). Postsynaptic currents were evoked by local fiber stimulation with tungsten bipolar electrodes (MX21DBW; FHC Inc., Bowdoin, ME) while V1 layer II/III pyramidal neurons were voltage clamped at +40 mV.

Stimulating electrodes were placed ventral to the approximate recording area, at a depth corresponding to ~layer IV (300-450  $\mu\text{m}$  from the surface), and electrical stimuli (10-500  $\mu\text{A}$ , 0.1 ms duration) were applied at 0.033 Hz. For single pulse and temporal summation recordings GABA<sub>A</sub>-IPSC, AMPA-EPSC, and NR2B-EPSC were pharmacologically isolated with DNQX (50  $\mu\text{M}$ ), picrotoxin (100  $\mu\text{M}$ ; Tocris Bioscience) and Ro 25-6981 (1.0  $\mu\text{M}$ ), respectively. NMDA receptor contribution to the EPSC was calculated offline by subtracting AMPA current from total EPSC. NMDA receptor current temporal summation recordings consisted of a single pulse (0.1 ms duration) followed by a train of 40 pulses (0.1 ms; 40 Hz). All summation protocols were repeated 3 times at 1 Hz.

#### **4.3.5 Pharmacology**

A stock solution of Ro 25-6981 (1 mM; Sigma-Aldrich) was prepared in double-distilled water. The stock solution of DNQX (100 mM; Tocris Bioscience) was prepared in DMSO (100%). Each drug was further dissolved in the physiological solutions at the desired concentration and the final DMSO concentration never exceeded 0.1%. Each slice was used for electrophysiological recording until the application of Ro 25-6981. Once a slice was exposed to this antagonist, it was removed from the bath and a new one was transferred from the incubator chamber.

#### **4.3.6 Data Analysis**

All data are expressed as mean $\pm$ standard error of mean (S.E.M.) or standard error of the difference (S.E.D.) for repeated measures, within-subject factors. Postsynaptic currents were quantified by measuring the peak amplitude of each EPSC (using Axograph software) before and approximately 5 min after drug application. Changes in EPSCs were calculated as follows:  $[(\text{Peak amplitude}_{\text{drug}} - \text{Peak amplitude}_{\text{baseline}})/\text{Peak amplitude}_{\text{baseline}}] \times 100$ . Statistical

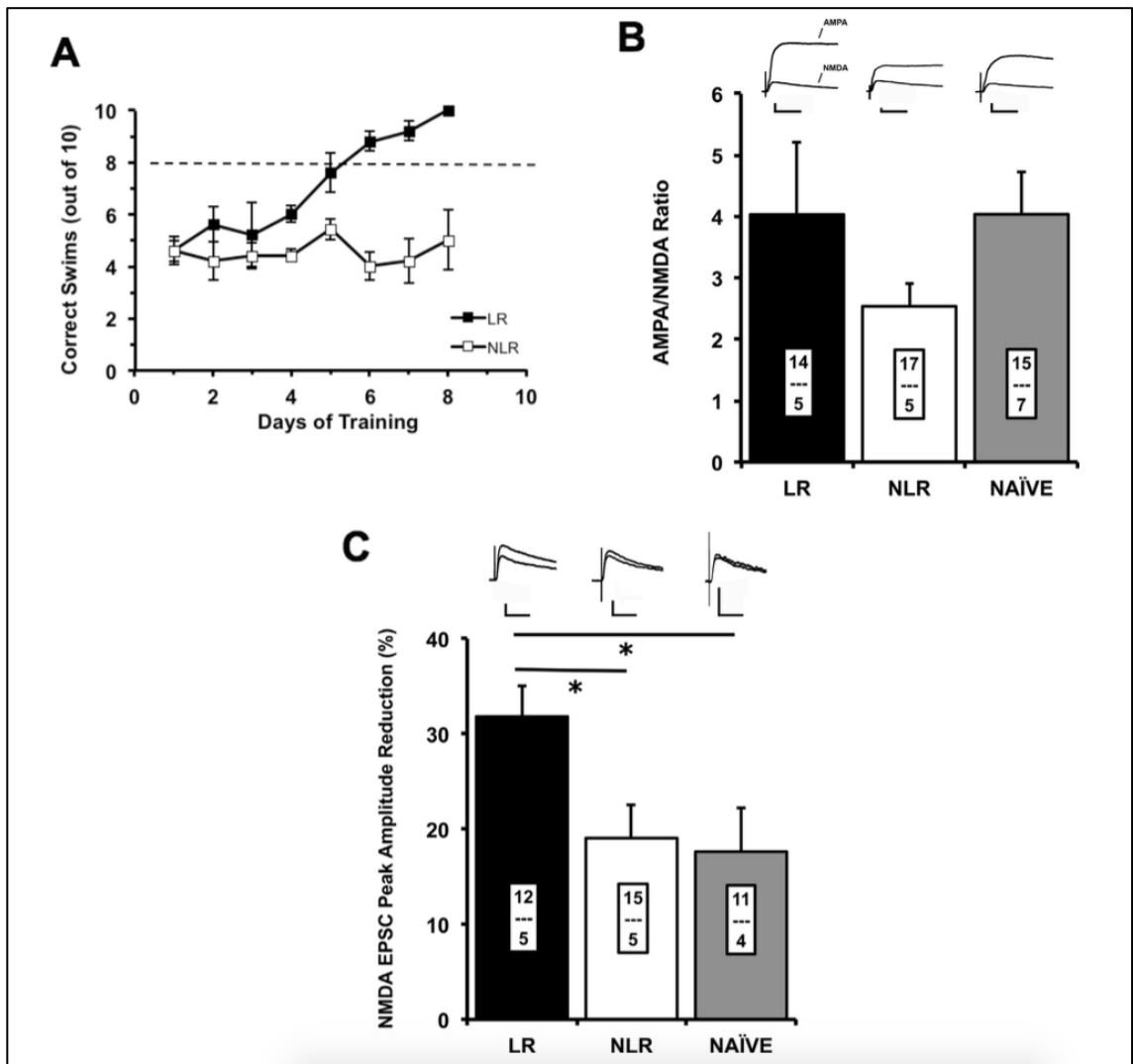
evaluations consisted of analyses of variance (ANOVA), performed using SPSS software (V.19.0; SPSS Inc., Chicago, IL). Post-hoc analyses (Tukey), polynomial contrasts, or simple effects tests using SPSS syntax were calculated to follow up significant interactions between variables when appropriate.

## **4.4 Results**

### **4.4.1 Visual discrimination training**

We assessed the effects of visual discrimination training on synaptic currents in layers II/III pyramidal cells of V1 studied under *in vitro* conditions, cells that were chosen based on the current-source density analysis reported in previous work (Heynen and Bear, 2001).

Two groups of animals were trained on the visual discrimination task, with one group serving as learning rats (LRs; trained to associate the platform with a visual cue; n = 5), while a second group acted as ‘no learning rats’ (NLR; random association of the visual cues with the platform location; n = 5). All LRs reached the predetermined acquisition criterion (8/10 correct daily trials for 3 consecutive days) after an average of 7.4 training days (Fig. 4.4A; range, 7-8 days). The NLR did not show increases in task performance (Fig. 4.4A), with correct trials around chance level (5/10 trials) throughout the entire training period (average of 8 training days; range 7-9 days).



**Figure 4.4.** (A) Correct swims (mean  $\pm$  S.E.M.) for learning rats (LR,  $n = 5$ ; trained with a consistent association of the escape platform and visual cues) and no learning rats (NLR,  $n = 5$ ; trained with a random association of the escape platform and visual cues). All LRs reached the acquisition criterion of at least 8/10 correct trials for three consecutive days; see dashed line). The NLRs performed at chance level throughout the entire training period. (B) Contributions of AMPA and NMDA currents (expressed as the AMPA/NMDA ratio) to excitatory postsynaptic currents (EPSCs; elicited by stimulation of the deeper gray matter) in layer II/III pyramidal neurons of V1 in LRs, NLRs, and task-naïve animals (top and bottom numbers in each bar of panels B and C indicate number of cells/slices [only one cell per slice was recorded] and animals, respectively). Ratios were calculated subtracting the NMDA component (smaller EPSC traces in insert in the presence of DNQX, 50  $\mu$ M bath application) from the raw currents (not shown), followed by division of the remaining current (large EPSC traces in insert) by the NMDA component. There were no significant differences ( $p > 0.1$ ) in AMPA/NMDA ratios among the three experimental groups. Traces depict typical EPSCs (AMPA and NMDA components, see

above) in LR (left), NLR (middle), and naïve animals (right; calibration bars are 200 pA and 50 ms). (C) Contributions of GluN2B subunits to NMDA receptor-mediated EPSCs, calculated by the reduction of peak EPSC amplitude following bath application of Ro 25-6981 (1.0  $\mu$ M). Drug application resulted in a significantly (\* indicate  $p < 0.05$ , Tukey post hoc tests) greater EPSC reduction in LRs relative to both NLR and naïve animals. Traces depict typical EPSCs before (larger amplitude) and after Ro 25-6981 application for LR (left), NLR (middle), and naïve animals (right; calibration bars are 200 pA and 50 ms).

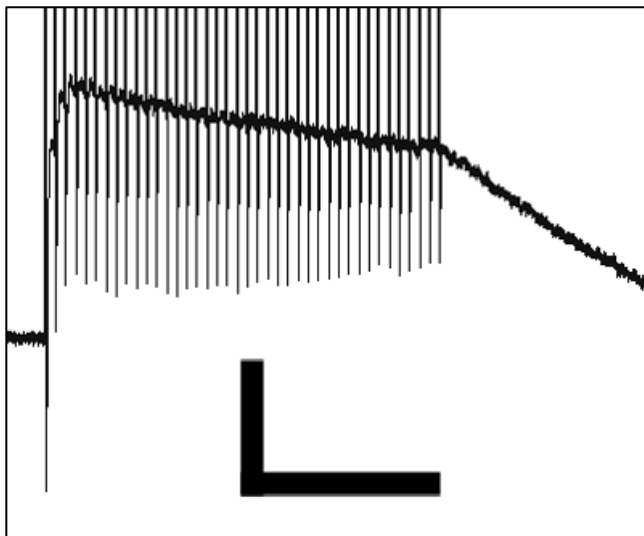
#### 4.4.2 Electrophysiology

NMDA Conductance in V1 Slices: One day following the last training session, rats were euthanized and slices of V1 (300  $\mu$ m; typically six slices for each animal) were obtained using standard sectioning procedures; an additional group of untrained, task-naïve rats ( $n = 7$ ) was also included for the *in vitro* experiments. A bipolar stimulation electrode was placed ventral to layer II/III, and a borosilicate glass tip pipette was used to obtain whole-cell voltage clamp recordings of visually identified pyramidal cells (Fig. 4.3) in cortical layers II/III (Fig. 4.2). Stimulation reliably elicited EPSC in these neurons, with membrane characteristics exhibiting no reliable differences for cells obtained from LRs ( $n = 21$  neurons), NLRs ( $n = 15$  neurons), and task-naïve rats ( $n = 17$  neurons); membrane capacitance:  $22.2 \pm 1.0$  pF in LRs,  $22.4 \pm 0.9$  pF in NLRs,  $22.5 \pm 1.1$  pF in naïve rats; series resistance:  $8.5 \pm 0.3$  M $\Omega$  in LRs,  $8.4 \pm 0.2$  M $\Omega$  in NLRs,  $8.9 \pm 0.3$  in naïve rats; input resistance:  $86.2 \pm 8.5$  M $\Omega$  in LRs,  $78.8 \pm 7.2$  M $\Omega$  in NLRs,  $91.0 \pm 6.3$  M $\Omega$  in naïve rats; all  $F$ 's  $< 0.9$ ;  $p$ 's  $> 0.5$ ).

The contribution of AMPA and NMDA components of EPSCs in neurons from LRs, NLRs, and task-naïve rats was examined by bath application of DNQX (50  $\mu$ M) to remove the AMPA component of the EPSCs. Neurons from LRs ( $n = 14$ ) and naïve rats ( $n = 15$ ) tended to exhibit larger AMPA/NMDA EPSC ratios ( $5.0 \pm 1.2$  and  $5.0 \pm 0.7$ , respectively) relative to cells from NLRs ( $n = 17$ ;  $3.5 \pm 0.4$ ; Fig. 4.4B); however, this difference did not reach statistical significance ( $F(2, 43) = 1.3$ ,  $p = 0.3$ ).

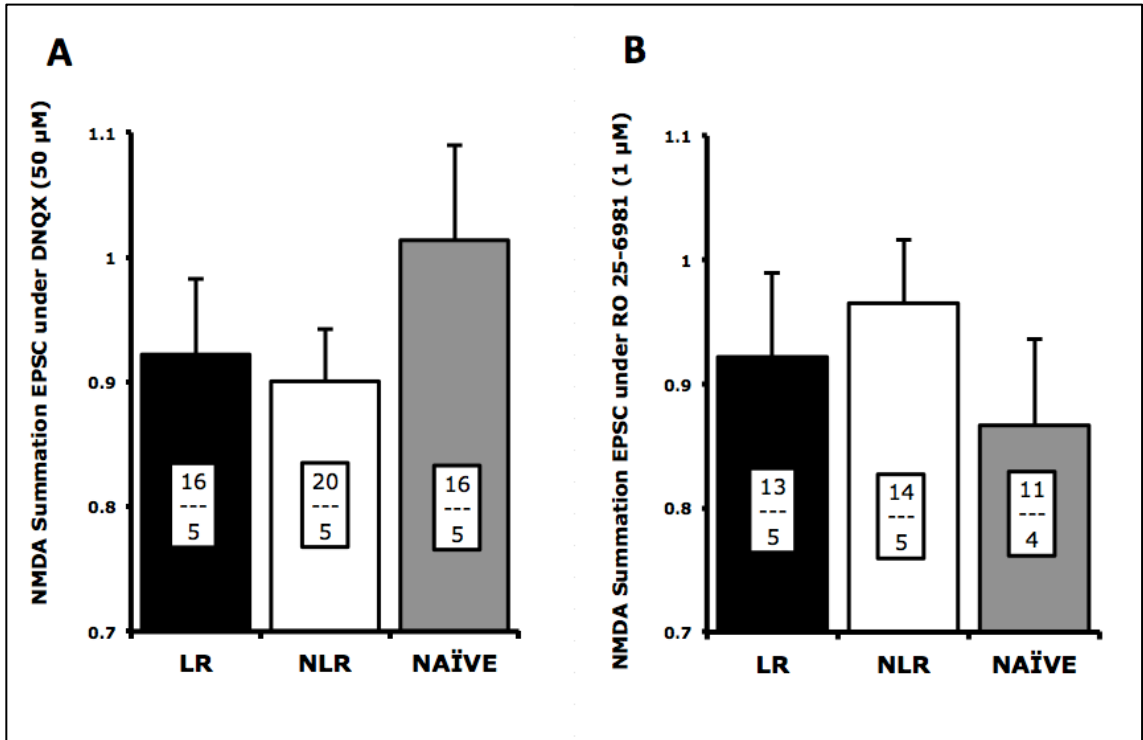
Once NMDA-mediated EPSCs were isolated, the contribution of NR2B subunits to these EPSC was examined by bath application of Ro 25-6981 (1.0  $\mu$ M). For all cells analyzed, drug application reduced the peak EPSC amplitude, indicative of a clear, NR2B-mediated component in these NMDA currents. In neurons (n = 11) from naïve rats or NLRs (n = 15 cells), Ro 25-6981 reduced EPSC amplitude by  $17.6 \pm 4.6\%$  and  $19.1 \pm 3.5\%$ , respectively (Fig. 4.4B). Interestingly, in neurons (n = 12) from LRs, Ro 25-6981 reduced EPSC amplitude by  $31.8 \pm 3.2\%$ , which was significantly greater than the suppression in naïve or NLRs (Fig. 4.4C;  $F(2, 35) = 4.2$ ,  $p = 0.02$ ). These results suggest that visual discrimination training increase the contribution of NR2B currents to the EPSCs of layer II/III pyramidal neurons of V1 *in vitro*.

Additionally, we analyzed the temporal summation of NMDAR currents (Fig. 4.5) before



**Figure 4.5.** Example of temporally summated NMDA-mediated excitatory postsynaptic currents (EPSCs; elicited by stimulation of the deeper gray matter) in layer II/III pyramidal neurons of V1 from LR animal. AMPA current component was blocked using DNQX (50  $\mu$ M; bath applied). Summation was achieved with train of 40 single pulses (0.1ms @ 40 Hz). Composite image represents the average of three separate sweeps, one every 60 seconds. Calibration bars represent 200 pA and 500 ms.

and after the application of Ro 25-6981 (1.0  $\mu\text{M}$ ; Fig. 4.6). It has been previously shown that even small differences in NMDA EPSC duration can manifest as substantial cumulative



**Figure 4.6.** Summation ratios of NMDAR-mediated excitatory postsynaptic currents (EPSCs; elicited by stimulation of the deeper gray matter) in layer II/III pyramidal neurons of V1 in LR, NLR, and task-naïve animals (top and bottom numbers in each bar of panels A and B indicate number of cells/slices [only one cell per slice was recorded] and animals, respectively). Summation ratios were calculated by dividing the peak amplitude following the 40th stimulation pulse by the peak amplitude after the 1<sup>st</sup> (but prior to the 2<sup>nd</sup>) stimulation pulse. (A) There were no significant differences ( $p > 0.3$ ) in summation ratios among the three experimental groups in the presence of DNQX (50  $\mu\text{M}$ ; applied to bath). (B) There were no significant differences ( $p > 0.5$ ) in summation ratios among the three experimental groups in the presence of Ro 25-6981 (1  $\mu\text{M}$ ; applied to bath).

changes in NMDA-mediated currents recruited through trains of stimuli delivered to V1 (Philpot et al., 2001). When we applied trains of stimulation delivered at 40 Hz, no detectable differences

were apparent in NMDA summation EPSCs between the LR, NLR and naïve groups (Fig. 4.6A;  $F(2, 50) = 1.047, p = 0.359$ ). The temporal summation protocol was repeated once more following the application of Ro 25-6981, also resulting in no detectable differences in NMDA-mediated EPSC summation (Fig. 6B;  $F(2, 37) = 0.598, p = 0.555$ ). These data demonstrate that NMDAR EPSC dynamics remain similar between LR, NLR and naïve animals when assessed using rapid-stimulus, temporal summation protocols.

#### **4.5 Discussion**

The present set of experiments demonstrates that visual discrimination training leads to a greater sensitivity of NMDA currents in V1 (layer II/III) pyramidal cells to the pharmacological blockade of GluN2B subunits. These data provide evidence for an experience-induced, metaplastic up-regulation of plasticity mechanisms in the mature V1 and occurs only in animals that have mastered the behavioral task. The NLRs were exposed to the same conditions as LRs except for learning to associate the visual cues with the platform location; however, NLR exhibited electrophysiological/pharmacological parameters similar to those seen in task-naïve rats.

The greater sensitivity of NMDA EPSCs to the application Ro 25-6981 observed in the V1 of LR is indicative of a greater contribution of GluN2B subunits to ionic conductance, a sensory area of the brain involved in early processing of visual stimuli. It is notable that previous work has shown that current sinks of cells located in layers II/III give rise to the negative-going thalamocortical field postsynaptic potentials (fPSPs) that can be recorded in the superficial V1 under *in vivo* conditions (Heynen & Bear, 2001). Principal cell activity at this location is the main source of currents contributing to fPSPs; hence we examined these cells for alterations to

NMDAR-mediated conductance following task mastery of the visual discrimination training paradigm.

The training-induced changes to NMDAR-conductance detected in the present experiments following the application of Ro 25-6981 may have manifested due to alterations in receptor expression, function, or location. For instance, additional NR2B subunits may have been expressed in pyramidal neurons of LR following mastery of the discrimination task. However, we currently do not have direct evidence for the upregulated expression of NR2B subunits in V1. Interestingly, Philpot et al. (2001) proposed that overexpression of the NR2B subunit does not necessarily alter plasticity or NMDA EPSCs in the visual cortex of mature, transgenic mice. The authors suggested that expression of NR2B protein might not have led to the incorporation of these receptors into the synaptic membrane, or to a possible endogenous saturation of NR2B mRNA levels in V1. In contrast, others have demonstrated that the overexpression of NR2B subunits in the hippocampus can result in enhanced memory function and CA1-LTP (Wang et al., 2009). Therefore, a detailed analysis of the expression pattern of NR2B subunits in V1 of LR and NLR would be an interesting follow-up study to the current work.

The NMDA EPSC changes detected in our experiments may also be due to changes in receptor function, such as seen following receptor subunit phosphorylation (Rosenblum et al., 1996). Alternatively, there is also the possibility of lateral mobility of NR2B-containing NMDA receptors between extrasynaptic and synaptic locations in LR rats (see Yashiro & Philpot, 2008). It has been suggested that visual deprivation can lead to the preferential trafficking of NR2B-containing NMDARs to extrasynaptic sites (Yashiro et. al., 2005). Therefore, it is tempting to speculate that training in the visual discrimination task, an experience somewhat opposite to visual deprivation, may have resulted in changes (increases) to synaptic NR2B-containing receptors (while extrasynaptic receptors remained unaltered), as deprivation, not learning

experience, has been previously shown to affect extrasynaptic NR2B receptors. This could account for the lack of group differences in the temporal summation (pulse trains) of EPSCs, and a differential response to Ro 25-6981 under lower stimulation intensities (single pulses). Therefore, our data fit with previous evidence that points to changes at synaptic NR2B receptors following experience. The data presented here also do not necessarily contradict theories about deprivation-induced changes at NR2B-containing synapses because the summation protocols could not detect changes at extrasynaptic sites in V1.

Despite the pronounced differences in the sensitivity of NMDA EPSCs to Ro 25-6981 following discrimination training, there were no detectable changes in the summation ratios before and after the pharmacological blockade of NR2B subunits. It has been hypothesized that high frequency stimulation is capable of recruiting extrasynaptic NR2B-containing NMDA receptors due to factors like glutamate spillover (see Yashiro et al., 2005). The pharmacological effect noted in LRs was manifested during low intensity stimulation, when glutamate spillover is unexpected, suggesting that the changes may have instead occurred within the synapses. However, it is unclear why NMDA summation ratios remained relatively similar between all of our tested groups. Interestingly, two previous studies assessed temporal summation of NMDAR EPSCs in V1 of transgenic and visually deprived adult rodents (Philpot et al., 2001; Yashiro et al., 2005). The stimulation parameters used in these experiments differed from those in our study; in general, our temporal summation paradigm was more intense, containing more pulses per stimulation train. This may have overemphasized the contribution of extrasynaptic NR2B to the summated EPSC responses, possibly accounting for a lack of detectable effects between the three experimental groups (LR, NLR, and naïve rats). To add, the experiments by Philpot et al. (2001) and Yashiro et al. (2005) were conducted on mice, as opposed to rats, further complicating the comparison of the different patterns of results obtained in these studies.

It is well established that increased GluN2B expression or functioning can enhance plasticity by several mechanisms, including greater calcium influx into the postsynaptic neuron, as well as greater affinity of GluN2B relative to GluN2A subunits for calcium/calmodulin-dependent protein kinase II, one of the dominant signaling molecules involved in plasticity induction (Carmignoto & Vicini, 1992; Monyer et al., 1994; Flint et al., 1997; Barria & Malinow, 2005; Kopp et al., 2007). The molecular mechanisms that allow behavioral training and task mastery to enhance GluN2B functioning in V1 remain unknown. It is of interest to note, however, that GluN2B expression is dynamic and appears to be governed by activity-dependent processes involving calcium-triggered signaling pathways (cAMP, PKA, and CREB), leading to the production of new GluN2B subunits that are delivered to the synapse (Zhuo, 2009). It is tempting to speculate that visual training and some reward-dependent signal activated by successful task performance converge to activate this signaling cascade, ultimately resulting in the up-regulating of GluN2B-NMDA receptor functioning.

The voltage clamp recordings of V1 pyramidal neurons did not reveal obvious differences between LRs and NLRs in basic membrane characteristics or changes in the ratio of AMPA to NMDA receptors, a common measure used to detect synaptic strengthening (Nicoll & Malenka, 1999; Hayton et al., 2010). These data indicate that training had relatively little or no effect on synaptic connectivity itself. Rather, training resulted in a priming of NMDA receptor-dependent plasticity mechanisms that revealed itself during the subsequent application of TBS to induce LTP (Gagolewicz & Dringenberg, 2011), and the reduction of NMDA EPSCs with Ro 25-6981 (current experiments). As such, the phenomenon described here satisfies the definition of “metaplasticity” as put forward by Abraham and Bear (Abraham & Bear, 1996; Abraham, 2008) as a process whereby a temporally remote priming event affects plasticity induction at a later time point without itself altering synaptic strength. Further, the behavioral specificity of the effect

described here indicates that this phenomenon does not constitute a generalized plasticity enhancement like that seen with environmental enrichment or exercise (Rosenzweig and Bennett, 1996; van Praag, 2009; Simpson & Kelly, 2011; Sale et al., 2014). Previous work has shown that rats experienced with a visual discrimination task (i.e., they have learned to discriminate two cues) show much faster acquisition of a second, novel cue set (Hager & Dringenberg, 2010a). Thus, it is tempting to ask if the plasticity enhancement we observed contributes to the parallel improvement in behavioral performance in experienced animals, a question that was addressed in the following chapter of this thesis.

## Chapter 5

### **The behavioral significance of NMDA receptor GluN2-B subunits in visual discrimination performance of mature rats**

*The following experiments have not been published at this time.*

#### **5.1 Abstract**

Mounting experimental evidence suggests that GluN2B-containing NMDARs can facilitate synaptic plasticity induction through metaplastic mechanisms following various types of experience throughout the brain, including the primary visual cortex. Here, we examined whether the antagonism of GluN2B subunits influences the behavioral performance of adult rats during sequential visual discrimination training (VDT), requiring animals to undergo two successive episodes of training, each one using two distinct visual cues. Rats were initially trained to swim to a hidden platform (indicated by visual cues) in a modified Morris Water Maze containing a Y-maze insert, this task being the initial visual discrimination training (iVDT). Upon meeting the performance criterion, rats were trained on a second (novel) set of visual cues to test whether the initial learning experience influenced acquisition of the second cue set (subsequent visual discrimination training; sVDT). All rats successfully completed the iVDT. Compared to vehicle-treated rats, administration of the GluN2B-selective antagonists Ro 25-6981 (5 mg/kg, i.p.) impaired performance during sVDT. However, this effect was not replicated with treatment of another GluN2B-selective antagonists, ifenprodil (5 mg/kg, i.p.). Probe trials administered at the conclusion of the sVDT revealed that neither sVDT, nor any of the drugs tested influenced the retention of the iVDT. In conclusion, the present experiments provide mixed support for a role of

GluN2B-subunits in some types of learning, such as the sequential acquisition of visual information during two rounds of VDT. The results from our experiments may be of interest to the rapidly expanding field of the behavioral consequences of previous experiences on subsequent learning and plasticity induction in the central nervous system.

## **5.2 Introduction**

Previous experience can shape the behavior of mammals, including rodents, and it can also influence the plasticity in the underlying neuronal circuitry of their brains. Whereas a good share of research attention has been devoted to understanding how experience can influence plasticity throughout the mature cortex (Lendvai et al., 2000; Malenka & Bear, 2004; Rema et al., 1998), less work has focused on understanding how a prior experience can influence the behavioral response to a subsequent one.

The idea that a previous experience, or priming activity, can alter plasticity during a subsequent experience has been termed metaplasticity (Abraham & Bear, 1996), alternately known as the “plasticity of plasticity”. A crucial feature of metaplasticity is a persistent alteration of plasticity mechanisms of synapses elicited by a priming event that itself does not alter synaptic connectivity. One focus of the work on metaplasticity so far has been attempting to describe this phenomenon through synaptic-level events, including long-term potentiation, and long-term depression (LTP; LTD; respectively; Malenka and Bear, 2004). For example, certain types of experiences such as dark rearing of an animal (priming) can subsequently expand the amount of LTP that can be expressed at V1 synapses (Kirkwood et al., 1996; Maffei et al., 2006; Philplot et al., 2003). Interestingly, other types of behavioral experiences may also facilitate subsequent LTP induction. For example, housing rats in enriched environments facilitates subsequent LTP

induction in V1 (Sale et al., 2007; Mainardi et al., 2010). Similarly, sound discrimination training has also been shown to enhance LTP in the primary auditory cortex of rodents (Zhang et al., 2013). Finally, visual discrimination training (VDT) facilitated subsequent LTP induction in the thalamocortical visual system of adult rats (Hager & Dringenberg, 2010a). Interestingly, this enhancement of LTP after training was attributed to the activation of GluN2B subunits of N-methyl-D-aspartate (NMDA) receptors in V1 (Gagolewicz & Dringenberg, 2011). This research was further expanded to show altered GluN2B-mediated conductance in V1 layer II/III pyramidal cells (Hager et al., 2015), suggesting that VDT can lead to alterations of principle cells in early visual processing areas. However, at present, it is not known whether GluN2B-mediated priming of plasticity induction in LTP has behavioral consequences, such as the facilitation of acquisition of visual information.

In light of the above-summarized findings, we decided to examine the effect of GluN2B subunit antagonists in situations where prior, behavioral experiences exert a facilitating effect on subsequent learning. To test this, we utilized a modified Morris Water Maze (Hager & Dringenberg, 2010a; also see Prusky et al., 2000; Dotigny et al., 2008) and subjected rats to two rounds of VDT, using novel sets of visual stimuli for each round of training. Further, we tested two different GluN2B-specific antagonists with daily, systemic injections prior to training to discriminate the second set of visual cues. We hypothesized that learning of the second set of cues would be facilitated by the initial training experience, and that this behavioral facilitation would be impaired by GluN2B antagonist treatment, in line with the role of these subunits in the metaplastic LTP enhancement in V1 following visual training.

## **5.3 Materials and methods**

### **5.3.1 Subjects**

Experiments were conducted on adult (200-400 g) male Long-Evans rats (Charles River Laboratories, Saint-Constant, Quebec, Canada). The animals were singly housed in a colony room under a reversed 12:12-h light cycle (lights off between 07:00 and 19:00 h), with food and water access *ad libitum*. Experiments were conducted between 08:00 and 18:00 h. All procedures were conducted in accordance with guidelines of the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee. Each animal was used for one experiment.

### **5.3.2 Visual discrimination training**

Behavioral training was conducted in a modified Morris Water Maze containing a Y-maze insert. The water maze consisted of a cylindrical pool (180 cm in diameter, and 60 cm in height, filled with water to a height of 40 cm). The water was maintained at a temperature of  $22 \pm 1$  °C, and rendered opaque by the addition of white, non-toxic paint. The pool contained a clear Plexiglas Y-maze insert (height, 60 cm; length, 140 cm; width, 50 cm at the proximal release site and 80 cm at the distal goal arms), which was kept in the same position throughout the entire training period. A black Plexiglas divider (height, 50 cm; length, 60 cm) separated the two goal arms. A clear Plexiglas rectangular platform (height, 38 cm; width, 12 cm; length, 36 cm) was placed 10 cm from the end of one of the goal arms, 2 cm below the water surface. The water opacity ensured that the platform was not visible.

Two sets of distinct visual cues were used for discrimination training. Cues were printed on white sheets of paper (21.5 x 28 cm) and made water-resistant with transparent laminate. The first set of cues consisted of either three black, horizontal bars (width, 3 cm; length, 15 cm;

spaced 3 cm apart) or three vertical bars (same dimension and spacing as above). The second set consisted of a black plus symbol (area = 135 cm<sup>2</sup>), and a black circle (diameter 16.7 cm, area = 222 cm<sup>2</sup>). The visual cues were mounted at the end of the two Y-maze goal arms, 1 cm above the water line.

Discrimination training was divided into four phases. For all phases, animals were released into the Y-maze insert facing the pool wall and required to swim towards the goal arms in order to reach the hidden escape platform located in one of the arms.

Phase 1 (Habituation): The initial training phase (1 day) served to familiarize animals with swimming in the pool and finding the hidden platform without the use of visual cues in the maze. Each animal was released into the pool and given a maximum of 90 s to find the platform. If an animal failed to do so, it was manually guided to the platform by the experimenter. Animals remained on the platform for 15 s before commencement of the next trial. Trials were repeated (the platform remained in the same arm) until an animal performed five consecutive correct responses, or a maximum of 10 trials, whichever came first (this constituted a 'trial block'). Correct trials were scored when an animal entered the correct goal arm and mounted the platform; errors were scored when an animal entered the incorrect goal arm with at least half of its body. Each trial block was followed by a rest period of ~5 min, during which animals were returned to a holding cage with holes in the bottom to allow water to drain. Following the rest period, the next trial block was administered with the same procedures as above, with the exception that the platform was moved to the opposite goal arm. A total of four trial blocks were administered, each followed by a 5-min rest period. After completion of training, animals were placed under a heat lamp for a minimum of 15 min before being returned to their home cage.

Phase 2 (Non-random visual discrimination training): The same training procedure as that outlined above was used for phase 2 (one day), with the exception that two distinct visual cues were placed at the end of the goal arms, one indicating the presence of the platform (P+), and the other indicating its absence (P-). The assignment of cues as P+ and P- was counterbalanced across rats. Platform location and associated visual cues remained unchanged during each trial block (10 trials), but alternated between blocks (four blocks in total), with the platform-cue association being kept constant for all blocks.

Phase 3 (Randomized visual discrimination training): The final training phase commenced on day three and continued until animals reliably discriminated the two visual cues. Procedures were the same as for phase 2, with the following exceptions. On each day, animals received 10 training trials, each followed by a 30 s rest period in a holding cage. For each trial, platform location was randomly assigned to one goal arm. For all animals, daily training continued until an animal reached a criterion of at least 80% correct (i.e. 8/10 daily trials) over three consecutive days.

Phase 4 (Second set visual cues): One day following successful completion of phase 3 (i.e., rats reliably discriminated between P+ and P-, the animals were randomly assigned to one of three treatment groups: control (saline), Ro 25-6981, and ifenprodil. Training was continued using two novel visual cues, acting as P+2 and P-2. The same procedure as in phase 3 was carried out with these novel cues, however training concluded at the end of day five. On day six, a probe trial was conducted with the original set of visual cues (P+ and P-) in order to assess whether the extended training period and/or subsequent learning experience influenced the retention of the original set of visual cues.

### **5.3.3 Pharmacology**

To investigate the roles of GluN2B receptor subunit populations during training with the second set of visual cues, three independent groups of animals received one of the following drug treatments: physiological (0.9%) saline; [R-(R\*,S\*)]- $\alpha$ - (4-hydroxyphenyl)- $\beta$ -methyl-4-(phenylmethyl)-1-piperidinepropanol hydrochloride (Ro 25-6981; Tocris Bioscience, Ellisville, MO, USA); or  $\alpha$ - (4-Hydroxyphenyl) - $\beta$ -methyl-4-benzyl-1-piperidineethanol (+)-tartrate salt (Ifenprodil; Sigma-Aldrich). Ro 25-6981 is a potent antagonist of NMDA receptors containing the NR2B subunit, with a >5000-fold selectivity for NR2B over NR2A subunits (Fischer et al., 1997). Ifenprodil also acts as an antagonist of GluN2B-containing NMDA receptors (Carter et al., 1988; Chenard & Menniti 1999). Drug concentrations were chosen on the basis of previous work demonstrating effective GluN2B antagonism for electrophysiological LTP and behavioral experiments (Gagolewicz & Dringenberg 2011, Dalton et. al. 2011). Ro 25-6981 (5mg/kg) was dissolved in saline (0.9%), while ifenprodil (5mg/kg) was dissolved in a mixture of saline and DMSO (60/40%). All drugs, including saline, were administered intraperitoneally (i.p.), at a volume of 1 ml/kg, 30 minutes prior to the start of behavioral testing.

#### **5.3.4 Data analysis**

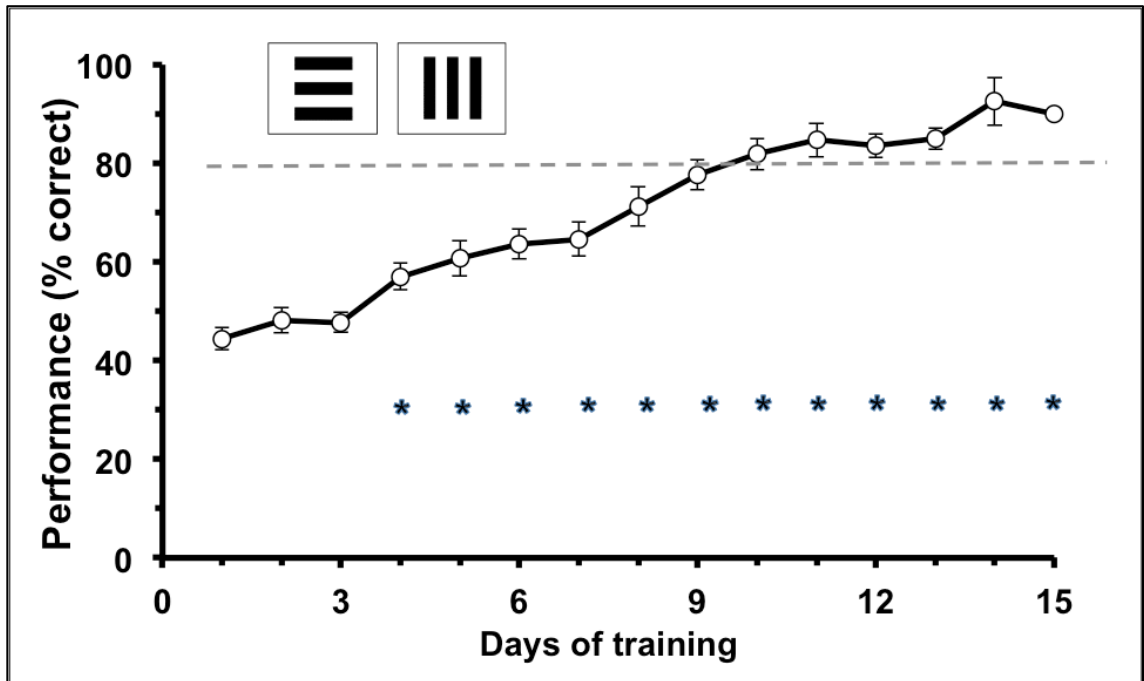
Behavioral data are presented as mean  $\pm$  S.E.M. and were analyzed utilizing a repeated-measures analysis of variance (ANOVA) and followed up, where statistically appropriate, with unpaired Student's t-tests using SPSS software (v. 15.0; SPSS, Colorado Springs, CO, USA). Probe trial data were analyzed using one-way ANOVA. The analysis was conducted with time as the within-subjects factor and group (i.e., drug condition) as the between-subjects factor. The Greenhouse-Geisser correction was applied in all cases when the assumption of sphericity was violated, as tested by the Mauchly's test of sphericity (using SPSS software).

## **5.4 Results**

In a previous set of experiments (see Hager & Dringenberg, 2010a, Gagolewicz & Dringenberg, 2011), an initial round of VDT led to accelerated learning of a novel set of visual cues, as well as facilitated LTP at thalamocortical synapses. In light of these observations, we tested whether past visual experience would affect the efficiency of encoding novel visual features, and whether GluN2B are involved in the possible enhancement of performance by prior VDT.

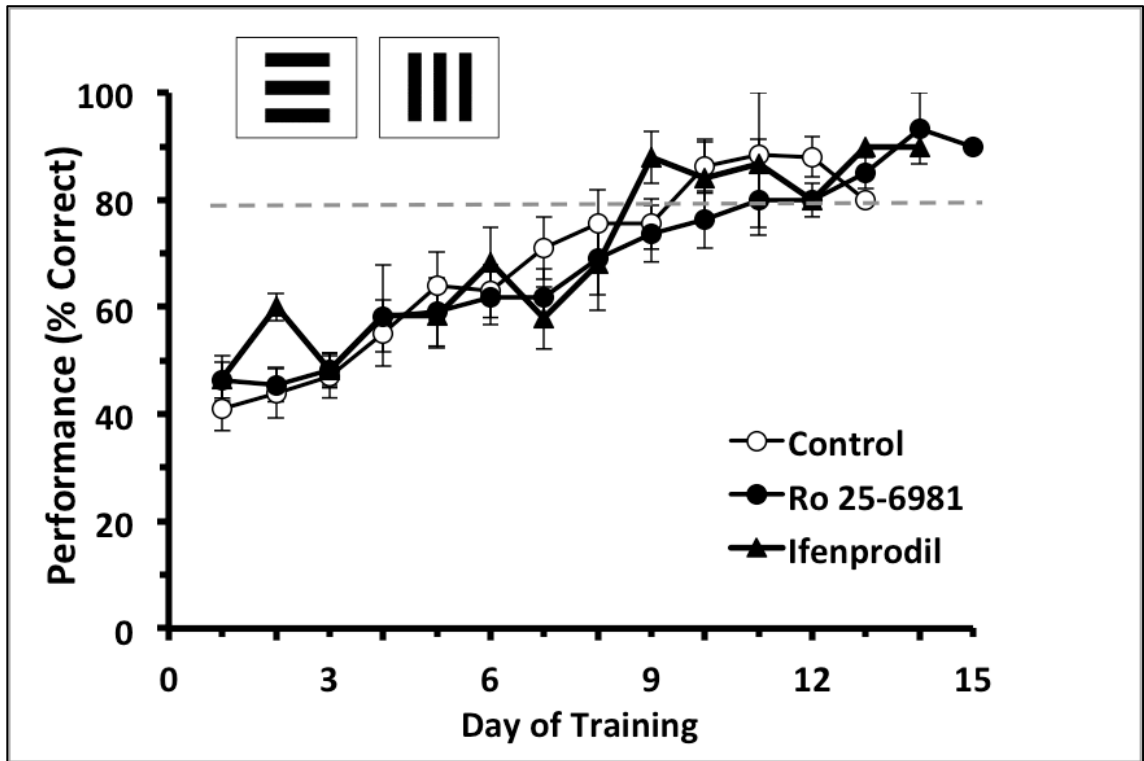
### **5.4.1 Visual discrimination training**

Animals ( $n = 27$ ) were trained to associate visual cues with the location of the escape platform in the water maze. Animals reached the predetermined acquisition criterion (80% correct arm entries per day for 3 consecutive days) after an average of eleven training days (Fig. 5.1; range, 6-15 days), as represented by a significant effect of time  $F(4, 96) = 6.554, p < 0.001$ . Successfully trained animals were subsequently assigned to one of three groups for another round of VDT. The three groups of animals performed similarly throughout the acquisition of the first



**Figure 5.1.** Average ( $\pm$  SEM) correct goal arm entries for all trained ( $n = 27$ ) animals during discrimination training using the first set of visual cues. Trained animals took an average of 11 days to meet the training criterion ( $\geq 80\%$  correct trials for three consecutive days; see dashed line). Rectangular figures near top left depict visual cues used. The \* indicate significant ( $p < 0.05$ ) improvement in performance from training days 1, 2, 3.

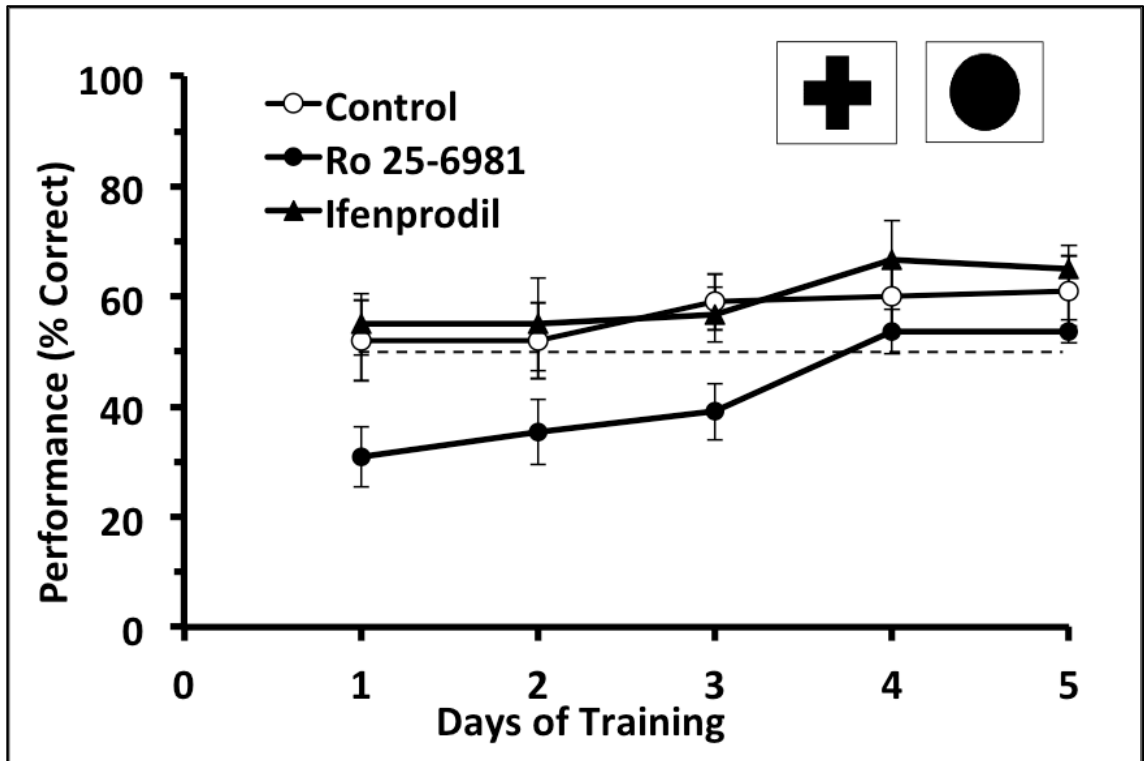
set of visual cues (Fig. 5.2), as confirmed by a lack of statistical main effect of time by group interaction or group (Fig 5.2; all  $p > 0.5$ ).



**Figure 5.2.** Average ( $\pm$  S.E.M.) correct goal arm entries during the first round of VDT for rats subsequently assigned to receive injections of either saline, Ro 25-6981, or ifenprodil, (n = 10, 11, 6, respectively). Dashed line indicates the performance criterion of 80% correct daily trials for three consecutive days.

#### 5.4.2 Second set of visual cues

Next, the animals underwent VDT on a second, novel set of visual cues (Fig. 5.3). Prior to each daily training session, separate groups of rats received injections of either Ro 25-6981 (n = 11, 5 mg/kg), ifenprodil (n = 6, 5 mg/kg), or saline (n = 10, 0.9% saline at a volume of 1ml/kg). The drugs were administered daily throughout the five days of training using the novel visual cues, 30 minutes prior to each training session.



**Figure 5.3.** Average ( $\pm$  S.E.M.) correct goal arm entries for animals receiving either control (0.9% saline at 1 ml/kg volume;  $n = 10$ ), Ro 25-6981 ( $n = 11$ ; 5mg/kg), or ifenprodil ( $n = 6$ ; 5 mg/kg), prior to each daily test session with a second, novel set of visual cues. The dashed line represents chance performance level. Rectangular figures in top right depict visual cues used.

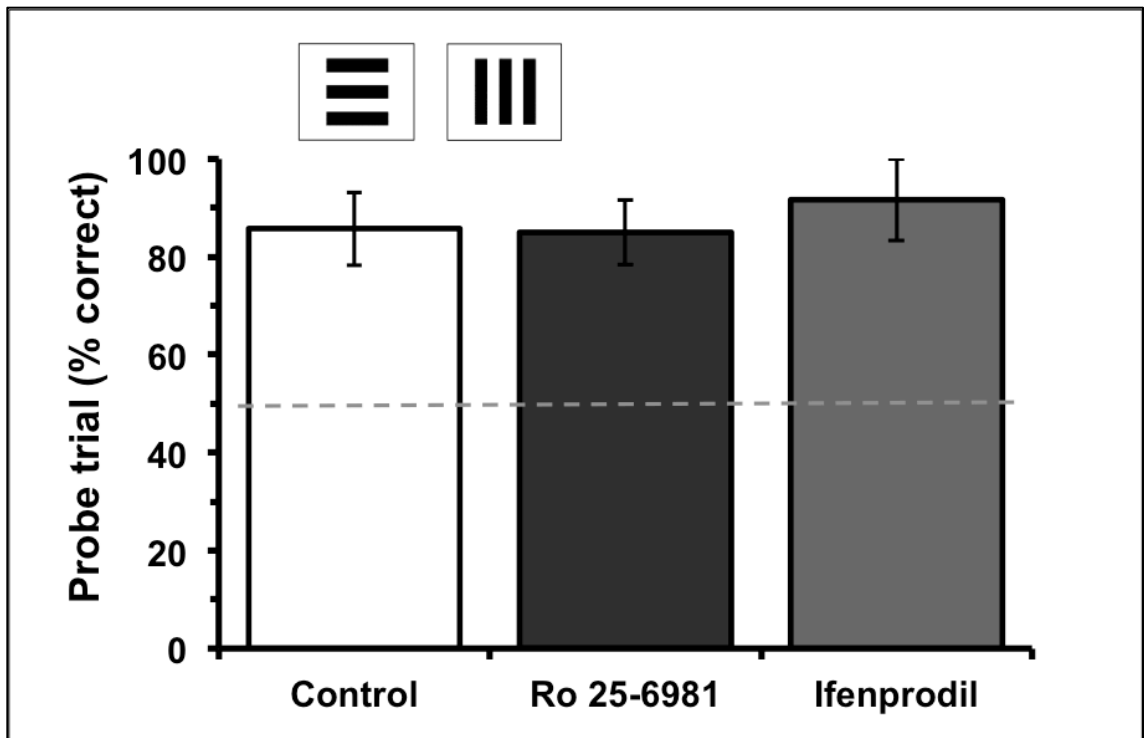
A repeated measures ANOVA revealed a main effect of time  $F(4, 96) = 6.554, p < 0.001$ , and group  $F(2, 24) = 4.501, p < 0.025$ , but failed to detect a significant interaction  $F(8, 96) = 0.826, p > 0.55$ . As expected, both control and ifenprodil (avg.; 52%, 55%; correct swims; respectively) groups performed at chance levels during the first day of visual discrimination of the second set of cues. However, after five days of training, these groups showed very little improvement (avg.; controls =  $61 \pm 6\%$ , ifenprodil =  $65 \pm 4\%$ ; correct swims).

Interestingly, the group of animals receiving Ro 25-6981 performed below chance levels during day one of the extended training (avg., 31%, correct swims), as well as during days two and three (avg.; 35%, 39%; correct swims; respectively). This group improved in performance to

just above chance levels during the fifth, final day of extended VDT (avg.,  $54 \pm 2\%$ , correct swims).

### 5.4.3 Probe trials

One day following the end of extended VDT, the three groups of animals received probe trials utilizing the first set of visual cues. On average, all groups of animals were able to correctly discriminate the original cues (Fig. 5.4,  $87 \pm 16\%$  correct). This was supported by a lack of statistically detectable differences in the mixed two-way ANOVA. Main effect of; trial  $F(3, 60) = 0.828$ ,  $p = 0.483$ ; trial x group  $F(6, 60) = 0.802$ ,  $p = 0.572$ ; or group  $F(2, 20) = 0.372$ ,  $p = 0.694$ . These observations suggest that exposing rats to a second set of visual cues and treating them with the different, pharmacological compounds do not interfere with the retention of the original set of visual cues.



**Figure 5.4.** Average percent ( $\pm$  S.E.M.) of correct goal arm entries during probe trial testing for rats previously given either saline, Ro 25-6981, and ifenprodil ( $n = 7$ ;  $n = 10$ ;  $n = 6$ ; respectively) during visual discrimination training using the second set of visual cues. Note that, for probe trials, the original (first) set of visual cues was used. Dashed line represents chance performance level. Rectangular figures near top left depict visual cues used.

## 5.5 Discussion

The above-summarized set of experiments revealed that, following an initial experience of VDT, the systemic administration of the GluN2B receptor antagonist Ro 25-6981 produced an impairment in performance throughout the second round of VDT. During the first few days of training on the second, novel set of visual cues, animals receiving Ro 25-6981 consistently selected the visual cue that was not associated with the escape platform. On the other hand, systemic administration of the GluN2B receptor antagonist ifenprodil did not produce detectable impairments in visual discrimination performance on the second, novel set of visual cues when compared to rats receiving saline injections. Additionally, probe trials administered at the conclusion of all discrimination training revealed that none of the systemically administered compounds affected the ability of rats to discriminate the first, initially learned set of visual cues. This suggests that, following treatment with Ro 25-6981 or ifenprodil, previously learned visual information that guides behavior remains preserved. Interestingly, the performance of saline-administered control rats during the second round of visual discrimination training implies that previous, related training may not necessarily lead to the improvement of subsequent visual discrimination performance, at least under the current experimental conditions.

The literature containing descriptions of metaplastic alterations at cortical synapses amid two distinct, but related (in our experiments, the general rules for both learning episodes were the same, but different visual cues were utilized) sequential experiences is steadily increasing.

Priming (see Introduction) rat V1 synapses with VDT results in a facilitation of subsequently induced LTP, while LTD remains unaffected (Hager & Dringenberg, 2010a). Interestingly, this form of metaplastic-enhancement of LTP was reversed in the presence of the GluN2B-selective receptor antagonist Ro 25-6981 (Gagolewicz & Dringenberg, 2011). Cortical metaplastic priming during previous experiences not only manifests as alterations to the subsequent induction of synaptic plasticity, but also as changes in behavioral responses during subsequent training. For example, evidence exists that implicates GluN2B receptors in regulating behavioral flexibility, or the capability to alter behavior to changing environmental rules. When Ro 25-6981 was systemically administered to rats, it disrupted their ability to suppress a previously acquired prevailing response during simple visual discrimination tasks (Dalton et al., 2011). In other words, the animals found it difficult to learn a new task under a different set of rules, when previously trained on a similar task. Under these conditions, blocking GluN2B subunits disrupted the capacity of past experience to improve subsequent, similar discrimination task performance. The authors highlighted the relatively high expression levels of GluN2B-containing NMDA receptors in the frontal cortex, and the similarity between behavioral flexibility deficits after focused lesions within these areas and those seen following the systemic administration of Ro 25-6981. This suggests that disruption of GluN2B subunit function could lead to alterations in synaptic metaplasticity, a process that may have behavioral significance during increasingly complex episodes of successive experiences. In related experiments, Ro 25-6981 blocked the induction of LTD at hippocampal synapses following the experience of spatial learning in the Morris water maze (Dong et al., 2012). Here, the initial experience of spatial training represented the priming at hippocampal synapses, while the induction of LTD can be understood as the synaptic equivalent of a subsequent experience. In this case, the metaplastic effect was again blocked in the presence of Ro 25-6981.

Interestingly, the administration of ifenprodil during training on the second set of cues did not result in below-chance visual discrimination performance, and animals in this group selected visual cues at levels that were similar to those seen in control (saline-treated) rats. Ifenprodil and Ro 25-6981 are both classified as selective, non-competitive GluN2B receptor antagonists (Fischer et al., 1997, Lynch et al., 2001). However, the potency of Ro 25-6981 as an antagonist at GluN2B subunit-containing NMDA receptors has been reported to be 25 times greater than that of ifenprodil (Fischer et al., 1997). Additionally, Ro 25-6981 has a much lower affinity than ifenprodil at other receptors, including adrenoceptors and serotonin receptors (Pinard et al., 2001; Mutel et al., 1998). Even though ifenprodil is commonly used as a selective-GluN2B receptor antagonist, it actually has a higher affinity for binding to a subtype of adrenoceptors than to GluN2B-containing NMDA receptors (Chenard & Menniti, 1999). These factors may contribute to the differences we observed in behavioral performance between the two drug-treatment groups.

Another unexpected outcome of our experiments was the lack of facilitation of visual discrimination performance using the second, novel set of cues by the control group of animals. Previous research using this experimental apparatus has shown that rats are significantly faster at learning to discriminate a second set of cues following initial VDT (Hager & Dringenberg, 2010a). One factor that differed between these two sets of experiments is the systemic administration of compounds by daily injections prior to training on the second set of visual cues; Hager and Dringenberg (2010a) did not use pharmacological treatments in their behavioral experiments. There are a number of well-documented pieces of evidence for the impact of stress on multiple learning and memory brain systems (see Shors, 2004). The animals in this current study were not habituated to receiving daily injections, and the performance differences between the present and past experiments may be due to differences in stress induced by the injection

procedure. It has been shown that acute, uncontrollable stress can impair decision-making performance in rodents (Graham et al., 2010). A single, stressful experience could potentially impair an animal's performance in a relatively simple discrimination task. However, the effects of stress on training can be complex, and also depend of the brain systems involved, and the types of tasks being experienced. A more thorough investigation of the neural networks involved in the currently described visual discrimination training will help to clarify some of these uncertainties.

To summarize, our findings reveal that systemic administration of Ro 25-6981 results in a pronounced impairment of discrimination performance during a second learning episode employing a novel set of visual cues. Learning of the initial discrimination task was preserved at the end of all training phases and drug treatments. A second antagonist, ifenprodil, was ineffective in producing this effect. The impairment of visual discrimination performance by Ro 25-6981 highlights the importance of GluN2B receptors in metaplastic effects that influence performance at the behavioral level. Clearly, this type of research is important in order to further expand our understanding of the dynamic interactions occurring between sequential, related experiences.

## Chapter 6

### General discussion

Synaptic plasticity in the mature visual cortex is remarkably sensitive to a variety of environmental influences and experimental manipulations. The mammalian V1, an area traditionally believed to serve as a basic, early processor of modality specific sensory information, is now recognized as a dynamic region capable of synaptic reorganization, both throughout development and also following its maturation. In the large majority of mammals, neuronal connections in V1 are organized into anatomically and functionally related columns, which are common throughout various sensory areas of the mammalian neocortex (Mountcastle, 1997). Interestingly, this columnar organization is not present in the rodent V1, which exhibits a more scattered, irregular arrangements of functional processing units, such as groups of neurons responding to a particular orientation of a visual stimulus, or neurons showing preferential (ocular dominance) responses to one of the two eyes (Ohki et al., 2005).

Regardless of these differences in the anatomical and functional organization of V1 among various species, the precise patterns of cellular and network connectivity are continuously adjusted by changes in synaptic strength. For example, synapses along the thalamocortical visual system readily exhibit LTP in response to high, correlated levels of synaptic activity, e.g., by means of electrical stimulation (Heynen & Bear, 2001) or during visual stimulation and learning (Sale et al., 2011). Importantly, synaptic strengthening and weakening themselves are under regulatory or modulatory control by a number of factors, such as the history of activity at the specific synapse, or the present neurochemical milieu.

## 6.1 Summary of the main results

The series of experiments presented in this thesis examined the roles of serotonergic neuromodulation and NMDA receptor subtypes in the regulation of LTP at synaptic connections in the V1 of mature hooded rats. A summary of the main experimental findings, as related to each chapter of this thesis, is provided below:

To begin, the neuromodulatory roles of 5-HT were studied during plasticity induction at V1 synapses *in vivo*. Cortical application of 5-HT at the site of recording in V1 was found to be ineffective in modulating LTP induced by TBS of the LGN. However, pharmacological blockade of the 5-HT<sub>1A</sub> receptor subtype resulted in the facilitation of LTP in adult rats without altering ongoing cortical oscillatory activity, as sampled by the ECoG recorded in V1. Surprisingly, 5-HT<sub>1A</sub> antagonism impaired LTP in V1 of juvenile rats, suggesting that 5-HT<sub>1A</sub> receptors play a differential role in LTP regulation in juvenile and adult animals, that is, they facilitate synaptic plasticity in earlier life, but then assume an inhibitory role over LTP and act to stabilize patterns of synaptic connectivity.

Next we examined the roles of glutamatergic receptors in governing metaplastic changes at V1 synapses following visual discrimination training. Here, the GluN2B receptor antagonist Ro 25-6981, applied at the cortical recording site in V1, blocked the facilitation of LTP observed following visual discrimination training. The training-dependent facilitation of LTP was not dependent on the activation of metabotropic glutamate receptors. Thus, the GluN2B subunits of ionotropic NMDA receptors appear to provide a critical contribution to the facilitation of LTP following behavioral training.

Subsequent experiments expanded on the work described in chapter three, utilizing the same water maze visual discrimination-training task. Here, a series of *in vitro* experiments

assessed layer II/III pyramidal cells for possible, training-induced changes in NMDA receptor mediated responses in the V1 of trained, and untrained (swim control and naïve) rats. Using whole-cell patch clamp recordings of V1 neurons, it was demonstrated that pharmacologically isolated NMDA currents exhibited an increased sensitivity to GluN2B blockade in rats trained to discriminate visual cues compared to control animals.

Lastly, we assessed the behavioral consequences of blocking GluN2B receptor subunits during visual discrimination training in adult rats. Here, the systemic administration of Ro 25-6981 resulted in impaired behavioral performance compared to vehicle treatment when rats were required to discriminate a novel set of visual cues following an initial learning episode. Interestingly, probe trials utilizing the first set of visual cues suggest that the original discrimination strategy was preserved following treatment with Ro 25-6981.

Taken together, these data suggest that certain types of visual training readily induce metaplastic-priming of LTP at V1 synapses. This effect appears to depend on the activity of GluN2B receptor subunits, which may function at upper (II/III) layers of cortical pyramidal neurons. Systemic administration of Ro 25-6981, which should block GluN2B receptors on these pyramidal neurons in V1 and reverse the enhanced LTP following initial learning, impairs discrimination performance using a novel set of visual cues. Finally, while behavioral experience appears to prime or enhance LTP, 5-HT<sub>1A</sub> receptor activation inhibits plasticity induction in the adult brain, thus acting to stabilize the synaptic connectivity of V1 circuitry.

## **6.2 Oscillatory ECoG activity and neuromodulation by 5-HT**

Based on prior research, one would predict that increasing levels of 5-HT in the neocortex should activate, that is, increase high-frequency activity in the ECoG (Dringenberg &

Vanderwolf; 1995; 1997). One unexpected finding in my experiments was the lack of influence of 5-HT and 5-HT receptor antagonists on oscillatory activity in V1. It is possible that the extracellular neurochemical milieu found in the *in vivo* preparation may have contributed to the lack of serotonergic effects on ECoG activity in my experiments. There is evidence that 5-HT exists at detectable levels in the neocortex under deep urethane anesthesia (Dringenberg et al., 2003). Therefore, it is possible that endogenous 5-HT may have exerted maximal neuromodulatory effects in the urethane-anesthetized brain. Additionally, deep urethane anesthesia produces regular, slow coherent oscillatory activity in the neocortex (Steriade et al., 1993; Dringenberg & Olmstead, 2003), with spectral power focused around the delta frequency band, observations that agree with the ECoG data obtained during the experiments reported in this thesis. Interestingly, there is some evidence that urethane is capable of interacting with the serotonergic system and 5-HT antagonists in the neocortex (Dringenberg & Vanderwolf; 1995, 1997). Hence, recording under deep anesthesia may result in observations different from those in brain slices or unanaesthetized, freely-moving preparations.

It is also possible that, for neuromodulators to influence cortical state and ECoG activity, their release and action must occur throughout large areas of cortical (and perhaps subcortical) tissue. Thus, it is possible that the utilization of a reverse microdialysis probe to deliver 5-HT and various drugs locally into a relative small portion of V1 is insufficient to influence a large-enough cortical area to perturb ongoing oscillations in V1. Thus, this method of drug delivery may have contributed to the discrepancy between the current experiments and prior research on the role of 5-HT in the regulation of the ECoG.

### 6.3 Synaptic plasticity in the rodent V1

The V1 of rodents participates in various types of visual processing, including contrast and edge detection (Sprague et al., 1981; Stone & Derher, 1982). Initially, visual information arriving at the cortex from the LGN is processed by spatial frequency-detecting mechanisms, as opposed to primary pattern and form analysis (Sefton & Dreher, 1995). Throughout the visual cortex, principal neurons have finely tuned receptive fields sensitive to a small set of stimuli in the external visual field (Montero & Scott, 1981; Shaw et al., 1975; Griman et al., 1999). In rodents, the receptive field properties of V1 pyramidal cells for spatial frequencies and orientations form around the time of eye opening (Espinosa & Stryker, 2012), and are subsequently refined by an assortment of experience-dependent plasticity mechanisms (Fagiolini et al., 1994). The various forms of plasticity (LTP/LTD, homeostatic, non-Hebbian) operating at developing and mature V1 synapses are influenced by patterns of sensory input, but are also sensitive to changes in the neurochemical milieu under distinct emotional and behavioral states of the animal (Bear & Singer, 1986; Gu, 2002; Hu, 2007; Kang & Vaucher, 2009). Furthermore, specific psychological and neurophysiological states may “gate” adult cortical plasticity (Fontanini & Katz, 2008). This property of cortical circuits is consistent with the experimental observations of this thesis (Chapter 2). Here, 5-HT<sub>1A</sub> receptors appeared to mediate a tonic gating of LTP induction in V1, with enhanced potentiation apparent following the local blockade of 5-HT<sub>1A</sub> receptors in V1. This facilitation of plasticity by blockade of 5-HT<sub>1A</sub> receptors could potentially influence visual perception and/ or performance of visual tasks, although the links between synaptic changes and behavior are not always clear or linear. However, there is some evidence that stimulation of 5-HT<sub>1A</sub> receptors can impair visual learning in rats, (Cassaday et al., 2000), an effect that is consistent with the inhibitory role of these receptors in plasticity induction in adult rats noted in the present thesis.

A further, important aspect of visual processing in the rodent V1 is the contribution of the lateral aspect of V1 to binocular vision. Here, cortical plasticity mechanisms control the integration of visual inputs from both eyes, giving rise to binocular receptive fields (Domenici et al., 1992). Elegant studies have implicated serotonergic neuromodulation, albeit through the action on inhibitory cortical networks, in restoring juvenile-like ocular dominance plasticity in the mature V1 of adult rodents (Maya Vetencourt et. al., 2008). It would be tempting to examine the involvement of the direct modulation of LTP by 5-HT<sub>1A</sub> receptors in binocular visual processing.

It is important to note that experimental evidence suggests that there are important differences in plasticity mechanisms and between adult and immature animals (Griffen et al., 2012). Factors that act to influence (e.g., gate) the degree of synaptic plasticity change throughout development, including levels of neuromodulatory neurotransmitters, cortical GABAergic inhibition, and activity-dependent NMDA receptor subunit composition (Edagawa et al., 2001; Huang et al., 1999; Williams et al., 1993). The present experiments described a developmental shift in the role of 5-HT<sub>1A</sub> receptors, from LTP enhancement in juvenile rats to LTP inhibition in adult animals. It is possible that this effect may be a general, developmental mechanism of plasticity regulation during the maturation of cortical circuits. As cortical circuits develop, they first require the ability to adapt to the surrounding sensory environment. Over time, as an organism develops its role in the environment and has sampled the sensory environment in sufficient detail, cortical circuits that process sensory stimuli require less flexibility. Altering levels of plasticity observed at cortical synapses parallel this time-course of sensory and behavioral maturation. Initially, when synaptic plasticity is high, cortical circuits may rearrange to drive various behaviors. As nervous systems mature over time, plasticity levels generally decline (Burke & Barnes, 2002). Relating these ideas back to the experiments on the role of 5-HT<sub>1A</sub> receptors in LTP gating in V1, it appears that these receptors facilitate plasticity in

immature V1 circuits by allowing certain synapses to gain additional efficacy. However, later in life, as the organism matures, activation of 5-HT<sub>1A</sub> receptors suppresses plasticity, thereby acting to stabilize the existing, synaptic circuitry. Thus, this influence of 5-HT<sub>1A</sub> receptors upon cortical LTP fits with our understanding of a developmental decline of synaptic plasticity in sensory fields of the mammalian neocortex. In other words, 5-HT<sub>1A</sub> receptors participate both in the maintenance of network stability in adult animals, but also the heightened levels of circuit flexibility during development. Of course, other mechanisms will also contribute to these developmental changes in plasticity in V1 and elsewhere, including non-Hebbian, structural, and metaplasticity (Karmarkar & Dan, 2006; Feldman, 2009).

#### **6.4 Plasticity and metaplasticity at V1 synapses**

Synaptic plasticity of V1 circuits is not only governed by the neurochemical milieu (Chapter 2), but also by events occurring earlier in time (Chapter 3). Hence, activity-dependent changes in neural functions can modulate subsequent synaptic plasticity; this is referred to as metaplasticity or the “plasticity of plasticity” (Abraham & Bear, 1996). One of the novel findings of the experiments conducted for this thesis was the discovery of a facilitation of LTP in V1 following behavioral training, which could be reversed by the blockade of GluN2B subunits of the NMDA receptor (Chapter 3). To the best of my knowledge, these experiments were the first to demonstrate the metaplastic, NMDA-dependent (Ro 25-6981-sensitive) facilitation of LTP in V1 following visual discrimination training, even though work exists documenting NMDA-dependent impairments of LTP following electrical stimulation in area CA1 of the hippocampus (Huang et al., 1992). Notably, these impairments of LTP were transient, lasting between 50 to 80 minutes following tetanus priming activity. Prior to our experiments, it was understood that

increased synaptic activity, such as seen following electrical stimulation or various types of behavioral training, may result in the ‘occlusion’ of subsequent LTP at the same set of recruited synapses (Cooke & Bear, 2010; Sale et al., 2011). In contrast to this occlusion effect, LTP at the synapses studied in my experiments was not occluded, but facilitated following behavioral training. Further, activity-dependent metaplasticity rules would predict an increase in the GluN2A/B ratio at V1 synapses following high levels of synaptic activity (Quinlan et al., 1999; Philpot et al., 2007), an effect that is opposite to the GluN2B-dependent facilitation of LTP in my experiments. Thus, it appears that the metaplastic changes that were observed in V1 following discrimination training (Chapters 3 & 4) are unlike those occurring following the impairment of LTP seen in other work.

An unanswered question concerns the relative contribution of different plasticity mechanisms and different loci of plasticity induction in the thalamocortical visual system. We have demonstrated that one modulatory locus exists directly in V1, at the site of drug application (Ro 25-6981, WAY 100635). A second locus may exist extracortically at synapses within the LGN, the stimulation site for our LTP experiments. Prior work on LTP in the thalamocortical somatosensory system has shown that disinhibition at the level of the thalamus can lead to LTP induction in the primary somatosensory cortex of rats (Hirata & Castro-Alamancos, 2006). The contributions of cortical and subcortical mechanisms to LTP induction between LGN and V1 were not resolved for the current set of experiments. Thus, a possible follow-up experiment would be to apply an NMDA antagonist at the stimulation site in the LGN to examine the involvement of thalamic mechanisms in naïve animals and animals trained in the visual discrimination task. It is noteworthy that cortical Ro 25-6981 application eliminated the training-induced enhancement of LTP, but LTP still occurred (Chapter 3). Testing for multiple modulatory loci would help to clarify if synaptic plasticity (LTP) and metaplasticity (the

facilitation of LTP) are both occurring in V1, since the action of Ro 25-6981 was limited to cortical NR2B receptors.

The alternative interpretation of this form of V1 metaplasticity is that homeostatic changes may have occurred at synapses during the time of the behavioral training and the subsequent electrophysiological recordings. It is known that homeostatic plasticity mechanisms, such as synaptic scaling, operate in the rodent visual cortex to maintain a set level of activity in neuronal circuits (Hengen et al., 2013; Keck et al., 2013). In the rodent V1, there is evidence for up-scaling of synaptic strength in response to deprivation (Goel & Lee, 2007; Hengen et al., 2016), as well as down-scaling during sleep (Diering et al., 2017). Since rats are largely nocturnal, animals used for the present experiments were housed under a reverse light cycles, allowing for behavioral and electrophysiological procedures to be conducted during the active/wake hours of the animals. Following the completion of visual discrimination training, all of the animals had one evening (i.e., light cycle) of rest, which allowed for the animals to obtain sleep prior to the commencement of electrophysiology on the next day. It is feasible that V1 synapses may have undergone LTP in response to visual discrimination training during active/wake hours, hence becoming ‘occluded’, and these same synapses were subsequently down-scaled during sleep. This process cannot be ruled out, as electrophysiological experiments were never conducted immediately following the final episode of visual discrimination training.

Interestingly, Sale et al. (2011), using similar training and visual discrimination apparatus, reported ‘occlusion’ of LTP in V1 at vertical and horizontal intracortical synapses in layers II/III. Important differences between this work and my experiments in Chapter 3 were that this ‘occluded’ LTP was assessed *in vitro* and, interestingly, immediately following the last episode of training. To contrast, my *in vivo* assessment of LTP was conducted one day following the completion of behavioral training. The GluN2B-dependent facilitation of thalamocortical LTP

described in this thesis may be a remnant, metaplastic-tag following neural circuit reorganization in response to the multi-day behavioral training. Currently, additional experiments designed to explore these possibilities are required.

## **6.5 Functional implications of neuromodulation and metaplasticity in V1**

In contrast to the neuromodulation of synaptic plasticity, one distinguishing feature of metaplasticity is that it can be elicited by priming activity, that is, activity that occurred in the past (Abraham, 2008). The metaplasticity described in this thesis occurred in response to a (presumed) high level of processing of visual information that held behavioral significance for the animal. This priming activity likely resulted in molecular tags at the activated synapses, eliciting a metaplastic upregulation of LTP. Manifestations of this presumed mechanism were detected in my experiments in the form of the GluN2B-dependent facilitation of LTP (Chapter 3), as well as an increased sensitivity of pharmacologically isolated NMDA currents to GluN2B blockade in V1 pyramidal cells (Chapter 4).

Interestingly, these alterations at the NMDA receptor following successful learning of a discrimination task do not appear to fit the current understanding of activity-dependent metaplastic changes in V1. The upregulation of GluN2B subunits is commonly reported following deprivation of experience, including dark-rearing (He, 2006; Kopp, 2007), while increased synaptic activity (e.g., during sensory processing or perceptual learning) generally results in an increase in the contribution of GluN2A subunits to NMDA receptor functioning (see Bear, 2003). However, as mentioned previously in Chapter 3, some reports have documented enhanced GluN2B subunit functioning following certain types of dietary and hormonal manipulations (Coutrap et al., 2008; Snyder et al., 2011). It is also known that neurotrophins,

particularly brain-derived neurotrophic factor (BDNF), can facilitate certain types of LTP in layers II/III of rodents (Akaneya et al., 1997; Huber et al., 1998). BDNF has been demonstrated to upregulate the phosphorylation of GluN2B-containing hippocampal NMDA receptors (Crozier et al., 1999, Xu et al., 2006). Thus, it is tempting to speculate that the facilitation of LTP (metaplasticity) in rats trained to visually discriminate cues may be due to a BDNF-mediated phosphorylation of GluN2B subunits (priming) in V1; however, direct evidence for this link is currently unavailable.

Metaplastic-tags can represent the history of previous synaptic activity. Hence, metaplasticity has also been considered to function as a type of memory trace at neuronal synapses (Abraham and Philpot, 2009). The primary sensory cortical areas of adult rodents participate in the feature detection of sensory stimuli, but are also the site of plasticity and certain types of information storage (Chubykin et al., 2013; Cooke & Bear, 2014; Weinberger & Bakin, 1998). This is particularly evident at NMDA receptor-containing glutamatergic synapses throughout V1, which are capable of experience-dependent LTP and metaplasticity. The fact that mature sensory cortices are highly plastic is reminiscent of the classic work by Karl Lashley (1931), who proposed that the entire neocortex participates equally in information storage. More recently, Alain Berthoz (2000) proposed that memory serves as a tool for prediction, allowing the anticipation of future events, which may also aid with future learning. Support for this prediction has, amongst others, been provided with the visual discrimination task used for the present experiments. Hager and Dringenberg (2010a) showed that, after an initial episode of visual discrimination learning, future learning of novel visual cues is accelerated, together with the enhancement of LTP that follows the first learning experience. Thus, it is tempting to speculate whether the plasticity enhancement is related to the improved ability to acquire novel visual cues, a hypothesis that was examined in the Chapter 5 of my thesis. It is also likely that the

modifications at V1 synapses following training take part in a larger, more distributed memory process or schema that is acquired during training (Iran-Nejad, 2000; Tse et al., 2007), operating throughout the cortical mantle. For example, it is likely that the hippocampal formation is involved in the type of learning examined here, and changes in hippocampal functioning may have occurred during visual training, a possibility that could be examined with further experiments.

## **6.6 General conclusions**

This thesis has demonstrated that the primary sensory cortex retains a remarkable degree of flexibility, or plasticity, as observed in the adult lab rat. Experimental evidence has highlighted the versatility of V1 as not only an early processor of sensory information, but also as a brain area involved in information storage.

As the 21<sup>st</sup> century unfolds, we are progressing towards an improved understanding of the links between physiological changes occurring at synapses and observed behavior. Modern neuroscientific research is rapidly expanding the predictive power for changes that occur throughout the brain following various types of experience. Consideration of metaplasticity and plasticity variables, such as history of activity at the synapse and the neurochemical milieu, is paving the road for advanced predictive power of changes in connectivity within neuronal circuits where serotonin or NMDA receptors are found. The precise targeting of neuromodulatory systems may allow for real-time, non-invasive manipulations of visual experience or cortical plasticity, and can be recruited for recovery of visual response strength, selectivity, and spatial acuity in adult amblyopes (Bavelier et al., 2010). Similarly, the enhancement of synaptic plasticity in the visual cortex paired with perceptual learning from visual training may improve

recovery in severely amblyopic adults (Eaton et al., 2016). Further, the possibility of selective NR2B receptor upregulation may have therapeutic potential for memory problems, including phobias, post-traumatic stress disorder, and various modality-specific learning impairments. This knowledge will also be exploited for preventing age-related cognitive decline, thereby improving the quality of life of elderly populations. It may also provide unexpected avenues for technological advancement, including enhanced designs of artificial neural networks for machine learning, leading to improved mimicry of biological nervous system capabilities.

In closing, while the concept of neuromodulation of plasticity has been explored extensively, metaplasticity is a relatively newer, but nonetheless important, addition to our understanding of the roles of synaptic alterations in learning, memory, and behavior. While advancements in these fields may create many exciting opportunities for human enrichment, it may be that rodents still have a thing or two to teach us about the workings of learning and memory.

It is a widely accepted dogma that synapses get strengthened during learning, and that their strengthening is important for creating memories. It is less well understood that learning and the formation of memories might also involve the weakening of certain neuronal connections, a process that may be equally important for the reorganization of brain circuitry in response to experience. While LTP and LTD are occurring, neuromodulators and metaplasticity regulate the degree of change at cortical synapse in response to experience. The regulation of plasticity by state-dependent neuromodulators and history-dependent metaplasticity is perhaps the most up-to-date understanding of the intricate level of control over circuits of the brain at the scale of synapses. My research has highlighted the importance of serotonin receptors at early sensory cortical processing zones, expanding the description of the role of 5-HT<sub>1A</sub> receptors in the control of LTP. Neurons containing the glutamatergic NMDA receptor are also sensitive to the history of

activity at the synapse, or metaplasticity. At V1, certain types of training endows, or primes specific NMDA-containing synapses with the capacity for enhanced LTP. This effect may be a process that V1 synapses go through following visual learning, or it may contribute to improved performance on subsequent, related visual tasks. Regardless of which holds true, this research has highlighted the importance of examining experience-dependent tasks at various time points following training, and some of the behavioral consequences of these changes at early visual processing areas in the neocortex.

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