TRAINING-INDUCED PLASTICITY IN THE VISUAL CORTEX OF ADULT RATS FOLLOWING VISUAL DISCRIMINATION LEARNING

by

Audrey Marion Hager

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Abstract

Synaptic plasticity plays a key role in processes of learning and memory. Long-term potentiation (LTP) is a relatively stable enhancement of synaptic transmission following specific patterns of electrical stimulation. Some types of learning (e.g. motor learning, fear conditioning) result in LTP-like changes at synapses. However, no studies have examined LTP-like plasticity in the visual cortex as a result of visual discrimination learning.

A visual discrimination task was used to examine changes in LTP in the primary visual cortex (V1) of adult rats. Rats were placed in a Y-shaped water maze and required to swim to one choice arm containing a hidden platform. Distinct visual cues indicated the presence (CS+) and absence (CS-) of the platform. Rats learned to reliably discriminate the visual cues to successfully navigate the maze. Control rats received the same procedure, but the visual cues did not have a predictive relation with the platform. Following training, trained, control, and task-naïve rats were anesthetized and visual evoked potentials (VEPs) in V1 were recorded in response to CS+, CS-, and novel stimuli.

Results indicate that, in both task-naïve and control animals, all visual stimuli elicit VEPs of similar ($p > 0.05$) amplitude. In contrast, trained animals show significantly larger amplitude VEPs to stimuli encountered during training relative to novel stimuli, regardless of whether stimuli act as CS+ or CS-. In addition, trained animals show 71% and controls 47% potentiation ($p < .05$) that was induced by electrical (theta-burst) stimulation of the lateral geniculate nucleus (LGN) indicating greater plasticity of thalamocortical synapses following training. There were no differences between controls and task-naïve animals. This facilitation of LTP was shown to allow past visual experience to influence the efficiency of encoding novel visual features.

These experiments demonstrate that visual discrimination learning might involve stimulus-selective facilitation of neuronal responses at early stages of visual processing (LGN, V1). The effect requires that stimuli carry some significance to the animal, while exposure to stimuli with no significance does not result in the same level of neuronal enhancement. Further, visual experience alters the plasticity properties of V1 (metaplasticity) by facilitating LTP along thalamocortical sensory fibers.
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## List of Abbreviations

A1 – Primary Auditory Cortex  
ACh – Acetylcholine  
AP – Anterior-Posterior  
Ca²⁺ – Calcium  
CS⁺ – Positive Conditioned Stimulus  
CS⁻ – Negative Conditioned Stimulus  
HFS – High Frequency Stimulation  
fEPSPs – field Evoked Postsynaptic Potential  
fPSPs – field Postsynaptic Potential  
L – Lateral  
LGN – Lateral Geniculate Nucleus  
LTD – Long-term Depression  
LTP – Long-term Potentiation  
NMDA – N-methyl-D-aspartate  
SEM – Standard Error of the Mean  
TBS – Theta Burst Stimulation  
V – Ventral  
V1 – Primary Visual Cortex  
VEPs – Visual Evoked Potentials
Chapter 1

Introduction

Each new experience and processing of sensory information can modify the structure and function of neural circuits over various timescales. This experience may be something trivial, such as remembering where you left the car keys, or more life threatening such as driving cautiously through a particular turn because of the fender bender you had last week. These new experiences (such as the fender bender) may affect how we interpret future sensations and stimuli (being more cautious when driving). How these experiences and stimuli are stored in neural circuits is a central question being asked in neuroscience today. Elucidating the underlying mechanisms of neuronal plasticity seeks to answer this question. Neuroplasticity may be defined succinctly as the ability of the nervous system to undergo changes in its anatomical, chemical and physiological and functional properties. Plasticity occurs throughout the entire nervous system from the spinal cord to higher cortical areas and involves different mechanisms at the structural, cellular and molecular level. One such mechanism in the nervous system is long-term potentiation (LTP). This study examined LTP as a mechanism of neuroplasticity and whether it forms a direct link to memory storage and consolidation in the brain.
Chapter 2
Literature Review

2.1 Long-Term Potentiation

LTP was first discovered by Bliss and Lomo in 1973 and is a relatively stable enhancement of synaptic transmission that typically follows specific patterns of electrical stimulation (Bliss & Collingridge, 1993). Long-term potentiation has been studied extensively for the past 35 years, primarily in the hippocampus as it is readily induced there. The reason why an extensive amount of research has focused on LTP stems back to Donald Hebb, who laid the foundation for modern thinking about memory and the neural mechanisms involved in memory storage in the brain. Hebb’s postulate for learning states that:

“When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased.”
(Hebb, 1949, p. 17)

This is often summed up simply as ‘Neurons that fire together, wire together.’ The types of synaptic changes that Hebb described above are the same changes that appear to underlie LTP. There are several, distinct temporal domains encompassed by the phenomenon of LTP: initial induction (learning), stabilization, and long-term maintenance (memory). Most work to date has emphasized LTP induction mechanisms and the changes that take place within cells that lead to an increase in synaptic efficiency.

Typically, LTP is a glutamate N-methyl-D-aspartate (NMDA) receptor and calcium (Ca^{2+}) dependent process. In order for LTP to occur, there must be NMDA receptor activation in a postsynaptic neuron. The NMDA receptors have a unique property in that they are both ligand and voltage gated. That is, in order for the ion channel to open, glutamate binding has to occur
together with depolarization of the postsynaptic membrane. If these two conditions are met, NMDA channels are effectively opened, thus allowing $\text{Ca}^{2+}$ entry into the neuron. This increase in $\text{Ca}^{2+}$ leads to activation of $\text{Ca}^{2+}$ dependent enzymes and various protein kinases, which ultimately results in a number of changes that increase the responsiveness of the postsynaptic cell to synaptic inputs (e.g., phosphorylation of existing receptors, insertion of new receptors into the membrane, changes in spine density and shape) (Bennett, 2000).

Properties of LTP include that it is input specific, or LTP at one synapse does not spread to other synapses. The induction of LTP occurs with a strong stimulation of a single pathway or through stimulation of many weaker pathways creating an additive effect. Often LTP is induced using high frequency electrical stimulation (HFS) and the subsequent magnitude of LTP is used as an index of the overall amount of plasticity that is available in a particular brain region, as well as the ability of neurons to maintain synaptic enhancements for prolonged time periods (LTP maintenance). Both LTP induction and maintenance are further influenced by neuromodulators and other cellular mechanisms (Lynch, 2004).

Most research to date on LTP has been performed in the hippocampus as this is where LTP was first discovered. Work done in the neocortex has primarily been examined in slices allowing for a very controlled environment. Though the neuronal and cellular mechanisms of LTP have been extensively studied, the role and significance of LTP in normal brain plasticity and behavior is still in question.

## 2.2 Learning and LTP

Long-term potentiation is widely believed to be the mechanism by which neurons store and encode memories in synapses (Martin & Morris, 2002). However, to date, very few studies have examined a direct link between LTP and learning/memory encoding at a behavioral level (Rioult-Pedotti et al., 2000; Rogan et al., 1997; Whitlock et al., 2006). Two studies in particular,
which address this question, have examined LTP in the motor cortex and hippocampus following motor and inhibitory avoidance learning, respectively.

Rioult-Pedotti et al., (2000) examined LTP and its counterpart long-term depression (LTD) in the primary motor cortex in vitro following acquisition of a motor task (skilled reaching) in rats. Training resulted in increases in strength of local synapses in the motor cortex. At the same time, electrically induced LTP was diminished, while LTD was enhanced in the brains of trained animals. The fact that the magnitude of LTP measured in the motor cortex was reduced suggests that the same mechanisms mediating electrically induced LTP were engaged (and, therefore, partially used-up) while learning the task. However, since LTD was enhanced, the overall synaptic modification range was the same in trained and untrained animals. This suggests that the overall amount of plasticity available in the motor cortex following learning remains the same.

Whitlock et al. (2006) used a different type of learning, inhibitory avoidance learning, to examine LTP in area CA1 of the hippocampus in vivo. Again, training produced a potentiation of synapses in CA1, and LTP following HFS was lower compared to control animals not undergoing the learning procedure. The authors conclude that, since learning-induced synaptic potentiation occluded or used up HFS-induced LTP, inhibitory avoidance training induces LTP in CA1.

These and similar studies (Rogan et al., 1997; Clem et al., 2008; Sacchetti et al., 2002) have provided evidence to support the notion of a link between various types of learning and LTP-like plasticity. Uniformly, the available literature suggests a decrease in the magnitude of LTP after learning, and this “trade-off” is interpreted as supporting a link between learning and LTP. However, at the same time, the sets of data are also somewhat counter-intuitive to what is known about the rules and principles of basic learning and memory processes. For example, there appears to be no obvious limit or saturation point for memory, that is, one can always learn new
things and it seems that there is no end to the depth of memory. In fact, often it appears that learning one skill or set of information can facilitate future learning, a fact that is not readily compatible with the notion that each learning experience uses up LTP and plasticity in the brain. As such, the question remains that once learning occurs, is plasticity used up, such as using up memory on the hard disk of a computer, or can it be facilitated, thus aiding the encoding of new information? The present study seeks to extend previous work and address this fundamental question by examining LTP in the adult visual cortex following visual discrimination learning.

2.3 Plasticity in the Visual Cortex

The visual cortex serves as a primary region to study and elucidate plasticity mechanisms in the brain. Much has been explained about plasticity and the underlying mechanisms involved in the developing visual cortex of young animals since Hubel and Wiesel’s ground breaking work in the 1960’s and 70’s. One of these underlying mechanisms is thought to be LTP since it appears to reflect naturally occurring mechanisms of experience-dependent synaptic modification in the developing rodent visual cortex (Kato et al., 1991; Kirkwood et al., 1995). Hubel and Wiesel (1970) originally thought that once the critical period of development closed in young animals, the visual cortex was no longer susceptible to plasticity induced by visual deprivation. However, more recent studies suggest that this is not the case and that, indeed, the adult visual cortex remains plastic (Hofer et al., 2006; Karmarkar & Dan, 2006). Not only is the adult brain not set in stone but the changes that take place appear to involve and reflect LTP mechanisms.

In the adult visual cortex (V1), LTP can be induced in vivo through the use of theta burst stimulation (TBS) (a type of HFS) of the lateral geniculate nucleus (LGN) of the thalamus. Theta-burst stimulation induces long-lasting, NMDA receptor-dependent enhancement of field potentials in V1 elicited either by electrical stimulation of the LGN, or visual stimuli flashed to the retina of anesthetized animals (Heynen & Bear, 2001). This stimulation paradigm indicates
that LTP mechanisms are functional, even though little is known with regard to the role served by these mechanisms in the adult V1. There are many factors that influence LTP, such as attention, arousal, and the related neuromodulators such as acetylcholine. In adult rats, stimulation of the basal forebrain or the application of cholinergic agonists in V1 has been shown to facilitate LTP (Dringenberg et al., 2007). Active learning through visual discrimination training which requires attention, such as used in this study may provide a naturalistic paradigm to assess LTP in behavioral contexts.

There are several methodologies available to study plasticity in the visual system, which include the use of HFS (described above), ocular dominance plasticity and visual deprivation (monocular or binocular). One study, which took a different approach, examined LTP in the visual cortex not through deprivation but through the use of repeated or extended visual experience. Frenkel et al. (2006) used a paradigm in which mice were repeatedly exposed to visual stimuli consisting of dark bars of specific orientations. After multiple days of repeated exposure, these researchers noted a potentiation of V1 evoked responses that were selective to the previously encountered orientation. This synaptic potentiation is somewhat surprising since the animals were not required to acquire or learn any task, or pay particular attention to the visual stimuli. However, these results do indicate that responses of neurons in the mature V1 are plastic and can change their responsiveness with visual experience.

### 2.4 The Present Study

As discussed, there is evidence that visual experience can result in LTP-like changes in V1. However, the concept of “visual experience”, as used in much of the previous work, has often been quite non-naturalistic. Extended periods of deprivation or looking at specific grating patterns for many hours each day is not a common visual experience for most mammalian species. Further, it seems that other studies have examined the two ends of the “experience spectrum”, one
being lack of experience through deprivation and the other being extended visual experience through repeated exposure to some visual stimuli. The present study employs a different type of visual experience through the use of a simple visual discrimination task, providing a more naturalistic paradigm of visual learning processes.

The two central questions asked are: (a) do neurons in the visual cortex change their responsiveness to visual stimuli following visual discrimination training? and (b) is there a difference in the amount of potentiation/LTP in V1 as a result of visual discrimination training? To address these questions, a visual discrimination task was used that required rats to recognize, remember, and discriminate two visual cues in order to find a hidden escape platform in a modified water maze apparatus. Following training, visual evoked potentials (VEPs) were recorded using local field potentials from the surface of V1, while different (familiar and novel) visual stimuli were repeatedly displayed before the animals. It was hypothesized that there will be a difference in VEPs between trained and naïve rats, and familiar and novel visual stimuli.

Subsequently, using the same animals, high frequency, TBS of the LGN was used to measure the magnitude and duration of LTP that could be induced in V1. It was hypothesized that, consistent with the reduced LTP following learning seen in previous studies (see above), trained rats will show less LTP relative to untrained rats.
Chapter 3
Materials and Methods

3.1 Subjects
All experiments were conducted in accordance with published guidelines of the Canadian Council on Animal Care and approved by the Queen’s University Animal Care Committee. The experiments were conducted on adult (340-620 g), male Long-Evans rats (Charles River, Canada) housed in a colony room (12/12 h reversed light cycle) with free access to food and water. All behavioral tests and electrophysiological procedures took place during the day, typically between the time of 10:00 and 17:00 h.

3.2 Y-Maze Visual Discrimination Apparatus
The visual discrimination task was performed using a modified water maze which included a Y maze insert and distinct visual cues. The water maze (Figure 1) was a circular pool (diameter 180 cm, height 60 cm) made of white Perspex. It was filled with water (21 +/- 1 °C) to a depth of 40 cm. The Y maze insert (height 61 cm, length 140 cm, width 51 cm and 81 cm), constructed of clear Plexi-glass, was placed inside the water maze with the same orientation for all trials. A black divider (length 50 cm, height 61 cm) separated the two goal arms. The rectangular platform (width 12 cm, height 38 cm, length 36 cm) of clear Plexi-glass was positioned 10 cm from the end of one of the two goal arms of the Y maze and kept submerged 2 cm below the water surface. Non-toxic, white tempera paint was added to create milky water to insure the platform was not visible. The fluorescent lighting in the room was muted to avoid glare on the pool surface and visual cues.
Figure 1: Y-maze apparatus.

The Y maze apparatus and subsequent dimensions used during visual discrimination training.

3.3 Visual Cues

Computer-generated visual cues were printed on white sheets of paper (28 cm x 21.5 cm) and consisted of the following for the trained and control groups: 3 black vertical bars (length 15 cm, width 3 cm) spaced at equal 3 cm distances (area=135 cm²); 3 black horizontal bars (length 3 cm, width 15 cm) spaced at equal 3 cm distances (area=135 cm²); a black diamond (15 cm x 15 cm) (area=225 cm²) (Figure 2 A).

Three additional visual cues were included in a group of animals that received extended training on multiple sets of stimuli (see below). These new pictures included a black plus symbol (area=135 cm²), a black circle (diameter 16.7 cm, area=222 cm²), and a black X symbol (area=135 cm²) (Figure 2 B). Cues were mounted at the ends of the goal arms 1 cm above the water surface.
Figure 2: Visual Cues.

Visual cues used during visual discrimination training. (A) Images used for the trained and control animals during visual discrimination procedures. For individual animals, one of the images served as CS+ (platform), CS- (no platform), and novel (counterbalanced among subgroups). (B) Additional images used for animals receiving a second set of visual cues for extended, multiple visual discrimination training. Images above are 10% of the size of the actual stimuli used.

For trained animals the pictures were randomly assigned the designation of positive conditioned stimulus (CS+), negative conditioned stimulus (CS-) or novel. Throughout training CS+ indicated the arm where the platform was located, CS- the incorrect arm and the novel cue was never seen during training. Visual cues were counterbalanced between subgroups of trained animals so that no cue was presented more often than another.

Control animals received similar training procedures with visual stimuli; however the visual stimuli remained stationary while the platform was randomly assigned a goal arm. In this way the visual stimuli did not cue or predict the location of the platform.

3.4 Visual Discrimination Training

Visual discrimination training was divided into 3 phases: habituation, non-random acquisition, and random acquisition. In all phases animals were released into the maze facing the
pool wall, away from the goal arms. During rest periods between training trials (approximately 5 minutes) animals were returned to a temporary cage (length 45 cm, width 24 cm, height 20 cm) with holes in the bottom to allow water to drain. After completion of daily training, animals were placed under a heat lamp for a minimum of 15 minutes before being returned to their home cage.

3.4.1 Phase 1: Habituation

Phase 1 coincided with the first day of training and involved habituating animals to swimming in the pool and finding the hidden platform without the use of explicit visual cues. For each trial, a rat was released into the maze and allowed to swim towards the goal arms of the maze. The animal was given a maximum of 200 seconds to explore the maze and find the hidden platform. If it failed to do so, the rat was manually guided by the experimenter to the platform. Animals remained on the platform for 15 seconds before commencement of the next trial. Trials were repeated until a rat performed five consecutive correct responses, or a maximum of ten trials, whichever came first. This constituted a “trial block”. After each trial block, rats were allowed a 5 minute rest period in the temporary cage before receiving the next trial block, for a total of four trial blocks on the habituation day. The platform location remained constant for all trials within a given trial block. However, for each new trial block, the platform was moved to the goal arm that did not previously contain the platform.

The following information was manually recorded for each trial: platform location, escape latency (i.e., the number of seconds from release to mounting the platform, as measured manually with a stopwatch), and whether the response was correct or incorrect. A correct response occurred when the arm containing the platform was entered and the platform mounted; a trial was deemed incorrect if more than half of the animal’s body passed the divider into the arm not containing the platform.
3.4.2 Phase 2: Non-Random Visual Discrimination Training

On day 2, the same training procedure as that outlined above was used, with the exception that visual cues were added to the apparatus. For animals receiving visual discrimination training, two distinct visual cues were used, which acted as CS+ and CS-, indicating the presence and absence of the hidden escape platform, respectively. As on day 1, a total of four trial blocks were conducted, with the platform and corresponding visual cues alternating between the two goal arms on successive blocks. For control animals, the same visual cues were present, but they did not predict the platform location. That is, the platform was moved for each new trial block (see above), while the visual cues remained stationary throughout the entire training procedure, thus lacking consistent CS+ and CS- associations with the escape platform. The experimenter manually recorded the same information as stated above for Phase 1 of the training procedure.

3.4.3 Phase 2: Randomized Visual Discrimination Training

This phase took place from day 3 of training until training was completed (i.e., the pre-performance criterion was reached, as outlined below). In this phase, the location of the escape platform was randomly assigned for each new trial and rats completed ten trials per day, with 30-second rest intervals between trials. This procedure was continued on consecutive days until a rat reached a criterion of at least 80% correct (i.e., 8/10 daily trials) over three consecutive days. Again, for rats receiving visual discrimination training, the CS+ and CS- stimuli consistently indicated the presence and absence, respectively, of the escape platform. In contrast, control animals received the same procedures as the trained animals, with the exception that visual stimuli remained stationary within the maze, thus lacking a consistent association with the platform. For control animals, this procedure was repeated for nine consecutive days, as this was the average time required by visually trained rats to reach the performance criterion outlined
above. Throughout training, the experimenter manually recorded platform location and correct and incorrect responses per trial for all animals.

3.5 Training on Multiple Visual Cue Discriminations

An additional group of animals was trained on multiple sets of visual cues. Initially, animals in this group underwent the same procedures described above for Phases 1-3 of the training regime. After successful completion of Phase 3 (i.e., rats reliably discriminated between CS+\(^1\) and CS-\(^1\)), training was continued using two novel visual cues, acting as CS+\(^2\) and CS-\(^2\). The same procedure as in Phase 3 was carried out with these novel cues, until the same performance criterion was reached. On the final day, a probe trial was conducted with the original set of visual cues (CS+\(^1\) and CS-\(^1\)) in order to assess whether the extended training period and/or subsequent learning experience influenced the retention of the original set of visual cues.

3.6 Surgical Preparation

Electrophysiological procedures were performed on the day following completion of training. Experimental procedures were carried out under deep urethane anesthesia (1.5 g/kg, intraperitoneal [i.p.]), administered in three 0.5 g/kg doses every 20 minutes. Typically, urethane supplements were in the range of an additional 0.5 g/kg and were administered prior to the onset of data collection. This dosing regiment is sufficient to ensure that spontaneous high-frequency, low-amplitude activation in the electrocorticogram does not occur during the course of the experiment (Kuo & Dringenberg, 2008).

Rats were placed in a stereotaxic apparatus and body temperature was maintained between 36 and 37 °C by an electrical heating blanket and additional insulating material wrapped around the animal. The right eye was closed and the left eye to which visual stimuli were presented was kept open using a small hemostat. Tear-gel was applied to keep the open eye
lubricated. The skull was exposed, and small skull holes were drilled to allow access to V1 (anterior-posterior (AP) $+7.5$ mm, lateral (L) $-3.5$ mm, ventral (V) $-0.5$ to $-1.0$ mm), and the ipsilateral lateral geniculate nucleus (LGN; AP $+3.9$ mm to $+4.3$ mm, L $-3.9$ mm, V $-4.6$ mm to $-5.0$ mm). Final placements of LGN stimulation and V1 recording electrodes (*Figure 3*) were adjusted to elicit maximal amplitude field postsynaptic potentials (fPSPs) and augmented responses (at 100 ms interpulse intervals) in V1 in response to LGN stimulation.

**Figure 3: Electrode Placements.**

Schematic representation of electrode placements in the lateral geniculate nucleus (LGN, *left*) (anterior-posterior (AP) $+3.9$ mm to $+4.3$ mm, lateral (L) $-3.9$ mm, ventral (V) $-4.6$ mm to $-5.0$ mm) and primary visual cortex (V1, *right*) (AP $+7.5$ mm, L $-3.5$ mm, V $-0.5$ to $-1.0$ mm). Diagrams adapted from the atlas by Paxinos and Watson (1998).

### 3.7 Electrophysiology

#### 3.7.1 Visual Evoked Potentials (VEPs)

Following the final adjustments of electrodes, visual stimuli were presented to the animals. A liquid crystal display (LCD) computer monitor (model Acer, size 44 cm) was positioned 55 cm in front and 8 cm away from the midline of the animal, towards the open eye (*Figure 4*). DirectRT Precision Timing Software (version 2004.3.0.27, Empirisoft, New York,
NY) was used to present the visual stimuli and trigger the electrophysiological recording set-up. Visual stimuli were bitmap images (resolution 621 x 480) of the same visual cues used for behavioral training. The dimensions of the visual stimuli used for the electrophysiological experiments were identical to those of the visual cues presented during visual discrimination training.

The VEPs, recorded as local field potentials in response to visual stimuli, were differentially recorded (Teflon insulated stainless steel wire, 125 -µm tip diameter) against a ground screw placed in the bone overlying the cerebellum. The signal was amplified, digitized (10 kHz), and stored for subsequent offline analysis using the PowerLab system (PowerLab/16 s system with ML 180 Stimulus Isolator, ADInstruments, Toronto, Canada).

For each rat, VEPs were elicited by successively presenting the three distinct visual stimuli. Two stimuli were those encountered during behavioral training (CS+, CS-). An additional visual stimulus was presented to elicit a novel VEP response and was never encountered during the behavioral training phase (novel stimulus). An initial test was conducted by presenting these stimuli 15 times (5 times per stimulus, 5000 ms duration/stimulus) in order to test that all equipment and electrode placements were functional. Subsequently, formal data collection commenced by successively presenting a total of 300 stimuli (100 times/stimulus, 5000 ms duration) and recording VEPs elicited by each stimulus. For all rats, this procedure was performed twice for a total of two blocks of 300 waveforms. For one of these two blocks, a barrier was placed between the computer monitor and the animal to block the image from being projected onto the retina of the open eye. This control procedure was included to assess whether the visual stimuli, or some potential electrical and/or magnetic interferences were responsible for eliciting, or contributing to the waveforms recorded in V1. The order of the two blocks (with and without barrier) was counterbalanced across different animals.
The same electrophysiological procedures described above for rats that had received training in the water maze apparatus were also carried out in a group of animals that had not undergone any behavioral training (task-naïve group). Obviously, for these animals, all three visual stimuli used to elicit VEPs were novel.

For rats that had undergone multiple visual cue discrimination training, the procedures outlined above were modified in that 6 distinct visual cues were presented to elicit VEPs (CS$^{+1}$, CS$^{-1}$, CS$^{+2}$, CS$^{-2}$, novel$^1$, novel$^2$). These stimuli were sequentially presented for a total of 600 times (100 times for each stimulus). The same control procedure as described above using a barrier was implemented to ensure the visual stimuli were responsible for eliciting VEPs recorded in V1.

![Figure 4: VEP Recording Apparatus.](image)

A schematic of the stereotaxic apparatus and monitor set up used when recording VEPs in V1 in response to visual stimuli displayed on the computer monitor.
3.7.2 Thalamocortical LTP Experiments

Immediately after completion of the VEP experiments, the animals underwent assessments of the effectiveness of high-frequency stimulation of the LGN to elicit long-lasting enhancement of thalamocortical synapses in the form of LTP. Stimulation of the LGN (0.2 ms pulses every 30 seconds, intensity adjusted to yield 50 - 60% of maximal fPSP amplitude) was provided by a concentric bipolar electrode (Rhodes Medical Instruments Series 100, David Kopf, Tujunga, CA) connected to a stimulus isolation unit providing a constant current output (PowerLab/16 s system with ML 180 Stimulus Isolator, ADInstruments, Toronto, Canada). For each experiment, 60 initial baseline fPSPs were recorded every 30 seconds, followed by TBS of the LGN to elicit LTP. This stimulation of the LGN was applied in the form of repeated bursts, with each burst consisting of 5 pulses at 100 Hz, and bursts being repeated for a total of 10 times at 5 Hz; (pulse duration and intensity same as above) (Figure 5). These parameters were chosen based on previous work demonstrating robust, near-maximal LTP with this stimulation protocol (Dringenberg et al., 2007). Recordings of fPSPs (every 30 seconds) continued for 1 hour following the TBS, after which TBS was repeated and recordings continued for another 60 minutes. Thus, a typical recording consisted of a 30-minute baseline followed by 2 hours of recording following TBS.
Figure 5: Stimulation Protocol.

Baseline consisted of 60 single pulses every 30 seconds for a total of 30 minutes. This was followed by TBS which consisted of bursts of 5 pulses/burst at 100 Hz, which were repeated 10 times at 5 Hz. Post-TBS recording consisted of 120 single pulses every 30 seconds for a total of 60 minutes. This was followed by a second TBS and hour recording.

At the end of the experiment, rats were perfused through the heart with 10 % formalin, their brains were removed, and standard histological techniques were used to verify all electrode placements. Data obtained with inaccurate placements were excluded from the data analyses.

3.8 Data Analysis

Data are expressed as mean ± standard error of mean (SEM) when statistically appropriate. Within subject design and repeated measures tests do not require reporting SEM error bars in figures. All analyses were performed using SPSS (version 15.0, SPSS Inc., Chicago, IL). Behavioral data were examined comparing the number of days to reach successful completion of the task among subgroups using one-way analyses of variance (ANOVA). For
multiple visual cue discrimination a repeated measures t-test is used to examine differences in training time of the two sets of visual cues.

For each animal, VEPs elicited by each visual stimulus (i.e., 100 waveforms/stimuli) were averaged. The maximum amplitude of the positive peak of the VEP was computed offline using Scope Software (version 3.6.5, ADInstruments, Inc., Colorado Springs, CO). These amplitude values obtained for each visual stimulus (CS+, CS-, novel) were then compared within each group of animals using separate repeated measures analyses of variance (ANOVA). In one case the visual stimuli seen during training (CS+, CS-) were averaged to create a familiar stimuli VEP measure and compared to novel stimuli using a repeated measures t-test. In addition, some between group comparisons (trained, control, naïve) using ANOVAs were also performed, as outlined below.

For thalamocortical LTP experiments, the fPSPs were stored and the maximal amplitude of the negative-going peak of each fPSP was computed offline. The amplitude values for each animal were then averaged over 10-minute intervals, and these averages were normalized by dividing them by the mean baseline (i.e., pre-TBS) amplitude of that animal. Mixed two-way ANOVAs were used to examine differences in fPSP amplitude before and after TBS within groups. LTP was then indicated when a significant increase in the fPSP amplitude after TBS occurred. Between groups analysis (trained, control, naïve and multiple trained) was also examined using the mixed two-way ANOVA. For all ANOVAs, post-hoc, pairwise comparisons or simple effects tests were performed, as statistically appropriate.
Chapter 4
Results

4.1 Acquisition of Visual Discrimination Learning

Rats that received training (n=24) in the visual discrimination task required an average of 9 days (range 6 to 13 days) to reach the performance criterion of at least 80% correct trials on 3 consecutive days (Figure 6). There were no significant differences in the number of days to reach criterion between different subgroups of animals (i.e., animals that encountered different pairs of visual cues during training; ANOVA; $F(2, 15) = .458, p > .05$), indicating that all visual stimuli used were discriminated with similar levels of efficiency. Only two animals failed to successfully complete the task (did not reach criterion after fifteen days of training) and were excluded from the study. Control animals (n=18) that underwent water maze training without consistent pairing of visual cues to platform location failed to learn the task, as expected (Figure 6). The average response of control animals was consistently just below chance (50% of trials correct). This is believed to be a result of the training procedures and process of randomization of platform location during the training and did not affect further data collection and analyses. Again, there were no differences in performance among the different subgroups of control animals.
Figure 6: Average Behavioral Responses.

Average (± SEM) number of correct responses in the Y maze for visual discrimination training during the random acquisition phase. Correct responses for both trained (n=24) and control animals (n=18) are shown. Dashed line indicates the criterion set for successful completion of the task.

4.2 Characteristics of VEPs

Typical VEPs recorded from the surface of V1 using field recordings in response to the visual stimuli consisted of a biphasic wave, with an initial positive-going potential, followed by a negative-going deflection. Latencies to the peaks of these two VEP components were approximately 250 ± 50 ms and 700 ± 50 ms, respectively (Figure 7). The barrier control procedure did not produce VEPs in response to potential electrical and magnetic interferences.
Thus, it can be established that the electrophysiology recording procedure itself did not contribute to the VEPs recorded from V1.

To be certain that the VEPs were not affected by repeated exposure to the visual stimuli on the day of recording (displaying enhanced potentials as a result of viewing the stimuli repeatedly over time, see Frenkel et al., 2006), averages were computed and compared for the first and last 10 minutes of stimulus presentation. There was no clear trend of potentials increasing or decreasing as a result of simply viewing the visual stimuli over time during recording.

![Figure 7: Typical VEP.](image)

A typical VEP elicited by a familiar visual stimulus seen during training (average of 100 waveforms evoked in response to stimulus presentation). Arrow indicates approximate stimulus onset. Amplitude peak value of positive-going potential was used in further analyses. Barrier control reflects activity in V1 during presentation of a visual cue with the computer monitor blocked to prevent the animal from seeing the cue. Note that, in this condition, a VEP was not elicited.

### 4.3 Effect of Different Visual Stimuli on VEPs in Task-Naïve Animals

Initially, VEPs in response to visual stimuli used during water maze procedures were recorded in task-naïve, untrained animals in order to establish whether there were systematic differences in visual responses evoked by these different stimuli. There were no significant amplitude differences in VEPs elicited by these different stimuli when viewed by naïve rats.
(n=21) (Repeated ANOVA; $F(2, 38) = .102; p > .05$). This suggests that the design and elements (such as light/dark contrast) of the visual stimuli themselves had no significant influence on VEPs (Figure 8).

![Graph of VEPs in Task-Naive Animals](image)

**Figure 8: VEPs in Task-Naive Animals:**

Amplitude of VEPs elicited by three, distinct visual cues in rats (n=21) that had not undergone behavioral training. There were no significant differences in amplitude among the three stimuli (note that all cues are novel to these animals).

As a further control to verify that there were no systematic differences in VEPs elicited by the different stimuli used, comparisons of VEPs obtained in response to novel visual stimuli in the different experimental groups were also made. That is, each group that underwent water maze
procedures was presented with one novel stimulus during the VEP recordings, which differed among the subgroups, due to the counterbalancing procedure. A comparison of these VEPs plus all of those recorded in naïve rats (which only experienced novel stimuli) was performed and found that the VEPs in response to novel stimuli resulted in no significant differences across all groups (trained, control, naïve) (ANOVA; $F(2, 64) = .345; p > .05$) (Figure 9). This analysis further confirms that all visual stimuli used in these experiments elicited comparable VEPs under the current experimental conditions.

![Figure 9: VEPs in Response to Novel Stimuli.](image)

Comparison of VEP amplitude (± SEM) elicited by novel visual stimuli in the different groups of rats (task naïve n=21, control n=18, trained n=24). There were no significant differences in VEP amplitude between the three groups.
4.4 Visual Discrimination Procedures Had No Effect on VEPs

The training procedures of swimming and simply being exposed to the visual cues did not affect the VEPs of stimuli encountered during training procedures compared to novel stimuli (Figure 10). This swim task control accounts for factors such as increased stress, enriched experience, exercise or any other variables associated with the visual discrimination procedures. In control animals, all visual stimuli whether or not seen during the swim task elicited VEPs of similar amplitude (Repeated ANOVA; $F(2, 34) = 1.127; p > .05$).

![Figure 10: VEPs in Control Animals.](image)

Amplitude of VEPs elicited by the visual cues in control animals ($n=18$) that underwent swimming without visual stimuli predicting the platform location. Note that these animals
showed no difference among VEP amplitude to stimuli present in the maze, and a novel stimulus not encountered during training (Left/Right indicate stationary position of the stimuli in the Y maze during swim training).

4.5 Learning the Visual Discrimination Task Results in Enhanced VEPs

Trained animals learned to reliably discriminate between visual cues to find the hidden platform. VEPs were recorded using visual stimuli CS+, CS- and novel. A repeated measures ANOVA revealed a significant main effect of stimulus on VEP amplitude; \((F(2, 46) = 3.246; p < .05)\) (Figure 11). Pairwise comparisons using LSD (least significant differences, no adjustments made for multiple comparisons) showed that CS+ stimuli produced a significantly larger amplitude VEP compared to that seen in response novel stimuli \((p = .031)\), while the difference between the VEP in response to CS- and novel stimuli only approached significance \((p = .079)\). There was no significant difference in the VEP evoked by CS+ compared to CS- \((p = .689)\).

When familiar stimuli (CS+ and CS-) were pooled and compared to novel stimuli, there were significant differences, with a larger amplitude VEP seen for familiar stimuli (repeated measures t-test; \(t(23) = 2.258; p = .034\)). Consequently, it appears that the main effect of stimulus noted above was due to the fact that VEPs to stimuli encountered during training tended to elicit larger amplitude VEPs than novel stimuli.
Figure 11: VEPs in Trained Animals.

Animals in the visually trained group (n=24) that learned to reliably distinguish between CS+ and CS- showed significantly greater VEP amplitude to those stimuli encountered during training compared to novel stimuli; large asterisk refers to significant ($p < 0.05$) main effect of visual stimulus; small asterisk refers to significant ($p < 0.05$) pairwise comparison between CS+ and novel stimuli.

4.6 Characteristics of fEPSPs in Response to TBS of Thalamocortical Synapses

Following VEP recordings, fPSPs in V1 were recorded in response to stimulation of the LGN. Single-pulse stimulation of the LGN elicited a negative-going fPSP with a latency to peak
of about 15-20 ms (*Figure 12*). This potential reflects a current sink in the upper (mostly II-III) cortical layers of V1 in the rat, as shown by Heynen and Bear (2001).

![Figure 12: Typical fEPSP.](image)

**Figure 12: Typical fEPSP.**

Typical recording of fEPSPs in V1 in response to single-pulse stimulation of the LGN before (grey) and after (black) TBS. Note the increase in fEPSP amplitude following thalamic TBS.

### 4.7 Potentiation of V1 after TBS as a Result of Training Compared to Controls

A Mixed Two-Way ANOVA was performed between groups and across time. There was a main effect of time ($F(14, 294) = 52.025; p < .01$) and an interaction of time x group ($F(28, 294) = 1.802; p < .01$). Looking at between-subjects effects of group there was a main effect ($F(2, 21) = 3.555; p < .05$). Subsequent comparisons of naïve (n=7) and control (n=10) groups in post-hoc tests using LSD showed no significant differences. Trained animals (n=7) approached significance (naïve compared to train, $p = .064$, control compared to train, $p = .081$) (*Figure 13*).
Figure 13: Potentiation After TBS.

Amplitude of fEPSP (± SEM) before and after TBS of the LGN in the three groups: task-naïve (n=7), swim control (n=10), and trained animals (n=7) that underwent visual discrimination training. The first three data points represent amplitude during a 30-minute baseline period. This was followed by two episodes of TBS (indicated by arrows) at 0 and 60 minutes. No significant differences occurred between task-naïve and control animals. Trained animals displayed a significant facilitation of LTP. Normalized amplitude during the final 30 minutes of the experiment for each group was: task-naïve = 1.4, control = 1.47 and train = 1.71 increases over baseline.
4.8 More Efficient Learning of Multiple Visual Cue Discriminations

Since there was a facilitation of LTP as a result of the visual discrimination training, will past visual experience affect the efficiency of encoding novel visual features? An additional group of rats (n=9) underwent extended visual discrimination training, first learning one set of visual cues followed by a second set of visual cues. Rats were divided into two subgroups counterbalancing the first and second set of visual stimuli. A probe trial using the 1st set of cues was conducted one day following successful completion of learning the 2nd set of visual cues. All animals but one obtained a probe trial response of 80 % or more (8/10 correct trials) with an average probe trial response being 90 % correct. The average number of days to learn the first set of stimuli was 8.7 days. This is consistent with the animals in the trained group which were only required to learn one set of visual cues. The average number of days to learn the second set of visual cues during training was 5.1 days. A repeated measures t-test showed a significant difference of the days to learn the second set of visual stimuli compared to the first ($t(8) = 2.354; p < .05$). As expected, animals were more efficient at learning and discriminating the second set of stimuli than the first (Figure 14).
Figure 14: Behavioral Responses on Two Sets of Stimuli.

Animals (n=9) learned to discriminate between a 2nd set of visual cues more quickly than initially learning a 1st set of visual cues.

When recording VEPs for animals that received multiple visual cue training a total of six visual stimuli were used (Figure 2), two cues from the 1st set, two cues from the 2nd set and two novel visual stimuli. When analyzing the VEPs elicited by these six visual stimuli no significant differences occurred (repeated measures ANOVA; $F(5, 40) = .280, p > .05$) (data not shown). This could be due to the relatively low number of subjects. No further LTP was present in animals that received training on multiple sets of visual cues compared to those that received single visual cue discrimination. This is demonstrated by a mixed two-way ANOVA between
groups (multiple discrimination set trained vs. single discrimination set trained) and across time (Figure 15). There was a main effect of time ($F(14, 182) = 24.428; p < .05$) however no interaction of time x group ($F(14, 182) = .490; p > .05$) as well as no between-subjects effects of group ($F(1, 13) = .033; p > .05$). Though animals learned to discriminate the 2nd set of visual cues more quickly they did not display a greater amount of LTP.

**Figure 15: Potentiation After TBS After Multiple Discriminations.**

Amplitude of fEPSP (± SEM) before and after TBS of the LGN in the two groups: rats trained on multiple sets of visual discrimination cues (n=8), and animals previously trained on only one set of visual discrimination cues (n=7) (see Figure 13). No significant differences occurred between
animals trained on one or two sets of visual cues. Amplitude during the final 30 minutes of the experiment was: trained on two sets = 58 %, trained on one set = 71 %.
Chapter 5
Discussion

The present study demonstrated that visual discrimination learning results in stimulus-selective facilitation of neuronal responses at early stages of visual processing (V1). In trained animals, familiar stimuli encountered during training elicited larger VEPs than novel stimuli. Although control animals received passive exposure to the stimuli they did not exhibit neuronal enhancement to stimuli encountered during training compared to novel stimuli. Thus, it appears this effect required that stimuli carry some significance (e.g., indicating the platform location). Contrary to the hypothesis presented above and previous studies (Rioult-Pedotti et al., 2000; Whitlock et al., 2006), visual discrimination learning did not impair subsequent LTP induction; rather, it altered the plasticity properties of V1 neurons by facilitating LTP.

5.1 Enhanced VEPs

The present experiments confirm that a significant degree of plasticity is present in the mature V1 of adult rodents and can be detected as a result of visual discrimination learning. Animals exhibited enhanced VEPs to learned compared to novel stimuli. The VEPs analyzed here, recorded close to the cortical surface (i.e., layer I), consisted of a positive going potential, which has been characterized in a previous study using a current source density analysis (Heynen & Bear, 2001). This analysis showed that the positive going wave elicited by visual stimuli reflects a (passive) current source in layer I (i.e., positive current moving out of the cell into the extracellular space) driven by an active sink (i.e., positive current entering the cell) in layers II/III. Thus, the change in VEP amplitude seen here can be interpreted in at least two ways: (1) there is a greater number of layers II/III neurons recruited when viewing learned stimuli compared to novel, thus creating an overall larger evoked potential, or (2) there is a greater
depolarization of the same set of layer II/III neurons, thus creating greater current flow in the extracellular space when viewing learned stimuli. Future work employing intracellular recordings of V1 neurons, or extracellular ensemble recordings of large groups of V1 cells would be useful to further characterize and distinguish the underlying mechanisms mediating the effects noted in the present study.

It is worthwhile noting that the changes in VEP amplitude after discrimination learning were generally very small. However, the experimental design employing a within-subjects statistical design (i.e., trained rats saw CS+, CS-, and novel stimuli) provided a highly sensitive measure to detect small, but reliable differences between different visual stimuli (Howell, 2007).

Although changes in VEP amplitude were detected in V1, the actual plastic changes mediating this effect do not necessarily occur at synapses in this area. For example, synaptic changes may occur earlier in the visual pathway, such as in the LGN which has been shown to exhibit learning-related changes in neuronal discharge (Albrecht et al. 1990). Alternatively, higher visual (V2, V3, etc.) or other cortical and subcortical areas (e.g., basal forebrain, amygdala) could provide modulation to cortical excitability, resulting in a highly selective enhancement of visual responses to specific stimuli (Gu, 2003; Bear & Singer, 1986; Murakoshi, 1995). It is likely that any experience-induced learning process involves plasticity at many loci throughout the brain, and measuring an enhanced response at a specific site might reflect changes elsewhere.

It is tempting to say that the enhanced VEPs to familiar stimuli constitute the memory ‘engram’ for those visual cues. The concept of engrams (i.e., the neuronal substrate of memory) is considered to be the totality of neural changes that comprise a memory (Weinberger, 2003). Thus, despite the fact that there are enhanced VEPs to learned stimuli in V1, this does not necessarily indicate the ‘engram’ or memory for that particular stimulus is stored in V1, since it is possible...
that the enhancement is merely an expression of an ‘engram’ stored elsewhere (Martin and Morris, 2002; Neves et al., 2008).

Interestingly, there is a large body of literature strongly supporting the notion that large-scale changes occur in primary sensory cortices as a result of experience and associational learning. Studies in the rat auditory cortex have found experience-induced enhancements of neuronal responses to particular acoustic stimuli. Using conditioned learning paradigms, the pairing of a sound of a specific frequency with an appetitive or aversive stimulus resulted in an increase in the tonotopic representation of that frequency in the primary auditory cortex (A1), a phenomenon known as receptive field plasticity (Weinberger, 2003). Consistently, studies have shown that these experience-induced shifts in the tonotopic arrangement of A1 are only found when some importance or significance is associated with the specific sound frequency, such as indicating an aversive or appetitive event, while mere passive exposure to a tone does not result in the same augmentation of neuronal responses (Kilgard & Merzenich, 1998; Weinberger, 2003). These data are consistent with the results of the present experiment in that control animals exposed to visual stimuli that did not indicate the presence and absence of the escape platform did not display enhanced VEP responses to these stimuli. Thus, adult sensory cortices exhibit plasticity as a result of learning about biologically significant events and environmental features, but are resistant to change in response to apparently neutral, “meaningless” stimuli encountered in the environment.

In contrast to the present study and literature reviewed above, Frenkel et al. (2006) found that mere passive exposure to visual stimuli resulted in enhanced VEPs recorded in V1. Mice exposed to bar grating over a number of days exhibited stimulus-selective enhancement of VEP amplitude with repeated, passive exposure. Importantly, mice were not required to attend to, or learn visual cues since the only experimental manipulation involved exposing mice to these cues.
At present, the apparent discrepancy between Frenkel et al. (2006) and the data presented here (as well as the work on A1) cannot be explained. It is possible that different plasticity mechanisms could be involved as a result of extended, repeated, passive exposure of visual stimuli relative to those manipulations involving active learning (see below for a possible mechanism and explanation of the current finding.) Further, the Frenkel study elicited VEP enhancement by a non-naturalistic paradigm of restraining mice in a fixed position and repeatedly displaying visual stimuli in sessions over multiple days. As such it is not clear as to its significance and whether it may occur as a result of everyday, naturalistic mouse experience.

5.2 Facilitation of LTP

The initial hypothesis that was tested with regard to thalamocortical LTP was that visual discrimination learning should result in a form of “endogenous LTP” and, consequently, less LTP might occur in response to electrical stimulation of the LGN. This hypothesis follows directly from previous work, demonstrating a trade-off between learning-induced synaptic potentiation and electrically induced LTP in cortical and subcortical systems (motor cortex, hippocampus, amygdala; Rioult-Pedotti et al., 2000; Whitlock et al., 2006; Rogan et al., 1997). In contrast to this hypothesis, the present experiments revealed a clear facilitation of LTP in V1 as a result of visual discrimination learning. There are many differences between this study and the preceding research that may account for the data presented here and this major discrepancy, some of which may bring insight into the underlying mechanisms mediating experience-dependent plasticity.

Similar to the enhancement of VEPs, this facilitation of LTP was only seen in those that received visual discrimination training. Control animals that received passive exposure to the stimuli did not demonstrate the same results. Consequently, it again appears that stimuli require some sort of significance to the animal (in this case indicating the escape platform location) in order for the LTP facilitating effect to occur. In order for an animal to form the association
between the visual cues and the platform location, it would first have to actively attend to the cues. This active attention to the visual stimuli may be the primary difference between trained and control animals.

One of the important, underlying neural mechanisms of ‘attention’ in learning and behavior is activation of the basal forebrain and the subsequent release of acetylcholine (ACh) in the cortex (Muir et al., 1994; Dalley et al., 2001; McGaughy et al., 2002). ACh is a neuromodulator and has been shown to be important for plasticity (Gu, 2003) and facilitation of LTP induction in the visual system (Dringenberg et al., 2007). In the auditory cortex, activation of the nucleus basalis (part of the basal forebrain), when paired with a tone, was sufficient to induce large-scale receptive field plasticity to the particular tone (Kilgard & Merzenich, 1998) and specific, associative behavioral memory (Weinberger, 2003). These data suggest ACh might play a role in some of the effects noted here, and future studies are needed in order to determine if, indeed, activation of the basal forebrain and ACh release is the mediating factor behind the enhanced VEPs and the facilitation of LTP seen here.

Previous studies exhibiting a reduction in LTP as a result of learning, all used learning paradigms different from those employed here. Two previous studies have used conditioning paradigms involving aversive stimuli (inhibitory avoidance learning; Whitlock et al., 2006; and contextual fear conditioning; Sacchetti et al., 2002), while a third investigation used motor skill learning (Rioul-Pedotti et al., 2000). These types of learning may involve differences in attentional demands and the subsequent involvement of the basal forebrain cholinergic system when compared to visual learning. In order to appreciate the important contributions of attention to visual processing, one only has to remember the classic study ‘Gorillas in our midst’ where many participants failed to perceive a man in a gorilla suit directly in their visual field because their visual attention was consumed by a difficult task (Simons & Chabris, 1999).
In addition, different sensory modalities and cortices may exhibit different plasticity properties and make use of different mechanisms of plasticity. Visual learning is likely different from motor learning and hippocampal-dependent, associational learning. Anecdotally, since many motor skills, once they are acquired, appear to be maintained for very long (some perhaps lifelong) time periods, one would expect the synaptic structure of the motor cortex to be fairly stable, likely displaying long-lasting LTP maintenance. After all, once you learn to ride a bike you always know how to ride a bike. In contrast, other types of memory encoding, including sensory-related learning, may be subject to more rapid forgetting, perhaps indicative of less stable changes in synaptic connectivity. For example, it is rare that one can accurately recall details of the visual components of particular scenes, or describe detailed features of paintings one has encountered. Plasticity in the auditory sensory modality may also differ from plasticity mediating visual or motor memory. For example, quite often one has to hear only the first two or three notes of a song to recognize it. One can also be subject to ‘ear worms’ or songs that stick in your head for hours on end, playing over and over again. It seems clear from many anecdotal reports that different sensory modalities involve different properties of memory. These may involve different mechanisms of plasticity and different types of LTP. In summary, the complex interactions between differences in the forms of learning, the synaptic organization of brain areas involved, and the modulatory influence of attention-related systems may account for some of the discrepancies noted between the current and previous studies.

In addition to the interactions of attention and learning discussed above, it is also important to consider whether a particular type of learning is dependent on the hippocampus. The specific role of the hippocampus was not examined in this study, as previous studies provide evidence of its involvement in visual discrimination learning and memory. A similar apparatus and training procedures were used to explore the role of the hippocampus in visual non-spatial
recognition memory (Prusky et al., 2004; Epp et al., 2008). Epp et al., (2008) tested the retention of visual memories after hippocampal damage in rats. If learning occurred and was followed by damage to the hippocampus, severe, persistent retrograde amnesia for the discriminations occurred. However, through extended training, hippocampal-damaged rats could successfully relearn the task. Thus, it appears that the hippocampus is involved in the acquisition and long-term storage of visual discrimination learning, even though its integrity is not a necessary requirement for this learning to take place (Epp et al., 2008). Clearly, learning and memory within the brain is a complex process that involves multiple, and perhaps all structures that compose the nervous system (McDonald et al., 2007).

5.3 Facilitation of LTP \( \Rightarrow \) Facilitation of Learning?

If LTP is a mechanism by which synapses encode learning and memory, then perhaps greater LTP leads to a facilitation of learning. As seen in this study, there was a facilitation of LTP in the visual cortex as a result of visual discrimination learning. One might expect past visual experience to affect the efficiency of encoding novel visual features. To test this hypothesis, an additional group of rats underwent extended training using multiple sets of visual stimuli. Rats were more efficient at learning to discriminate the 2\(^{nd}\) set of stimuli compared to the 1\(^{st}\). Indeed, this facilitation of learning as a result of some prior learning experience fits with much of what is known about processes of learning and memory in general. For example, one learns how to play a 2\(^{nd}\) musical instrument more quickly as a result of learning the first instrument. This is because the framework and rules for how to play the instrument are already in place. Clearly, past experience can exert a profound influence on the rate of learning new information.

Since there was a facilitation of LTP after learning the 1\(^{st}\) set of stimuli compared to control animals, one might expect an even greater facilitation of LTP after learning the 2\(^{nd}\) set of
stimuli. Contrary to this hypothesis, rats that learned to discriminate between two sets of stimuli exhibited the same amount of LTP as rats that learned to discriminate one set of stimuli. One theory to explain these results is that the initial learning of the visual discrimination task provided the maximal amount of general, procedural information to form an optimal framework of rules and guidelines for how to complete the task. In this case, the strategy is already in place for how to accomplish the visual discrimination task and all that is needed is to modify the discriminating rule to fit the 2nd set of visual cues. Perhaps only a few synapses are needed to be modified because the framework has already been set up, consequently, greater LTP is not exhibited as a result of further learning.

5.4 Future Directions and Open Questions

As mentioned throughout the Discussion, the data presented here provide novel and exciting results, contributing towards a more complete understanding of the synaptic and neuronal mechanisms of plasticity that underlie the learning of a simple visual discrimination task. The enhanced VEP data should be further examined using more refined electrophysiology measures (i.e., intracellular recordings of V1 neurons or extracellular ensemble recordings). Such techniques will aid in determining more specifically the nature of synaptic and cellular changes that appear to occur in layers II/III of V1. Using these approaches can aid in answering questions such as “Are more neurons recruited, or are the same set of synapses modified in layers II/III?” thus producing a greater current flow in the extracellular space as a result of this learning.

The facilitation of LTP following visual discrimination learning that was revealed in this study is a truly novel discovery that adds to the sparse literature on the link between LTP and learning. Consequently, a great deal of research is required to further characterize this phenomenon. Based on these present findings, it is hypothesized that the cholinergic system may play a mediating role in the expression of the enhanced VEPs and LTP. If this is the case, this
type of learning may be dependent on multiple neurochemical systems, possibly including NMDA receptors and cholinergic inputs to V1 and other areas of the brain.

If LTP is a mechanism for learning and memory, then learning a task should be related to and change the amount of LTP. Inversely, the amount of potential LTP that can be induced might predict the rate and/or ease of new learning. These experiments demonstrate that rats learn a 2nd set of stimuli more rapidly than the 1st set. The question remains if they could learn a 3rd set of stimuli quicker than the 2nd? With the current simple visual discrimination task, one might expect a ceiling effect for behavioral performance. Interestingly, as outlined above, it appeared as if LTP magnitude reached a ceiling after only one set of visual discrimination learning, an observation that would predict that future learning rates should also reach a ceiling. This may be examined in the future by including additional sets of stimuli in the visual discrimination task, through the use of other visual learning paradigms, or by employing more difficult learning tasks such as a radial arm maze.

A further extension of the present work would be to test whether the facilitation of LTP seen in V1 after training can be replicated in other sensory cortices (e.g., the auditory cortex). Clearly, it is important to determine if various sensory modalities exhibit similar or differing mechanisms of plasticity, as they may display different characteristics of learning and memory process at the behavioral and cognitive level. This may be done behaviorally by training rats in a variety of tasks (a motor, visual, auditory, olfactory, or spatial task). Of course, these tasks would need to be kept as similar as possible to eliminate confounding variables. Across these tasks, one could examine differences in learning acquisition compared to LTP induction, and memory maintenance compared to LTP maintenance in different brain systems mediating these different types of learning.
It is clear that LTP plays a role within the framework of synaptic modification as a result of learning. However, the role of LTD in these processes is still in question. The BCM theory of learning and synaptic modification in the visual cortex was developed in 1982 by Bienenstock, Cooper and Munro (Bienenstock et al., 1982). This theory is a modified version of the Hebbian learning rule and requires that both LTP and LTD (increases and decreases in synaptic strength) occur depending on the level of postsynaptic response activity. The crossover point in which a synapse exhibits LTD or LTP is known as the sliding threshold, which varies as a result of previous activity or experience. Evidence for this theory exists in computer simulations (Shouval et al., 1996) and in slice work of young rats (Kirkwood et al., 1996). Future studies similar to the present study will examine whether LTD is enhanced or diminished in V1 as a result of visual discrimination learning. This will address questions regarding the overall synaptic modification range and the sliding threshold hypothesis of the BCM theory of memory.

5.5 Conclusions

This study compliments numerous other findings indicating that response properties of neurons in the mature V1 are plastic and can change with visual experience. After visual discrimination training, there was stimulus-selective facilitation of neuronal responses in V1, while passive exposure to the same stimuli did not elicit facilitation of these neuronal responses. This effect may be mediated by attentional resources and the cholinergic system as the stimuli acquire some significance to the animal. Contrary to previous studies (Rioult-Pedotti, et al. 2000; Whitlock, et al., 2006), there was a greater level of LTP-like plasticity following training. It was hypothesized that this facilitation of LTP may lead to more efficient learning. Indeed, further experiments demonstrated that animals learned to discriminate between a 2\textsuperscript{nd} set of visual stimuli more quickly than a 1\textsuperscript{st} set. Thus, visual experience alters the physiological properties of neurons
in the primary visual cortex, and LTP in V1 is facilitated as a result of a simple visual discrimination task.

The present finding that visual discrimination learning results in stimulus selective enhancement of visual evoked potentials and that LTP is facilitated as a result of this learning may contribute to determining underlying mechanisms of learning and memory. A significant research effort investigating the neuronal mechanisms of learning and memory has taken place over the last 40 to 50 years. Some may become discouraged about the ‘slow’ progress of this research relative to other fields (brain imaging, neurophysiology, cellular and molecular neuroscience). Learning and memory in the brain may not be as clear-cut as was originally thought many years ago. Indeed, a major purpose of the brain is to encode, store, and make sense of the information about the environment around us. As such, the processes underlying learning and memory and synaptic encoding are bound to be complex in nature. It may be best to view the brain and the incredibly complex biological processes of learning and memory as a dynamical system. Within this system, seemingly simple processes of neuronal firing, synaptic strengthening and weakening occur and build on one another to create the emergent property of memory. It is through rigorous experimental procedures and analyses and similar experiments to those presented above that one can be confident we are closer than ever to solving the mystery of memory.
References


