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Mechanisms of Human Perceptual Learning:
Interference, Consolidation, and Transfer

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Neuroscience

by

Shao-Chin Hung

December 2013

Dissertation Committee:
      Dr. Aaron R. Seitz, Chairperson
      Dr. John Andersen
      Dr. Sara C. Mednick

The Dissertation of Shao-Chin Hung is approved:

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                                                                    Committee Chairperson

University of California, Riverside

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Last, I would like to mention one of the most important people in my life, my boy friend Pin-Chao. Thank you for accompanying me more than eight years and half. Life has never been easy for this long-distance relationship in the past five years. I really appreciate for having this boy friend who supported me to pursue my dream oversea, and I'm also grateful for seeing this long-distance relationship worked on both of ours consideration, comprehension, and trust. Thank you for all of people (Aaron, my parents, my friends, and Pin-Chao) I mentioned. I couldn't complete my PhD without any of you in my life. ☺

ABSTRACT OF THE DISSERTATION

Mechanisms of Human Perceptual Learning:
Interference, Consolidation, and Transfer

by

Shao-Chin Hung

Doctor of Philosophy, Graduate Program in Neuroscience
University of California, Riverside, December 2013
Dr. Aaron R. Seitz, Chairperson

Human perceptual learning is the process of improving in basic sensory discriminations.

This process may involve visual, auditory, tactile, and olfactory systems and forms

essential foundations of human cognitive abilities. To understand neural correlates

underlying this process and to improve learning in applied sensory domains, this

dissertation discusses three aspects of mechanisms in human visual perceptual learning –

interference, consolidation, and transfer. A number of studies have reported that

perceptual learning is highly specific to trained stimuli features. These findings are often

taken as evidence that the learning takes place at primary sensory areas. Of note, a study

has shown that interference of the visual hyperacuity task occurs when two similar

stimuli are learned sequentially. However, this was under the debate that some

researchers' findings failed to support this effect and claimed that retrograde interference

doesn't exist. The first chapter addresses this controversy by replicating both patterns of

results, and demonstrates that retrograde interference in perceptual learning does occur

when subjects' eye-movements are tightly controlled in a peripheral visual task. The existence of retrograde interference suggests that visual perceptual learning requires a period of stabilization before being interfered with by a second stimulus. Therefore, the second chapter investigates memory consolidation, a period that stabilizes memory traces after initial acquisition. This chapter aims to investigate the effects of caffeine and nicotine on memory consolidation of implicit and explicit learning and to discuss the role of a neurotransmitter, acetylcholine (ACh), in consolidation. This dissertation then moves on to the third chapter, which discusses the mechanism of retinotopic specificity in perceptual learning. Whether the perceptual gain results from synaptic changes in early sensory cortices, or whether changes in higher brain areas should also be taken into account still remains the central debate in this field. Notably, a novel double-training paradigm has revealed diminished spatial specificity when multiple stimuli were trained at different locations. To resolve this controversy, the third chapter discusses the roles of stimulus representation and training stimuli's precision in learning effects under double training, and finds that learning specificity depends highly upon particularities of the training procedure.

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INTRODUCTION

Perceptual learning (PL) is a process to gain improvements in sensory discriminations after repetitive practice. Human visual perceptual learning can involve various primitive discriminations such as visual acuity (Poggio, Fahle et al. 1992; Seitz, Yamagishi et al. 2005), contrast (Adini, Sagi et al. 2002; Xiao, Zhang et al. 2008), orientation (Schoups, Vogels et al. 1995; Schoups, Vogels et al. 2001), texture (Karni and Sagi 1991), and motion direction (Ball and Sekuler 1982) of stimuli. Perceptual learning can occur within and between training sessions, and the improvement can last for months or even years depending upon the task (Karni and Sagi 1993). Studies on basic visual discriminations typically show that perceptual learning effects are very specific to trained features (Karni and Sagi 1991; Poggio, Fahle et al. 1992; Crist, Kapadia et al. 1997; Seitz, Yamagishi et al. 2005). For instance, perceptual gain on a vertical Vernier stimulus trained at one quadrant won't be transferred to another quadrant or orientation unless subjects perform additional trainings on the desired condition. The location and orientation specificities are often taken as evidence that perceptual learning takes place at early visual cortices (e.g., V1 and V2), where retinotopic organization and orientations are still retained and processed separately.

In line with this argument, electrophysiological studies have shown corresponding plasticity occurred in primary sensory areas (Recanzone, Schreiner et al. 1993; Schoups, Vogels et al. 2001; Bao, Chang et al. 2004). Schoups et al. reported changes in the properties of neurons, such as an increase of neurons representing trained parameters or an increase selectivity of neurons in V1 or V2 after monkeys performed an orientation

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identification task. Evidence from fMRI studies also implied that perceptual learning is a low-level learning (Schwartz, Maquet et al. 2002; Yotsumoto, Watanabe et al. 2008). However, after people dug more deeply, controversy arose in regards to the locus of perceptual learning. Many studies have shown that the learning-related neuronal changes are not only confined to the primary sensory areas (Yang and Maunsell 2004; Law and Gold 2008; Adab and Vogels 2011), and receptive field modification of neurons in places where changes have been found are too small to explain behavioral improvements even in the same task (Ghose, Yang et al. 2002). Moreover, the degree of specificity depends on the difficulty of the training conditions. Learning of easy trials may lead to better transfer and learning of harder trials retains more specificity to the trained orientation and location (Ahissar and Hochstein 1997). On another hand, a recent double-training study revealed a possibility that learning specificity may be due to insufficient attention to untrained locations or features (Xiao, Zhang et al. 2008). Xiao et al. (2008) reported that the additional location training enabled a complete transfer of feature learning to the second location, arguing that perceptual learning involves higher nonretinotopic brain areas. These results, taken together, suggest that perceptual learning requires a better model or theory.

Notably, Dosher and Lu's " channel-reweighting model" emphasized the influence from the higher-level readout stages (Dosher and Lu 1998). They interpreted improvement of PL through multiple intermediate weighting inputs to the decision-making area, and changes in the readout are already sufficient to explain specificity. More importantly, the Reverse Hierarchy Theory (RHT) links dynamics of perceptual

learning and the underlying neural sites (Ahissar and Hochstein 1997; Ahissar and Hochstein 2004). RHT asserts that perceptual learning is a top-down guided process, which begins at high-level areas of the visual system and progresses backwards to input levels (low-level). Learning of easy trials showed faster improvement and transferred to other orientations, indicating that easy tasks were learned at higher visual areas where neurons have broader tuning curves across orientations and retinal locations. On the other hand, improvement of difficult trials occurred later and was both orientation and position specific, suggesting that difficult trials were learned at low-level areas. Thus, in the temporal domain, this learning cascade proceeds from easier to more difficult conditions and acts as a countercurrent along the cortical hierarchy. Regarding the transfer in perceptual learning, an Integrated Reweighting Theory (IRT) proposed by Dosher et al (2013) explains location and feature transfer by incorporating higher-level location-independent representations into a multi-level learning system (Dosher, Jeter et al. 2013). The IRT includes both location-independent representations and location-specific representations. This model suggests that learning reweights the connections from representation activations to a decision unit and the location transfer is mediated through reweighting of broadly tuned location-independent representations.

In addition to models or theories that link between high-level areas and input levels in perceptual learning, the learning improvements have been thought to occur only when persistent and intensive attention is focused on the learned stimuli (task-relevant learning) (Shiu and Pashler 1992; Ahissar and Hochstein 1993). However, studies have found that learning also takes place without selective attention (task-irrelevant perceptual learning,

TIPL) (Watanabe, Nanez et al. 2001; Seitz and Watanabe 2003; Seitz and Watanabe 2009). Therefore, it becomes important to investigate the mechanism that gates attentional systems and learning signals (reinforcement system) during perceptual learning. A model of task-relevant and task-irrelevant learning suggests that learning can be gated by high level processing (Seitz and Watanabe 2009). The learning signals (e.g. reinforcement signals) and attention can boost sensory stimulation to pass the learning threshold. Once signals pass the learning threshold, learning can occur on both task-irrelevant and task-relevant stimuli, which are thought to induce low-level plasticity. Also, task-relevant stimulus can trigger attentional selection that inhibits learning of task-irrelevant signals and promotes learning of task-relevant stimulus.

In addition, what makes learning susceptible? Research has shown that learning of an immediate similar task will interrupt the previous learning (Brashers‑Krug, Shadmehr et al. 1996; Seitz, Yamagishi et al. 2005). Seitz et al. (2005) reported that learning of a three-dot hyperacuity task was disrupted by an immediate task with similar stimuli (Seitz, Yamagishi et al. 2005; Hung and Seitz 2011), whereas a temporal delay of 1 hour can stabilize the initial learning. In addition, perceptual learning is unable to manifest without a night of sleep (Karni, Tanne et al. 1994; Stickgold, James et al. 2000). These imply that learning must undergo a process to become solid and manifest improvement after initial acquisition, a concept referred to as "memory consolidation." Recently, memory consolidation has been refined to acknowledge that it consists of different stages, including stabilization and enhancement that depend upon specific states during the sleep-wake cycle (Walker, Brakefield et al. 2003). It has been suggested that the

stabilization stage can occur within several hours after the initial acquisition of learning, while the enhancement stage is considered "sleep-dependent memory processing." However, to date, the factors that mediate or influence this dynamic process, as well as the underlying mechanism, remain largely unknown. To further investigate memory consolidation, the second chapter will discuss the possible neural mechanism and the factors that influence this process in implicit and explicit learning. Furthermore, to resolve the controversies between retinotopic specificity and transfer in perceptual learning, the third chapter will study how stimuli representation and training stimuli's precision influence learning transfer under double training.

CHAPTER 1

Retrograde Interference in Perceptual Learning of a Peripheral Hyperacuity Task

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Abstract

Consolidation, a process that stabilizes memory trace after initial acquisition, has been studied for over a century. A number of studies have shown that a skill or memory must be consolidated after acquisition so that it becomes resistant to interference from new information. Previous research found that training on a peripheral 3-dot hyperacuity task could retrogradely interfere with earlier training on the same task but with a mirrored stimulus configuration. However, a recent study failed to replicate this finding. Here we address the controversy by replicating both patterns of results, however, under different experimental settings. We find that retrograde interference occurs when eye-movements are tightly controlled, using a gaze-contingent display, where the peripheral stimuli were only presented when subjects maintained fixation. On the other hand, no retrograde interference was found in a group of subjects who performed the task without this fixation control. Our results provide a plausible explanation of why divergent results were found for retrograde interference in perceptual learning on the 3-dot hyperacuity task and confirm that retrograde interference can occur in this type of low-level perceptual learning. Furthermore, our results demonstrate the importance of eye-movement controls in studies of perceptual learning in the peripheral visual field.

Introduction

Consolidation, a process that stabilizes memory or skills after initial acquisition, has been studied over a century as a central issue in learning and memory (Müller and Pilzecker 1900). While consolidation involves multiple sub-processes (Walker, Brakefield et al. 2003), a key aspect of consolidation involves building up a resistance from interference of new learning. This process of stabilization has been studied in learning of word lists (Müller and Pilzecker 1900), motor learning tasks (Brashers-Krug, Shadmehr et al. 1996; Shadmehr and Brashers-Krug 1997; Walker, Brakefield et al. 2003; Caithness, Osu et al. 2004; Osu, Hirai et al. 2004), and perceptual learning (Yu, Klein et al. 2004; Kuai, Zhang et al. 2005; Petrov, Dosher et al. 2005; Seitz, Yamagishi et al. 2005; Otto, Herzog et al. 2006; Zhang, Kuai et al. 2008; Sasaki, Yotsumoto et al. 2009; Tartaglia, Aberg et al. 2009; Aberg and Herzog 2010; Been, Jans et al. 2010; Mednick 2010), and across these disciplines it has been observed that practice with two tasks (Task A and then Task B) in close temporal proximity can result in interference from Task B on Task A. Furthermore, a number of studies (Müller and Pilzecker 1900; Brashers-Krug, Shadmehr et al. 1996; Shadmehr and Brashers-Krug 1997; Walker, Brakefield et al. 2003; Caithness, Osu et al. 2004; Osu, Hirai et al. 2004; Seitz, Yamagishi et al. 2005; Zhang, Kuai et al. 2008) demonstrate that a temporal interval between the practicing of two tasks can ameliorate this interference. These behavioral investigations, along with neuroscientific research of stabilization at the synaptic level (e.g. l-LTP (Frey, Huang et al. 1993)) have led to broad agreement that initial learning is liable to interference and that stabilization processes can protect learning from later interference.

However, while there is broad agreement that in many tasks interference of learning can occur, and that there exists processes of stabilization, the time-course and mechanisms by which stabilization occurs at a behavioral level are heavily debated. Early, studies of word learning found that stabilization occurred in a period of 6 minutes (Müller and Pilzecker 1900), studies of perceptual learning show that stabilization can occur over an hour in some settings (Seitz, Yamagishi et al. 2005), or within a few minutes in others (Zhang, Kuai et al. 2008), and studies of motor learning show that in some cases stabilization occurs over 4-6 hours (Shadmehr and Brashers-Krug 1997), and in others 24 hours is not sufficient (Caithness, Osu et al. 2004). These divergent findings bring into question whether there are common mechanisms of stabilization that are involved in different experimental domains, and, in some cases, bring into question the veracity of certain findings.

Indeed, in the case of perceptual learning, a controversy has arisen regarding whether interference of learning occurs in both a retrograde fashion (i.e. between different blocks of trials) and a trial-wise (i.e. rapidly interleaved trials of different types) basis. This has led to two published studies that used qualitatively similar methods and observed divergent results. In the case of Seitz et al (Seitz, Yamagishi et al. 2005), disruption of learning for a hyperacuity task occurred if a sequential session with an opposite offset side was performed immediately after the first training session. Moreover, a 1-hour temporal delay of the second session was sufficient to restore learning. This study suggested that visual perceptual learning also requires a stabilization process to consolidate before being interfered by a second stimulus, and this interference is highly

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specific to the orientation of stimulus. However, a recent study by Aberg and Herzog (Aberg and Herzog 2010) conducted five experiments testing for retrograde interference in a variety of hyperacuity stimulus sets that produced interference on a trial-wise basis. Four of experiments involved line bisection tasks presented at the fovea, and, one of the experiments tested was modeled after Seitz et al (Seitz, Yamagishi et al. 2005). These authors found no retrograde interference in any of their experiments. The divergent findings of Seitz et al (Seitz, Yamagishi et al. 2005) and Aberg and Herzog (Aberg and Herzog 2010) makes it uncertain whether retrograde interference truly occurs in the peripheral 3-dot hyperacuity task.

To address this controversy, we decided to replicate our initial finding of retrograde interference for 3-dot hyperacuity. To improve the validity of our findings, we rewrote the experimental code from scratch and ran the experiment on different equipment, in a different lab, and with a different subject population than was used in Seitz et al (Seitz, Yamagishi et al. 2005). Also, to ensure tight experimental control we ran the experiment with and without an eye-tracker, which was integrated into the program to create a gaze-contingent stimulus presentation that enforced fixation while subjects performed the task. Of note, neither Seitz et al (Seitz, Yamagishi et al. 2005) and Aberg and Herzog (Aberg and Herzog 2010) employed an eye-tracker, although both studies instructed subjects to maintain fixation during task-performance. The use of the eye-tracker was important in our task where subjects were asked to fixate a central cross while task-relevant stimuli were always presented in the lower-right peripheral visual field. As we discuss below, the

use of an eye-tracker can be important in tasks where peripheral targets are presented in a

predicable manner.

Material and Methods

<u>Participants</u>

Thirty subjects who were naïve to research purpose participated and received payment for their participation in the experiment. An extra bonus was given based upon good performance to all subjects who completed all 5 sessions. All subjects reported normal (or corrected-to-normal) binocular visual acuity. Informed consent was obtained from all the subjects and the experiments were conducted in accordance with the IRB approved by the Human Research Review Board of University of California, Riverside.

<u>Apparatus</u>

The stimuli were presented using Psychophysics Toolbox (Brainard 1997; Pelli 1997) for MATLAB (The MathWorks, Natick, MA) on a Mac mini computer. The stimuli appeared on a 24" SonyTrinitron CRT monitor with resolution of 1600 x 1024 pixels and a refresh rate of 100Hz. ViewPoint Eye Tracker system running at 220Hz (USB-220^{TM}, Arrington Research ®) and head positioner including chin rest were used to facilitate the eye fixation at the center throughout the entire experiment. Layout of eye tracker system was displayed on PC, the Mac and PC computers communicated through a direct, Ethernet line. The eye-tracker system was programmed so that new trials start once when subjects fixate at the center (within a 2 degree radius fixation window) for 300ms. If an eye-movement outside of this window was detected at any point after the trial started, which was rare due to the rapid stimulus presentation, then that trial was aborted (and excluded from the analysis) and a new trial was initiated.

The stimuli used were the same as previously reported (Seitz, Yamagishi et al. 2005) (see Figure 1). A white, vertical three-dot stimulus was presented on a black background on the monitor. Each dot had a radius of $2'$ (arc minute), and the distance between the top and bottom dots was $20'$. Each trial consisted of one aligned three-dot stimulus, and one offset stimulus with the middle dot offset to the right or left. We used the same set of offset variables that represented 5 different difficulties ($0.9'$, $1.8'$, $2.7'$, $3.6'$, and $4.5'$).

Procedure

Subjects were trained on the three-dot hyperacuity task using the gaze-contingent display that enforced fixation (Figure 1). A central fixation cross was presented on the screen for 300ms at the beginning of every trial, but to ensure subject's fixation, the stimuli wouldn't appear if subjects didn't fixate at the center. Two stimuli – one aligned and one offset three-dot – were presented successively in the bottom right visual field ($7.5°$ in the periphery). The presentation of each stimulus was 50ms, separated by an inter-stimulus interval (ISI) of 400ms. After each trial, subjects had 2 seconds to indicate whether the first stimulus or the second one was offset with a key-press (1 or 2) on the keyboard. Feedback was given as a flash of green cross at the center if the answer was correct, or a flash of red central cross if it was incorrect.

The entire task consisted of 5 training sessions, with each session being conducted at the same time on separate days. The task was typically performed on 5 consecutive days, however in a couple cases there were 1 or 2 days off between sessions. Each training session had 400 trials, divided into 20 blocks (4 blocks per offset size given 5 different

offsets), with each block containing 20 trials of the same offset. The order of blocks was randomly mixed in each session, and breaks allowing subjects rest their eyes were given every 5 blocks (every 100 trials).

Subjects were run in one of three conditions. In the A-group (n=12) subjects conducted 5 sessions on 5 different days in which session (400 trials) involved training with a single offset side. For the AB-group, subjects (n=12) performed an additional training session B (400 trials) immediately after they completed training session A. Training session B had the same vertical three-dot stimulus as the one in session A, except that the offset side of stimulus in session B was opposite to that presented in session A. Note the offset side used in session A and B were counterbalanced across subjects for all experiments. For both the A-group and the AB-group the eye-tracker was employed with the gaze-contingent display. In the AB-gazefree group, the paradigm was the same as for the AB-group, except that the eye-tracker was not employed.

Results

We first verified that training on a single condition (A) would produce learning. The results from the A-group are shown in Figure 2. Indeed we found significant learning between the first and fifth sessions for this group $(F(1,11)=7.02, p=.023)$. These results demonstrate that our training procedure is effective.

To verify whether immediate training with a task can interfere a previously learned task, we examined learning for the AB-group. The results for AB-group can be seen in Figure 3. For this group no significant learning was found $(F(1,11)=0.013, p=0.91)$. These results replicate our previous finding (Seitz, Yamagishi et al. 2005) that retrograde interference can occur in this type of perceptual learning.

So far, we've replicated the results of Seitz et al. (Seitz, Yamagishi et al. 2005) . To address the controversy raised by Aberg and Herzog (Aberg and Herzog 2010), another group of subjects was run without eye-tracker. In the AB-gazefree group, six subjects completed five training sessions, just like the AB-group, however without the use of the eye-tracker. It should be noted that subjects were told to fixate at the central cross throughout the experiment with their head positions stabilized with a chin rest. The results in the AB-gazefree group are shown in Figure 4. Significant learning was found when performance was compared between day 5 and day 1 $(F(1,5)=6.93, p=.046)$. While these results showed significant learning, we found that at least some subjects did not consistently maintain fixation in later sessions (this is a particular problem in a condition where the location of the stimulus is predictable as it was in this study). These results are

comparable to those of Aberg and Herzog (Aberg and Herzog 2010), who claim that perceptual learning does not suffer from retrograde interference of task B on task A either in visual hyperacuity task or bisection task.

We also examined performance in the B condition for the AB-gazefree group (Figure 5a) and the AB-group (Figure 5b). No significant learning was found for the B condition in the AB-gazefree group $(F(1,5)=.89, p=.39)$, nor for the B condition in the AB-group $F(1,11)=0.56, p=0.47$). The poor performance in the B group was also observed in Seitz et al. [7] and may simply reflect fatigue. However, in the AB-group, performance was below chance for the smallest offsets. This may represent anterograde interference and suggests that subjects were processing the aligned stimuli (for the smallest offsets) as being offset to the side consistent with the A training.

Discussion

Our results confirm that interference of learning on task A can occur if a subsequent task B is performed immediately after task A. The control group performing only task A showed a significant improvement after training. Moreover, a group run without the eye-tracker showed no retrograde disruption of task B on task A, similar to the findings of Aberg and Herzog (Aberg and Herzog 2010). These results suggest that visual perceptual learning of peripheral 3-dot hyperacuity can suffer from retrograde interference when subjects' eye-movements are controlled.

To address why the AB training with and without the eye-tracker gives rise to two opposite outcomes, it must be considered the stimuli type presented in hyperacuity task. In visual hyperacuity learning being studied here, the three-dot stimuli were constantly presented in the lower-right visual field, a location that was highly predictable. These stimuli were presented on a mostly blank screen, other than the fixation point, and the sudden onset of the 3-dots can serve to draw eye-movements. In addition, unlike other studies of perceptual learning, such as the classic texture discrimination task (Karni and Sagi 1991), there was no central task to facilitate subjects' fixation. Therefore, it is difficult for subjects to maintain fixation throughout the experiment without an aid. While subjects in the gaze-free group were told to strictly conduct eye fixation at the central cross throughout the experiment, a number of subjects expressed that they tried their best, but that they inadvertently made occasional eye-moments towards the target stimuli. In these cases, when subjects foveated the target, the stimuli would be straightforward to discriminate, even at the hardest condition. Accordingly, two subjects,

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who were dropped after first day, exhibited evenly high accuracy (around 90%) among different offsets (data not shown).

So, why was learning found in the gaze-free group of the present study, and in Aberg and Herzog (Aberg and Herzog 2010), but not in Seitz et al (Seitz, Yamagishi et al. 2005), when none of these experiments employed an eye-tracker? A hint that eye-movements may have occurred in all of these studies is that the gaze-free group, and both Seitz et al (Seitz, Yamagishi et al. 2005) and Aberg and Herzog (Aberg and Herzog 2010), showed above chance performance in the hardest conditions, whereas this was not observed in the fixation conditions of the present study (even for the A-only group). While it is difficult to speculate regarding the extent to which subjects in Seitz et al (Seitz, Yamagishi et al. 2005) and Aberg and Herzog (Aberg and Herzog 2010) did or did not maintain fixation, we suspect that at least some of the differences found between these studies may be due to the extent to which subjects maintained fixation during those experiments. We thus postulate that subjects in Seitz et al [7] were better at maintaining fixation. However, this speculation cannot be proven given that no eye-tracking data exists from those experiments. While it could be interesting to perform a new experiment to address our claim that eye-movement strategies change during the learning process, the primary goal of the present manuscript was to see if results of retrograde interference could be replicated under controlled conditions, which we have done.

It is difficult to determine precisely the key factors that contributed to possible eye movement differences, and otherwise, to the divergent findings across these experiments, however, there were a number of differences between our studies and that of Aberg and

Herzog (Aberg and Herzog 2010). For example, instructions were different in Aberg and Herzog (Aberg and Herzog 2010), in that they explicitly informed subjects of the offset side in each condition, whereas we did not. Furthermore, their stimuli looked qualitatively different in that there appeared to be some apparent motion for the central dot between the two presentation intervals, whereas this was not observable in our experiment (Seitz's personal observations). In Seitz et al (Seitz, Yamagishi et al. 2005) and Aberg and Herzog (Aberg and Herzog 2010) breaks were given every 20 trials, whereas in the current study breaks were given every 100 trials. Also, our present study and Seitz et al (Seitz, Yamagishi et al. 2005) employed chinrest/forehead restraints, however, one was not used by Aberg and Herzog (Aberg and Herzog 2010). Furthermore, different subject populations were used and different experimental equipment was employed. How these factors, and the numerous other experimental differences that are unaccounted for, play a role in the observed findings remains a target of further research.

Given that even an occasional lapse in fixation can cause a large difference in observed results, we suggest that it is imperative to control subjects' eye movements in visual perceptual learning tasks that involve predictable presentation of peripheral stimuli. In our study, the eye-tracker ensured that subjects strictly performed fixation at the central cross when stimuli were presented on periphery; the trial wouldn't start if subjects didn't fixate at the center, and any eye movement deviating away the fixation cross during stimuli presentation was caught by eye-tracker and the trial was skipped. Under the control of eye-tracker, we assume the chance of subject cheating in this experiment has been reduced to minimum, and the entire task was performed exactly on subject's

peripheral vision instead of foveal vision. It is important to note, that the lack of eye-movement control in our gaze-free group provides ambiguity regarding the true nature of the learning in that study. It may be that case that retrograde interference occurred but we failed to observe it due to contamination from eye-movements. Further research is warranted to determine whether eye-movements in the gaze-free group actually prevented retrograde interference from occurring or merely reduced our ability to detect such interference.

Furthermore, it is important to note that the general observation by Aberg and Herzog that retrograde interference is not ubiquitous in perceptual learning is not called into question by our findings that retrograde interference can occur in perceptual learning. Notably, we only dispute the conclusion regarding one of the five studies included in the Aberg and Herzog (Aberg and Herzog 2010) paper. Their other studies were run in central vision and are unlikely to have been impacted by subjects' eye-movements. While a variety of perceptual learning paradigms do demonstrate signs of retrograde interference (Petrov, Dosher et al. 2005; Seitz, Yamagishi et al. 2005; Sasaki, Yotsumoto et al. 2009; Been, Jans et al. 2010; Mednick 2010), Aberg and Herzog's study make clear that retrograde interference is not ubiquitous in perceptual learning.

In conclusion, we suggest that retrograde interference is a common process across studies in perceptual learning (Petrov, Dosher et al. 2005; Seitz, Yamagishi et al. 2005; Sasaki, Yotsumoto et al. 2009; Been, Jans et al. 2010; Mednick 2010; Sotiropoulos, Seitz et al. 2011) and that it may share processes with retrograde interference in reading (Müller and Pilzecker 1900) and motor learning tasks (Brashers-Krug, Shadmehr et al.

1996; Shadmehr and Brashers-Krug 1997; Walker, Brakefield et al. 2003; Caithness, Osu et al. 2004; Osu, Hirai et al. 2004). However, retrograde interference may not be ubiquitous (Aberg and Herzog 2010) and it certainly depends upon the details of the training task. Future research is definitely needed to gain a greater understanding of the processes that lead to interference of perceptual learning.

Furthermore, we conclude that taking advantage of eye-tracking technologies to not only track, but also to control for, eye-movements can provide needed clarity in studies of perceptual learning, particularly those involving presentation of stimuli in the visual periphery. Eye-tracking in peripheral perceptual tasks is very important because eye-movements, even on a small percentage of trials, can turn a difficult peripheral task into an easy foveal task. These occasional lapses can have a profound effect on measures of performance that emulate sensitivity changes and confound results. While the impact of eye-movements will have a greater impact in some studies (in particular studies employing peripheral tasks) than others, it is likely that eye-movements played a role in a large number of studies reported in the literature, including both Seitz et al (Seitz, Yamagishi et al. 2005) and Aberg and Herzog (Aberg and Herzog 2010), and that without being measured and controlled for, readers are left guessing how they impacted the results of those studies.

Figures and Tables
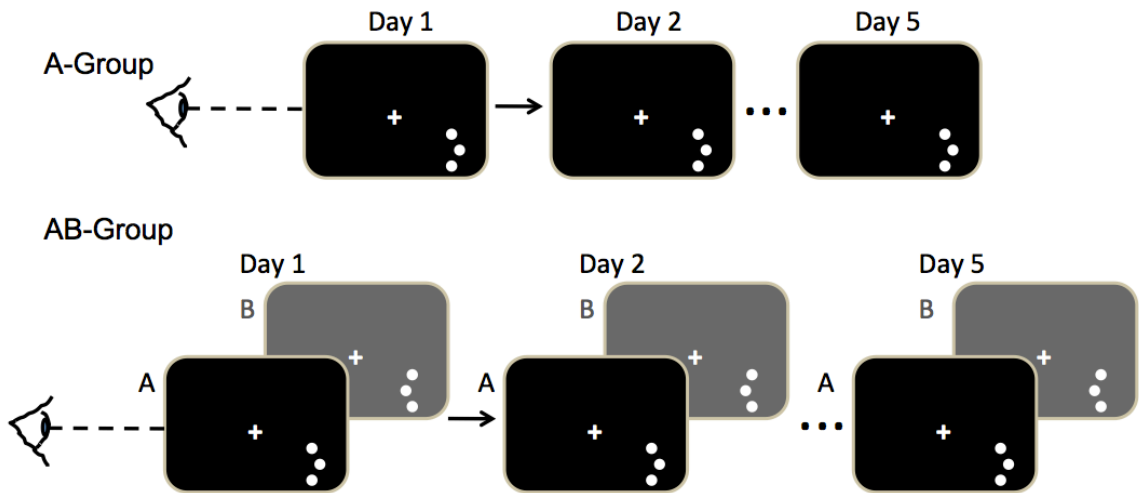
Figure 1. Stimuli and procedure. In the A-group, subjects (n=12) performed one single session (400 trials) with a single offset side for 5 days. In the AB-group, subjects (n=12) performed an additional training session B (400 trials) immediately after session A. The three-dot were identical except that the offset side in training session B was opposite to that presented in session A. For both these groups, the eye-tracker was employed with the gaze-contingent display. Note the offset side used in session A and B were counterbalanced across subjects for all experiments. The AB-gazefree group used the same paradigm as for the AB-group except that the eye-tracker was not employed.
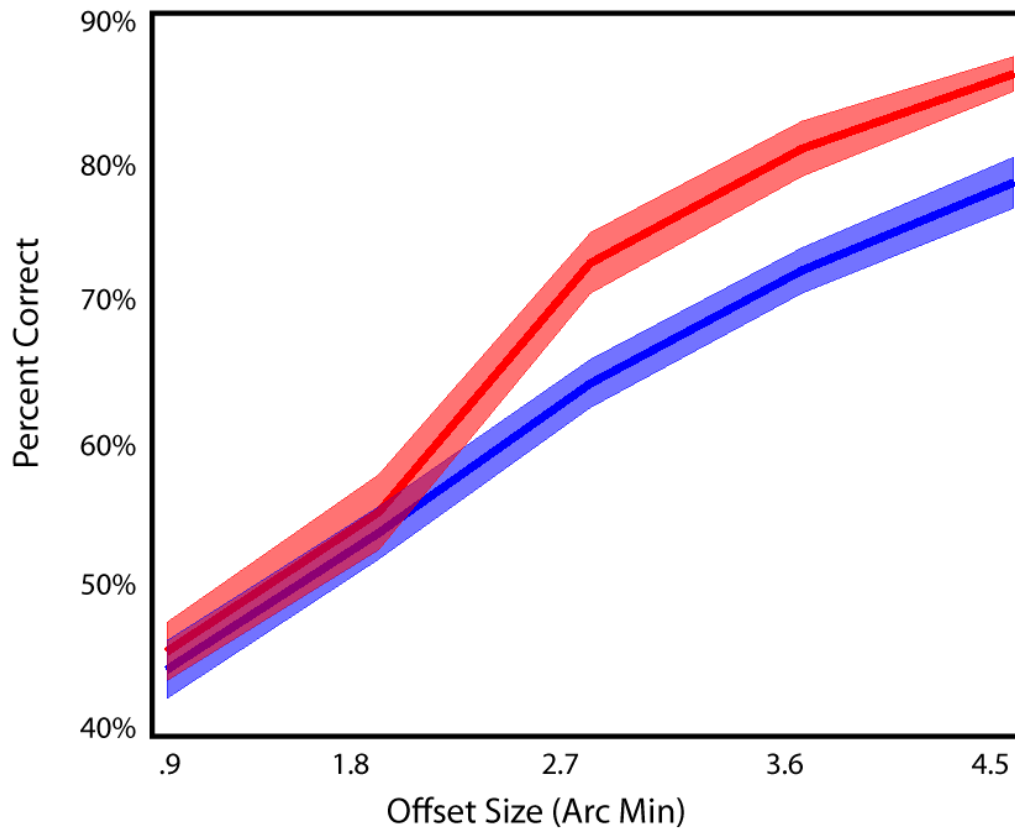
Figure 2. Results from training in the A-group. Pretest (blue) posttest (red). After performing the task with only one offset side for 5 days, subjects showed significant learning that was most prominent in the 2.7′, 3.6′, and 4.5′ offset size conditions. Shaded regions represent standard error.
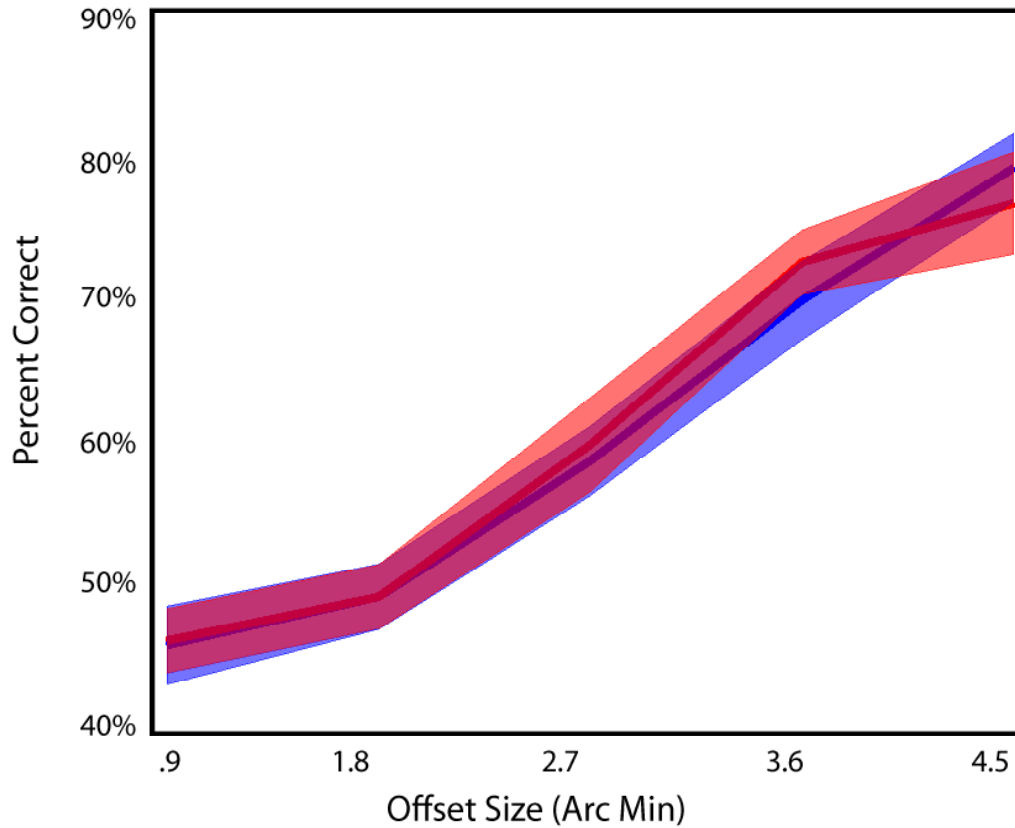
Figure 3. Results from training in the AB-group. Pretest (blue) posttest (red). Subjects performed an additional training session B immediately after session A; offset sides were opposite in session A and B. After 5 days, there was not significant learning found for offset side A. Shaded regions represent standard error.
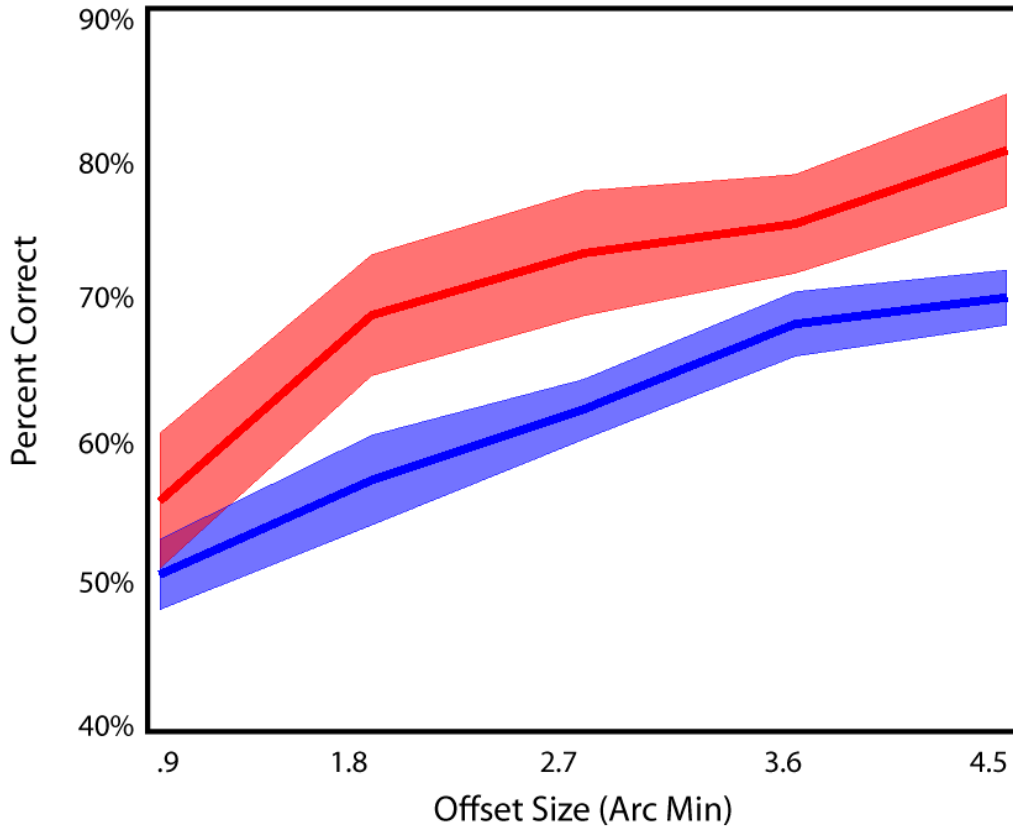
Figure 4. Results from training in the AB-gazefree group. Pretest (blue) posttest (red).
Without the use of eye-tracker, subjects showed significant improvement in offset side A
after 5 days of AB training. Shaded regions represent standard error.
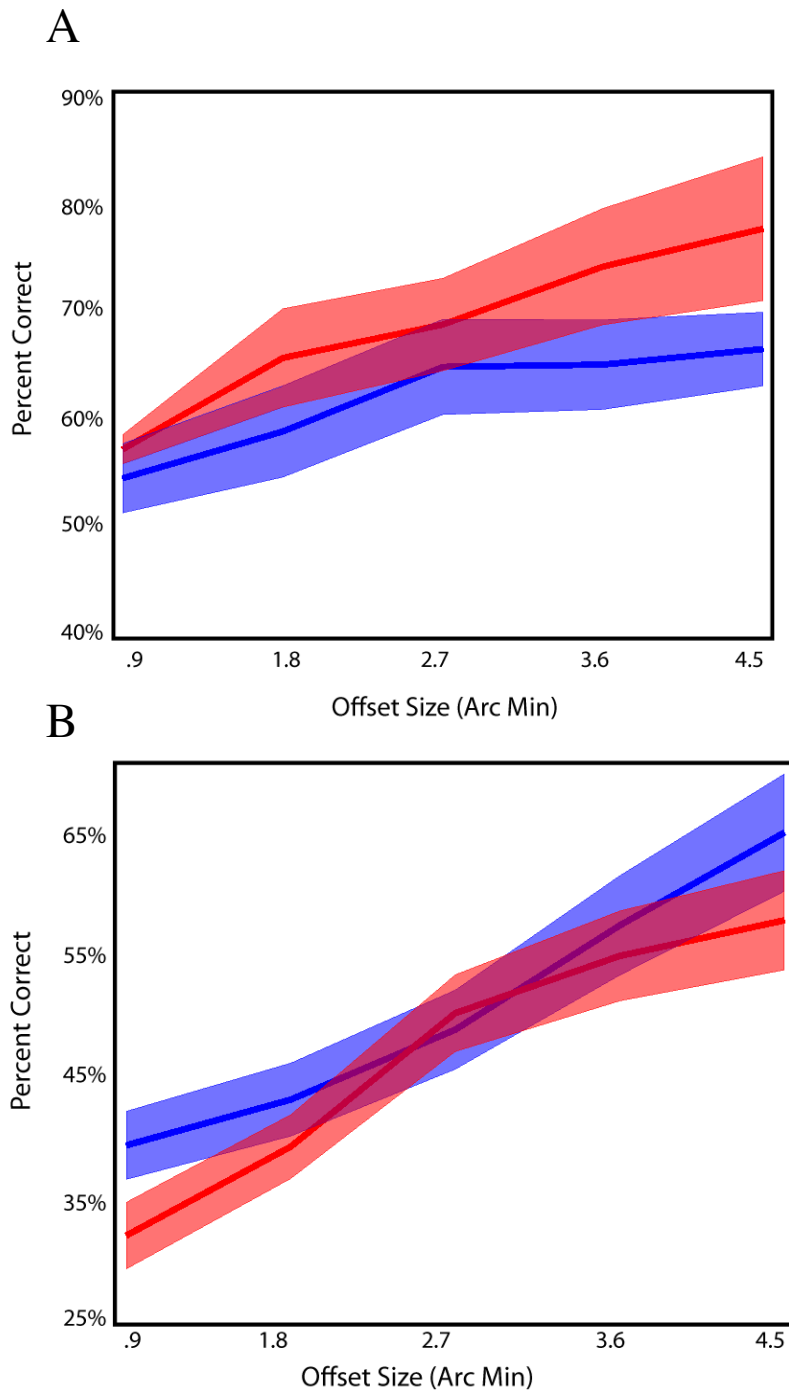
Figure 5. Results from the B condition. A, data from the AB-gazefree group. B, data from the AB group. Pretest (blue) posttest (red). Shaded regions represent standard error.

| Group	Condition	Day	Offset				
			0.9	1.8	2.7	3.6	4.5
A	A	D1	0.45 (0.02)	0.54 (0.02)	0.65 (0.02)	0.73 (0.02)	0.79 (0.02)
A	A	D5	0.46 (0.02)	0.56 (0.03)	0.73 (0.02)	0.82 (0.02)	0.87 (0.01)
AB	A	D1	0.46 (0.03)	0.49 (0.02)	0.59 (0.02)	0.70 (0.03)	0.80 (0.02)
AB	A	D5	0.46 (0.02)	0.49 (0.02)	0.60 (0.03)	0.73 (0.02)	0.77 (0.03)
AB-Gazefree	A	D1	0.51 (0.03)	0.58 (0.03)	0.63 (0.02)	0.69 (0.02)	0.71 (0.02)
AB-Gazefree	A	D5	0.56 (0.05)	0.70 (0.04)	0.74 (0.04)	0.76 (0.03)	0.81 (0.04)
AB-Gazefree	B	D1	0.55 (0.03)	0.59 (0.04)	0.65 (0.05)	0.65 (0.04)	0.67 (0.03)
AB-Gazefree	B	D5	0.57 (0.01)	0.66 (0.05)	0.69 (0.04)	0.74 (0.05)	0.78 (0.07)
AB	B	D1	0.39 (0.03)	0.43 (0.03)	0.49 (0.04)	0.58 (0.04)	0.65 (0.05)
AB	B	D5	0.32 (0.03)	0.39 (0.03)	0.50 (0.03)	0.55 (0.04)	0.58 (0.04)

Table 1. Accuracies (maximum=1.0) for different offsets (in arcmin) in the three-dot hyperacuity task. Values in parentheses represent within subject standard errors.

CHAPTER 2

Abstract

Memory consolidation, a process that stabilizes memory traces after initial acquisition, has been studied for over a century. However, to date, the factors that mediate or influence this dynamic process, as well as the underlying mechanism, remain largely unknown. Caffeine and nicotine are some of the most widely consumed stimulants in the world. Although these two drugs have been reported to have various effects on learning and memory, their effects on memory consolidation remain obscure. This chapter first examines how caffeine and nicotine influence memory consolidation in two types of learning - implicit and explicit – that depend upon different brain areas. Both caffeine and nicotine are potentially linked to the neurotransmitter acetylcholine (ACh) and have been shown to modulate the cholinergic system. Importantly, a number of studies have addressed the essential role ACh plays in memory consolidation. According to the two-stage model proposed by Hasselmo, lower levels of ACh might reduce interference from new information and is appropriate for consolidation (Hasselmo 1999; Hasselmo 2006). To examine ACh's roles, this chapter describes both the changes in the cholinergic tone in different stages of memory formation and the behavioral evidence supporting the importance of cholinergic fluctuation in learning and memory.

Introduction

Hippocampal-Neocortical Model of Memory Formation

Memory is a process that involves several stages including acquisition, consolidation, and retrieval. Memories can be initially formed by engaging with an object or an action, leading to the formation of a representation of the object or action in the brain. This stage is known as "acquisition" or "encoding." The two-stage model of memory formation states that after undergoing the initial encoding stage, memory can be stabilized during consolidation (Buzsaki 1989; Hasselmo 1999) (Fig 6). Both encoding and consolidation have been linked with specific behavioral states and electrophysiological evidence. It is suggested that initial encoding occurs during the waking state, and consolidation occurs during quiet waking or slow-wave sleep. During active waking, information acquired from sensory input flows through neocortex, entorhinal cortex and dentate gyrus into hippocampal CA3, and is encoded in the CA3 region in an intermediate-term representation (Hasselmo 1999). During quiet waking or slow-wave sleep, memories are reactivated in the region CA3 and information flows back through the CA1 region to the entorhinal cortex and the neocortex, enabling deeper consolidation to take place (Winson and Abzug 1978; Chrobak and Buzsaki 1994; Siapas and Wilson 1998).

Memory Consolidation and its Distinct Stages

Memory consolidation has been studied for over a century since it was first proposed by Müller and Pilzecker (Müller and Pilzecker 1900), though the mechanism underlying consolidation is still unclear. Consolidation refers to a process whereby a memory

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becomes increasingly resistant to interference from subsequent competing factors, and eventually becomes more stable (McGaugh 2000). Interestingly, more studies have refined this definition of consolidation and have suggested that consolidation can be determined by specific stages spent during the sleep-wake cycle (Karni, Tanne et al. 1994; Brashers-Krug, Shadmehr et al. 1996; Walker, Brakefield et al. 2003; Seitz, Yamagishi et al. 2005). Instead of being a single process, consolidation might consist of different stages, including stabilization (i.e. resistance to interference of new memory) and enhancement (i.e. exhibit learning effect or performance improvement). It has been suggested that stabilization could occur within several hours after the first training session (Brashers-Krug, Shadmehr et al. 1996; Seitz, Yamagishi et al. 2005), while the enhancement stage that improves performance on the task requires sleep to manifest (Karni, Tanne et al. 1994; Stickgold, James et al. 2000; Walker, Brakefield et al. 2003).

Memory Stabilization and Enhancement

Though studies have not produced a clear picture of the transition between the stabilization and enhancement stages, several pieces of evidence support memory stabilization. For instance, consolidation of one motor task was disrupted when a second task was performed immediately after the first, but there was no disruption if four hours elapsed between the two motor tasks, indicating a gradual consolidation might occur during this period (Brashers-Krug, Shadmehr et al. 1996). Similarly, Seitz et al. (2005) have reported that a disruption of hyperacuity learning occurred if a sequential session with an opposite offset side was performed immediately after the first training session,

while a 1-hour temporal delay of the second session was sufficient to restore learning performance.

Whereas the stabilization stage doesn't require sleep, the enhancement stage is considered "sleep-dependent memory processing " and can only manifest after a night of sleep or a nap. For example, improvement of visual perceptual learning tasks is dependent on the first night of sleep, and the subsequent sleep cannot replace the first night requirement (Stickgold, James et al. 2000). Meanwhile, studies show the importance of different stages of sleep such as rapid eye movement (REM) sleep or slow-wave sleep (SWS) in a variety of learning tasks. For example, selective disruption of rapid eye movement (REM) sleep resulted in no learning of perceptual skills while the same disruption of SWS had no effect on learning, indicating that perceptual learning might be REM-dependent (Karni, Tanne et al. 1994).

Recently, the role of slow-wave sleep (SWS) in different types of memories has been addressed (Walker 2009). Stimulation of SWS through transcranial application of slowly oscillating potentials boosted the performance of declarative and pair-associated task, while there was no enhancing effect on a non-declarative, procedural finger-tapping task and a procedural mirror-tracing task (Marshall, Helgadottir et al. 2006). This evidence suggests that SWS has a beneficial effect mainly on the retention of hippocampal-dependent memory.

In addition, studies suggest that a nap is as good as a night of sleep. For instance, it has been shown that the within-day deterioration of a texture discrimination task (TDT) (Fig 7) learning could be restored by a mid-day nap (Mednick, Nakayama et al. 2002).

Moreover, the improvement of TDT can be accomplished in humans by brief naps (60-90 min) containing both SWS and REM sleep, while the nap group with SWS but without REM sleep shows neither deterioration nor improvement in TDT (Mednick, Nakayama et al. 2003). These results suggest that both the stabilization and enhancement stages are important in the processing of newly acquired information in order to exhibit the long-term effect of learning and memory. While there are numerous factors that may influence these stages, the extensive use of the psychoactive stimulants caffeine and nicotine makes them interesting subjects for research into their effects on memory consolidation.

Caffeine and Nicotine in Learning and Memory

Caffeine, the most widely used stimulant in the world, is a psychoactive ingredient consumed by 90% of North Americans. The increasing daily use of caffeine suggests the importance of researching its effects on learning and memory. Although a number of studies have examined the benefits of daytime caffeine consumption, such as enhancement of mood, alertness and attention (Lieberman, Wurtman et al. 1987; Zwyghuizen-Doorenbos, Roehrs et al. 1990; Kaplan, Greenblatt et al. 1997), the effects of caffeine on learning and memory is still controversial. For instance, it has been reported that long-term consumption of low-dose caffeine slowed down hippocampus-dependent learning in rats (Han, Park et al. 2007), and caffeine appears to impair subjects' declarative verbal memory compared with subjects who received a nap (Mednick, Cai et al. 2008).

Nicotine is known as the main factor responsible for dependence-forming properties during cigarette smoking and is an agonist of nicotinic acetylcholine receptors. In terms of the effect of nicotine on learning and memory, it has been shown that nicotine improves cognition for prolonged periods (i.e. 24 hours later when no nicotine remains in the body) in non-human primates (Buccafusco and Jackson 1991). Also, nicotine causes protracted improvement in spatial working memory in rats demonstrated by the radial-arm-maze task (Buccafusco, Letchworth et al. 2005). Although there are various studies reporting caffeine and nicotine's effects on learning and memory, to date, only a few of these studies are focused on these two psychoactive stimulants and their impacts on memory consolidation.

Acetylcholine in the Hippocampal-Neocortical Model

To study memory consolidation, an important issue is to understand how different neurotransmitters mediate this process. Both caffeine and nicotine are potentially linked to the neurotransmitter acetylcholine (ACh) and have been shown to modulate the cholinergic system. Importantly, Hasselmo's hippocampal-neocortical model suggests the essential role ACh plays in memory encoding and consolidation (Hasselmo 1999; Hasselmo 2006). ACh's role in memory is implied by the fact that cholinergic fluctuation in the hippocampus is highly correlated to different stages of waking and sleep. Microdialysis measurements revealed that acetylcholine levels in the hippocampus of rats and cats is higher during active waking, while there is a reliable decrease in acetylcholine levels during quiet waking and slow-wave sleep (Kametani and Kawamura 1990;

Marrosu, Portas et al. 1995). The changes of cholinergic tones in the hippocampus allow an inference that acetylcholine might have modulatory effects on particular dynamic states in the two-stage hippocampal-neocortical model. As physiological evidence suggests, acetylcholine might enhance the initial encoding of memory by enhancing the influence of feedforward afferents to the cortex (Gil, Connors et al. 1997; Giocomo and Hasselmo 2005; Hasselmo 2006). This allows cortical circuits to respond to sensory stimuli, while inhibiting the excitatory feedback network, which mediates consolidation and retrieval (Herreras, Solis et al. 1988; Hasselmo, Schnell et al. 1995; Vogt and Regehr 2001). While the enhancement of feedforward afferents seems to be mediated by nicotinic cholinergic activation (Hasselmo 2006), the inhibition within the feedback network is suggested to involve the muscarinic cholinergic pathway (Hasselmo and McGaughy 2004).

Therefore, these results reveal the possible role that acetylcholine plays during memory formation – higher levels of acetylcholine during active waking might provide dominant feedforward flow of new information to the hippocampus in memory encoding, while suppressing feedback connections within the hippocampus (Chrobak and Buzsaki 1994; Hasselmo 2006). However, lower levels of acetylcholine during quiet waking or slow-wave sleep might release this inhibition and facilitate feedback effects appropriate for consolidation (Hasselmo 1999).

The Role of Acetylcholine in Learning and Memory

There are a number of studies that have demonstrated the influence of acetylcholine in learning and memory. For instance, local infusion of cholinergic antagonist scopolamine into the hippocampus impairs spatial encoding in the rat (Blokland, Honig et al. 1992), and activation of nicotinic receptors by drugs also facilitates the encoding of new memory (Buccafusco, Letchworth et al. 2005; Levin, McClernon et al. 2006). In addition, since lower levels of acetylcholine during quiet-waking and slow-wave sleep could lead to a dominant feedback flow of information appropriate for consolidation, this predicts that consolidation should be impaired by an increase in acetylcholine levels. Indeed, studies looking at the infusion of cholinergic agonist carbachol into the medial septum (i.e. an area hypothesized to modulate whether the hippocampus engages in retrieval or encoding processes) in rats, and the application of cholinesterase blocker physostigmine in humans, both resulted in impairments of consolidation (Bunce, Sabolek et al. 2004; Gais and Born 2004).

Notably, changes of cholinergic tone are also reflected during different stages of sleep, implying that the role of sleep might be partially mediated by acetylcholine. It is known that the level of ACh, within the hippocampus, drops to a minimum level during SWS, but rises back during REM sleep to an equivalent level as seen in active waking (Marrosu, Portas et al. 1995). Both REM and SWS are potentially involved in sleep-dependent consolidation of learning and memory. It has been reported that low acetylcholine during SWS is essential for the consolidation of declarative memory (Gais and Born 2004). Gais and Born (2004) showed that elevation of acetylcholine via a

cholinesterase inhibitor, during SWS, specifically impaired declarative memory for word pairs (hippocamapal-dependent), but not nondeclarative memory (hippocamapal-independent), revealing the important role of cholinergic processing in the hippocampus. Higher level of ACh during REM sleep, as suggested to provide a new feedforward flow in the hippocampal-neocortical model (Hasselmo, 1999), seems to be critical for enhancing performance in the procedural task. Indeed, a combined blockade of muscarinic and nicotinic receptors during REM sleep specifically impaired procedural memory (motor finger-tapping task), whereas word-pair memory remained unaffected (Rasch, Gais et al. 2009). These pieces of evidence indicate that a change of cholinergic system is essential in memory processing, nevertheless, how acetylcholine influences different types of memory formation, which relies on various brain areas, is an issue not yet addressed.

Consolidation in Implicit and Explicit Learning

Taken together, these results demonstrate that the process of consolidation might be complex and that distinct stages involved in consolidation need to be elucidated. Research that explores either the different stages in memory consolidation (e.g., stabilization and enhancement) or how the cholinergic system mediates these stages may shed light on the process of consolidation. The widely used stimulants, caffeine and nicotine, have known to have effects on cholinergic modulation. Nicotine is a specific ligand of nicotinic acetylcholine receptor (nAChR), and caffeine has also been shown to enhance acetylcholine release in the hippocampus via antagonism of local adenosine A1

receptors (Carter, O'Connor et al. 1995). To see if different types of memory formations share the same mechanism in consolidation, our first goal is to examine caffeine and nicotine's effects on the stabilization stage in consolidation in two kinds of learning that presumably relies on distinct brain areas. Thus, the explicit word learning (i.e. word pair task) that's shown to depend on the hippocampus (Mayes, Holdstock et al. 2004; Gold, Hopkins et al. 2006) and the implicit perceptual learning (i.e. texture discrimination task) that is implied to result in plasticity in visual cortices (Karni and Sagi 1991; Schwartz, Maquet et al. 2002; Yotsumoto, Watanabe et al. 2008) will be used to examine two drugs' effects on memory consolidation.

Materials and Methods

We recruited 81 participants (54 males and 27 females) over the age of 18 and did not suffer from withdrawal symptoms when refraining from caffeine and nicotine for 36 hours. A phone survey (see Appendix A) was conducted before the study and all qualified subjects must meet criteria (i.e. suffers withdrawal ratings of either drug greater than 3 or suffers from risk conditions) before taking part in this study. Experiments were conducted as a two, 1 hour, testing sessions separated by 24 hours. To control the experimental time, research was conducted during 3:00 to 6:00pm every day. Subjects were asked to refrain from consuming caffeine and nicotine since the beginning morning of first session until the end of second session. Participants were trained on day1 for two tasks – texture discrimination task (TDT) and word pair association task (WPA). The order of two tasks was counterbalanced. At the end of the day 1 session, depending on the assigned condition, subjects received a single dose of caffeine (200mg; equivalent to 2 cups of coffee; in a form of gum), nicotine (2mg; equivalent to two cigarettes; in a form of lozenge), a combined dose of both (200mg caffeine + 2mg nicotine), a placebo (gum, lozenge, or both) or nothing. Participants were asked to have a full night sleep before the second day, and improvement of learning performance was analyzed after completion of experiment after the second session. A post-experiment questionnaire (see Appendix B) was also conducted to assess physical symptoms and psychological effects (if any) after drug administrations and evaluate subjects' feedbacks in this experiment. The following are description of TDT and WPA tasks.

Texture discrimination task

The texture discrimination task developed by Karni and Sagi (Karni and Sagi 1991) has been known that it results in long-term change in sensitivity. The behavioral improvement of this task is often shown to be restricted to the retinotopic location of trained stimuli. fMRI studies have revealed an increase of brain activity occurred in V1 area during the initial stage in the texture discrimination task, without involving a significant change in other brain areas such as V2, V3, or attention-related areas (Schwartz, Maquet et al. 2002; Yotsumoto, Watanabe et al. 2008). The texture discrimination task used in this project was similar to that developed by Karni and Sagi (Karni and Sagi 1991) and identical to that utilized by Mednick (Mednick, Arman et al. 2005; Mednick, Cai et al. 2008). Subjects were asked to discriminate two stimuli every trial: a central letter ("T" or "L") presenting in any orientation and a peripheral array composed of three diagonal lines (arranged in vertical or horizontal orientation) in one of the four quadrants (see Fig 7), which were counterbalanced when assigned to subjects. The peripheral array either with vertical orientation or horizontal orientation was presented against a background of horizontally orientated bars, which generated a texture difference between the stimuli and the background.

Each experimental trial followed a sequence: central fixation cross, target screen for 26ms, blank screen for a duration between 50 and 600ms (called the interstimulus interval, ISI), and a mask for 13ms followed by a response time interval for 2 seconds. Subjects pressed two keys ("1" or "2") to report both central letter ("T" or "L") and the

orientation of peripheral array (vertical or horizontal). Feedback was given as a green central cross if the answers were correct, or a red cross if they were wrong.

Before the actual task, subjects performed the initial training that consisted of 25 trials per block (ISI of 321, 593, 728, 868, 1000ms) for five blocks. This training ensured that subjects understood this task and was able to respond at least 80% correct in a single block. During the actual task, each block consisted of 50 trials with the same ISI. A threshold was determined from the performance across 13 blocks, with a progressively shorter ISI, starting with 600ms and ending with 0ms (ISI of 600, 500, 400, 300, 200, 160, 120, 100, 80, 60, 40, 20, 0ms). A psychometric function of percent correct for each block was fit with a Weibull function to determine the ISI at which performance yielded 80% accuracy.

Word Pair Association

The word pair association task was similar to what was reported by Mednick et al. (Mednick, Cai et al. 2008). On day 1, subjects were shown 48 pairs of words in the training session and were asked to memorize each word pair as best as they can. Word lists were matched for recallability, word length, concreteness, and imagery. An immediate recall, composed of 40 word pairs excluded the initial and last four word pairs shown during the training, was given followed by the training session. Subjects needed to type the missing word to complete the word pair, and every typed letter was replaced with asterisks (*) as a control for visual cues to avoid memorization between visual

patterns instead of words. After each answer, a correct word was shown on screen for one second as the feedback.

Subjects were asked to conduct a delayed recall after 24 hours on day 2, without a display of complete set of word pairs at the beginning. They were shown one word from the 40 word pairs they had seen yesterday excluded the initial and last four word pairs, and were asked to type the corresponding word in each pair. Every typed letter was also replaced with asterisks, but no correct words were given after each response in the delayed recall.

Results

We first examined the results of the texture discrimination task (TDT) in five groups of subjects (Fig 8). A between- and within-subject variables (mixed) ANOVA revealed a significant effect between days (i.e. within-subject variable, $F_{(4,75)}=1.985$, $p<0.001$), and a near significant effect of group (i.e. between-subject variable, $F_{(1,75)}=1.985$, $p=0.11$). There was a significant interaction between days and groups ($F_{(4,75)}=2.511$, $p<0.05$), indicating that some groups learned and that others did not.

Following up on this ANOVA, we found that subjects in the caffeine, placebo and nothing groups showed significantly improvement in thresholds on day 2 ($p<0.05$, one-tailed, paired t-test). Notably, subjects in the caffeine group showed a greater improvement than the placebo group (threshold difference between two days was 104±13.5ms in the caffeine group versus 30.8±12.6ms in the placebo group, $p<0.001$, two-sample t-test). In contrast to the caffeine group, the nicotine group showed no learning in TDT (threshold change was -2±40ms, $p=0.53$, one-tailed, paired t-test). The nicotine plus caffeine group showed an intermediate effect (threshold change was 43.4±36.2ms), consistently as what was seen in the two separate groups, however learning in this group was non-significant ($p=0.132$, one-tailed, paired t-test). Therefore, caffeine seems to have a beneficial effect on consolidation in perceptual learning, while nicotine-related groups show no learning effect in this task.

An important concern about the current dataset is that subjects started off at different baselines (blue bars in Fig 8), which is a potential confound in our study. To control this variance, we also compared subjects' TDT performance across five groups at more

similar baseline levels (i.e. 150-300ms). Subjects' day 1 thresholds were selected between 150ms and 300ms and results are shown in Fig 9. A between- and within-subject variables ANOVA revealed an effect between days ($F_{(1,39)}=14.87$, $p<0.001$), and a nearly significant effect of group ($F_{(4,39)}=1.925$, $p=0.13$). The interaction between days and groups didn't reach significance ($F_{(4,39)}=1.649$, $p=0.18$), which might be due to an insufficient power (note that the total subject number went down from eighty to forty-four in Fig 8 and 10, respectively). In addition, results of five groups were consistent with what was shown in Fig 8. There were significant improvements found in caffeine, placebo, and nothing groups ($p<0.05$ for all three groups, one-tailed, paired t-test). Moreover, caffeine facilitated learning of TDT compared to the placebo group ($p<0.001$, two-sample t-test). These results provide evidence that caffeine benefits memory consolidation in implicit learning whereas nicotine seems to impede this process.

Notably, another research group just demonstrated that nicotine facilitated memory consolidation in perceptual learning (Beer, Vartak et al. 2013), which is in stark contrast to our finding. A key difference was that subjects recruited in Beer et al. study were all non-smokers, while subjects in this study included not only smokers but also people who had past intake of nicotine. In order to provide a comparable comparison between these two findings, we conducted another analysis splitting subjects into two groups - habitual smokers (people who smoke 1-5 cigarettes a day) and occasional/non-smokers (people who smoke less than 1 cigarette a day or who don't smoke) based upon their smoking habits. People who smoke more than 5 cigarettes a day were considered heavy smokers and were excluded from the analysis. The results are shown in Figure 10 (habitual

smokers) and Figure 11 (non-habitual smokers). A between- and within-subject variables ANOVA showed no group difference in either of these two subject pools ($F_{(4,41)}=0.866$, p=0.49 in Fig 10 and $F_{(4,24)}=1.299$, p=0.3) in Fig 11), but a significant effect between days ($F_{(1,41)}=4.601$, $p<0.05$ in Fig 10 and $F_{(1,24)}=28.638$, $p<0.001$ in Fig 11). The interaction between groups and days neared significance in habitual smokers ($F_{(4,41)}=2.53$, p=0.055) but was non-significant in non-habitual smokers ($F_{(4,24)}=1.133$, p=0.36).

An important difference between Fig 10 and 12 was revealed in the nicotine group. Results in Figure 10 showed that the nicotine intake in habitual smokers resulted in no learning in TDT (threshold difference was -57.9 ± 57.2ms, p=0.82, one-tailed, paired t-test). Different from this result, administration of nicotine in occasional/non-smokers (Fig 11) nearly boosted learning of TDT (87.1 ± 41.8ms, p=0.053, one-tailed, paired t-test), similarly to what was found in Beer et al. study. Although the beneficial effect of nicotine intake in non-habitual smokers seemed consistent, we failed to replicate the full scope in Beer et al. study because of insignificant learning in the placebo group (p=0.18, one-tailed, paired t-test), possibly due to a smaller sample size. The performance difference observed in two subject pools was also reflected in the caffeine plus nicotine group. Threshold differences in this group were 12.1 ± 53.1ms (p=0.43) and 98.4 ± 50ms (p=0.053) in Fig 10 and 12, respectively. A mixed ANOVA comparing days (within-subject variable) and two nicotine-treated groups in habitual versus non-habitual smokers (between-subject variable) didn't reveal an effect either on days ($F_{(1,12)}=0.078$, p=0.78) or groups ($F_{(1,12)}=0.011$, p=0.92), however, the interaction between days and groups

neared significance ($F_{(1,12)}=2.875$, $p=0.12$). Results in the other groups (caffeine, placebo, and nothing) remained comparable with previous figures (Fig 8 and 10) and were not affected by subjects' smoking habit. These results suggest that people's smoking habit should be taken into account while using nicotine to study behavioral learning performance on smokers (see discussion).

In regards to the drugs' effects on memory consolidation in the word pair association task (WPA) (Fig 12), we conducted the between- and within-subject variables ANOVA and found a significant effect between days ($F_{(1,76)}=5.93$, $p<0.05$) but not in groups ($F_{(4,76)}=0.34$, $p=0.85$). Additionally, the interaction between days and groups ($F_{(4,76)}=1.11$, $p=0.36$) didn't reach significance. Subjects' word recall differences on day 2 were 1 ± 1.3 in the caffeine group, 1 ± 1.2 in the nicotine groups and 0.6 ± 1.3 in the placebo group, however none of the groups showed a significant difference between days ($p=0.44$, 0.41 and 0.66 for the caffeine, nicotine and placebo groups, respectively, one-tailed, paired t-test). It should be noted that the combined administration of both caffeine and nicotine seemed to have a synergistic effect and facilitated subjects' performance in the WPA (words recalled difference was 3.4 ± 0.9, $p<0.01$). Of note, result patterns of WPA between habitual and non-habitual smokers were indifferent with each other (data not shown).

Discussion

The results in this chapter suggest that nicotine and caffeine may exert different functions during the post-training period in implicit learning. Caffeine appears to enhance implicit learning performance while nicotine negatively impacted learning during a period in which learning is stabilized. Consolidation in the word-pair recall task was not significantly impacted by administration of either caffeine or nicotine, however the combined usage of both drugs may have facilitated this learning. While results here are inclusive, further research will be required to validate these findings and to better understand the roles of caffeine and nicotine in memory consolidation.

One key issue in this study is the opposite effects exhibited by the administration of nicotine on habitual smokers and non-habitual smokers. It should be noted that nicotine can both activate and desensitize nicotinic acetylcholine receptors (nAChRs). Prolonged exposure to an agonist will lead to an agonist-induced conformational change in the receptor, known as receptor desensitization. Therefore, smoking in chronic smokers may lead to nAChR desensitization that can alter downstream neuronal function and result in behavioral changes (Brody, Mandelkern et al. 2006). For instance, chronic exposure of nicotine experienced by smokers will desensitize α4β2 nAChRs in the ventral tegmental area (VTA) in the brain and cause a shift in the functional output in the VTA which in turn influences the downstream nicotine-mediated neurotransmitter release (Picciotto, Addy et al. 2008). It is known that on smokers, the simultaneous activation and desensitization of nAChRs in response to administration of nicotine will lead to increased dopamine (DA) release in the brain compared to non-smokers (Iyaniwura, Wright et al.

2001). It is possible that the increased downstream responses altered behavioral responses after nicotine administration, resulting in divergent learning performance we observed in two groups of subjects possessing different smoking habits. This may also explain different consequences observed in perceptual skill stabilization reported here and in Beer et al. study (Beer, Vartak et al. 2013).

The cholinergic system has been implicated to play an essential role in memory consolidation (Hasselmo 1999; Hasselmo 2006). Studies have shown that elevation of acetylcholine during sleep specifically impaired declarative memory for word pairs, but not non-declarative memory (Gais and Born 2004). Combined blockade of cholinergic receptors during wakefulness significantly facilitated consolidation of declarative memory, while consolidation of procedural memory remained unaffected (Rasch, Born et al. 2006). While the two studies (Gais and Born 2004; Rasch, Born et al. 2006) only examined consolidation during sleep or during the period 30minutes after training, our study specifically targeted the stabilization stage immediately after the training in the word pair task and non-declarative implicit learning –perceptual learning. Results here reveal that caffeine has a beneficial effect on implicit memory stabilization, whereas nicotine impairs the same process, indicating that facilitation of cholinergic system during stabilization might be harmful to perceptual learning. Note this finding is in line with Mednick et al. study, in which they found a moderate but non-significant improvement of TDT within one day through a post-training administration of caffeine (Mednick, Cai et al. 2008). It is known that the texture discrimination task won't manifest until undergoing a daytime nap or a night of sleep (Stickgold, James et al. 2000;

Mednick, Nakayama et al. 2003). Given that the paradigm used for TDT in Mednick et al. study didn't undergo a nap or a night of sleep, the two-day design of the present study is likely to allow the enhancement stage in perceptual memory consolidation to take place and thus demonstrates significant learning benefits of caffeine in TDT.

Future Directions

Although the current study investigated two drugs' influence in memory consolidation and inferred a potential role that cholinergic system may play in this process, we didn't specifically address how changes of cholinergic levels would influence this process as suggested by Hasselmo's hippocampal-neocortical model (Hasselmo 1999; Hasselmo 2006). Elevation of ACh after caffeine administration through adenosine receptors is indirect (Carter, O'Connor et al. 1995), and facilitation of the cholinergic system through specific ligands may trigger other downstream neurotransmitters' releases in addition to modulations of cholinergic systems. Additionally, these two drugs may also influence other aspects of brain and body activities (i.e. increase of attention, heart rate, or metabolic rate) that need to be taken into account. To further examine whether lower level of acetylcholine is essential in memory consolidation (either during slow-wave sleep or quiet waking), more specific drugs such as physostigmine (cholinesterase inhibitor) will be suggested to modulate cholinergic level in order to provide a clearer indication of the role of acetylcholine during consolidation. Previous study has indicated that an increase of cholinergic level through physostigmine in human subjects impaired consolidation of declarative memories (Gais and Born, 2004). It will be more convincing

to examine whether similar interruptive results would occur in learning performance of TDT and WPA through drug administration during the stabilization stage.

Another important question is, how long is it required to stabilize memory? The time course across different learning seems to vary. Seitz et al. reported that a temporal delay of one hour is sufficient to resist interruption from new information (Seitz, Yamagishi et al. 2005), while Zhang et al. found that four hours is required for learning of multi-level stimuli to consolidate in perceptual learning (Zhang, Kuai et al. 2008). To specifically address the temporal window of stabilization, drugs that are known to impair consolidation reported in previous findings can be administrated to participants at a delay of 1hr and 6hr after the end of TDT and WPA trainings. Presumably, learning of two tasks should not be impacted by drug administration if stabilization is already completed. While the time courses of stabilization in different tasks are still unclear, the impacts of distinct cholinergic receptors on memory stabilization also need to be elucidated. Both nicotinic and muscarinic cholinergic receptors (nRs and mRs) can modulate the central cholinergic system. The extensive evidence that memory is influenced by post-training cholinergic treatments via mRs indicates that muscarinic cholinergic activation is a critical component in modulation of memory consolidation (Power, Vazdarjanova et al. 2003). For instance, post-training infusions of mR agonists enhances long-term memory (LTM) in rats (Vazdarjanova and McGaugh 1999), while inactivation of mRs through antagonists impaired LTM (Izquierdo, da Cunha et al. 1992). These pieces of evidence indicate that the muscarinic cholinergic pathway may differ from the one recruiting nicotinic receptors. In addition, physiological studies also revealed different

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involvements of two cholinergic receptors in the hippocampal-neocortical model (e.g. activation of feedforward afferents through nicotinic receptors and inhibition of feedback connections through muscarinic receptors) (Gil, Connors et al. 1997; Hasselmo and McGaughy 2004; Giocomo and Hasselmo 2005; Hasselmo 2006). Administrations of drugs manipulating muscarinic pathway, such as the mR agonist oxotremorine or the antagonist scopolamine, in human subjects will shed light on muscarinic cholinergic effect on learning and memory, as well as the distinct roles of nicotinic and muscarinic cholinergic pathways in memory consolidation.
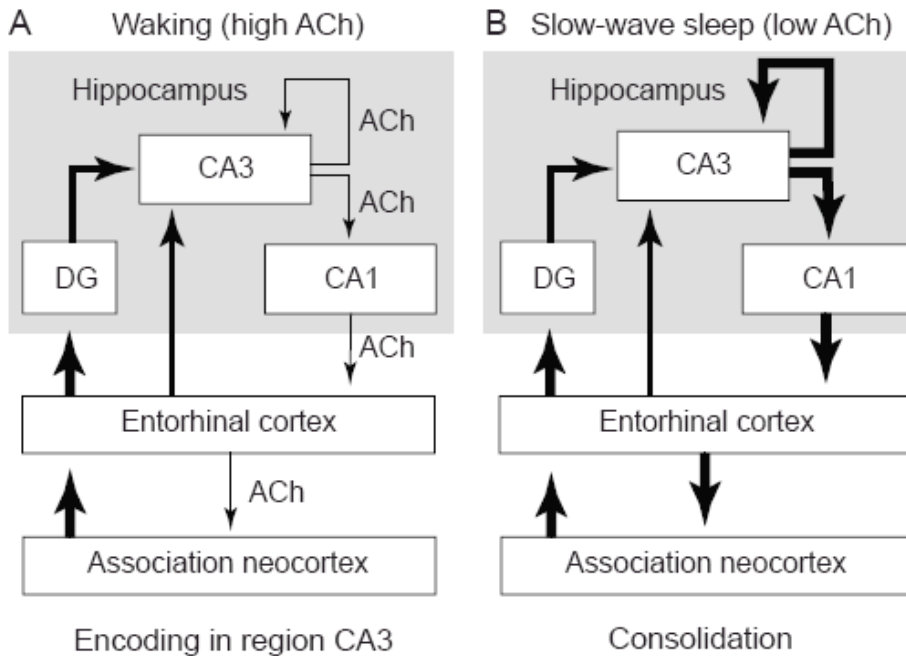
Figures and Tables

Figure 6. Two-stage model of long-term memory formation. (A) During active waking, information acquired from sensory input flows through the neocortex, entorhinal cortex and dentate gyrus into the hippocampal CA3, and is encoded in the CA3 region. Connections suppressed by ACh modulation (thin arrows) to the region CA1, entorhinal cortex and association cortex do not overwhelm the feedforward connectivity. (B) During quiet waking or slow-wave sleep, memories are reactivated in the region CA3 and information flows back through the region CA1 to the entorhinal cortex and neocortex, enabling deeper consolidation (Hasselmo, 1999).
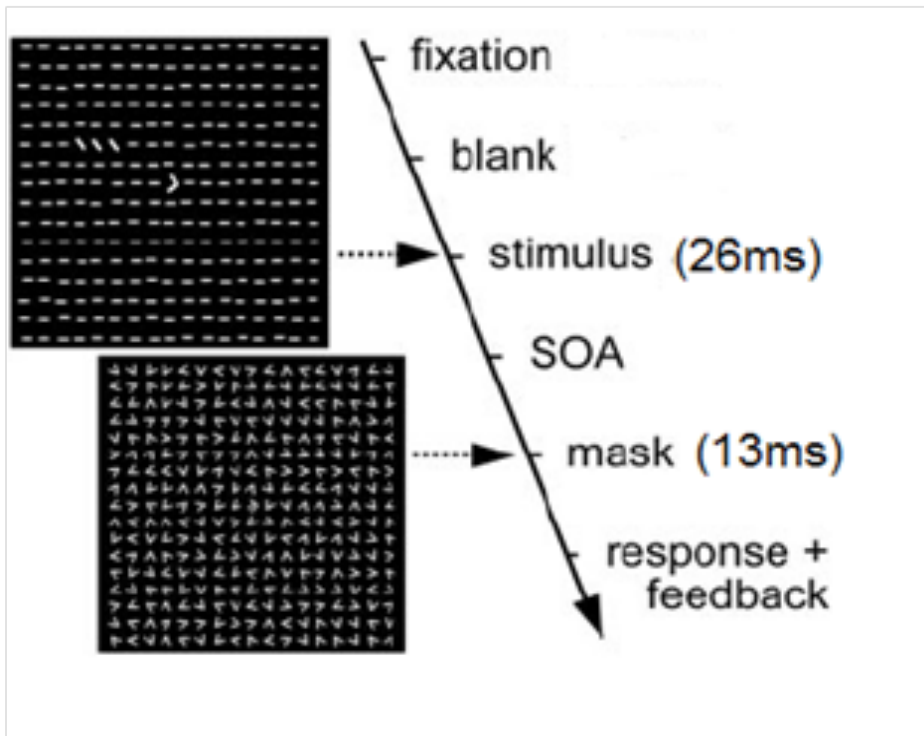
Figure 7. Texture discrimination task. Participants were asked to discriminate two stimuli on target screen per trial: a central letter (''T'' or ''L'') and a peripheral line array (vertical or horizontal orientation) in one of quadrants) by two key presses. Each trial began with a central fixation cross, target screen for 26 ms, an interstimulus interval (ISI) between 50 and 600ms, and a mask for 13 ms followed by the response time interval of 2 seconds. A feedback was given at the end of trial as a green or a red cross at the center for correct or incorrect answers, respectively (modified from Schwartz, Maquet et al., 2002; Copyright (2002) National Academy of Sciences, U.S.A.).

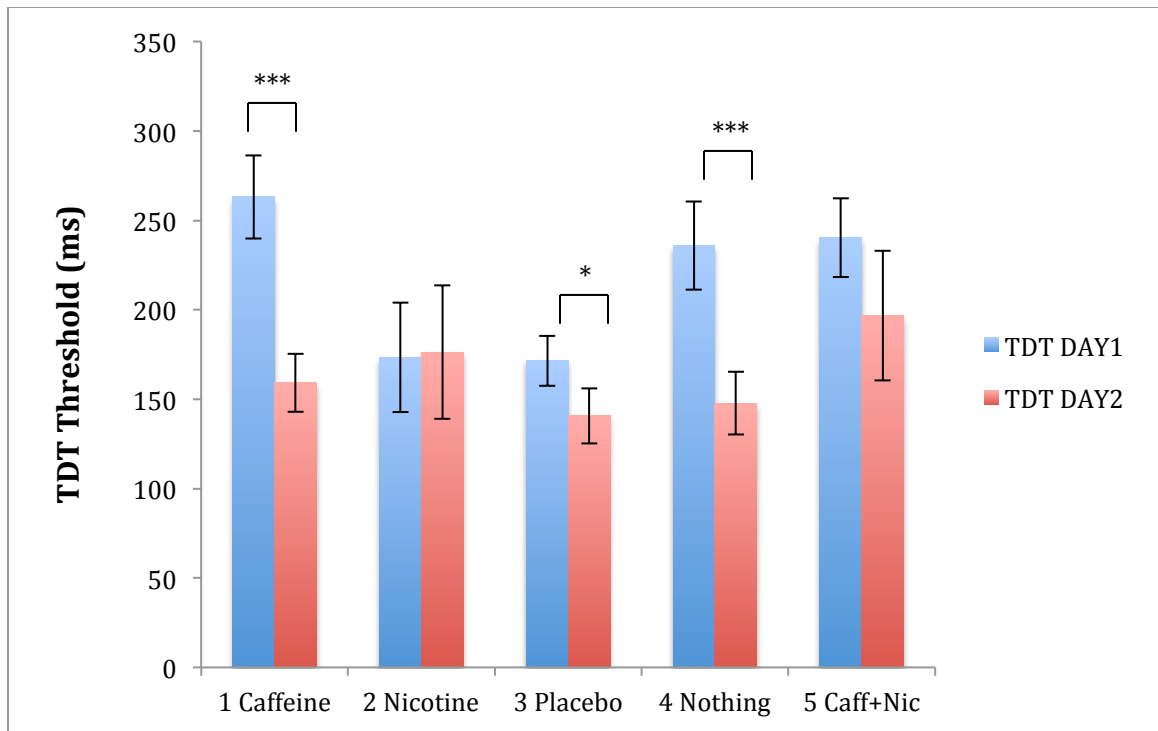Figure 8. TDT threshold results - all subjects.

Number of subjects in each group: caffeine, 16; nicotine, 16; placebo, 17; nothing, 16; caffeine/nicotine, 16. (*, p<0.05; **, p<0.01; ***, p<0.001)
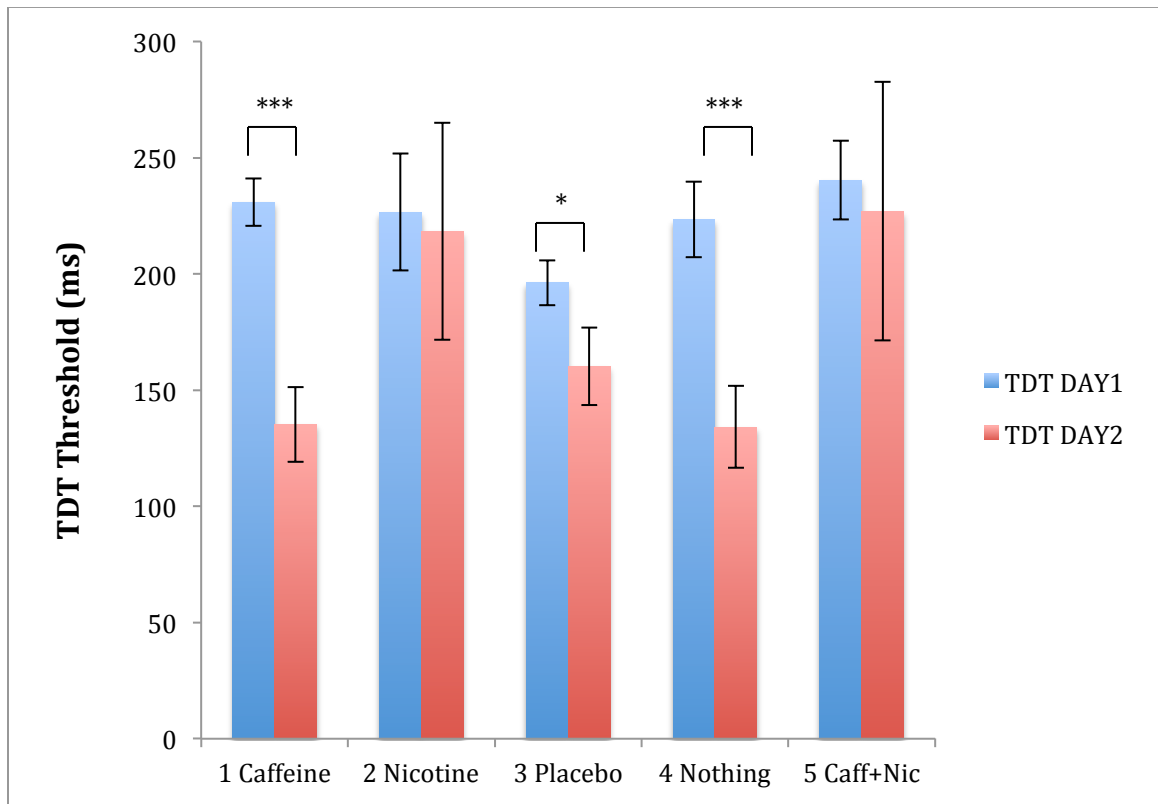
Figure 9. TDT threshold results – controlled baselines.

Number of subjects in each group: caffeine, 9; nicotine, 4; placebo, 12; nothing, 10; caffeine/nicotine, 9. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)
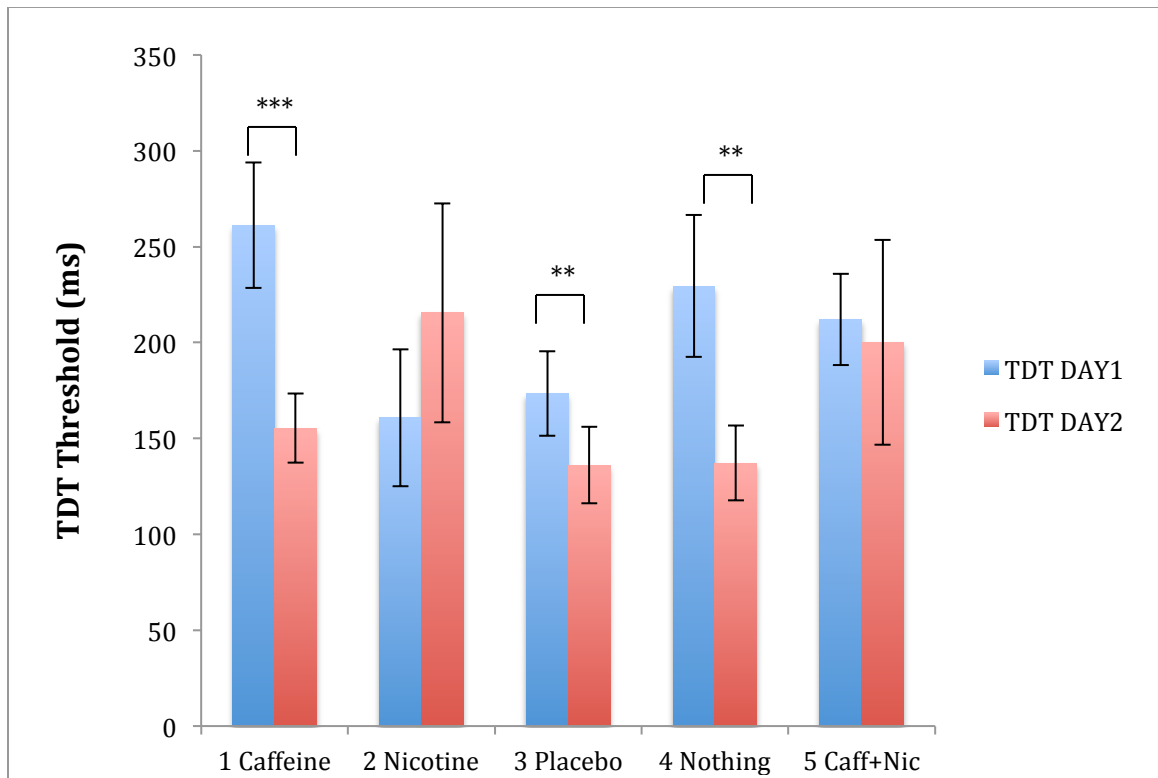
Figure 10. TDT threshold results - habitual smokers.

Number of subjects in each group: caffeine, 10; nicotine, 8; placebo, 9; nothing, 10; caffeine/nicotine, 9. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

Figure 11. TDT threshold results – non-habitual smokers (occasional smokers and non-smokers).

Number of subjects in each group: caffeine, 5; nicotine, 7; placebo, 7; nothing, 5; caffeine/nicotine, 6. (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$)

Figure 12. WPA results –all subjects.

Number of subjects in each group: caffeine, 16; nicotine, 16; placebo, 17; nothing, 16; caffeine/nicotine, 16. (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$)
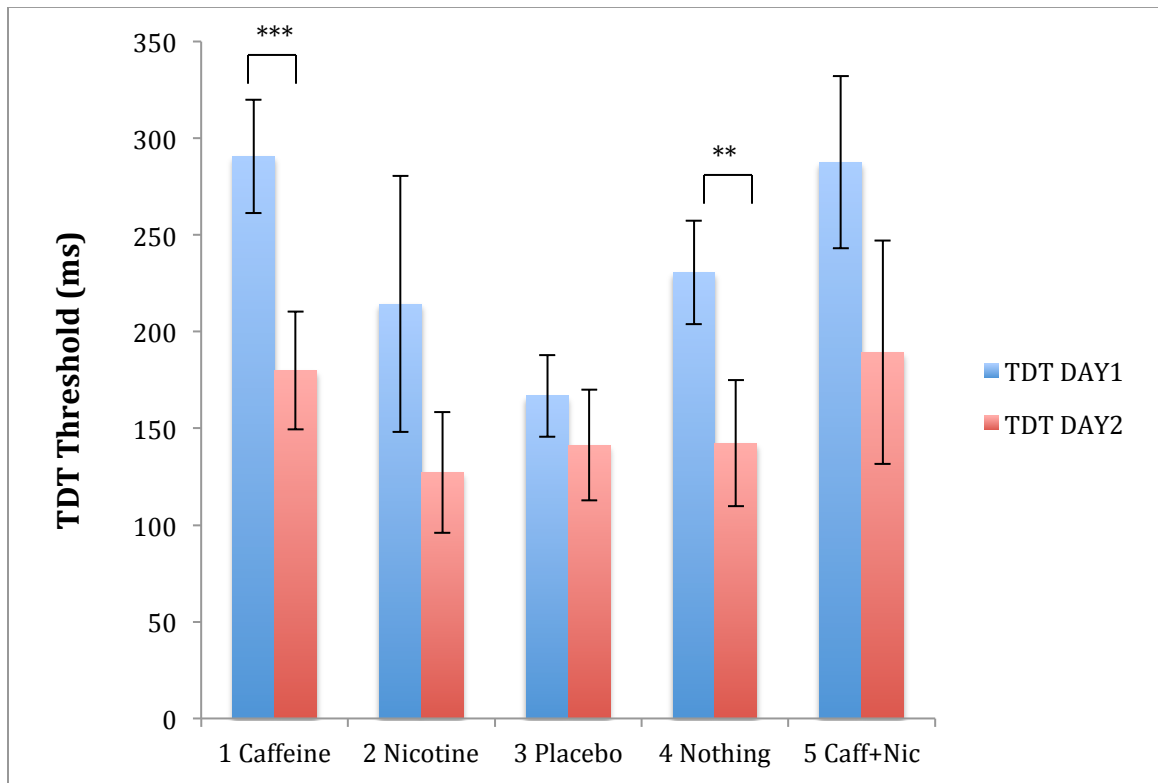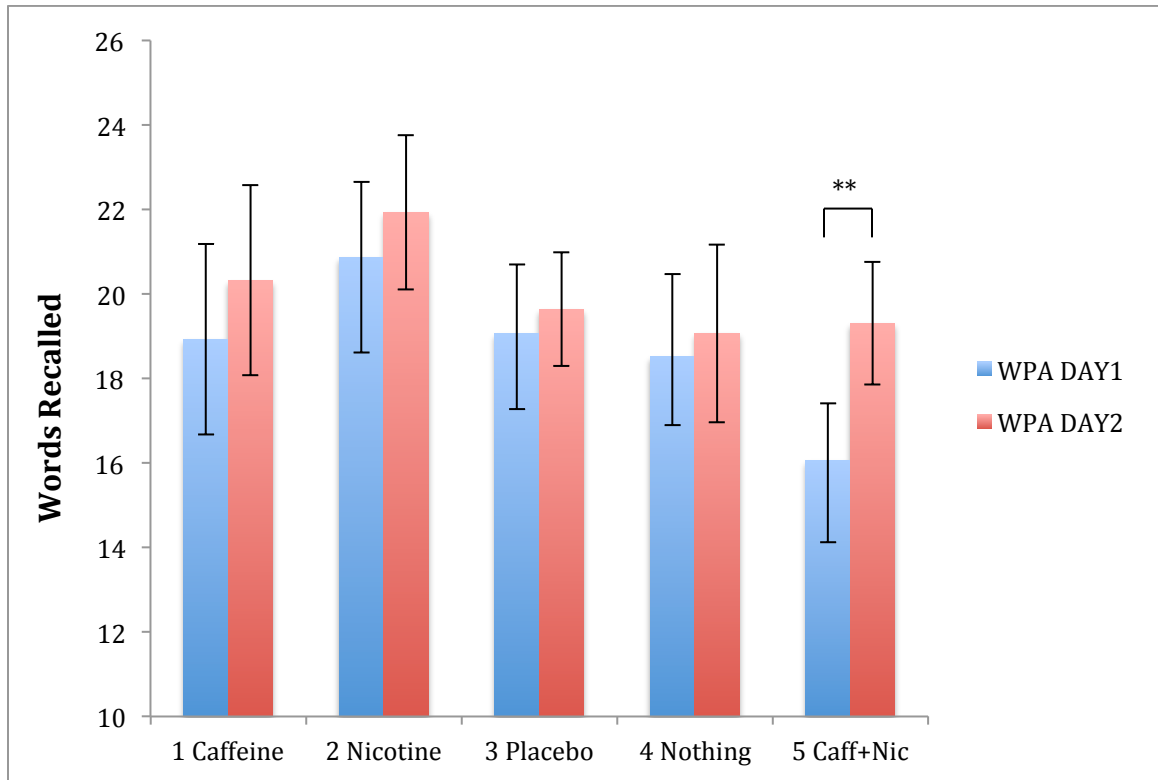
Task	Subs	Day	Caffeine	Nicotine	Placebo	Nothing	Caff+Nic
TDT	All	D1	263.13 (23.26)	173.43 (30.61)	171.46 (13.95)	235.96 (24.67)	240.19 (22.04)
TDT	All	D2	159.13 (16.15)	176.34 (37.30)	140.66 (15.50)	147.77 (17.61)	196.79 (36.27)
TDT	Controlled baseline	D1	230.84 (10.13)	226.63 (25.09)	196.17 (9.62)	223.48 (16.26)	240.41 (17.02)
TDT	Controlled baseline	D2	135.27 (16.09)	218.33 (46.69)	160.23 (16.63)	134.13 (17.62)	227.02 (55.66)
TDT	Habitual	D1	261.24 (32.79)	160.69 (35.61)	173.38 (22.13)	229.50 (36.96)	212.08 (23.80)
TDT	Habitual	D2	215.54 (57.18)	136.06 (19.88)	137.15 (19.44)	200.03 (53.43)	215.54 (57.18)
TDT	Non-habitual	D1	214.32 (66.20)	166.83 (21.15)	230.64 (26.73)	287.68 (44.60)	214.32 (66.20)
TDT	Non-habitual	D2	127.18 (31.18)	141.40 (28.61)	142.28 (32.64)	189.32 (57.83)	127.18 (31.18)
WPA	All	D1	20.31 (1.76)	19.06 (1.64)	18.63 (1.82)	16.44 (1.31)	20.31 (1.76)
WPA	All	D2	21.31 (1.82)	19.65 (1.34)	19.00 (1.97)	19.88 (1.45)	21.31 (1.82)

Table 2. Subject performance in five drug conditions. This table shows thresholds (in ms) for TDT and number of words recalled for WPA. Values in parentheses represent within subject standard errors.

CHAPTER 3

Resolving Controversies of Retinotopic Specificity of Perceptual Learning; Precision of Training Stimuli Determines Transfer Under Double Training

*This study is under revision after submission for publication

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Summary

Perceptual learning is classically thought to be highly specific to trained stimuli's retinal location (Karni and Sagi 1991; Poggio, Fahle et al. 1992; Crist, Kapadia et al. 1997; Seitz, Yamagishi et al. 2005). Together with evidence that specific learning effects can result in corresponding changes in early visual cortex (Karni and Sagi 1991; Schoups, Vogels et al. 2001; Yotsumoto, Watanabe et al. 2008; Adab and Vogels 2011), researchers have theorized that specificity implies regionalization of learning in the brain. However, other research suggests that specificity can arise from learning read-out in decision areas (Dosher and Lu 1998; Law and Gold 2008) or through top-down processes (Li, Piech et al. 2004). Notably, recent research using a novel double-training paradigm (Xiao, Zhang et al. 2008) reveals dramatic generalization of perceptual learning to untrained locations when multiple stimuli are trained. These data provoked significant controversy in the field and challenged extant models of perceptual learning. To resolve this controversy, we investigated mechanisms that account for retinotopic specificity in perceptual learning. We replicated findings of transfer after double training, however, we show that training with more precise stimuli (i.e. Vernier stimuli with fine offset difference) preserves location specificity (when double training occurred at the same location [Wang et al., in submission] or sequentially at different locations (Xiao, Zhang et al. 2008)). This shows that retinotopic specificity depends highly upon particularities of the training procedure. We suggest, perceptual learning can arise from decision rules,

attention learning, or representational changes, and small differences in the training

approach can emphasize some of these over the others.

Materials and Methods

<u>Participants and Apparatus</u>

Subjects who were naïve to research purpose participated and received payment for their participation in experiments. All subjects reported normal or corrected-to-normal binocular visual acuity. Informed consent was obtained from all subjects and experiments were conducted in accordance with the IRB approved by the Human Research Review Board of University of California, Riverside.

The stimuli were presented using Psychophysics Toolbox (Brainard 1997; Pelli 1997) for MATLAB (The MathWorks, Natick, MA) on a Mac mini computer with a 24" SonyTrinitron CRT monitor with resolution of 1600 x 1024 pixels and a refresh rate of 100Hz. A ViewPoint Eye Tracker system running at 220Hz (USB-220™, Arrington Research ®) and head positioner including chin rest were used to ensure eye fixation at the center of the display throughout each trial in the experimental sessions. Layout of eye tracking system was displayed on PC through an Ethernet communication between PC and Mac. All experiments were performed with a gaze-contingent display in which the eye-tracker enabled new trials to start only when subjects fixated at the center (within a 2 degree radius fixation window). If an eye-movement outside of this window was detected at any point after the trial started, then that trial was aborted (and ignored in the analysis) and a new trial was initiated.

<u>Stimuli</u>

Stimuli used were a pair of identical Gabor patches (Gaussian windowed sinusoidal gratings) in the Vernier task and a single Gabor patch in the orientation task presented at

5° retinal eccentricity on a grey background. The Gabor in the orientation discrimination task had spatial frequency of 3 cpd, standard deviation of 2λ, and contrast of 0.45. In addition, the two Gabors in the Vernier task had a center-to-center distance of 4λ, with other parameters being the same as described in the orientation task. The position of Vernier stimuli jittered 0.25° eccentricity (horizontally for vertical stimuli and vertically for horizontal stimuli) across trials to prevent subjects from using external cues such as the edge of monitor to judge stimuli's offset.

For the 3-dot hyperacuity task, a white vertical three-dot stimulus was presented at 7.5° retinal eccentricity on a black background. Each dot had a radius of 2′ (minute of arc), and the distance between the 1st and 3rd dot was 20′. Each trial consisted of one aligned three-dot stimulus, and one offset stimulus with the middle dot offset to the right or left. We used offset variables that represented 5 different difficulties (0.9′, 1.8′, 2.7′, 3.6′, and 4.5′). The viewing distance was 59 inches in all experiments.

Procedure

In the Vernier task, Vernier stimulus was presented for 200ms, and the subject's task was to judge whether the lower Gabor was to the right or left versus the upper one for a vertical Gabor pair, or whether the right Gabor was higher or lower than the left for a horizontal Gabor pair. In the orientation discrimination task, stimuli were presented in two intervals (92ms each) separated by an inter-stimulus interval of 600ms. One reference stimulus (36° or 126°) and one target stimulus (reference + clockwise offset) were presented in a sequential order, and subjects were asked to indicate whether the 1st or the 2nd interval contained the target stimulus. A central fixation cross was presented for

400ms before stimulus onset in both tasks and stayed through the trial. Both tasks were performed under a 3-down-1-up staircase rule and the step size was 1 arcmin in the Vernier task and 1 degree in the orientation task. All stimuli's parameters and the step size in the orientation task were the same as previous publications (Xiao, Zhang et al. 2008; Zhang, Zhang et al. 2010) to provide comparable comparison between two labs' results. Each block contained 20 reversals or 100 trials depending on whichever reached first, and the threshold was calculated from the last ten reversals in each block.

For the 7-day study, subjects were tested on vertical or horizontal Vernier stimuli at each of four quadrants (one block each condition) on their first and last day and received 5 double-training sessions on days 2-6. Double training of Vernier and orientation tasks was initiated by the Vernier task and followed by the orientation task in alternating blocks (eight blocks for each task). Threshold in the training session was only acquired from the 1st block of each task to avoid within-session learning and to provide comparable data to that in testing sessions.

In the 3-dot hyperacuity task, each trial began with a fixation period of 300ms followed by the stimuli presentation. Two stimuli, one aligned and one offset three-dot (50ms each), were presented successively separated by a 400ms inter-stimulus interval. Subjects had to indicate whether the first stimulus or the second one was offset. Threshold in this task was estimated by a power function ($f(x)=ax^n$, where a is a constant and n is a real number) where subjects achieved 75% accuracy.

The testing session of 3-dot hyperacuity task consisted of 800 trials equally distributed between eight different conditions (e.g. three-dot stimulus with left or right

offset at each of 4 quadrants). The 100 trials in each condition were divided into 20, 5 trial mini-blocks; 4 mini-blocks of each the 5 offset-sizes. Blocks of 50 trials (10 mini-blocks, two for each offset-size) were randomized across locations and breaks were given between blocks.

The double training session of the 3-dot hyperacuity task consisted of 400 trials in the same condition (i.e. single location and orientation) divided into 8 blocks (50 trials each block with 10 trials per offset size), with each block followed by one block of the orientation task.

In all experiments, subjects had 2 seconds to answer by a key-press, and feedback was given as a flash of green cross at the center if the answer was correct, or a flash of red central cross if it was incorrect. The location and orientation of trained stimuli were counterbalanced across subjects.

Results

We first replicated the most counter-intuitive example of double-training, namely the "piggybacking effect," where learning of a peripheral Vernier hyperacuity task transfers to another spatial location after training an orientation discrimination task at the same location (Zhang et al., VSS 2011; Wang et al., in submission). The Vernier hyperacuity task has been shown as a location specific task (Xiao, Zhang et al. 2008) and this "piggybacking effect" was initially examined using tasks under an exact replication as previously reported (Xiao, Zhang et al. 2008; Zhang, Zhang et al. 2010). We did so under very tight experimental control, using an eye-tracker to create gaze-contingent displays where trials were aborted as soon as subjects made any eye-movement. In that way, we assured that the training and testing stimuli were always at the intended retinotopic locations (Hung and Seitz 2011). Six subjects participated this 7-day study and were tested on Vernier stimuli at the trained and untrained locations (see Experimental Procedures) on their first and last day. On days 2-6, they performed five double-training sessions (2 hours each day) that consisted of Vernier and orientation discrimination tasks at the same location in alternating blocks (Figure 13A). After training, we found significant learning in the Vernier task at the trained location (Figure 13B and C; Δ Ver_loc1, Mean Percent Improvement [MPI]=33.5 ± 4.5%, p=0.003, one-tailed, paired, t-test), with a gradual, non-significant learning in the orientation task (Δ Ori_loc1, MPI= 16.1 ± 11.5%, p=0.12). More importantly, Vernier learning also transferred to the untrained orthogonal location (Δ Ver_loc2, MPI=24.5 ± 11.6%, p=0.04). These data confirm the "piggybacking effect" and suggest a broad spatial transfer of feature learning

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(e.g. Vernier task) when paired with a second, location-unspecific training (e.g. orientation task) at the same location.

To examine how ubiquitously double training can lead to retinotopic transfer, we examined the "piggybacking effect" using a different hyperacuity task (i.e. 3-dot hyperacuity task). Seven subjects participated in this experiment in which they performed alternating blocks of a 3-dot hyperacuity task and the orientation task, both trained at the same location (Figure 14A). Notably, while we found significant learning in both the 3-dot hyperacuity task and the orientation task (Figure 14B and C; Δ Dot_loc1, MPI=30.2 ± 8.3%, p=0.007 and Δ Ori_loc1, MPI=31.3 ± 7.8%, p=0.003), perceptual learning in the 3-dot hyperacuity task did not transfer to the untrained quadrant (Δ Dot_loc2, MPI= -9.4 ± 10.5%, p=0.67). These results demonstrate that double training does not ubiquitously lead to retinotopic transfer in perceptual learning.

We next sought to understand why double training led to transfer in the Vernier task but not in the 3-dot hyperacuity task. We observed that Vernier training employed multiple short staircases, while the 3-dot hyperacuity training employed the method of constant stimuli. This methodological difference, in which the former included many easy trials (above threshold), while the latter included many hard trials (at or below threshold), leads to a difference in task-difficulty and stimulus precision during the training of the two tasks. Of note, while these repeated short-staircases are typical of the double training studies that found transfer (Xiao, Zhang et al. 2008)(Wang et al., in submission), many studies finding specificity have relied on a single staircase per session (Schoups, Vogels et al. 1995; Jehee, Ling et al. 2012; Le Dantec and Seitz 2012). We

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thus hypothesized that the difficulty/precision of the training stimuli may be key to driving specificity (Ahissar and Hochstein 1997; Jeter, Dosher et al. 2009).

To test this hypothesis, we replaced the repeated short staircases (i.e. a larger portion of trials above threshold) used in the Vernier training with a single staircase (i.e. a larger portion of trials at threshold; Figure 15A). Six subjects were recruited and trained in the single staircase condition. Here we found that training with more difficult/precise stimuli restored location specificity in the Vernier task (Figure 15B and C; Δ Ver_loc1, MPI=34.7 ± 7.7%, p=0.008 and Δ Ver_loc2, MPI= -1.0 ± 14.2%, p=0.41). These results are in stark contrast to those found in the Vernier task trained with multiple short staircases, even though all other aspects of the experiment were preserved. These results support our hypothesis that perceptual learning with more difficult tasks/precise stimuli is more likely to retain specificity, even under double training.

To confirm the finding that training stimuli's precision influences transfer in perceptual learning, we replicated these results by applying the single staircase to the two-location sequential double-training paradigm that was first reported by (Xiao, Zhang et al. 2008). Eleven new subjects participated in this 13-day study and were sequentially trained on two different orientations of the Vernier stimuli, each at a different location. In the first stage of double training (i.e. days 2-6), they were trained with orientation 1 at location 1 (ori1_loc1), and in the second stage (i.e. days 8-12) were trained with the orthogonal orientation at the diagonal location (ori2_loc2). On days 1, 7, and 13, subjects received pre-, mid-, and post-training testing sessions, respectively. All training sessions employed the single staircase method. After successive training at ori1_loc1 (Figure 16A,

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blue circles; Figure 16B, left blue bar, MPI= 34.1 ± 7.7%, p<0.001), contrary to prior results (Xiao, Zhang et al. 2008), sequential double training did not lead to improvement of stimuli at ori1_loc2 after training on ori2_loc2 (Figure 16A, the second and third green circles; Figure 16B, right green bar, MPI= -4.2 ± 9.2%, p=0.62). This further supports our hypothesis that training with more difficult/precise stimuli leads to the retention of retinotopic specificity when subjected to double training.

However, a complexity in our data is that we observed substantial transfer of learning to the untrained location in the mid-testing session (Figure 16A, first two green circles; Figure 16B, left green bar, MPI=19 ± 9.7%, p=0.01). Further observation of the data indicated that there were significant individual differences in the degree of specificity observed after ori1_loc1 training, an effect recently reported by (Zhang, Cong et al. 2013). Thus, to determine if the lack of transfer under double training was resultant from the level of initial transfer, we split the eleven subjects into a "specificity" group (N=6) and a "transfer" group (N=5) based upon the retinotopic transfer observed in the mid-testing session (Figure 16C and E). A transfer index (TI) was calculated as the mean percentage improvement (MPI) at the untrained location (ΔVer_ori1_loc2) divided by the MPI at the trained location (ΔVer_ori1_loc1). The "specificity" group had TI= -0.12 indicating no spatial transfer, and the "transfer" group had TI=1.16, which indicates complete transfer. We next investigated whether the degree of transfer induced by double training was consistent between these two groups. We found that neither the "specificity" group nor the "transfer" group showed any measureable, additional transfer to the second location after sequential double training (Figure 16C and E, the second and third green

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circles). MPIs were -5.2 ± 13.7% (p=0.67) and -2.9 ± 13.6% (p=0.47) for the "specificity" group and the "transfer" group, respectively (Figure 16D and F, green bars in the post-test).

Taken together, we found no evidence that neither simultaneous nor sequential double training induced retinotopic transfer of perceptual learning in the single staircase condition. As such, retinotopic specificity of perceptual learning is not ubiquitously undone by double training and instead depends on the difficulty/precision of the trained stimuli.

Discussion

In this study, we provide a simple and elegant solution to when and why double training will lead to transfer and when it will not. We first replicated the most counter-intuitive example of double-training, namely the "piggybacking effect," in which peripheral hyperacuity of a Vernier task transfers to other spatial locations after training an orientation discrimination task at the same location (Wang et al., in submission). We then moved on to show that this "piggybacking effect" is not ubiquitous, even to hyperacuity stimuli, by showing that the same secondary task (i.e. an orientation task) did not induce any location transfer for the 3-dot hyperacuity task. To elucidate the mechanism of learning specificity, we employed a single staircase per session during training to increase difficulty/precision of the training stimuli and found preserved retinotopic specificity in the Vernier task after double training. These experiments were done under a gaze-contingent display since subjects' eye movements can confound experimental results when a task is performed in the periphery (Hung and Seitz 2011).

A consideration is whether there is a relationship between the degree of learning in the transfer-inducing task and the amount of transfer that is unlocked by double training. Of note, in figures 13B and 15B, learning of the orientation identification task was improved but not significantly so. To test if there was a relationship between learning in the orientation task and the extent of transfer, we compared the correlation between the degrees of orientation learning and transfer of the Vernier task. There was no significant correlation between these variables ($r= 0.09$, $p= 0.73$). Further analysis also revealed no correlation between improvements of the transfer-inducing task (i.e. orientation task in

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figures 13-15 and Vernier task in orthogonal orientation and location in Fig 16) and transfer of the feature task (r=0.14, p=0.47) for all subjects. Another concern is whether the blockwise training of two different tasks would interfere results due to the roving effect. However, given that roving is ineffective if each stimulus is presented for five or more consecutive trials (while ours contained 80-100 trials per block) (Zhang, Kuai et al. 2008) and takes place when employing distinct stimuli in the same task (while ours used different tasks) (Tartaglia, Aberg et al. 2009), this should not be a factor that confounds our study.

It is notable that there were substantial individual subject differences in the degree of transfer after initial training, even when training with the single-staircase (figures 16C and E). Individual differences in the degree of transfer have often been observed in studies of perceptual learning (Aberg and Herzog 2009), and the portion of subjects in our "transfer" group versus the "specificity" group are comparable with the psychophysical data from a recent ERP study examining mechanisms underlying these individual subject differences (Zhang, Cong et al. 2013). However, critical to the point of the current study, the degree of initial transfer was independent of the effects of double training.

Our results provide a powerful demonstration that subtle changes to the training procedure can result in profound differences in the observed learning effects. To answer the central debate in the field about whether perceptual learning is due to representational changes (Karni and Sagi 1991; Schoups, Vogels et al. 2001; Yotsumoto, Watanabe et al. 2008; Adab and Vogels 2011), attentional factors (Moran and Desimone 1985; Luck and

Hillyard 1995; Li, Piech et al. 2004), decision rules (Xiao, Zhang et al. 2008; Zhang, Zhang et al. 2010; Kahnt, Grueschow et al. 2011), or weight changes in readout (Dosher and Lu 1998; Law and Gold 2008), we suggest a simple answer: all of the above. However, importantly, the distribution of learning across the neural system depends upon the fine details of the training procedure. Changing the difficulty/precision of the training stimuli (Ahissar and Hochstein 1997; Jeter, Dosher et al. 2009), whether a pre-test is employed (Zhang, Xiao et al. 2010) or whether multiple stimuli are trained (Xiao, Zhang et al. 2008), can have a profound effect on the distribution of learning across the system. Likely, numerous other undiscovered methodological details (such as design of training/testing sessions, settings and subject instructions etc.) might play critical roles in interpreting the characteristics of perceptual learning (unfortunately even those that some researchers think not important to report in their papers).

So, why does higher stimuli precision restore task specificity? The Reverse Hierarchy Theory (Ahissar and Hochstein 1997; Ahissar and Hochstein 2004) suggests that harder trials trigger early input levels and make learning more specific to trained features in visual perceptual learning. Training with more precise stimuli increased task difficulty and thus is more likely to have neurons tuned to the trained location, resulting in specificity. Notably, recent models of perceptual learning show promise in explaining how these different training approaches account for differences in learning (Dosher, Jeter et al. 2013). An Integrated Reweighting Theory (IRT) proposed by Dosher et al. (2013) explains location transfer by incorporating higher-level location-independent representations into a multi-level learning system. The double-training procedure may

weight more location-independent representations to the decision unit and thus results in location transfer, while an increase of stimuli precision reweights location-specific representations and thus restores location specificity of the task.

We believe that our study both helps settle the debate regarding whether perceptual learning involves retinotopically specific mechanisms (i.e. it can or can not, depending on the training methods) and serves as a call to the field to consider how subtle differences in training can yield dramatic differences in what is learned. To advance our understanding of perceptual learning, the field must move towards understanding individual, and procedurally induced, differences in learning and how multiple neural mechanisms may together underlie behavioral learning effects.

Figures and Tables

A
Double Training   Transfer

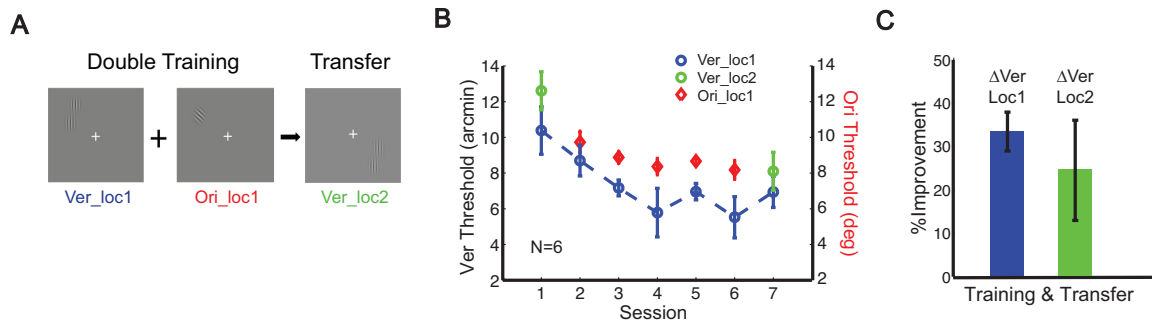Ver_loc1   Ori_loc1   Ver_loc2

B

C

Figure 13. Transfer of Vernier Learning After Double Training at the Same Location.

(A) Stimulus configuration. Double training of Vernier and orientation discrimination tasks was performed at the same location (loc1), and transfer of Vernier learning was tested at the untrained diagonal location (loc2). (B) Mean session-by-session threshold analysis for Vernier learning (blue circles) and orientation learning (red diamonds). Transfer of Vernier learning was tested before and after double training (green circles). The practice of location-unspecific orientation learning piggybacked Vernier learning to the untrained location. (C) Mean percent improvement for Vernier learning at the trained (loc1, blue bar) and untrained (loc2, green bar) location. Vernier learning significantly transferred to the untrained location (loc2) after double training. Error bars represent within subject standard error.
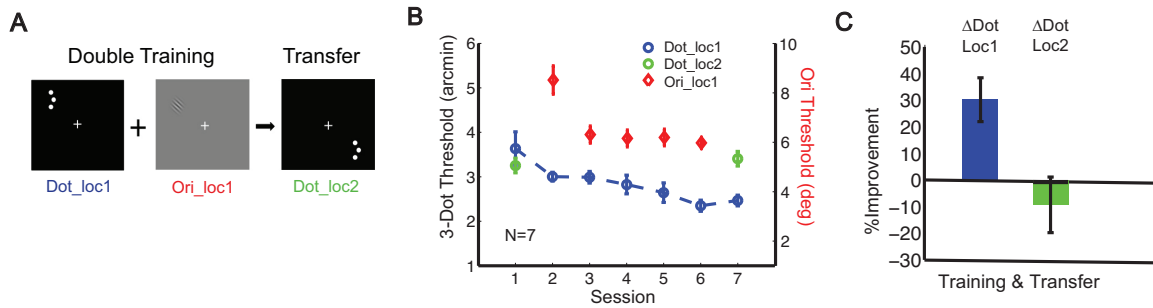
Figure 14. Retinotopic Specificity in the 3-Dot Hyperacuity Task After Double Training. (A) Double training of 3-dot hyperacuity and orientation discrimination tasks was performed at the same location (loc1), and transfer of 3-dot hyperacuity learning was tested at the untrained diagonal location (loc2). (B) This plot shows session-by-session thresholds for 3-dot hyperacuity learning (blue circles) and orientation learning (red diamonds) and transfer results of 3-dot hyperacuity task (green circles). Improvement of the 3-dot hyperacuity task did not transfer to the untrained location (green circles). (C) Mean percent improvement of 3-dot hyperacuity learning at the trained (loc1, blue bar) and untrained (loc2, green bar) location is shown. The 3-dot hyperacuity task retained retinotopic specificity after double training.
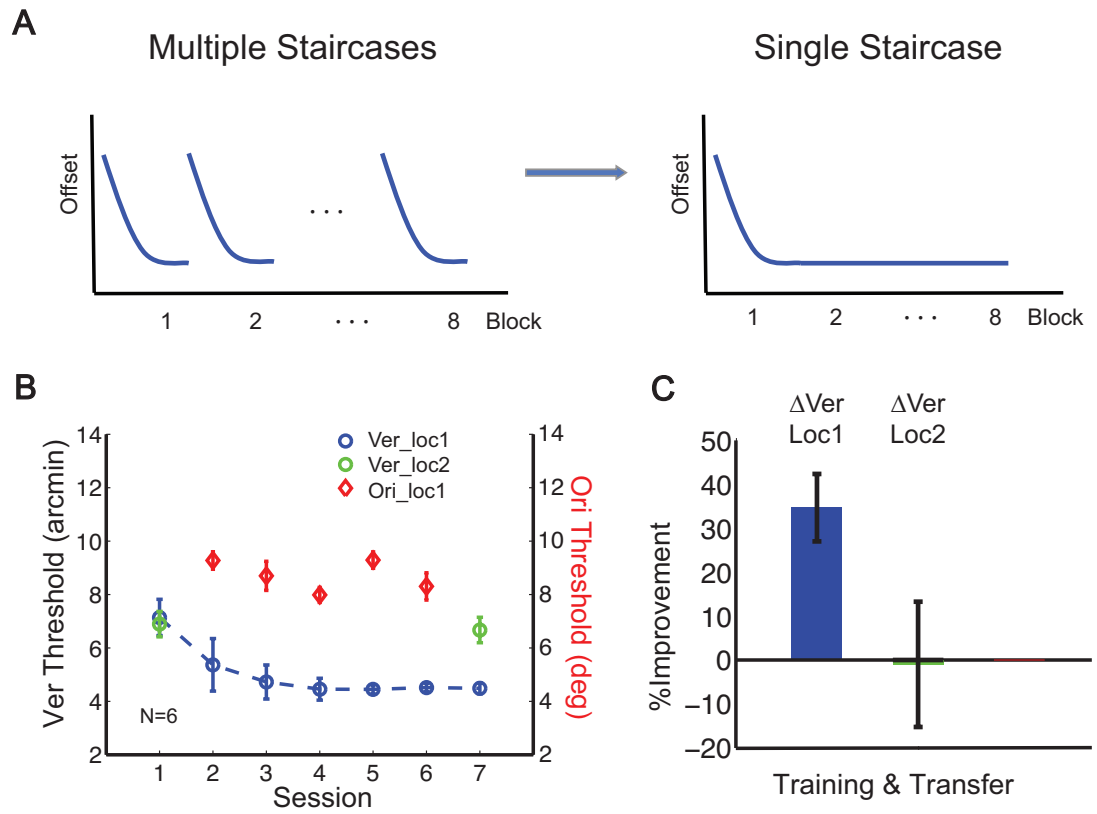
Figure 15. More Precise Training Stimuli Restored Spatial Specificity of Vernier Learning.

(A) Comparison between the multiple short staircases and the single staircase. Conventional multiple staircases return back to stimuli's original offset and contain many easy trials as the new block begins. The single staircase remains around subjects' threshold for almost the entire training session. (B) Session-by-session threshold analysis for Vernier learning (blue circles) and orientation learning (red diamonds) in the single staircase condition. Transfer of Vernier learning was shown in green circles. Double training at the same location with more precise stimuli did not enable transfer of Vernier learning to the untrained location (green circles). (C) A summary of percent improvement of Vernier learning at the trained (loc1, blue bar) and untrained (loc2, green bar) location in the single staircase condition.
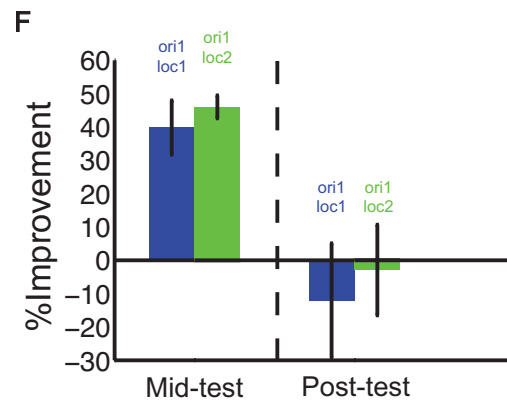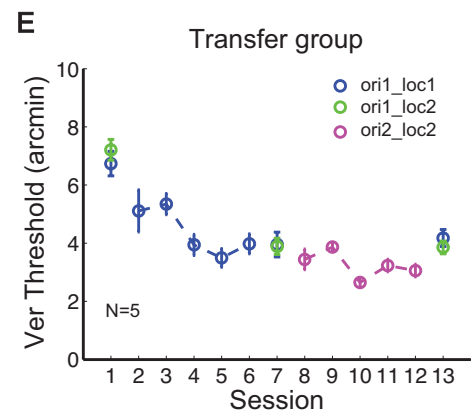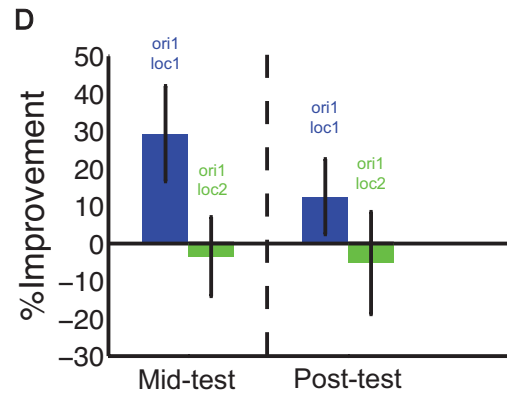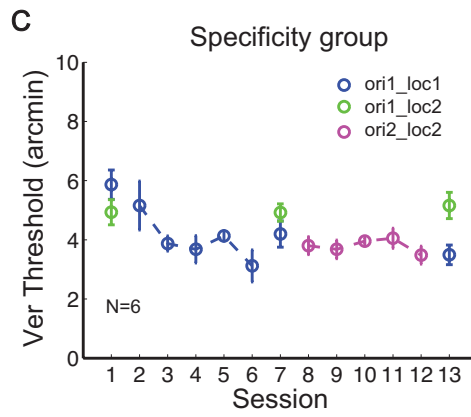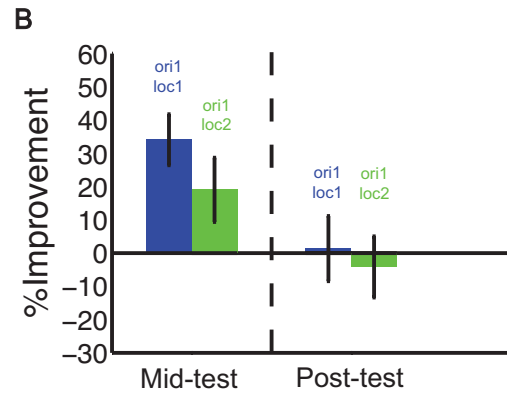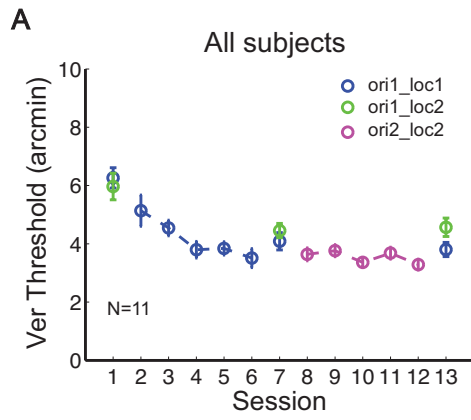
Figure 16. Sequential Double Training with Precise Stimuli in Vernier Discrimination.

(A and B) Sequential double training for all subjects. Vernier discrimination was first trained at ori1_loc1 (blue circles), and then sequentially trained at ori2_loc2 (magenta circles). All training sessions employed the single staircase method. Transfer of Vernier learning was measured at ori1_loc2 in pre-, mid- and post-testing sessions (green circles). Sequential location training (ori2_loc2) did not induce transfer of Vernier learning to untrained locations (ori1_loc2, second and third green circles in A; right green bar in B). Bars in the mid-test and post-test indicate threshold differences between the pre- versus mid-test, and mid- versus post-test, respectively. (C and D) Sequential Vernier training in "specificity" group. Vernier learning showed location specificity in this group (first two green circles in C; left green bar in D). Double training didn't enable transfer of Vernier learning (ori1_loc1) to ori1_loc2 (second and third green circles in C; right green bar in D). (E and F) Sequential Vernier training in "transfer" group. Initial training of ori1_loc1 resulted in transfer of learning to ori1_loc2 (first two green circles in E; left green bar in F). However, the second location training (ori2_loc2) didn't promote additional transfer to ori1_loc2 (the second and third green circles in E; right green bar in F).

Day	Exp I			Exp II			Exp III		
	Ver_l oc1	Ver_l oc2	Ori_l oc1	Dot_l oc1	Dot_l oc2	Ori_l oc1	Ver_l oc1	Ver_l oc2	Ori_l oc1
D1	10.38 (1.33)	12.61 (1.07)	−	3.63 (0.38)	3.25 (0.16)	−	7.14 (0.68)	6.89 (0.48)	−
D2	8.70 (0.86)	−	9.73 (0.62)	3 (0.10)	−	8.5 (0.58)	5.37 (0.98)	−	9.27 (0.33)
D3	7.17 (0.44)	−	8.88 (0.33)	2.99 (0.13)	−	6.31 (0.35)	4.72 (0.64)	−	8.9 (0.54)
D4	5.79 (1.36)	−	8.36 (0.46)	2.83 (0.21)	−	6.16 (0.35)	4.45 (0.41)	−	7.98 (0.28)
D5	6.96 (0.46)	−	8.67 (0.20)	2.64 (0.22)	−	6.19 (0.34)	4.44 (0.18)	−	9.29 (0.31)
D6	5.53 (1.16)	−	8.18 (0.53)	2.35 (0.13)	−	5.97 (0.23)	4.52 (0.16)	−	8.3 (0.51)
D7	6.94 (0.87)	8.1 (1.07)	−	2.47 (0.12)	3.41 (0.16)	−	4.49 (0.20)	6.17 (0.48)	−

Table 3. Subject thresholds in simultaneous double-training experiments. This table shows thresholds for the Vernier Gabor task (in arcmin) and the orientation identification task (in degree) in Experiment I (Ver + Ori, multiple short staircases), Experiment II (Dot + Ori), and Experiment III (Ver + Ori, single staircase). Values in parentheses represent within subject standard errors.

Day	Exp IV								
	All			Specificity			Transfer		
	ori1_l oc1	ori1_l oc2	ori2_l oc2	ori1_l oc1	ori1_l oc2	ori2_l oc2	ori1_l oc1	ori1_l oc2	ori2_l oc2
D1	9.16 (0.51)	8.73 (0.67)	–	8.58 (0.72)	7.21 (0.62)	–	9.85 (0.62)	10.55 (0.52)	–
D2	7.51 (0.78)	–	–	7.55 (1.21)	–	–	7.47 (1.06)	–	–
D3	6.64 (0.39)	–	–	5.67 (0.37)	–	–	7.82 (0.54)	–	–
D4	5.55 (0.42)	–	–	5.38 (0.67)	–	–	5.76 (0.53)	–	–
D5	5.62 (0.34)	–	–	6.05 (0.26)	–	–	5.11 (0.47)	–	–
D6	5.13 (0.48)	–	–	4.56 (0.79)	–	–	5.82 (0.50)	–	–
D7	5.98 (0.43)	6.51 (0.36)	–	6.14 (0.65)	7.20 (0.43)	–	5.78 (0.63)	5.69 (0.41)	–
D8	–	–	5.32 (0.32)	–	–	5.56 (0.45)	–	–	5.04 (0.52)
D9	–	–	5.50 (0.31)	–	–	5.38 (0.46)	–	–	5.65 (0.20)
D10	–	–	4.92 (0.22)	–	–	5.79 (0.18)	–	–	3.87 (0.21)
D11	–	–	5.38 (0.30)	–	–	5.92 (0.50)	–	–	4.73 (0.32)
D12	–	–	4.81 (0.27)	–	–	5.09 (0.44)	–	–	4.47 (0.32)
D13	5.56 (0.36)	6.68 (0.46)	–	5.11 (0.48)	7.55 (0.65)	–	6.11 (0.43)	5.63 (0.32)	–

Table 4. Subject thresholds (in arcmin) in the sequential double-training experiment (Experiment IV) employed with the Vernier Gabor task. Values in parentheses represent within subject standard errors.

CONCLUSION

This dissertation examined three aspects in the mechanisms of human perceptual learning – interference, consolidation, and transfer. The first chapter settled a debate regarding the interference in the three-dot hyperacuity learning. Following this chapter, this dissertation then moved on to discuss a process required for memories to stabilize before being interfered, a concept referred to as "memory consolidation". In order to understand the mechanisms of retinotopic specificity in perceptual learning, the third chapter investigated how stimuli representation and training stimuli's precision influence learning transfer under double training.

The first chapter, discussed interference of perceptual learning, showing that learning of basic visual stimuli can be overwritten by new learning of similar stimuli immediately after the initial learning (Hung and Seitz 2011). In addition, this chapter addressed the importance of eye-fixation in a peripheral perceptual task. Retrograde interference was found when eye-movements of subjects were tightly controlled using a gaze-contingent display, whereas no such interference occurred without this fixation control. This chapter provides a possible explanation of why divergent results were found for retrograde interference in the 3-dot hyperacuity task (Seitz, Yamagishi et al. 2005; Aberg and Herzog 2010) and confirms the existence of retrograde interference in this type of low-level perceptual learning.

The retrograde interference in a 3-dot hyperacuity task, along with the previous finding that a 1-hour delay can restore learning of this task (Seitz, Yamagishi et al. 2005),

indicates that learning of visual discriminations must undergo a period before it stabilizes. In the second chapter, we conducted a study using two commonly used stimulants – caffeine and nicotine – to investigate their effects on memory stabilization during consolidation in implicit and explicit learning. A post-training administration of caffeine significantly enhanced learning of a texture discrimination task (i.e. implicit learning), however the nicotine group resulted in no learning in this task. These results indicate that caffeine and nicotine may exert their functions in implicit learning through different pathways during consolidation. On the other hand, the word pair association task (i.e. explicit learning) may have been facilitated by a combined administration of caffeine and nicotine, while a single dose administration of either caffeine or nicotine did not lead to such a beneficial effect. Given that caffeine and nicotine are both linked to a neurotransmitter, acetylcholine, which has been suggested to play an essential role during memory consolidation (Hasselmo 1999; Hasselmo 2006), this chapter further discussed potential future directions regarding drugs' administrations to modulate cholinergic levels or cholinergic receptors. Studies specifically targeting cholinergic levels or the specific receptor groups involved in distinct types of memories will shed light on understanding mechanisms of memory consolidation.

In addition to studies that examine interference and consolidation in perceptual learning, it is important to understand the mechanisms underlying learning specificity. The experiments conducted in the third chapter, made an effort to resolve controversies of retinotopic specificity of perceptual learning. Studies in this chapter first validated a "piggybacking effect", in which a hyperacuity perceptual learning transfers to untrained

locations, after pairing with an orientation learning at the same location. However, this "piggybacking effect" became diminished in a different hyperacuity task, under the same double-training paradigm, indicating that this effect is not ubiquitous. To narrow down which factor determines learning transfer and specificity, we increased training difficulty and found that learning, with more precise stimuli, preserved location specificity. Results in this chapter suggest that retinotopic specificity of perceptual learning is highly dependent upon particularities of the training procedure, and a simple change of stimuli representation, and task precision, can result in profound differences in the observed learning effects.

The findings in this dissertation suggested that fine details in the experimental procedures (e.g. a control of eye-fixation or the extent of stimuli precision) could produce divergent results found in different studies. However, some follow-up studies are suggested to overcome limitations in our findings. For instance, results of the gaze-free group in Chapter I lacked eye-tracking. A future study monitoring eye-movements in this group will provide more robust evidence and help clarify whether eye-movements in the gaze-free group actually prevented retrograde interference from occurring or just reduced subjects' ability to detect interference. The experimental design in Chapter II could have controlled subjects' activities for hours while the drugs' effects were taking place. If caffeine and nicotine interfered consolidation presumably mediated by low level of ACh, the results would be more solid if we controlled subjects at the quiet-resting state and prevent them from involving in the sate dominated by high level of ACh (e.g. active waking). To further confirm the role of stimuli precision in learning specificity,

experiments manipulating stimuli precision can be applied to other perceptual task such an orientation discrimination task that is known to be controversial in terms of its retinotopic specificity.

All in all, further research is required to gain greater understanding of the mechanisms of interference, consolidation and specificity in perceptual learning. The mechanisms of perceptual learning are complicated. We suggest that perceptual learning can arise from representational changes, attentional factors, decision rules, or weight changes in readout, and it can be difficult to disambiguate between contributions of these factors. To advance our understanding of perceptual learning, the field must move towards understanding procedurally induced differences in learning, how multiple neural mechanisms mediated by a variety of neurotransmitters contribute to behavioral effects, and models or theories that link between the high-level processing and low-level systems in perceptual learning.

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APPENDIX A

Phone Script

Thank you for your interest in our study on the effects of caffeine and nicotine on learning. Before we begin do you have any general questions regarding the research study or the requirements to participate in this study?

[answer basic questions. If questions are better addressed by later sections of the script then tell this to the potential subject].

First, I would like to check whether you are eligible for the study. To do this I will need you to answer the following questions. You are free to refuse to answer any of the questions, however, these questions are designed for the purpose of your safety and thus refusal to answer any question will make you ineligible to participate in the study.

Questions:

(1) Are you over the age of 18?

[If no, subject is ineligible]

(2) Do you smoke? If so how many Cigarettes (or equivalent) do you smoke on an average day?

(3) Do you smoke every day or do you sometimes stop smoking for a day or longer?

(4) On a scale of 1-5 (with 1 being without problem and 5 being with great discomfort), how do you feel when you go a day without smoking? How about 2 days? Please describe what withdrawal symptoms that you experience

(5) Do drink any caffeinated beverages? If so, what kinds and how much do you consume on an average day?

(6) Do you consume caffeine every day or do you sometimes stop for a day or longer?

(7) On a scale of 1-5 (with 1 being without problem and 5 being with great discomfort) How do you feel when you go a day without caffeine? How about 2 days? Please describe what withdrawal symptoms that you experience.

(8) Please let me know if you have any of the following conditions:
- o epilepsy
- o suffer from strokes
- o migraines
- o claustrophobia
- o Are you pregnant?
- o Do you have any other condition that may cause a negative reaction to responding to repetitive stimuli in a small dimly lit chamber?

o Do you have any condition or are taking any medications that may cause an

adverse reaction to caffeine or nicotine?

[**If subject does not meet criteria (i.e. suffers withdrawal ratings greater than 3 or suffers from risk conditions), thank the subject for their time and tell them that they are ineligible for the study**]

[If subjects meet criteria then continue with the script]

Thank you for answering these questions. Based upon the your responses you appear to be eligible to participate in the study. However please be aware that final eligibility will be determined when you show up for the first session. If you are deemed ineligible at that time you will still receive the $10 compensation for that session.

You will perform two, 1 hour, testing sessions (separated by 24 hours) where you will view visual stimuli, auditory stimuli or both. Auditory stimuli will be presented stimuli at a comfortable listening level either through speakers or headphones. Visual stimuli will be presented either on a computer monitor, projector, or head mounted display. You will be asked to make judgments and in some cases to remember what you see and hear. You may be video taped during the experimental sessions. Depending on your assigned condition you will be asked to take a single dose of caffeine (200mg; equivalent to 2 cups of coffee), nicotine (2mg; equivalent to two cigarettes), a placebo or nothing.

It is important that you get a full night's worth of sleep the night before the experiment and for the first night of the experiment. We require that you obtain 7-9 hours of sleep that night with a bedtime between 22:00 and 24:00 and a habitual wake time between 06:00 – 08:00. If you are unable to obtain sufficient sleep on these days then we ask that you reschedule for a more appropriate time.

We also require that you refrain from any caffeine or nicotine intake starting the day of the study and continuing until the final session. If you are unable to go without caffeine and nicotine for 48 hours then it is best that you do not participate in this study.

Do you have any questions?

Do you consent to these arrangements?

[If so then schedule the subject]

APPENDIX B

Post-Experiment Questionnaire

Subject #: _____   Date Visited: _____

(1) Were you comfortable during the experiment?

YES / NO

If you answered 'NO' please elaborate:

(2) Were there any distractions that diverted your attention away from the tasks?

YES / NO

If you answered 'YES' please elaborate:

(3) Did you have any difficulty with understanding or following the instructions given to you?

YES / NO

If you answered 'YES' please elaborate:

(4) Did you have any difficulty making your responses?

<div align="center">YES / NO</div>

If you answered 'YES' please elaborate:

(5) Please rate on a 0-4 scale how much, if any, you have experienced the following signs and symptoms since you took the medication /placebo yesterday.

0 = none

1 = mild, but it did not bother me *and* I did not experience it more than usual

2 = mild, but it bothered me *or* I experienced it more than usual

3 = moderate

4 = severe

_____	Decreased appetite	_____	Low energy
_____	Dizziness	_____	Nausea
_____	Dry Mouth	_____	Nervousness
_____	Elevated mood	_____	Paranoia
_____	Faintness/Lightheaded	_____	Poor mood
_____	Headache	_____	Runny nose
_____	Heart racing	_____	Sore throat
_____	Hunger	_____	Sweating
_____	Increased energy	_____	Thirst
_____	Irritability	_____	Tremors or shakiness

(6) Do you think that you received (circle one):

       Active medication   Placebo (no active medication)

        If active medication: Caffeine   Nicotine

(7) How confident, 0% - 100%, are you that your answer to (6) is correct?

(8) Did you sleep well last night?

<div align="center">YES / NO</div>

(9) Did you dream last night?

<div align="center">YES / NO</div>

(10) If you dreamed, what did you dream about (If you need more space, please use the back)?

(11) How sleepy do you feel right now?
       a. Extremely alert
       b.
       c. Alert
       d.
       e. Neither alert, nor sleepy
       f.
       g. Sleepy, but no difficulty remaining awake
       h.
       i. Extremely sleepy, fighting sleep

(12) How _____ do you feel? (Please mark on the line)

How **sad** do you feel?

Very Little ——————————————————————— Very Much

How **happy** do you feel?

Very Little——————————————————————— Very Much

How **calm** do you feel?

Very Little ——————————————————————— Very Much

How **anxious** do you feel?

Very Little ——————————————————————— Very Much

How **relaxed** do you feel?

Very Little ——————————————————————— Very Much

How **stressed** do you feel?

Very Little——————————————————————— Very Much

How **irritable** do you feel?

Very Little ——————————————————————— Very Much