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Associative Representational Plasticity in Primary Auditory Cortex: Importance of Learning Strategies in Acquisition and Maintenance

DISSERTATION

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by

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Dedicated to my grandfather, the first Dr. Gabriel Elias, thank you for inspiring me and making all of this possible

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ABSTRACT OF THE DISSERTATION

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By

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Professor Norman M. Weinberger, Chair

Classical views on the functional organization of the cerebral cortex portray primary sensory regions as passive recipients of sensory impressions. Over the past half century or so, a body of work has accumulated in opposition of this view, which endows primary sensory regions with cognitive functions, such as learning and memory, classically localized elsewhere. Most extensively studied in the primary auditory cortex (A1), learning has been repeatedly shown to coincide with the induction of neural changes that not only enhance coding of behaviorally relevant sounds, but alter the way auditory stimuli are processed in general. The term representational plasticity (RP) has been adopted to collectively refer to these learning related changes in the processing or representation of behaviorally relevant stimuli. Recently a number of studies have demonstrated that RP, induced during learning, dissipates with extended training without impacting a subject's performance. Such findings stand at odds with a body of evidence supporting a mnemonic function for RP but might be explained by when considered in light of learning strategy use. Learning strategy, i.e. the way animals use environmental cues to solve a problem, has been identified as a critical factor regulating the induction of RP. The first experiment sought to better understand the relationship between learning strategy and RP by

examining the effects of overtraining on strategy use and RP. During overtraining, map renormalization occurred and the degree of renormalization was correlated with the degree to which animals stopped utilizing a tone-onset-to-error (TOTE) strategy. We developed a novel behavioral analysis method to track and quantify strategy use during training. This revealed that strategy use was much more dynamic of a process than was previously realized. This prompted a critical test of the factor underlying the relationship between TOTE and RP. This test showed that use of the TOTE strategy alone was not sufficient to produce RP, indicating that RP was in fact related to learning. These results compelled a reconceptualization of learning strategy in a more general light. Collectively, the experiments in this dissertation provide the framework for a new understanding of the relationship between learning strategy and RP.

Chapter 1: Introduction

A Brief History of Cortical Functional Organization

A great deal of our current understanding of the brain's functional organization is attributed to research done in the 1800s. A debate that raged for much of the century concerned the question of whether the brain operated as a single, highly complex functional system or if different cognitive and psychological functions were compartmentalized to different regions of the brain. The rise and fall of the pseudoscience of phrenology, a view of extreme functional localization, soured many researchers to the idea of functional localization and caused many to be wary of any claims of localization well into the 1900s. Trepidations about repeating the failings of phrenology aside, by the turn of the century there was sufficient evidence demonstrating evidence for localization of function.

It was the report in 1861 by Paul Broca that is widely credited as the first major step towards a general acceptance of functional localization in the brain (described by Finger, 1994). Broca presented his case study of Monsieur Leborgne, a patient who had been transferred to his care. Leborgne had been hospitalized for 21 years due to epilepsy, hemiplesia (paralysis on one side of his body) and loss of speech (he had lost the ability to say anything other than the syllable 'tan'). Broca only attended to Leborgne for six days before he passed, but he took this opportunity to inspect Leborgne's brain afterward. A contemporary of Broca's, Jean-Baptiste Bouillaud, claimed that his patient records demonstrated that loss of fluent speech was invariably associated with extensive damage to the frontal lobes. Leborgne's brain provided Broca with an opportunity to verify Bouillaud's proposed connection between the frontal lobes and speech. At autopsy, Leborgne's brain revealed a large lesion in the ventral region of the frontal lobe,

supporting Bouillaud's view. This swayed Broca's opinion in favor of localization, and with his influence many others were swayed as well (Finger, 1994).

Further support for localization came from experimental research by Gustav Fritsch and Eduard Hitzig. In 1870 Fritsch and Hitzig discovered the motor cortex through electrical stimulation. In their experiments, they applied electricity directly to a specific region of the dog's exposed cerebral cortex. This stimulation elicited involuntary muscle contractions on the opposite side of the dog's body. When they moved their stimulation site they found that adjacent muscles would contract (described in Finger, 1994). This showed that motor functions were localized to a particular region of the brain. Moreover it showed that this region was very well organized, forming a map of the animal's body along the cortical surface.

David Ferrier replicated and extended the findings of Fritsch and Hitzig by examining the effects of stimulation on other parts of the cortex on all types of animals (summarized by Finger, 1994). Ferrier found that when he stimulated the regions of the brain now known as the auditory and visual cortices, his subjects would behave as if they had just heard a sound or seen a flash of light. Ferrier was chiefly interested in uncovering the regions of the brain responsible for sight and hearing. To see if these regions were necessary for hearing and seeing, he complemented his stimulation studies with lesion studies. Since stimulation of these regions was sufficient to produce behavioral indications of stimulus perception, he reasoned that their destruction should destroy that ability. Ferrier found great success with his stimulation and lesion studies. While his studies were not always perfect he was able to correctly identify those regions now considered the auditory and visual cortices.

By the end of the 1800s, the fundamental question of localization had changed. Rather than questioning whether functions were localized in the brain, the question became a matter of degree. To what degree are functions localized within particular regions of the brain? This question is still open today. It forces researchers to be mindful not only of the nature of localization but also of the merits of the functions they try to localize in the brain.

An influential monograph was published by Alfred Walter Campbell in 1905. The work synthesized a variety of clinical observations, experimental and developmental studies, along with Campbell's work delineating cortical regions based on architectonic boundaries. He aimed to identify and delineate different cortical regions not only on anatomical but also on functional grounds. The most enduring and influential aspect of Campbell's monograph was the functional organization he described for sensory regions. Each region of sensory cortex was divided into primary receiving areas and adjacent areas. Campbell ascribed the primary areas with the function of receiving sensory impressions while the adjacent areas served to provide meaning to the sensory impressions. This view that primary sensory areas functioned to identify and determine the physical qualities of sensory stimuli was held well into the 20th century.

Representational Plasticity: Early Indications of Plasticity in Sensory Cortices

Reports dating back to the middle of the 20th century demonstrated that sensory cortical areas held the capacity to change through experience even in adulthood. Galambos et al. (1956) demonstrated in cats that following the pairing of a click with a shock to the chest, the response evoked by the conditioned stimulus (CS) could be recorded from the auditory cortex and was enhanced as animals began to show signs of conditioned responding (CR). Following a period of extinction, this enhancement dissipated and response levels reverted back to baseline. Beck et al.

(1958) found that CS presentation developed an increase in “desynchronized” (low voltage, fast cycling waves) EEG activation immediately preceding the CR development. Conditioning also produced increases in evoked multi-unit activity following CS onset (Buchwald, Halas & Schramm, 1966; Halas, Beardsley & Sandlie, 1970).

These studies also suggested that the auditory cortex was sensitive to the learned importance of a stimulus. However, an unambiguous demonstration of this point was hampered by a host of confounds due to the standard methodology of the time. In many of these studies there were no controls for the possible effects of habituation or sensitization so it was unclear whether the observed neural changes were in fact related to learning. Even more problematic was the fact that all of the data collected in these studies were collected during the training sessions, producing a number of state-related confounds, as changes in arousal level have been shown to modulate activity in the auditory cortex (Murata & Kameda, 1963; Wickelgren, 1968). Additionally, changes in the effective stimulus intensity at the periphery could also result in augmented neural responses in cortex. Activation of the middle ear muscles can attenuate the effective sound level at the periphery (Galambos &Rupert, 1959). When these effects were controlled for it was demonstrated that neural responses in the auditory cortex were in fact learning-related changes (Oleson, Ashe & Weinberger, 1975) demonstrating that the auditory cortex can be modified by learning.

Reports of learning-related increases in CS-evoked activity did not reflect a significant departure from the classic notions of functional organization of the cortex. While they clearly demonstrated that neural activity changed with learning, these changes may have simply reflected an increased activation of CS pathways. However, an alternative view was also possible: that learning changed the way that the auditory cortex processed sounds.

Demonstration of this point would be a significant departure from the view that the primary auditory cortex functioned as a stimulus analyzer.

Representational Plasticity: Associative Learning and Memory

The critical test for this possibility came from the methodological marriage of sensory physiology and the neurobiology of learning and memory, which allowed for the comparison of receptive fields recorded before and after aversive classical conditioning. If learning resulted in the potentiation of a CS pathway through the auditory cortex, then the only change to a cell's receptive field should be an increase in responsiveness to the CS frequency. Bakin and Weinberger (1990) demonstrated that following conditioning, the best frequency (the frequency eliciting the greatest neural response) was changed such that the peak in the receptive field had shifted towards, or in some cases to, the CS frequency. This shift consisted not merely of an increase in responsiveness to the CS frequency, but in many cases resulted in a reduction in responding to the cell's pre-training best frequency as well. This demonstrated that learning changed the way that auditory stimuli are processed in A1, not simply that learning involved the potentiation of a particular pathway through A1.

A series of follow-up studies aimed to characterize the properties of these receptive field shifts. These studies demonstrated that shifts are associative in that they require that a predictive relationship be established between the CS and the US, and discriminative in that they develop for a CS+ but not a CS- (Murata and Kameda 1963; Bakin, Lepan & Weinberger, 1992; Kisley & Gerstein, 2001; Blake, Heiser, Caywood & Merzenich, 2006). Also, they are specific to the CS frequency (Bakin & Weinberger, 1990; 1992). They develop rapidly, are observed in as few as five CS-US pairings, occur relative to the first indication of a CR (Edeline, Pham &

Weinberger, 1993), and undergo consolidation, i.e., increase in strength in the absence of further training (Galvan & Weinberger, 2002). Shifts are also long lasting, the longest time point examined being eight weeks (Weinberger, Javid & Lepan, 1993).

These traits—associativity, discriminability, specificity, rapid induction, consolidation, and permanence in the face of time—are traits shared by both receptive field plasticity and associative learning. The term representational plasticity (RP) has been adopted to refer to both the potential for, and specific instances of, learning-related modifications to the neural processing and/or representation of sensory stimuli. That RP shares key characteristics with associative learning and has been observed across many different species in a wide variety of tasks and conditions (For review see: Weinberger, 2007) has led to the proposal that RP may serve as a memory trace, or part of the distributed engram for a learned experience. This idea reflects a significant departure from classical notions of A1's function.

Learning Strategy and Representational Plasticity

Recent studies have shown that the way an animal learns to solve a task is critically important in the development of RP. In a standard cued instrumental paradigm the tone offset demarcates a clear boundary between reward-generating versus error-generating responses. As animals learn about this boundary condition they cease responding past tone offset but it is ambiguous why they do so. They may have learned that the tone offset is a cue to cease responding and cease responding due to this knowledge. Alternatively, they may have learned that responding during silence is not rewarded and potentially results in some aversive contingency which is in place to promote stimulus control, i.e., an ITI extension or aversive

stimulus presentation. By maintaining tone offset as the boundary condition between reward and error, it is ambiguous why animals cease to respond.

Berlau and Weinberger (2008) attempted to disambiguate these two strategies using a modified instrumental paradigm. They trained rats on either a standard cued instrumental paradigm where tone offset was a clear boundary or a modified paradigm in which a 2-s "grace" period following tone offset was added. The grace period disconnected tone offset from the boundary between reward and error, which forced animals to rely upon generating errors to indicate when to cease responding during a trial. Thus, instead of using tone offset as a cue, animals trained with the grace period ignored it. At the end of training all animals underwent terminal mapping. Animals trained on the grace period protocol that used a tone-onset to error (TOTE) strategy displayed RP in A1. Compared with animals trained on the standard protocol as well as naïve animals, animals using a TOTE strategy showed CS-specific decreases in bandwidth (i.e., they were more selectively activated by the CS frequency) and threshold (i.e,. they showed increased sensitivity).

Bieszczad and Weinberger (2010b) compared two groups of rats on the same grace period protocol but differing in their level of motivation during training. One group was maintained at ~85% control body weight (moderate motivation) while another was maintained at ~70% control body weight (high motivation). As previous reports had indicated that the degree of RP is related to the degree of motivation during training (Rutkowski & Weinberger, 2005), it was expected that a similar relationship would be found here as well. However, the authors observed an absence of plasticity in A1 for those animals in the higher motivation group while those in the moderate motivation group displayed plasticity in A1. Upon inspection of their training data the authors found that the difference between the two was attributable to differences

in learning strategy use. Moderately motivated animals adopted the TOTE strategy. Highly motivated animals were more inclined to use the tone offset as a cue to cease responding, possibly because their high level of motivation caused the absence of reward during the grace period to be perceived as mildly aversive, thus essentially re-establishing the importance of the tone-offset as a boundary between reward and error.

In a final study Bieszczad and Weinberger (2010c) examined how increasing the use of the TOTE strategy affected RP. To further promote the use of a TOTE strategy the authors developed another instrumental paradigm in which, following tone offset, animals could respond one further time in order to obtain an additional reward. They found that animals trained on this protocol increased their use of the TOTE strategy dramatically compared to animals trained on the grace period protocol. At the end of training these animals did not show the same profile of plasticity as those trained on the grace period protocol. Rather than a CS-specific decrease in bandwidth and threshold, these animals displayed a significant increase in the amount of representational area for the CS in A1. Furthermore, the degree to which they displayed use of the TOTE strategy during a test session after training was significantly correlated with the amount of relative CS area in A1.

Concerns Regarding the Role of Representational Plasticity in A1

Recent studies have questioned the necessity of RP in ongoing behavior. Demonstrations that RP was absent after extensive discrimination training (Brown, Irvine & Park, 2004) conflict with previous findings that demonstrated a relationship between RP and improvements in discrimination abilities (Recanzone, Schreiner & Merzenich, 1993). Additionally, findings that plasticity is present during learning but not after periods of overtraining (Reed et al., 2011;

Takahashi, Funamizu, Mitsumori, Kose & Kanzaki, 2010; Takahashi, Yokota, Funamizu, Kose & Kanzaki, 2011) directly contradict notions that RP is long-lasting and serves as a memory trace for a learning experience.

These findings of RP dissipation with overtraining also conflict with the findings that A1 plasticity is correlated with memory strength as measured through resistance to extinction (Bieszczad & Weinberger, 2010a). Overtraining is generally considered to result in the transition from goal-directed behavior to response-directed, habit behavior. As habit strength is generally thought to increase monotonically with training (Dickinson, Balleine, Watt, Gonzalez & Boakes, 1995) , if A1 plasticity is correlated with memory strength then an animal has transitioned into habitual behavior after overtraining its resistance to extinction should be strengthened and therefore also show the greatest amount of A1 representational area. This account stands at odds with the reports above.

One possible explanation for this contradiction is that as animals change the strategies they use to solve the task with extended training. Animals trained in the T-maze use different strategies to solve the task early and late in training (Packard & McGaugh, 1996). Moreover, these different strategies are differentially reliant on distinct brain regions. Neither the Reed et al. (2011) nor Takahashi et al. (2010; 2011) studies attempted to assess how animals were solving their tasks. The present set of experiments were undertaken in order to develop a deeper understanding of the relationship between learning strategy and A1 representational plasticity by examining the potential role of learning strategy in the maintenance of plasticity.

Chapter 2: The Effects of Extended Training on Representational Plasticity and Learning Strategy

Introduction

Classical theories of cortical organization held that the function of primary sensory cortical fields is to analyze the physical properties of sensory stimuli (Campbell, 1905). The fact that associative learning can specifically modify the representation of sound in the primary auditory cortex (A1) argues against this classical notion. Shifts in frequency tuning strengthen the encoding of sounds that predict reinforcement (Bakin & Weinberger, 1990; Edeline & Weinberger, 1993) and can result in an increased representational area for a sound signal within the tonotopic "map" of A1 (Recanzone, Schreiner & Merzenich, 1993). This area gain can encode both stimulus importance (Rutkowski & Weinberger, 2005) and the strength of frequency-specific memory (Bieszczad & Weinberger, 2010c). The term representational plasticity (RP) has been adopted to refer to these learning-related changes in the coding and/or representation of a sensory stimulus or stimulus dimension. The fact that neural representations in primary sensory regions are sensitive to manipulations of the affective/psychological quality of a sensory stimulus is incongruous with the idea that they serve merely as sensory analyzers.

It has been proposed that RP serves as a memory code for a sensory experience (Weinberger, 2011). This proposal was based initially on a series of studies examining how tuning properties of cells in A1 change following classical conditioning, which revealed that RP shares many key characteristics with associative memory (reviewed in Weinberger, 2007). Subsequent investigations have shown that RP has been observed across species (including humans), types of learning, varieties of tasks, motivational valences, and other sound parameters (reviewed in Weinberger, 1995, 2004, 2007; Scheich, Brechmann, Brosch, Budinger & Ohl,

2007). Perhaps most striking is the finding that directly increasing A1 responses to a tone by pairing it with stimulation of the nucleus basalis (NB) (Bakin & Weinberger, 1996; Kilgard & Merzenich, 1998) implants specific behavioral memory (McLin, Miasnikov & Weinberger, 2002; Miasnikov,Chen, Gross, Poytress & Weinberger, 2008; Bieszczad, Miasnikov & Weinberger, 2013) by increasing its area of representation (Bieszczad, Miasnikov & Weinberger, 2013).

A critical factor identified in the development of RP is learning strategy. Learning strategy refers to the way an individual learns to solve a behaviorally relevant problem. That is to say that RP is sensitive to the way sensory objects are used by an individual during learning. For example, consider a standard instrumental task where an individual must learn to respond during tone presentation and withhold responses during silence. While this is a simple task, different strategies could be used to solve it. A tone has multiple physical components: an onset, a plateau (steady state), and an offset. In a standard instrumental task, the problem could be solved by starting to respond at tone onset and continue until tone offset. However, the same number of rewards could be obtained by responding from tone onset, past tone offset, until receiving an error signal. Representational plasticity in the primary auditory cortex is reliant upon use of this latter strategy, referred to as a tone-onset-to-error (TOTE) strategy (Berlau & Weinberger, 2008). Furthermore, the magnitude of RP is correlated with the degree of TOTE strategy use (Bieszczad & Weinberger, 2010a,b,c). Learning strategy therefore gates the induction of RP in that it modulates the location and degree of RP.

Although learning strategy has been identified as an important factor for the development of RP, its role in the maintenance of plasticity is unknown. This is particularly important because learning-related representational expansions in A1 can diminish or completely fade away under

certain circumstances, a process dubbed “renormalization” (Reed et al, 2011). The goal of the present experiment was to determine if the maintenance, or lack thereof, of representational plasticity is linked to the behavioral strategy employed in an auditory task.

Materials and Methods

Subjects

Male Sprague–Dawley rats (250–300 g, $n = 21$) from Charles River Laboratories (Wilmington, MA) were individually housed in a vivarium (temperature maintained at 22º C, 12/12 h light/dark cycle, lights on 7 am). Subjects were provided with *ad libitum* access to food and water before the onset of training. During training with water restriction (see *Behavioral training*), continuous access to water was restored on the weekends and supplements were provided after training sessions if necessary to maintain weight. All procedures were conducted with care to minimize pain or discomfort and were in accordance with the University of California, Irvine, Animal Research Committee and the NIH Animal Welfare guidelines.

Training apparatus and stimuli

The training apparatus and equipment used to generate auditory stimuli were the same as those used previously (Bieszczad & Weinberger, 2012). Training was conducted in a sound-attenuated instrumental conditioning chamber (H10-11R; Coulbourn Instruments, Whitehall, PA) fitted on one wall with a bar manipulandum, a water cup attached to a lever (H14-05R) that could deliver 0.02 ml of water to a small port 9 cm to the left of the bar (H21-03R), a speaker (H12-01R) 13 cm above the reward port, and an overhead house light (H11-01R). The chamber was enclosed in a sound-attenuating chamber (H10-24A).

All tone stimuli were generated using Tucker–Davis Technologies (TDT, Alachua, FL) System 3 (RP2.1 Enhanced Real-Time Processor) and RPvdsEx software. Tones were always 10 s in duration (70 dB sound pressure level [SPL]) and cosine-squared, gated with rise/fall time (10–90%) of 10 ms. Tone levels for all frequencies used in training were calibrated for three locations in the training chamber at animal head height and set to average 70 (±2) dB SPL.

Behavioral training

Upon arrival, all subjects were acclimated to the vivarium for 1–2 days after which they underwent 3 days of handling to familiarize them with the experimenter and movement to and from the vivarium. Subjects were then water-restricted to ~85% body weight of unrestricted litter controls where they were maintained throughout training. Next, they were shaped to bar-press (BP) for water reward (1:1 ratio) over four daily consecutive 60 min sessions. The dipper remained accessible in the port for 5 s to allow for complete consumption during which time additional BPs were ineffective. Thus only one reward could be obtained every 5 s. No tones were presented during the shaping period.

Animals then began three-tone discrimination training (TTD) consisting of trials comprised of a 10 s tone followed at offset by a 7 s silent "free" period (see below), separated by silent intertrial intervals (ITI; mean = 20 s, range = 12–28 s). Trial types were 70% CS+ (rewarded) trials (CS+ = 5.0 kHz, 70 dB SPL) and 30% CS– (non-rewarded) trials divided approximately equally between tones ± 1.25 octaves from the CS+ (Low CS– = 2.1 kHz, High CS– = 11.9 kHz, both at 70 dB). Trial types were intermixed on a random schedule. On CS+ trials, water rewards were given for BPs made during the presence of the 10 s CS+ and also for the first BP made during a 7 s free period that began at CS+ tone offset (maximum 3 rewards per

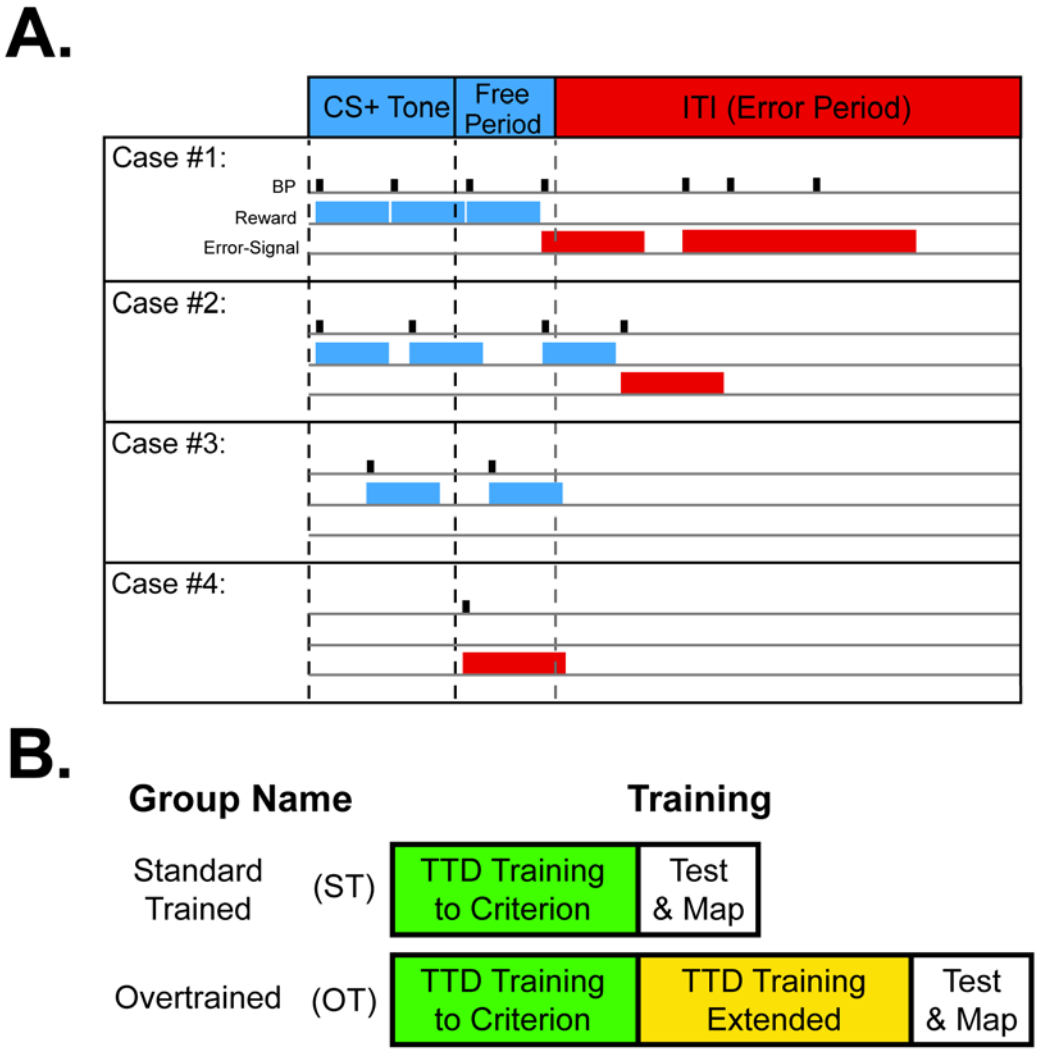


Figure 2.1. (A) Training protocol and four examples of possible outcomes on each CS+ trial. The CS+ tone (10 s duration) was followed by the Free Period (provided at least one bar-press (BP) had been made during the CS+) during which one BP was rewarded. During intertrial interval periods (ITI), all bar-presses were unrewarded and triggered an error signal (flashing light and 7 s time out period). Case #1 depicts a three reward outcome, two during the CS+ and one during the free period. Note that a second BP following the free period reward triggers an error signal. All BPs during the ITI triggered error signals that could cumulate. Case #2 depicts another three rewards scenario. Note that the BP during the free period is rewarded although it occurred at the same time as the first error generating BP in case #1, because it is the first BP during the free period in this case. Case #3 shows a two reward situation, one during the CS+ and one during the free period. Case #4 illustrates a trial on which no rewards were obtained because there were no BPs during the CS+. The bar-press made during the 7 s following tone offset was not rewarded because the functional free period was not in force due to the absence of at least one CS+ bar-press. (B) Experimental phases and time line. Following handing and habituation to the experimenters both the standard (ST) and overtrained (OT) groups underwent four sessions of bar-press shaping and were then trained to a criterion of performance (see text for details). The standard trained (ST) group then underwent a stimulus generalization session prior to A1 mapping while the over trained (OT) group was trained for an additional two weeks before stimulus generalization and A1 mapping.

CS+ trial; 2× during CS+, 1× during free period). Addition of this free period promotes the use of a tone-to-error (TOTE) learning strategy that is associated with specific expansions of CS+ area in A1 (Bieszczad & Weinberger, 2010b, 2010c) (see *Behavioral analysis*). The presence of the free period was contingent upon the animal making at least one BP during the preceding CS+ and concluded following either a BP yielding reward or the passing of 7 s without a BP. The free period was not present during CS– trials. Any BP made during the presentation of either CS– tone triggered a time out (7 s extension of the ITI) and an error signal (flashing house light for the remaining duration of the CS– tone). All BPs made during the silent ITI triggered a time out and 7 s error signal (Fig. 2.1A). Training was conducted five days per week (~60 min sessions).

All animals (n = 21) were trained to a criterion of stability of correct performance (see *Behavioral analysis*). The criterion was defined as three consecutive sessions during which the coefficient of variation was ≤ 0.10, where CV = standard deviation/mean of daily performance. Two groups were trained. One group underwent standard training until each subject attained the performance criterion (Group ST, n = 11). Another group was also trained to the same criterion, and then overtrained for up to three additional weeks (Group OT, n = 10). A few animals had difficulty in developing consistent BPs during tone presentation, and so were temporarily trained with shorter ITIs (mean = 8 s) and then shifted back to the normal ITI, averaging 20 s (ST = 2; OT = 1).

Upon completion of training, all subjects underwent a combined extinction/generalization session to determine the frequency-specificity of learning. Seven different frequencies were tested, including the frequencies used during training (1.4, 2.1 [CS–], 3.2, 5.0 [CS+], 7.8, 11.9 [CS–] and 18.3 kHz; all at 70 dB). This session began with twenty standard training trials (14 CS+, 6 CS–) to insure that performance was still stable, followed by 140 extinction trials without

reward. Test frequencies were presented in a pseudo-random order to yield 20 trials for each frequency. After the completion of the extinction/generalization session, subjects were returned to *ad libitum* access to water prior to physiological mapping of A1 (Fig. 2.1B).

Behavioral analysis

All stimuli and behavioral responses during training sessions were recorded using Graphic State II software (Coulbourn) and subsequently analyzed with custom MATLAB (MathWorks, Natick, MA) software. Daily task performance values were calculated as:

$$P = \frac{BPR^{CS+}}{BPR^{CS+} + BPR^{ER}} - \left(\frac{BPR^{ER}}{BPR^{CS+} + BPR^{ER}} \times \frac{BPR^{CS+}}{BPR^{CS+} + BPR^{ER}}\right) \times 100$$

where BPR^{CS+} was defined as the BP rate during CS+ presentation and BPR^{ER} was defined as the BP rate during CS– presentation plus BP rate during the silent ITI period. BP rates were calculated for each session as follows: BPR^{CS+} = (# BPs during CS+ tones) / (total amount of time during CS+ periods); BPR^{ER} = (# BPs during CS– and silent ITI periods) / (total amount of time during CS– and silent ITI periods). Thus perfect performance (BPs only to the CS+) would yield a value of 100 and the worst performance (BPs only during CS– trials, ITIs or both) would yield a value of 0.

To specifically examine discrimination performance, d' was calculated as well (Green & Swets, 1966; Talwar & Gerstein, 1999). Hit and false alarm rates were calculated based upon the percentage of trials in which at least one BP was registered during tone presentation. The discrimination performance measure d' was determined to be the difference between the z-normalized hit and false-alarm rates. Daily d' values were calculated comparing CS+ with each CS- individually as well as combined.

In addition to calculating performance measures, we also characterized the patterns of BPs on each trial to determine the strategies used during each session. Figure 2.2 provides examples of the three main patterns of behavior we used to classify behavior during training. The first pattern, prevalent early in training, was termed “non-stop”, because animals made at least one BP during the tone, during the free period, and during the intertrial interval, typically making repeated errors despite triggering the error signal and time out. Thus, time-out periods often accumulated.

The second pattern is termed the “tone-onset-to-error” (TOTE) pattern. TOTE was defined as consisting of at least one BP each during CS+ presentation during the free period and during the ITI period, provided that it was made within 5 s of the end of the free period. A key distinction between TOTE and NS is that in TOTE, all the ITI BPs occurred within the first 5 s of the ITI. As the minimal duration of the error signal was 7 s, this meant that on TOTE trials the animal triggered an error signal almost immediately after acquiring a free period reward and then ceased BPs after the error signal was triggered. Thus, in TOTE, the initiation of BPs was cued by tone onset and the cessation

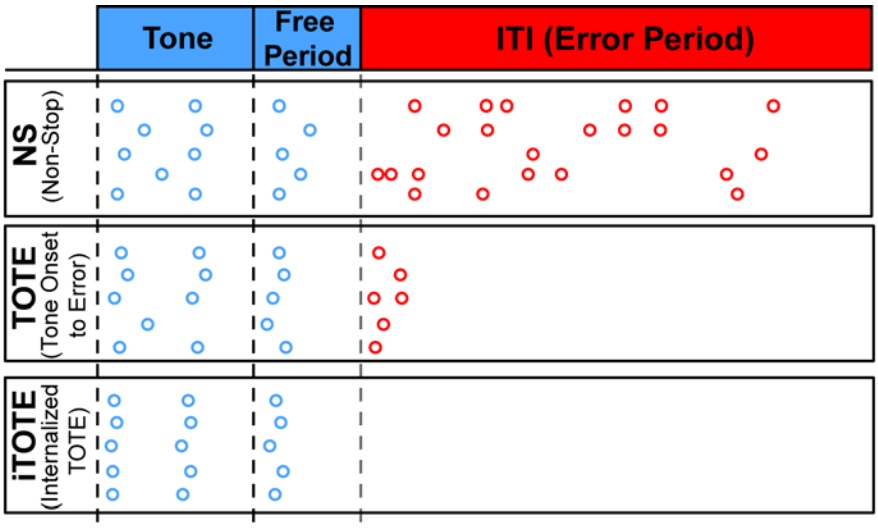


Figure 2.2 Examples of various trials categorized as NS, TOTE or iTOTE strategies. A trial was categorized as non-stop (NS) if the animal bar-pressed during the CS+ tone, as well as during the free period and the ITI period and continued bar-pressing after generating an error signal. A trial was categorized as tone-onset to error (TOTE) if the animal bar-pressed during the CS+ tone, the free period and the ITI period, but unlike the NS pattern, ceased responding after generating one error signal. The iTOTE strategy was defined as bar-pressing during the tone and the free period, but not during the ITI period.

of BPs was in response to the error signal.

The third pattern developed as a variant of TOTE and demonstrated that animals became able to predict the occurrence of the error signal, and avoided making errors. It is termed the "internalized tone-onset-to-error" (iTOTE) pattern. The iTOTE pattern was defined as consisting of at least one BP during CS+ presentation, at least one BP during the free period and no responding at all during the ITI period.

Strategy use per session was calculated as the percentage of trials that fell into each of these three behavioral patterns. Remaining (unclassified) trials occurred mainly during early training, as animals transitioned from BP rewards in silence during shaping to rewards based on the presence of the CS+ during tone training. Such response patterns appeared to be a continuation of behavior during shaping and were largely independent of tone presentations. They were classified as "Other".

Behavior during the extinction/generalization test was analyzed by constructing frequency generalization gradients from BPs performed during the 140 extinction trials. To control for different levels of baseline responding, each animal's generalization gradient was expressed as the proportion of total responding performed during presentation of each frequency.

Neurophysiological recordings

Complete mapping of A1 was performed 2–3 days following completion of the extinction/generalization session. Subjects were water deprived the night previous to surgery to help reduce salivary secretions. An additional group of untrained naïve animals (n = 6) was mapped as a control to determine the effects of training on A1 organization.

Methods were the same as previously reported (Bieszczad & Weinberger, 2012). Briefly, subjects were anesthetized (sodium pentobarbital, 0.1 ml/kg i.p., 55 mg/ml), with supplemental

doses administered as necessary to maintain a state of areflexia. Atropine sulfate (0.2 ml i.m., 0.54 mg/ml) was administered to minimize bronchial secretions. Body temperature was maintained at 37 ºC with the use of a homeothermic heating pad (Harvard Apparatus, Cambridge, MA), and ophthalmic ointment was applied to keep the eyes moist. Subjects were mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) inside a double-walled sound attenuated room (Industrial Acoustics, Bronx, NY). The scalp was resected after subcutaneous administration of lidocaine hydrochloride (AstraZeneca, Wilmington, DE). After clearing the calvaria, stainless steel screws were threaded into burr holes and cemented to a dental acrylic pedestal fixed to the frame. Once affixed to the frame the ear bars were removed leaving the ear canals unobstructed. A craniotomy was performed over the right auditory cortex and the cisterna magna was drained of cerebrospinal fluid to reduce brain pulsation. The dura was resected and warm saline was applied frequently to prevent desiccation. Photographs of the cortical surface were taken using a digital camera prior to each recording. These images were aligned using vascular landmarks to construct a relative map of each recording site.

Stimuli were delivered to the contralateral ear using an open field speaker positioned 2–3 cm away from the ear canal. Stimuli consisted of pure tone bursts (50 ms, cosine-squared gate with 8 ms rise/fall time, 0.5–53.8 kHz in quarter-octave steps, 0–70 dB SPL in 10 dB increments, 8 presentations of each stimulus). Stimuli were presented, pseudo-randomly, once every 700 ms using a TDT RX6 Multifunction Processor controlled by custom MATLAB software.

Extracellular recordings were made with a linear array of 4 parylene-coated microelectrodes (1–2 MΩ, FHC, Bowdoin, ME) lowered to the middle cortical layers (III–IV; 400–600 μm deep) via a microdrive (Inchworm 8200, EXFO Burleigh Instruments, Victor, NY). Neural activity was amplified (TDT RA16 Amplifier, TDT RZ5 Bioamp Processor) and stored

for offline analysis. Offline spike detection was performed using custom MATLAB software. Recordings were filtered (0.3–3.0 kHz) and multiunit discharges were characterized using temporal and amplitude criteria. Acceptable spikes were defined as waveforms with peaks separated by no more than 0.6 ms and with a threshold amplitude greater than 2.0 (for the positive peak) and less than 2.5 (for the negative peak) × RMS of 500 random traces from the same recording.

Neurophysiological analysis

Tone evoked activity was determined by subtracting the spontaneous firing rate (recorded during the 50 ms period prior to tone onset) from the firing rate during stimulus presentation. The mean evoked activity for each stimulus was used to construct frequency response areas (FRAs) for each recording site. Each FRA threshold was based upon its recorded spontaneous activity; only evoked responses greater than the mean +2 s.e. of spontaneous activity were considered true evoked responses. The FRAs were used in determination of the characteristic frequency (CF) for each site, defined as the stimulus frequency having the lowest threshold for an evoked response. If multiple frequencies were found having the same lowest threshold, the CF was defined as the geometric mean between these frequencies. Voronoi tessellations were constructed for all recording sites sampled during mapping. The primary auditory cortex (A1) was then physiologically defined and those tessellations were selected to determine CF area. A1 was identified as having a caudal–rostral, low–high frequency tonotopic organization (Sally & Kelly, 1988). Borders with the anterior (AAF) and ventral auditory fields (VAF) were identified through reversals in frequency tuning. The caudal border was identified by discontinuities in the tonotopic gradient while the dorsal border was identified by discontinuities in the tonotopic gradient as well as the presence of sites with multi-peaked FRAs and broad (>3 octaves) tuning.

Frequency area was quantified by determining the percentage of total A1 area occupied for each frequency band.

Statistics

All behavioral and CF area measures were analyzed using ANOVA ($\alpha = 0.05$) and post hoc analyses were performed using t-tests with bonferroni α correction for multiple comparisons. Brain–behavior relationships were assessed using Pearson's correlations.

Results

Discrimination performance

Animals in both groups solved the three-tone discrimination task, indicated by an increase in performance over days (Fig. 2.3A). Both groups reached criterion with a similar

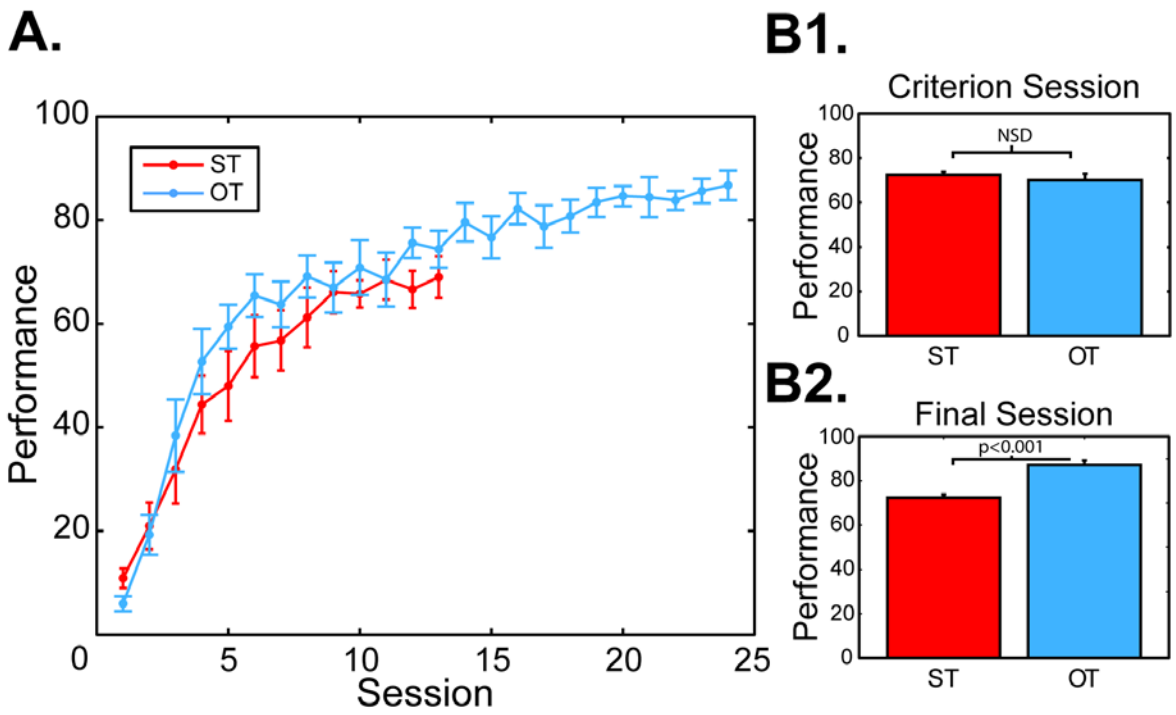


Figure 2.3: Performance data for groups ST and OT. (A) Performance functions: both groups learned to perform the three-tone discrimination task to a high degree. They reached criterion in a similar number of training sessions and at criterion their performance levels were not significantly different (B1) With continued training for two weeks, the OT group achieved a significant increase in performance (B2).

amount of training (ST = 10.3 ± 2.94 sessions; OT = 10.6 ± 5.38 sessions; $t_{(19)} = 0.127$, $p = 0.9$) and showed no difference in task performance or discrimination performance at criterion (Fig. 2.3B1). After reaching criterion the OT group received an additional two weeks of training during which time their task performance and discrimination performance significantly improved (task performance: ~75–86%; Figure 2.3.B2; d': ~2-3). Comparing the final training sessions for ST and OT groups indicated that task performance and discrimination performance were both significantly higher for the OT animals compared with ST (Task: $t_{(19)} = 6.16$, $p<0.001$; d': $t_{(19)}=3.63$, $p<0.01$).

Learning strategy during training

To understand how animals learned to solve the discrimination task, we examined behavior on a trial-by-trial basis. Specifically, we quantified the daily strategy use based upon the pattern of responding during CS+ trials (see *Behavioral analysis*). Fig. 2.4 shows an example of behavior on CS+ trials from a subject in the OT group. Note the general progression from the NS to the TOTE strategies leading up to criterion, which was reached on session 11. During additional training thereafter, strategy use shifted from majority TOTE strategy use near criterion to majority iTOTE strategy use towards the end of training. That is, after making a non-rewarded bar-press during the free period, the animal greatly reduced or eliminated bar-presses during the subsequent intertrial period.

At criterion, there was no difference in strategy use between groups (Figure 2.5 C1 and C2). Both groups used NS ($t_{(19)} = 0.76$, $p = 0.46$), TOTE ($t_{(19)} = 0.62$, $p = 0.54$) and iTOTE ($t_{(19)} = 0.97$, $p = 0.34$) equally. At this point TOTE was the dominant strategy used by both groups. With extended training, the OT animals came to replace TOTE use with iTOTE. During overtraining, the OT group significantly decreased their reliance on the TOTE strategy ($t_{(18)} =$

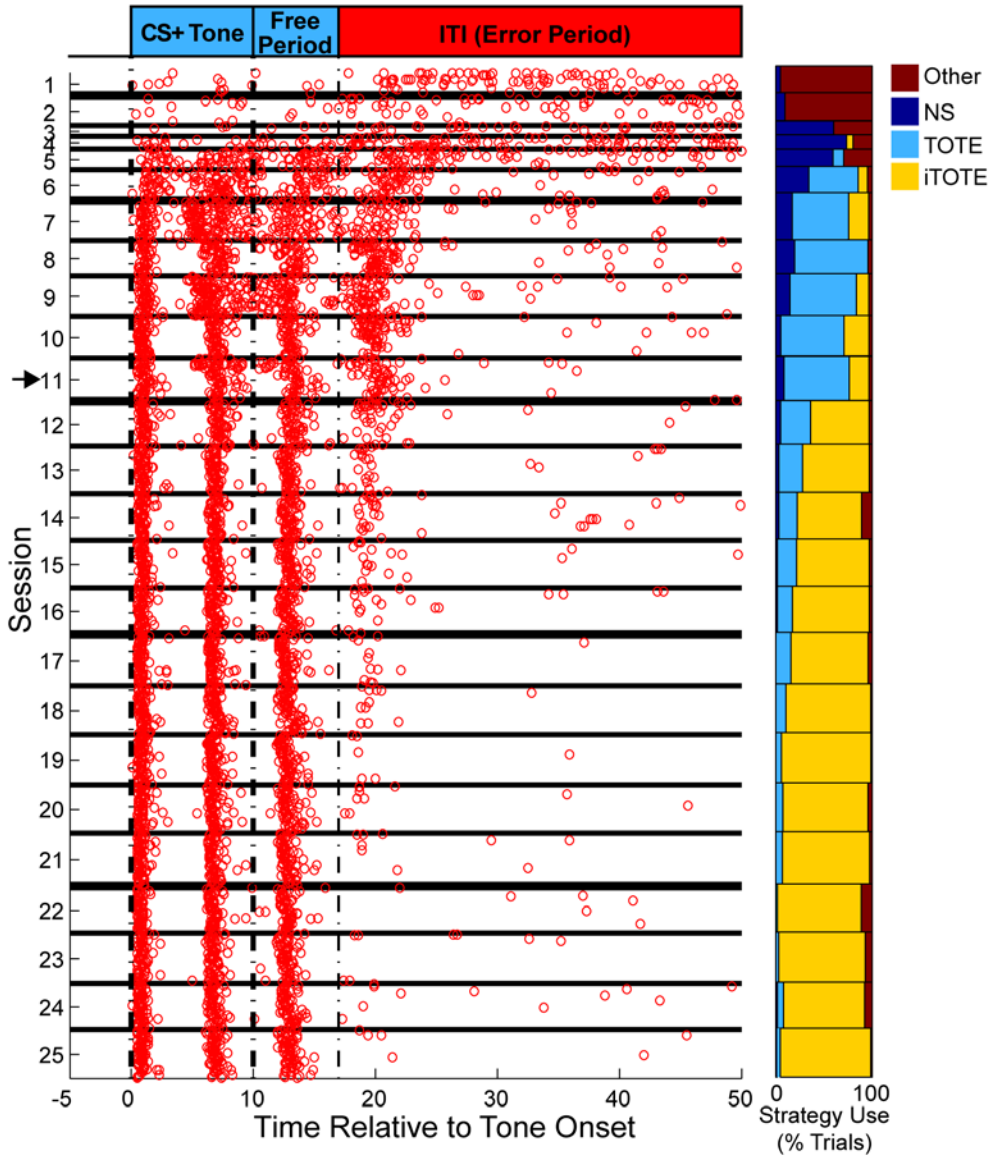


Figure 2.4 Example behavior and strategy use quantification for an animal from the OT group during training. Trials are arranged relative to tone onset. Vertical dashed lines denote tone onset, tone offset and the maximum length of the free period. Horizontal lines denote the boundary between sessions. Open circles represent BPs. The first 50s of CS+ trials is displayed here. The stacked bars on the right depict the relative amount of each behavioral pattern used during each session. During sessions 1 and 2, most BPs were made during the silent ITI periods, probably a continuation of BPs that had been rewarded during the preceding silent shaping to BP for water. As a result, no tone related strategies were identified during these sessions, which were classified as "Other." Note than sessions 3-5 have fewer trials, reflecting numerous BPs during ITIs producing cumulating time-outs, so that the next scheduled trial might not occur for 4-5 minutes. With continued training, ITI responding was reduced. This animal showed increasing use the error signal as a cue to stop bar-pressing during ITIs starting with the 6th session. Use of the TOTE strategy increased through session 11, after which the iTOTE strategy gradually replaced it almost entirely by the end of the two week period of overtraining. The arrow indicates that this animal reached the criterion on session 11.

5.25, $p<0.001$) and significantly increased their use of the iTOTE strategy ($t_{(18)} = 6.47$, $p<0.001$). Therefore, extended training resulted in a change of learning strategy from TOTE, to iTOTE.

High levels of iTOTE use were only observed in the OT group during overtraining. While the rise in iTOTE occurs as TOTE use decreases, the origins of iTOTE are unclear. It is possible that iTOTE reflects a refined version of the TOTE strategy. Alternatively, the iTOTE strategy may be independent from the TOTE strategy but increases in use at such a slow rate that it only becomes a dominant strategy after extensive training. To distinguish between these two alternatives, we examined each of the OT animals' strategy use individually, relative to the session during which they displayed the highest level of TOTE use. If iTOTE is independent of TOTE, then this alignment should reveal iTOTE use to increase prior to the session of peak TOTE use. Alternatively, if iTOTE reflects a refinement of TOTE use then it should only increase in use after the peak in TOTE use and increase in use at a rate similar to the rate of TOTE's decrease. Figure 2.5B shows TOTE and NS levels relative to each animal's peak TOTE use session. This revealed that iTOTE use increased only after TOTE use began to decline (Fig. 2.5B). It also revealed that the increase in TOTE use leading up to the peak was accompanied by a decrease in NS use. This indicates that not only did the iTOTE strategy emerge as a refinement of the TOTE strategy, but also that TOTE seems to reflect a similar refinement of the NS strategy.

Specificity of learning: frequency generalization gradients

To determine the frequency specificity of learning, subjects underwent a stimulus generalization session (during extinction) 24 h after their final discrimination session. The generalization gradients show a high degree of specificity with the peaks at the CS+ frequency

and negligible responses to the CS– tones. The ST and OT groups had the same gradients (two-way ANOVA; Frequency × Group: $F_{(6,133)} = 1.01$, $p = 0.42$), i.e., they had learned the same information about frequency (Figure 2.6).

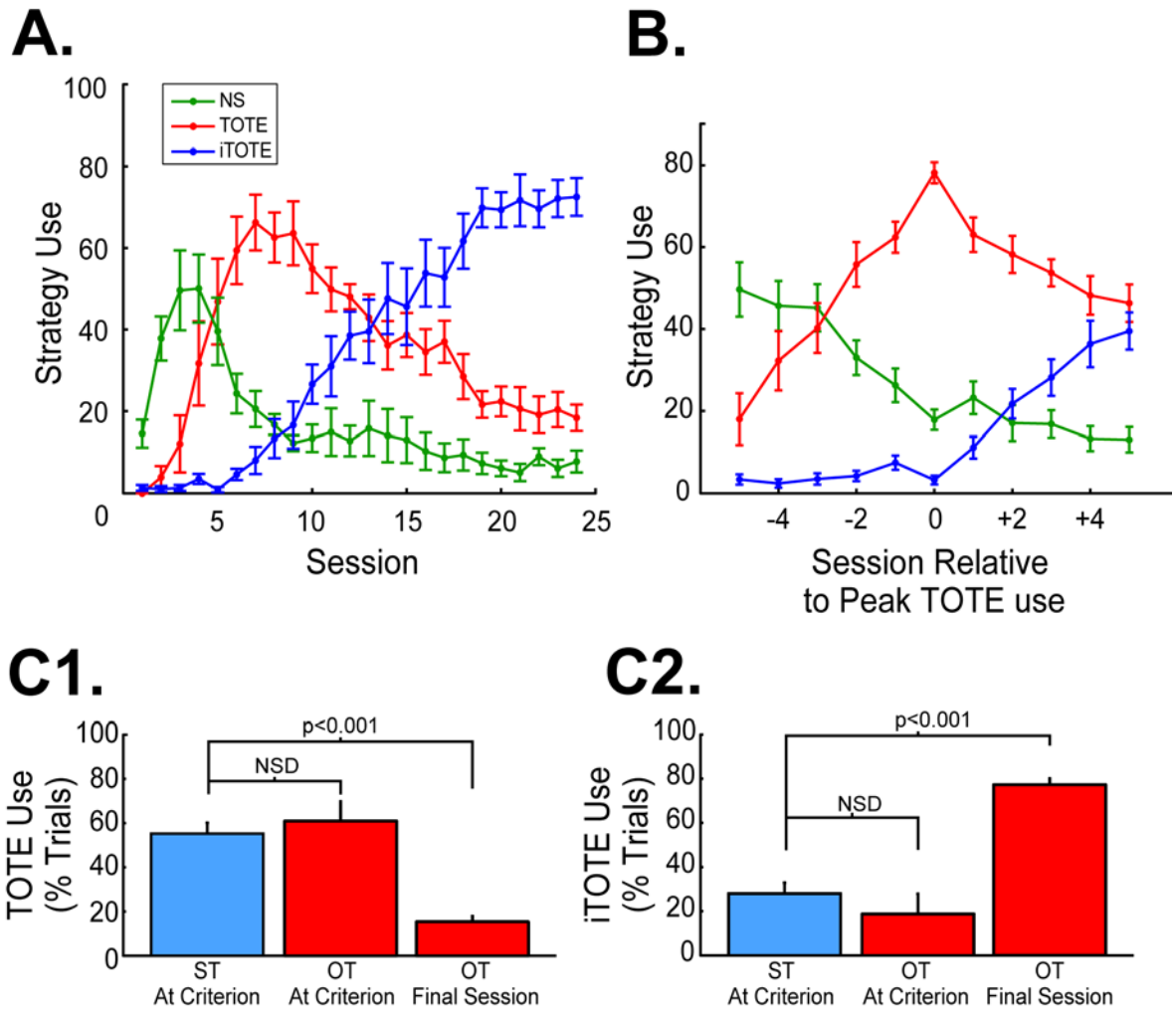


Figure 2.5(A) Strategy use over days for animals for the OT group. Animals progressed through stages where their behavior was dominated by one of the three learning strategies. Early sessions were dominated by the "non-stop" (NS) pattern which was replaced by TOTE as animals learned to use the error signal as a cue to cease responding during a trial. During overtraining, behavior became dominated by the iTOTE strategy, i.e., animals reduced or eliminated ITI responding. (B) Individual subjects progressed through these phases at different rates. To examine whether iTOTE reflected a refinement of the TOTE strategy or the emergence of an independent strategy, we aligned each animal's behavior relative to its maximum observed level of TOTE use. This alignment revealed that iTOTE use remained low prior to the peak in the use of the TOTE strategy and only increased following the beginning of its decline, indicating that iTOTE emerged as a refinement of TOTE. (C) At criterion both groups favored use of the TOTE strategy but with overtraining the OT group came to favor iTOTE over TOTE.(C1) TOTE use (C2) iTOTE use.

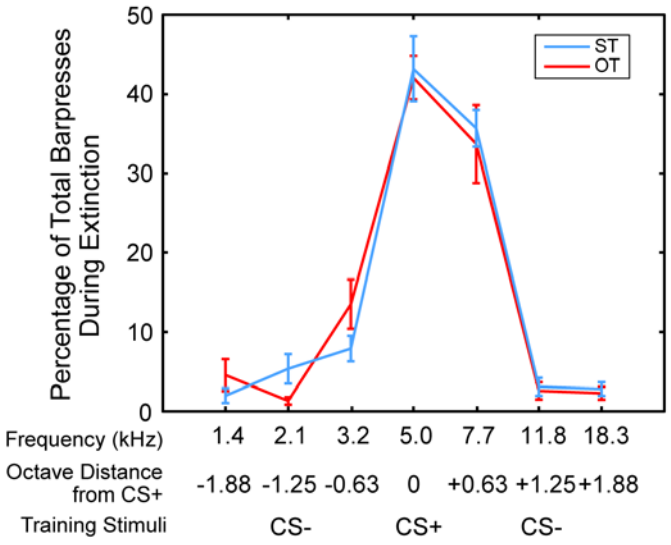


Figure 2.6 After their final TTD training session both groups underwent stimulus generalization to test the frequency specificity of their learning. The standard trained and overtrained groups had identical generalization gradients which indicates the frequency specificity of their learning was identical.

Effects of overtraining on representational plasticity

Both the ST and OT groups plus a group of Naïve rats underwent physiological mapping to determine whether RP occurred during training on the three tone discrimination protocol and if so, whether it was affected by overtraining. We were also interested in investigating whether representations for frequencies near the training stimuli were modified so we chose to focus our analysis on the representational area for the frequencies used during the stimulus generalization session. Figure 2.7 depicts each group's relative A1 area for the stimulus generalization frequencies as well as an example map from each group. A two-way ANOVA was used to compare group (ST, OT and Naive) vs frequency (non-overlapping frequency bins centered on each of the stimulus generalization frequencies, ~0.6 octaves wide). The effect of group was not significant ($F_{(2,189)}$=0.73, p=0.48) but there was a main effect of Frequency ($F_{(6,189)}$=17.74, p<0.001) and a significant Group X Frequency interaction ($F_{(12,189)}$=2.69, p<0.01). To determine what drove the interaction effect we ran one-way ANOVAs to compare the groups' representational area for each frequency bin individually. Across the seven frequency bins, only the representational area for the CS+ ($F_{(2,27)}$=5.25, p<0.05) and the highest

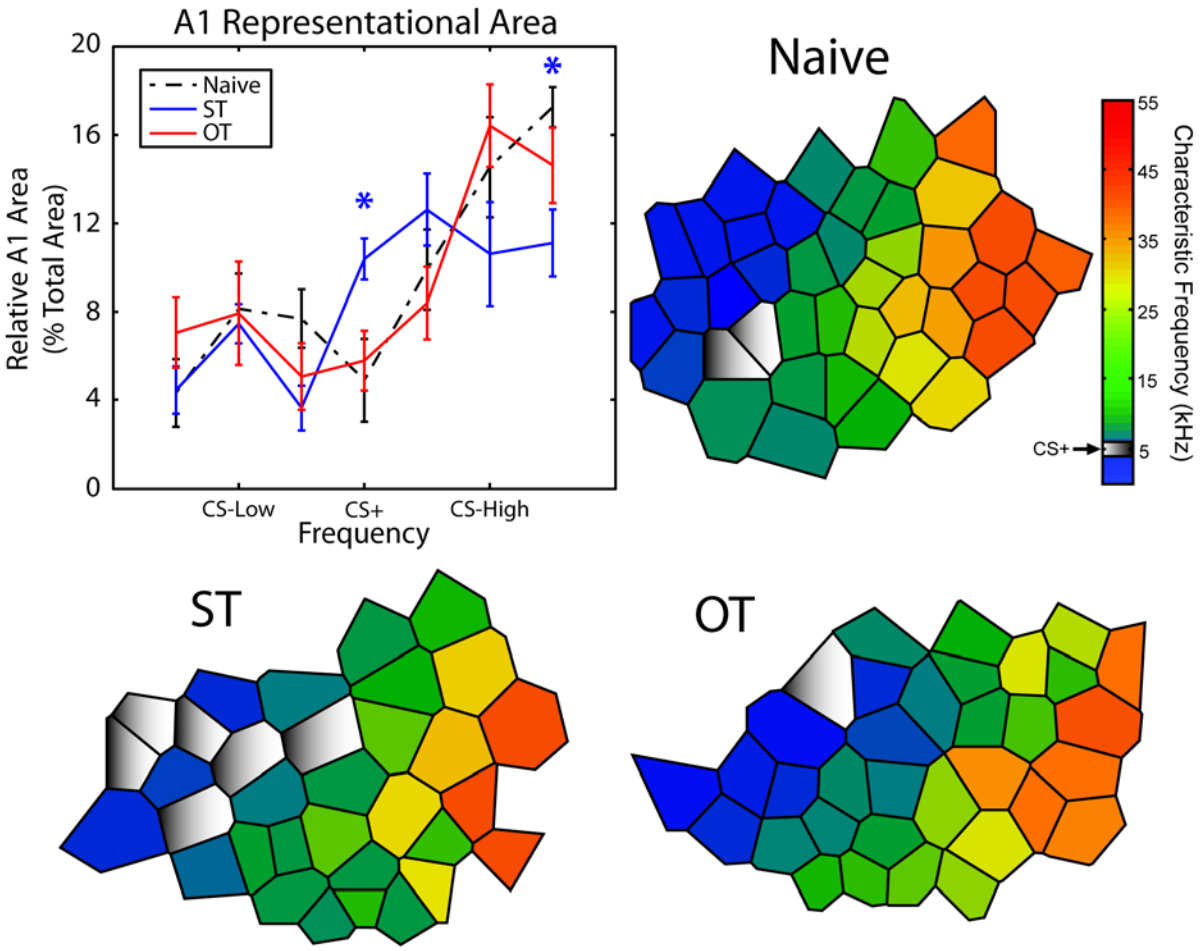


Figure 2.7 Representational plasticy was was in the standard trained (ST) but not the overtrained (OT) group indicating that overtraining produced map renormalization. *Top Left:* Group plots of relative A1 area (mean ± SEM) indicate that the ST group had a specific increase in CS+ representational area compared to naive and th OT group. In addition, the ST group had a significant decrease in representational area for the 18.3 kHz frequency. *Top Right:* Example map of a Naive animal and accompanying color map for all example maps. *Bottom Right:* Example map from an overtrained animal. *Bottom Left:* Example map from a standard trained animal. Note how the amount of CS+ area is significantly larger than the example Naive and OT animals.

generalization frequency representation, 18.3 kHz ($F_{(2,27)}$=4.97, p<0.05), had significant group effects. Post-hoc tests indicated that the group effect at the CS+ was driven by a significantly increased area for the ST group compared to the OT ($t_{(19)}$=2.98, p<0.01) and Naïve groups ($t_{(18)}$=2.94, p<0.01). In contrast, CS+ area for the OT group was not significantly different from Naïve ($t_{(17)}$ = 0.41, p=0.69). Post hoc analysis for the 18.3 kHz representational area indicated that this effect was driven by a significant reduction in area representing this region in the ST group compared to Naïve ($t_{(18)}$=3.45, p<0.01). While the OT group had, on average, less area

than the Naïve group and more than the ST group, neither of these comparisons were significant. Overall our analysis of representational area between the ST, OT and Naïve animals demonstrated that plasticity was present for the ST group but not the OT group. This finding indicates that additional training returned CS+ area back to naïve levels. This analysis also revealed that the ST group had a reduction in area for the 18.3 kHz frequency bin. As this change only manifested in the ST group and co-occurred with RP, it is possible that the reduction in area supports or is caused by the plasticity at the CS+.

We also investigated whether representational area was correlated with the animals' behavior. We first examined behavior during the generalization session and its relationship to the A1 representational area for each frequency but failed to detect any significant relationship between responses during generalization and A1 representational area for any of the frequencies tested (even for the CS+). We reasoned that if representational area was not related to behavior during the generalization session, perhaps it was related to the development and refinement of strategy use. For this analysis we used strategy levels from the final session of training for each animal, because this occurred at the time closest to mapping, and tested for correlations between strategy levels and representational area for each frequency. The ST and OT groups were combined for this analysis. This analysis revealed significant correlations between strategy use and representational area for the CS+ frequency as well as the adjacent frequency bin, 7.7 kHz. Figure 2.8 shows the correlations between strategy use and A1 area. The directions of the correlations were consistent along strategy lines. TOTE use was positively correlated with CS+ ($r = 0.48$, $p<0.05$; Figure 2.8A) as well as 7.7 kHz ($r = 0.51$, $p<0.05$; Figure 2.8C) while iTOTE use was negatively correlated with CS+ ($r = -0.56$, $p<0.01$; Figure 2.8B) and 7.7 kHz ($r = 0.48$,

p<0.05; Figure 2.8D). Therefore, it appears that type of learning strategy used to solve a problem impacts the maintenance of representational plasticity in A1.

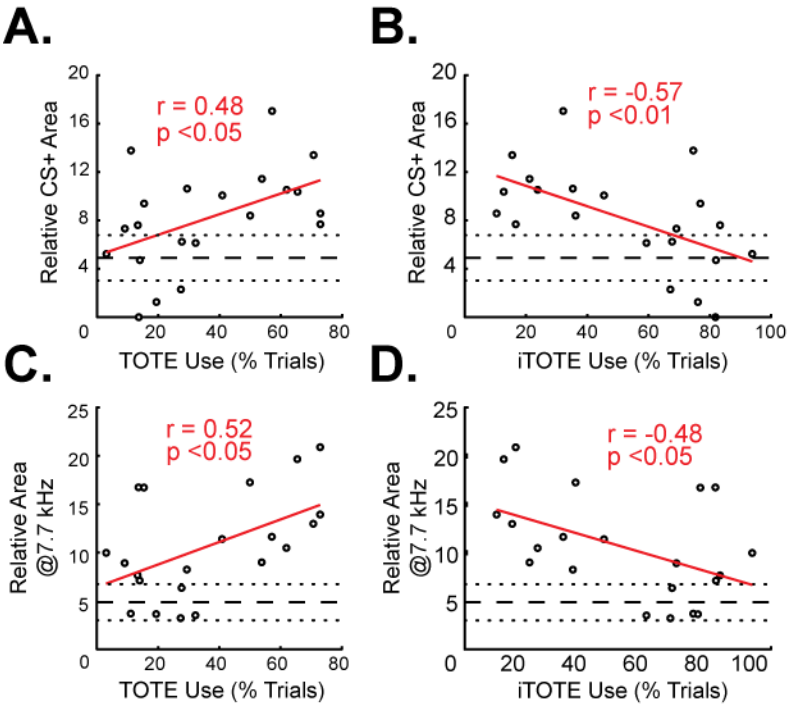


Figure 2.8 To determine whether representational area for any frequency bin was related to strategy use we correlated strategy use during each animal's final training session with each frequency bin. Only the CS+ and the the adjacent 7.7 kHz frequency bin were correlated with strategy use (all other correlations p>0.05). TOTE use was positively correlated with representational area for the CS+ (A) and the 7.7 kHz frequency directly adjacent to the CS+ (B). iTOTE use was negatively correlated with CS+ (B) and the 7.7 kHz (D) frequency bins. These relationships indicate that greater use of the TOTE strategy produces greater expansions of representational area. Moreover, the relationship with iTOTE indicates that strategy refinement and map renormalization are correlated.

Discussion

This experiment concerned the possible role of learning strategy in the maintenance of specific gains in the representation of acoustic signals during learning. Using a three tone discrimination protocol (5.0 kHz: CS+ rewarded, 2.1 and 11.9 kHz: CS- unrewarded), we observed that RP, in the form of a CS+ expansion, was present for animals examined after initially displaying proficiency on the TTD task (Group ST). If the animals were overtrained for 2-3 additional weeks (Group OT), A1 representational area returned back to naïve levels. This replicates previous results showing that map plasticity dissipates with continued training (Reed et al., 2011; Takahashi et al., 2010; Yotsumoto, Watanabe & Sasaki, 2008; Ma et al., 2010, Tennant et al., 2012)

To examine strategy use, we designed and implemented a novel behavioral analysis method based on characterization of an animal's pattern of responding on a trial by trial basis. Similar methods were used in previous investigations of strategy use (e.g. Bieszczad & Weinberger, 2010c) but the criteria used to determine strategy use were not as stringent as the criteria adopted here and the focus of the analyses was on the tone-onset to error (TOTE) strategy specifically while the present analyses investigated other strategies as well. We identified three main strategies that could account for the vast majority of an animal's behavior. These strategies are the non-stop (NS), TOTE and internalized TOTE (iTOTE) strategies. Throughout the course of training, an animal's behavior was characterized by different proportional use of these three strategies. Early in training, as animals learned to reliably utilize tone onset, NS was used most frequently. With continued training, NS use was replaced by TOTE use which, at criterion was the dominant strategy. Eventually, TOTE was replaced by iTOTE use but such a transition was only observed during overtraining. We found that TOTE and iTOTE use were significantly correlated with representational area for the CS+ and 7.7kHz frequency bins. TOTE was positively correlated with representational area for both frequency ranges while iTOTE was negatively correlated with representational area.

The present results expand upon previous studies that initially proposed a critical relationship between learning strategy use and cortical plasticity. While prior studies of learning strategy relied on single tone training, the present experiment expands the area of inquiry by utilizing a discrimination task. The presence of RP and its relationship to strategy use supports the idea that this relationship reflects a general principle regulating cortical plasticity.

These findings replicate those of Bieszczad and Weinberger (2010c), who had previously observed a relationship between TOTE use and representational area for behaviorally relevant

tones. Previous studies suggested that the degree of TOTE use was related to the form of RP. Modest TOTE use resulted in specific decrease in absolute threshold (increased neural sensitivity) and bandwidth (increased neural selectivity) at sites tuned to the CS+ (Berlau & Weinberger, 2008), while a specific gain in CS+ area resulted from greater TOTE use (Bieszczad & Weinberger, 2010c). The current study shows a similar effect. With overtraining, iTOTE came to replace TOTE as the dominant strategy and at the same time RP presumably dissipated. Bieszczad and Weinberger (2010c) proposed that the key aspect of the TOTE strategy that promotes plasticity in A1 is the strategy's reliance on tone onset. The observation that plasticity is present in the ST but not the OT animals stands in contrast with this proposal. Even though the two groups preferred different strategies, both strategies relied on the tone-onset as a cue to start barpressing during a trial. Bieszczad and Weinberger (2010c) also suggest that the reliance on tone onset is important only if it is accompanied by a reduction in reliance on tone offset as a cue. The ST group, who used TOTE, clearly did not rely on tone offset as a cue since they continued barpressing until triggering an error signal. The OT group, who preferentially used iTOTE, may have learned to avoid the error signal by becoming reliant upon the tone offset as a cue.

While it is fairly clear that the iTOTE strategy relies on tone onset as a cue to start barpressing, the exact cue utilized to signal when barpressing should be terminated is unclear. One possibility is that iTOTE relies on tone offset as a cue for the animal to barpress one more time and then wait for the next trial. If this were the case, the reduction in imbalance between utilization of onset and offset cues would explain the loss of plasticity during overtraining. However, it is also possible that iTOTE relies on the acquisition of the free period reward itself as a cue to cease further barpresses. In this case the animal would count the number of acquired

rewards since tone onset and stop barpressing once it acquired three. Of these two explanations, it seems more likely that the animal learns to count rewards from tone onset as doing so wouldn't require the animal to learn about a new cue (tone offset), and in that sense it would be more economical. Also, because the CS+ was 10s in duration and water reward was presented to the animal for 5s more often than not, the animal would be in the process of reward acquisition during tone offset. Not only would this arrangement reduce the animal's attention to tone offset but it would also require the animal to divide its attention in order to detect tone offset. Again, counting the number of acquired rewards from tone onset and stopping once the animal hit 3 is a more efficient strategy and therefore more likely.

Previous studies of renormalization

The loss of cortical map plasticity after initial learning has been termed "renormalization" (Reed et al., 2011). Several laboratories have reported renormalization of expanded cortical representations. A common characteristic seems to be that renormalization is found after training is continued for some period following initial learning of the task. Reed et al. (2011) used nucleus basalis (NB) stimulation to promote the learning of a two tone auditory discrimination in a Go–NoGo reward task, for ~270 trials per session twice daily, i.e., ~540 trials per day**.** The authors reported an increase in the ratio of the number of A1 sites responsive to the 19.0 kHz CS+ vs. the 2.0 kHz CS-. Although these data are not equivalent to the areas of representation of all frequencies in the tonotopic map, and the use of a ratio measure does not permit unambiguous conclusion of an actual gain in CS+ area, the dynamics of change over time are clear. The absence of cortical plasticity, while behavioral performance was maintained, was found in three groups after additional training for 10 days (~5400 trials), a 20-day break and then another 10

training days, the last five of which involved ~2700 trials (their Fig. 4*C*). Although the contribution to renormalization of a 20-day break in one group (during which groups heard tones with or without paired stimulation of the NB) is unclear, these subjects did receive additional training immediately before being mapped. Similarly, another group of animals in a related experiment within this overall study also exhibited a loss of plasticity after training that had culminated in 5 days of added training (their Fig. 5*C*). Therefore, four groups, all of which exhibited the loss of A1 plasticity, had received further training for at least 2500 trials each.

Takahashi et al. (2011) trained rats in an easier (non-discrimination) auditory task, to nose-poke during a tone to receive to a reward. Animals were trained for ten days (60 trials per day). Performance level was high by Day 4 (increasing "hit" rate, decreasing "false positive" rate) (their Fig. 1). Mapping of A1 at this point revealed an expanded representation for the trained frequency. Continuation of training for another six days (360 trials) resulted in a reduction of map plasticity (their Fig. 2) while behavioral performance was upheld. Insofar as animals had to attend to the tone to receive reward, their "hit" rate learning curve is perhaps more relevant than the reduction in "false alarms" as its slope change occurred on Day 5, suggesting that the additional five days of training was responsible for diminished plasticity.

Within the human primary visual cortex, Yotsumoto et al. (2008) reported the loss of a specific increased activation (fMRI) in a texture discrimination task, when training was maintained after initial acquisition. Thus, after the first ~10 days of training, during which the learning curve was steep, the BOLD response increased specifically in the visual quadrant of the target stimuli. However, after an additional ~12,160 trials of training over 14 days, activation was no different from baseline despite maintained behavioral improvement.

Beyond primary sensory cortex, additional training may have general relevance for the loss of cortical plasticity that develops during learning because it appears to account for at least one case of renormalization in the motor cortex. Ma and associates (2010) trained humans to perform complex digit patterns daily for four weeks. Correct performance increased from 37 sequences per minute to 70 per minute. The inter-regional connectivity between the primary motor cortex and the supplementary motor cortex was determined by fMRI at various stages. The learning curve (their Fig. 1) shows that the slope change occurred about Day 14. Recordings obtained at this time revealed an increase in functional connectivity. However, continued training for an additional two weeks produced a decrease in this cortical plasticity, again with maintenance of motor learning.

Additional training apparently cannot account for all dissipation of sensory or motor specific plasticity. For example, when stimulation of the cholinergic NB is used to promote two-tone discrimination learning, renormalization can occur after 2 weeks of a rest period (Reed et al, 2011). Additionally, shrinkage of initial expansion has been observed in forepaw motor representations within the primary motor cortex after eight days without additional training (Molina-Luna, Hertler, Buitrago & Luft, 2008). Also, a loss of plasticity has been shown to occur with experimental extinction, i.e., withdrawal of reward; the degree of retrenchment is greater for stronger extinction (Bieszczad & Weinberger, 2012). However in this case behavioral performance is not maintained because the tone's meaning has changed.

Mechanisms of renormalization with change in learning strategy

Renormalization has been attributed to a reduction of the number of learning-related neurons in A1, such that they are no longer detectable by mapping methods (Reed et al., 2011).

Alternatives should be considered, including a shift in the control of behavior to another brain system, which may or may not actively suppress the expression of plasticity in A1.

It has long been known that the amount of training can be an important factor controlling behavior. William James (1890) emphasized the distinction between “memory” and “habit”, the latter developing during “overtraining”, i.e, continued training after initial learning.. Moreover, the development and subsequent loss of learning-based cortical plasticity with maintained behavioral performance has been known for well over fifty years, starting with studies of conditioned electrocortical activation (John, 1961). More recently and generally, “systems consolidation” refers to the time-limited dependence of learning on a given brain structure, often initiated by prolonged training. For example, additionally training rats in a T-maze shifts learning strategy from *going to a place* to *making a right turn*, and in so doing, shifts the critical brain substrate from the hippocampus to the caudate nucleus (Packard & McGaugh, 1996). Shifts in the locus of behavioral control can also involve competition and inhibition between brain systems involving shifts in strategy (Coutureau & Killcross, 2003; Gold & Korol, 2012). Cognitive learning has been attributed to the cortex while habit-like behavior after additional training may become dependent on the striatum (e.g., Mishkin & Petrie, 1984; Yin & Knowlton, 2006). These possibilities warrant investigation.

Chapter 3: Representational Plasticity Maintenance in the Absence of Continued Training

Introduction

It is a widely held notion that learning related changes in behavior arise from concomitant changes in the brain. When a sound acquires behavioral relevance, cells in the primary auditory cortex shift their tuning, i.e., increase their firing rate, to that sound (Bakin & Weinberger, 1990). In aggregate, these tuning shifts result in an increased amount of representational area for the relevant sound within the tonotopic frequency map in A1 (Hui, Wong, Chavez, Leon, Robin & Weinberger, 2009; Recanzone, Schreiner & Merzenich, 1993; Rutkowski &Weinberger, 2005). Multiple lines of evidence support the view that A1 map plasticity is related to auditory learning. Not only has map plasticity been found to correlate with performance (Recanzone, Schreiner, & Merzenich, 1993) and memory strength (Bieszczad & Weinberger, 2010c) at the end of training, plasticity develops in tandem with learning during behavioral training (Edeline, Pham & Weinberger, 1993). Furthermore, induction of cortical plasticity through nucleus basalis stimulation can facilitate subsequent learning (Reed et al., 2011) and can itself induce behavioral changes in the absence of behavioral training (Bieszczad, Miasnikov & Weinberger, 2013; Weinberger, Miasnikov, Bieszczad & Chen, 2013). Moreover, disruption of mechanisms involved with the induction of cortical plasticity in A1 disrupts auditory learning (Letzkus et al., 2011). Thus it appears that auditory learning is directly related to plasticity in the primary auditory cortex.

However, it appears that map plasticity does not serve as a long term substrate for behavioral change. While behaviorally-induced (Weinberger, Javid & Lepan, 1993) and artificially induced (Reed et al., 2011) plasticity can persist for an extended period following induction, recent reports have observed plasticity detected during initial learning, to be absent

upon later investigation (Reed et al., 2011; Takahashi et al., 2010; Yotsumoto, Watanabe & Sasaki, 2008; Ma et al., 2010; Tennant et al., 2012). In each of these instances the subjects maintained high levels of task performance, even though the sensory map renormalized. While this may lead some to conclude that cortical plasticity is ultimately an epiphenomenon, this conclusion disregards the evidence which demonstrates a strong relationship between the induction of cortical plasticity and initial learning.

This supposition also implicitly disregards the possibility that the process of renormalization is itself related to the animal's behavior. In the previous experiment, renormalization of the tonotopic map in A1 was observed following extended training on a modified instrumental task. Animals that underwent extended training were observed to modify the way they solved the task. After initially displaying task proficiency, subjects tended to use a tone-onset-to-error (TOTE) strategy, characterized by use of the tone onset as a cue to initiate responding and reliance upon the external error signal as a cue to cease responding. Following extended training, animals tended to use an internalized tone-onset-to-error (iTOTE) strategy, characterized by use of the tone onset as a cue to initiate responding and the inhibition of responding after acquiring the final reward during a trial, prior to and presumably in anticipation of triggering an error signal. Across all animals, this strategy refinement was correlated with map renormalization. This finding suggests that, similar to the induction of map plasticity, the process of map renormalization is also related to the animal's behavior.

In the majority of studies that reported map renormalization, time is a confounding variable as behavioral training persisted in between when plasticity was initially detected and when it was found to be absent. In at least one case, however, plasticity was found to dissipate in the absence of continued training. Molina-Luna et al. (2008) observed changes in somatotopy in

the primary motor cortex, consisting of an enlarged forelimb representation, following learning of a forelimb reaching task. After a period in which the subjects did not receive training, the forelimb representation reverted to baseline levels while performance on the task remained unchanged. This suggests that map renormalization is not an active process, tied to the animal's behavior, but rather a passive process that occurs independently of the animal's experience.

Therefore, to address whether the map renormalization observed in the previous experiment resulted from the passage of time, we trained an additional group of rats, similar to those in the standard training (ST) group from Experiment #1. Upon attaining criterion, these animals (Group Long Term Maintenance, i.e. LTM) went untrained for 2-3 weeks, after which we tested their degree of task retention followed by stimulus generalization to test the specificity of their learning about frequency. Physiological mapping of A1 was then done 2-3 days after stimulus generalization to investigate representational plasticity (RP).

Materials and Methods

Subjects

Male Sprague-Dawley rats (250-300 g, $n = 6$) from Charles River Laboratories (Wilmington, MA) were individually housed in a vivarium (temperature maintained at 22º C, 12/12 h light/dark cycle, lights on 7 am). Subjects were treated identically to those in Experiment #1. *Ad libitum* access to food and water was available before the onset of training. During training with water restriction (see *Behavioral training*), continuous access to water was restored on the weekends and supplements were provided after training sessions if necessary to maintain weight. All procedures were conducted with care to minimize pain or discomfort and were in

accordance with the University of California, Irvine, Animal Research Committee and the NIH Animal Welfare guidelines.

Similarity to previous experiments

The training apparatus and stimuli were identical to those used in Experiment #1. Behavioral training and analysis methods were similar to those used in Experiment #1 and the differences are outlined below. Neurophysiological recording and analyses methods were identical to those used in Experiment #1.

Overview of unique behavioral methods

Figure 3.1 provides an overview of the experimental timeline for the long term maintenance (LTM) group. Subjects underwent daily three tone discrimination (TTD) training until they attained behavioral criterion defined as three consecutive sessions during which the coefficient of variation was ≤ 0.10, where CV = standard deviation/mean of daily performance. Upon reaching criterion, daily training sessions were discontinued for 2-3 weeks. The duration of the retention interval was chosen to approximate the length of extended training experienced by the overtrained group from Experiment #1 (group OT). During this period, animals remained in the vivarium and had *ad libitum* access to food and water. At the end of the retention interval,

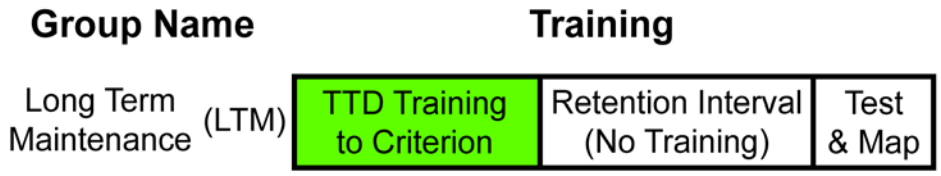


Figure 3.1 Long term maintenance (LTM) group trianing overview. Training on the TTD task continued until LTM animals reached criterion. They then received no furhter training for a period of 2-3 weeks, a duration comparable to the amount of overtraining used in experiment #1. After the retention interval the LTM animals underwent a single TTD training session to test for their retention of the TTD task. The following day they were the frequency specificity of their learning was tested with stimulus generalization followed by physioclogical mapping of A1 to examine representational plasticity 2-3 days after the generalization test.

subjects were water-restricted for 17-20 hours in preparation of behavioral testing the following day.

Behavioral testing

Following 2-3 weeks of rest, a single TTD training session was used to investigate whether the retention interval influenced strategy use and/or performance. The following day, subjects underwent a combined extinction/generalization session to test the frequency-specificity of learning. The procedures used for the generalization test were identical to those used for Experiment #1.

Neurophysiological recordings and analysis

Complete mapping of A1 was performed 2-3 days following completion of the extinction generalization session. Methods of neurophysiological recording and analysis were identical to those used in Experiment #1.

Statistics

All behavioral and characteristic frequency (CF) area measures were analyzed using ANOVA ($\alpha = 0.05$) and post hoc analyses were performed using t-tests with bonferroni α correction for multiple comparisons. Brain–behavior relationships were assessed using Pearson's correlations.

Results

In order to determine whether the passage of time was sufficient to produce map renormalization, we needed to determine whether the LTM group had developed RP. As the standard trained group (ST) from Experiment #1 had RP, we compared the LTM group with the ST group to determine how similar the two groups were behaviorally. Both groups learned to solve the TTD task by the end of TTD training as evident by both groups obtaining high performance levels by the time they reached criterion. Task performance levels were equivalent between the two groups (LTM: 75.95% ± 4.69% ; ST: 72.42% ± 1.4%; $t_{(15)}$=0.985, p = 0.34; Figure 3.2A), as was their discrimination performance as measured by d' (LTM: 2.47 ± 0.28; ST: 2.1 ±0.25; $t_{(15)}$=0.99, p=0.34; Figure 3.2B).

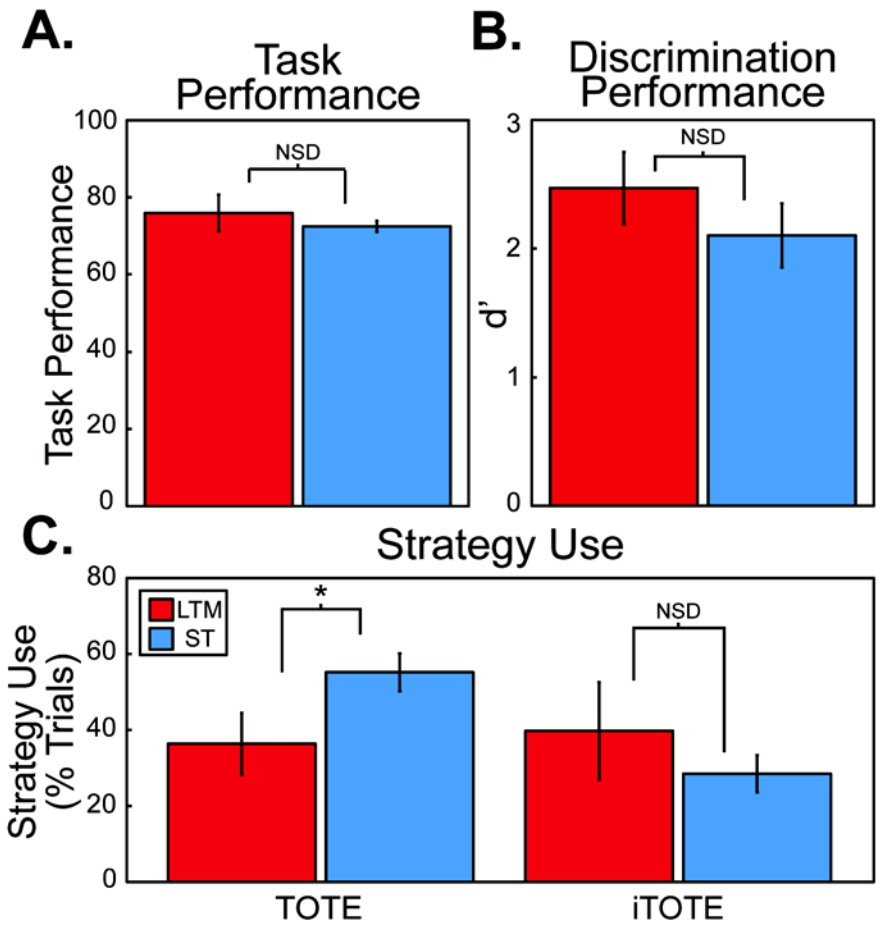


Figure 3.2: Comparison between the long term maintenance (LTM) and the standard trained (ST) group from experiment #1. The two groups were identical in terms of task performance (A) and discrimination performance measured by d' (B). In terms of strategy use, the ST group was observed to have significantly higher levels of TOTE use than the LTM group at criterion. Eventhough the groups did not differ significnatly in their levels of iTOTE use, the difference in TOTE use was determined to stem from the fact that many of the LTM animals had used iTOTE more frequently than TOTE, indicating they were further along in the process of strategy refinement (see text for details). * = $p<0.05$

These measures indicate that both groups learned the TTD task but provide no insight into how they solved the task. While the two groups had equivalent levels of performance, there were some differences in strategy use. Specifically, the LTM group used the TOTE strategy less frequently that the ST group did (LTM: 36.4% ± 8.11% during final training session; ST: 55.22% ± 4.95%; Figure 3.2C). This difference in TOTE strategy use is troublesome as it calls into question the assumption that the LTM group had plasticity at the end of TTD training before the retention interval. If the LTM group had not developed plasticity, there would be no plasticity to be maintained. Likewise, if the LTM group developed plasticity which subsequently renormalized by the end of training, that would also mean plasticity was not present during the retention interval.

To determine whether the LTM group had developed plasticity in the first place we investigated whether they had come to adopt TOTE as their main strategy prior to the end of training. Since TOTE use is thought to be a critical factor gating the induction of plasticity in A1 (see Chapter 1 for background and Chapter 2 for support of the relationship between TOTE and representational plasticity for TTD training), if the animals failed to adopted TOTE prior to the end of training that would be provide sufficient reason to doubt plasticity had been induced in the LTM animals. To determine whether the reduced TOTE use by the LTM animals reflected a failure to adopt TOTE, we examined the LTM group's use of the NS strategy during the final training session. Animals use the NS strategy early in training prior to adopting TOTE. Critically, NS is replaced by TOTE use as animals learn to rely on triggering an error signal to indicate when they should stop barpressing during a trial. If animals never adopted TOTE that should be indicated by high levels of NS use. Comparison of TOTE and NS use during the final session indicated that TOTE was used at significantly higher levels than NS (36% TOTE use;

16.38% NS use; $t_{(10)}$=2.31, $p<0.05$). This indicates that the lower levels of TOTE used at the end of training did not reflect a failure of the LTM animals to adopt TOTE use.

The fact that the LTM animals were not using the NS strategy indicates they had in fact adopted TOTE during training. Therefore, the lower levels of TOTE use in the LTM animals must stem from the LTM animals being farther along in the process of refining TOTE into iTOTE than the ST animals were. While levels of iTOTE use were not significantly different during the final session ($t_{(15)}$=1.07, $p=0.30$), the average amount of iTOTE was higher for the LTM group (39.18% ± 12.66%) than the ST group(28.06% ± 4.81%). In addition, the majority of animals in the LTM group used iTOTE more frequently than TOTE during the final training session (4/6) where these animals were a minority in the ST group (3/11).

While the LTM group was further along in the process of TOTE refinement than the ST group had been when they reached criterion, the similarities between the LTM and ST group suggests that they had not fully replaced TOTE with iTOTE use. To test this idea we compared the LTM animals with the overtrained group (OT) from Experiment #1 to see how similar these two groups were. The OT group used iTOTE much more frequently than the ST group ($t_{(14)}$ = 4.05, $p<0.01$) while the LTM group used TOTE much more frequently than the OT group ($t_{(14)}$=3.26, $p<0.01$). This confirms that even though the LTM group were further along in the process of refining TOTE into iTOTE than the ST group was at criterion, they had not completely replaced the TOTE strategy. Thus, it is reasonable to assume that the LTM group had developed RP by the end of TTD training.

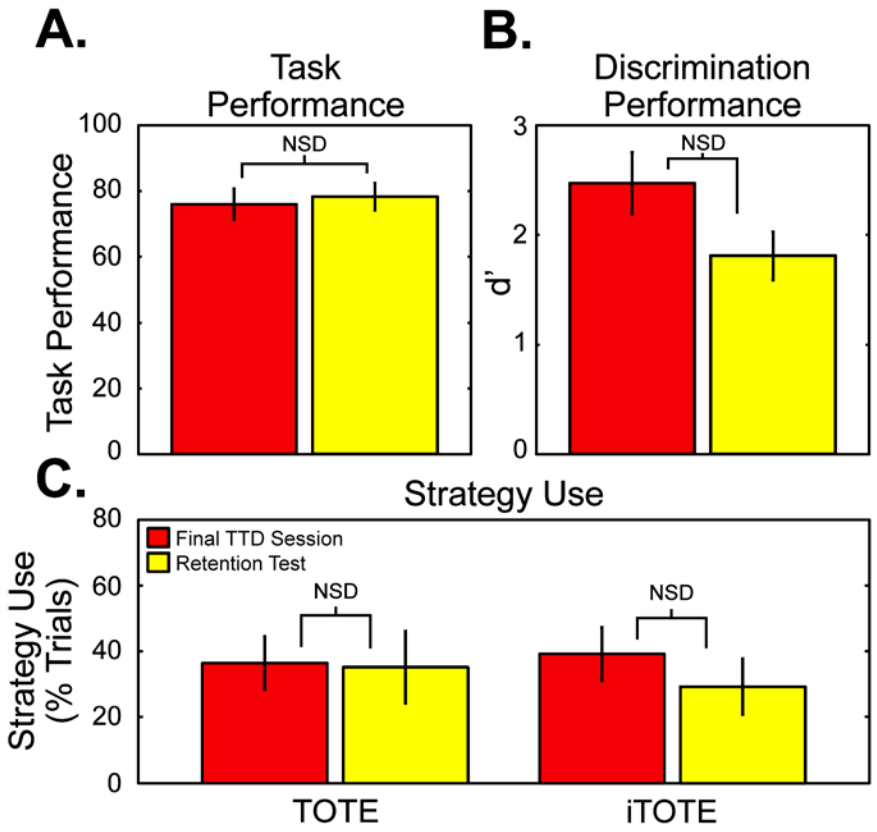


Figure 3.3: Task performance was well maintained across the retention intervan. No significant differences were found to result from discontinued training for 2-3 weeks. Levels of (A) task performance, (B) discrimination performance, and (C) strategy use during the reminder session were not different from the final TTD session.

Behavior following retention interval

After the LTM animals finished TTD training they were removed from the training schedule for 2-3 weeks. After the retention interval they underwent one further training session to assess how their behavior was affected by the time off. As shown in Figure 3.3, the LTM group did not show significant decreases in performance or strategy use measures as a result of the long retention interval. No significant differences were found between the final TTD session and the reminder session after the retention interval for measures of task ($t_{(10)}$=0.39, p = 0.69) and discrimination performance ($t_{(10)}$=2.04, p = 0.07). Levels of strategy use were similarly unchanged (TOTE: ($t_{(10)}$=0.1, p=0.92); iTOTE: ($t_{(10)}$=0.71, p=0.49)).

To examine the frequency specificity of their learning, LTM animals underwent a combined extinction/stimulus generalization test on the day following the reminder session. A two way ANOVA of Group X Frequency revealed a significant main effect of Frequency ($F_{(6,105)}$=122.26, p<0.001) but no effect of Group ($F_{(1,105)}$=0, p=1). Likewise, their interaction

was also non-significant ($F_{(5,105)}$=0.85, p=0.53). This indicates that the LTM and ST animals had similarly specific memory for frequency (Figure 3.4).

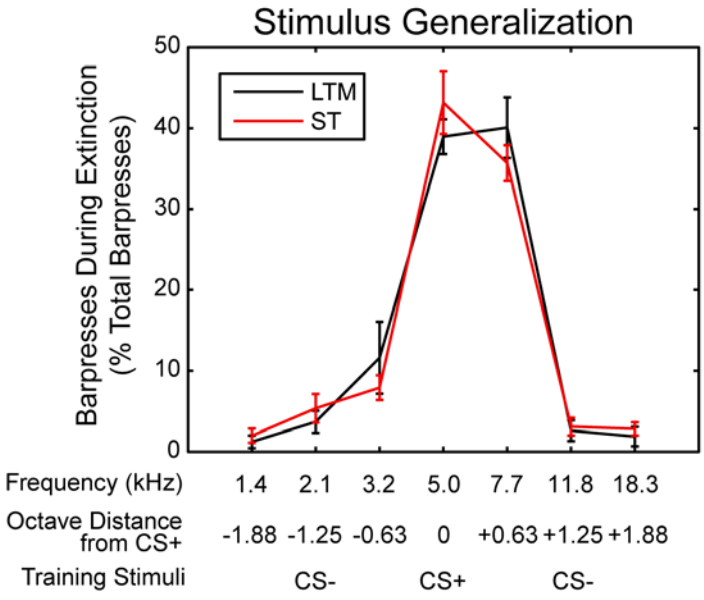


Figure 3.4: The generalization gradient for the LTM group was identical to that of the ST group indicating they had equally specific memory for frequency.

Neural analysis

Each LTM animal underwent physiological mapping 3-4 days following the stimulus generalization test. To investigate changes in representational area, we calculated the representational area for each of the stimulus generalization frequencies using non-overlapping frequency bins. Figure 3.5 shows example maps for two LTM animals as well as the average A1 representational area for the LTM, ST and Naïve groups. A two way ANOVA of Group X Frequency revealed a significant main effect of Frequency ($F_{(6,12)}$=21.98, p<0.001) as well as a significant Group X Frequency interaction effect ($F_{(12,166)}$=3.17, p<0.001) but not a main effect of Group ($F_{(2,161)}$=1.35, p=0.26). Each frequency was then examined individually using a one-way ANOVA to see what drove the initial interaction term to significance. Representational areas for the CS- frequencies were not significantly different between the groups (CS-Low: $F_{(2,23)}$=1.93, p=0.17; CS-High: $F_{(2,23)}$=1.01, p = 0.38), while representation area for the CS+ frequency ($F_{(2,23)}$ = 5.67, p<0.05) was different. This difference was driven by significantly increased area for the ST ($t_{(18)}$=2.94, p<0.01) and LTM ($t_{(13)}$=2.18, p<0.05) groups compared to naïve animals. This indicates that representational plasticity was in fact present in the form of a CS+ expansion in the LTM animals. This indicates that the loss of plasticity observed in the OT

group in Experiment #1 could not result simply from the passage of time and suggests that it is in fact related to the change in strategy use observed during overtraining.

We also observed changes in representational area for frequencies that were only experienced during the stimulus generalization test. Group effects were found for the highest generalization frequency ($F_{(2,23)}$=7.61, p<0.01) as well as the frequency directly above the CS+ frequency ($F_{(2,23)}$=2.53, p<0.05), between the CS+ and the high CS- frequency. Post hoc analysis revealed that the effect for the highest frequency was driven by a significant reduction in area for the ST group compared with Naïve ($t_{(18)}$=3.45, p<0.01) while area for this frequency was not different between LTM and Naïve animals ($t_{(13)}$ = 1.71, p=0.11). The effect at the test frequency

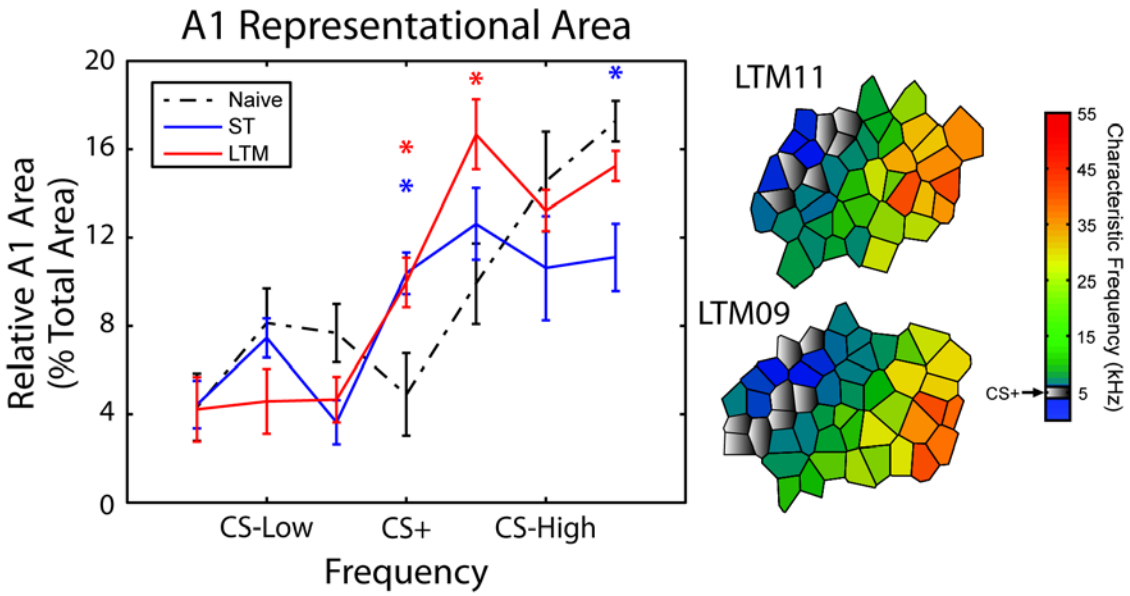


Figure 3.5: LTM animals had increased representational area for the CS+ as well as the adjacent, 7.7 kHz frequency bins compared to naive. Relative A1 area is plotted for the LTM, ST and naive groups are in the graph on the left. Asterisks denote frequency bins that differ significantly from naive (p<0.05) and the colors correspond to the group compared to naive. On the right are maps from two example LTM animals.

directly above the CS+ was driven by a significant increase in area for that frequency in the LTM group compared with Naïve ($t_{(13)}$=2.8, $p<0.05$, Figure 3.5).

Discussion

These findings show that representational plasticity is maintained for at least 3 weeks in the absence of further training after animals learned the TTD task. In Experiment #1, animals that received continued training during this period did not have representational plasticity (See Chapter 3). Task performance was well maintained across the retention interval, and the animals solved the task in the same way after the retention interval as they had before it. In contrast, animals that received continued training changed the way they solved the task. Taken together, these findings suggest that the observed renormalization was not simply due to the passage of time and support the hypothesis that they are instead due to the changes in learning strategy that occur with extended training.

These findings conflict with those of Molina-Luna et al. (2008), who reported map renormalization in the primary motor cortex (M1) in the absence of continued training. After learning a forelimb reaching task, rats developed RP in M1 in the form of an expanded forelimb representation. After a week with no further training, the somatotopic map in M1 renormalized with the dissipation of the forelimb expansion. While performance at the end of training was correlated with M1 plasticity, performance was not influenced by M1 renormalization. A number of differences between the two studies may explain the incongruous results, the most obvious being the different cortical areas under investigation.

It is possible that the principles underlying somatotopic renormalization differ from those for tonotopic renormalization. Somatotopic renormalization may be a passive process whereby

expansions dissipate at a set rate after initial induction unless actively maintained by the organism. In contrast, tonotopic renormalization may be an active process whereby expansions are maintained unless actively dissipated due to changes in the animal's behavior. There is no reason to believe, however, that these regions would display such differences in their maintenance of plasticity. It is just as likely that both regions are equally susceptible to passive and active renormalization but that passive renormalization did not occur under the current experimental conditions.

The greater degree of initial training in the current experiment may have rendered A1 less susceptible to passive renormalization. Molina-Luna et al. (2008) trained rats for 8 sessions lasting ~30min each session on their forelimb reaching task. In the current experiment, rats underwent 10-15 training sessions lasting ~60min each. The greater amount of experience, spread out over a longer time, may have strengthened RP in A1 against passive processes that produce renormalization.

Alternatively, active renormalization may have occurred in the Molina-Luna et al. (2008). It is possible that their subjects had practiced task-related movements in their home cage during the retention interval. While the authors state that they undertook measures to avoid such uncontrolled training, they did not elaborate on how they accomplished this beyond the use of "diet and feeding mechanisms…" designed to "[ensure] that the animals did not reach and grasp with their forelimb to obtain food" (Molina-Luna et al., 2008). Even with precautions in place to avoid practice holding food, the animals may have practiced portions of the task in their home cage. They could have practiced ways to better position their bodies in order to manipulate the task apparatus better and/or practiced grasping by reaching for and grasping home cage bedding. As the authors did not specify whether the animals were monitored in their home cage, the

possibility of covert practice through performance of behaviors approximating those required for the task cannot be completely ruled out.

The fact that performance levels remained high in Molina-Luna et al. (2008) does not rule out the possibility that the animal's behavior may have changed. Analyses that focus on macro-level behavior reliant upon the binary classification of correct/incorrect or successful/unsuccessful trials are adept at determining that learning has occurred but fail to capture how that learning has occurred. Classification of behavior based upon the subject's success at the task does not capture any of the micro-level behavioral changes that arise as the animal develops and optimizes a strategy to solve a task. Behavioral analysis of Molina-Luna et al. (2008) relied upon a macro-level behavioral analysis metric, percentage of correct trials per session, which did not account for possible micro-level behavioral changes that may have occurred as a result of the retention interval.

The results from the present experiment support the conclusion that A1 renormalization is an active process which results from the change in learning strategy during extended training. In previous studies of learning strategy and representational plasticity, use of the TOTE strategy has been identified as critical in the development of plasticity in A1 (Berlau & Weinberger, 2008; Bieszczad & Weinberger, 2010a,b). The experimental results thus far also support this conclusion, particularly the finding that the degree of tonotopic renormalization is correlated with the degree of learning strategy change (See Chapter 2). However, it is not clear whether the relationship between renormalization and learning strategy is driven simply by the process of behavioral optimization, which pushes the transition from TOTE to iTOTE, or whether it is the fact that the animals are no longer using the TOTE strategy specifically that is critical in causing renormalization.

Chapter 4: A Critical Test of the Relationship between TOTE use and A1 Representational Plasticity

Introduction

In a standard tone-based instrumental task, animals learn to make behavioral responses during presentation of a conditional stimulus (CS) tone and withhold them during silence. This relatively simple task can be solved in a number of different ways. At first glance, a sound may appear to be a unitary stimulus, but it actually consists of at least three distinct components: an onset, duration, and offset. Each these components can be used by animals to guide their behavior (Kehoe & Weidemann, 1999; Levis, 1966; 1971). For example, an animal may learn to use the tone onset as a cue to begin behavior, and the tone offset as a cue to stop. Alternatively, the animal may use the tone onset as a cue to initiate behavior, and instead of tone offset use an environmental cue such as the absence of reward or an explicit error signal as a cue to stop. These strategies are equally successful at acquiring reward and since they utilize the environmental cues in different ways, they are distinct solutions to the task.

Recent evidence has linked representational plasticity (RP) in primary sensory cortex, with the strategy an animal uses to solve a problem. Berlau and Weinberger (2008) trained two groups of rats on a tone instrumental task. The first group was trained using a standard instrumental paradigm (STD group) in which barpresses made during tone presentation triggered water reward while all barpresses made during the silent inter-trial interval (ITI) triggered a flashing light error signal and an ITI extension. The second group was trained on a modified instrumental protocol referred to as the Grace protocol (GRC group). Barpresses during tone presentation were rewarded in the same way as in the standard protocol. The GRC protocol

differed from the standard protocol in that, after tone offset, there was a 2s "grace period," such that the first barpress made during this period triggered neither water reward nor an error signal. All subsequent barpresses during the ITI triggered the flashing light error signal and an ITI extension.

Animals trained on the standard protocol quickly adopted a strategy in which they used the tone onset as a cue to start barpressing and the tone offset as a cue to stop barpressing. The authors referred to this as a tone duration (tDur) strategy. Animals trained on the GRC protocol adopted a strategy where they used the tone onset as a cue to start barpressing and continued to do so past tone offset until they triggered an error signal. The authors referred to this as a tone-onset-to-error (TOTE) strategy. After training both groups underwent physiological mapping of A1. Although neither group had significant changes in representational area compared to naïve animals, animals trained on the GRC protocol did show signs of CS specific plasticity. Compared to the STD or naïve animals, animals trained on the GRC protocol had specific decreases in threshold and bandwidth in cells tuned to the CS frequency. This indicates that cells tuned to the CS frequency became more sensitive to a narrower range of frequencies if the animal used the TOTE strategy. This was the first indication that RP in A1 was linked with use of a TOTE strategy.

A follow-up study investigated the effect of motivation on learning strategy and RP (Bieszczad & Weinberger, 2010a). The authors trained two groups of rats on the GRC protocol. One group (moderate motivation group; ModMot), was maintained at ~85% of control body weight while the other (high motivation; HiMot) group was maintained at ~70% of control body weight. As expected, the HiMot group learned the task faster and had a higher level of asymptotic performance than the ModMot group. However, the HiMot group adopted a tDur

strategy while the ModMot group adopted TOTE. Representational plasticity was found for the ModMot group but not the HiMot group further demonstrating that RP was only found for animals that used the TOTE strategy.

In A1, TOTE use has been proposed to influence not only the induction of representational plasticity, but also its form. The GRC protocol encouraged use of the TOTE strategy by essentially making tone offset an ambiguous cue in that it triggered neither water reward nor error signal. Bieszczad and Weinberger (2010c) recognized that this resulted in low levels of TOTE use and designed an instrumental protocol that would produce higher levels of TOTE use. This protocol, the Free Period (FrP) protocol, encouraged animals to use TOTE by rewarding animals for the first barpress made following tone offset. This modification incentivized animals to ignore the tone offset and produced significantly higher levels of TOTE use compared to the GRC protocol. Animals trained on the FrP protocol did not show the changes in selectivity and sensitivity seen in animals trained on the GRC protocol. Instead, FrP animals had an increased representational area for the CS frequency compared to Naïve animals. Furthermore, CS area was significantly correlated with TOTE use for animals trained on the FrP protocols. Collectively these findings indicate that RP in A1 develops if an animal uses the TOTE strategy, and that the degree to which they use TOTE influences the form of plasticity in A1.

The results from Experiments #1 (chapter 2) and #2 (chapter 3) replicate and extend the findings of Bieszczad and Weinberger 2010c by demonstrating that TOTE use regulates not only the induction and form of plasticity but its maintenance as well. In Experiment #1 two groups of rats were trained on the three-tone discrimination (TTD) task, a discrimination version of the FrP protocols employed by Bieszczad and Weinberger (2010c). One group received a standard

amount of TTD training (group ST), i.e., until they reached a behavioral criterion defined by stable levels of high performance. The second group was trained to the same criterion but then underwent 2-3 weeks of overtraining (group OT). At criterion both groups used the TOTE strategy, but with overtraining the OT group came to replace TOTE with a more refined strategy, internalized TOTE (iTOTE). Representational plasticity was found in the ST group using TOTE but not in the OT group, who were no longer using TOTE. Furthermore, CS+ area was found to be positively correlated with TOTE use and negatively correlated with iTOTE use. Experiment #2 found that the lack of plasticity in the OT group could not be accounted for by the passage of time alone, demonstrating that the loss of plasticity in the OT group was related to the change in strategy use that occurred during overtraining.

Bieszczad and Weinberger (2010c) have proposed that the key factor linking TOTE use with A1 plasticity is that TOTE causes animals to attend to and utilize tone onset while ignoring tone offset. As onset and offset responses in A1 emerge from different synaptic inputs (Scholl, Gao & Wehr, 2010), TOTE use should strengthen onset synapses while weakening offset synapses either due to lack of use or possibly homeostatic mechanisms. Therefore, the negative correlation between iTOTE use and CS+ area observed in Experiment #1 may have arisen because the iTOTE strategy reflects that the animal has learned to use both tone onset and tone offset. In this case, tone onset would be the animal's cue to start barpressing while tone offset would be the animal's cue to only barpress one more time to obtain the free period reward. From this perspective, as long as animals adopt a strategy in which they are simultaneously reliant on tone onset and ignore tone offset, plasticity should develop in A1.

An alternative explanation for the relationship between TOTE and A1 plasticity is that cortical plasticity is induced during problem solving and that TOTE is used by animals who are still engaged in this process. Numerous reports have indicated that RP observed in sensory and motor cortical regions observed after a subject learns a task dissipates without negatively affecting their performance on the task (Reed et al., 2011; Takahashi et al., 2010; Yotsumoto, Watanabe & Sasaki, 2008; Ma et al., 2010; Tennant et al., 2012). The common conclusion drawn from these studies is that map plasticity is involved in learning a task, but not in the long term retention of skilled performance. This suggests that plasticity is present while an animal is solving a task, but not once the task has been solved. The observation that strategy use changes from TOTE to iTOTE during overtraining indicates that learning still occurs after criterion, suggesting that TOTE use occurs while animals are still engaged in problem solving.

In order to test between these hypotheses it is necessary to dissociate the TOTE strategy, defined by the way animals utilize cues, from its role during the course of problem solving. We accomplished this by overtraining a group of rats on the TTD task and then reinstated TOTE use by training them further on a modified version of TTD task. The Variable Free Period (VFrP) task was identical to the TTD task except that the number of free period rewards available during each CS+ trial varied from trial to trial (0,1, or 2). This forced the animals to again rely on triggering the error signal as a cue to stop barpressing. This contingency made TOTE the most optimal solution to the VFrP task in the same way that iTOTE was the most optimal solution to the TTD task. Therefore, if reliance on tone onset and the error signal is sufficient to induce A1 plasticity, then reinstating TOTE use with VFrP training should rescue plasticity after map renormalization. However, if TOTE is reinstated and no plasticity is found, that would indicate that TOTE use alone is not sufficient for plasticity. This result would suggest that the previously

identified relationship between A1 plasticity and TOTE use occurred not because of the particular arrangement of cues that define the TOTE strategy, but rather because TOTE was used during auditory problem solving.

Methods

Subjects

Male Sprague-Dawley rats (250-300 g, n = 6) from Charles River Laboratories (Wilmington, MA) were individually housed in a vivarium (temperature maintained at 22º C, 12/12 h light/dark cycle, lights on 7 am). Subjects were treated identically to those in Experiment #1. *Ad libitum* access to food and water was available before the onset of training. During training with water restriction, continuous access to water was restored on the weekends and supplements were provided after training sessions if necessary to maintain weight. All procedures were conducted with care to minimize pain or discomfort and were in accordance

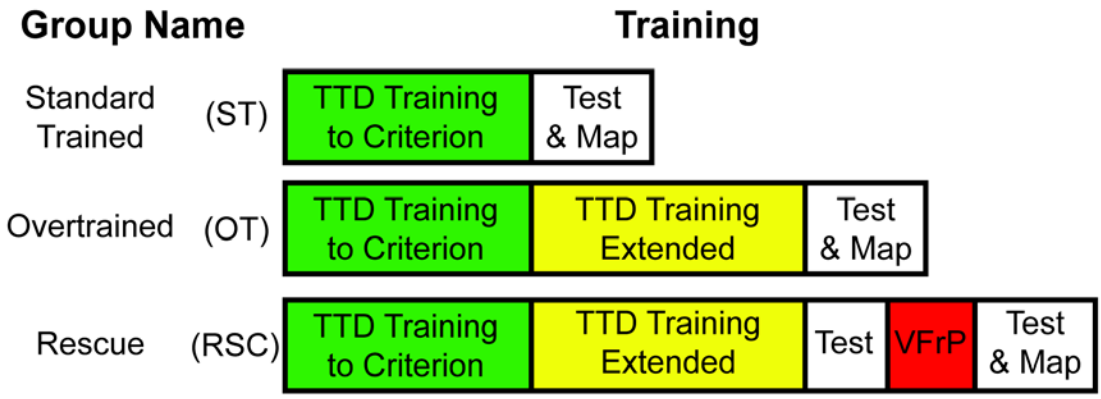


Figure 4.1: Experimental timelines for the rescue (RSC) group as well as the standard trained (ST) and overtrained (OT) groups described in experiment #1. After reaching criterion on the TTD task, the RSC group continued TTD training for 2-3 just as the OT group had. Following overtraining, the RSC group underwent the first of two stimulus generalization sessions to test the specificity of their learning about frequency. The next day they underwent the first of five sessions on the variable free period (VFrP) task. The day following the fifth VFrP session, the RSC group underwent their second stimulus generalization test. The two generalization tests were in place to determine whether training on the VFrP task affected the specificity of their learning about frequency. All animals underwent physiological mapping 2-3 days after the second generalization test. Training procedures for the ST and OT groups are described in chapter 2.

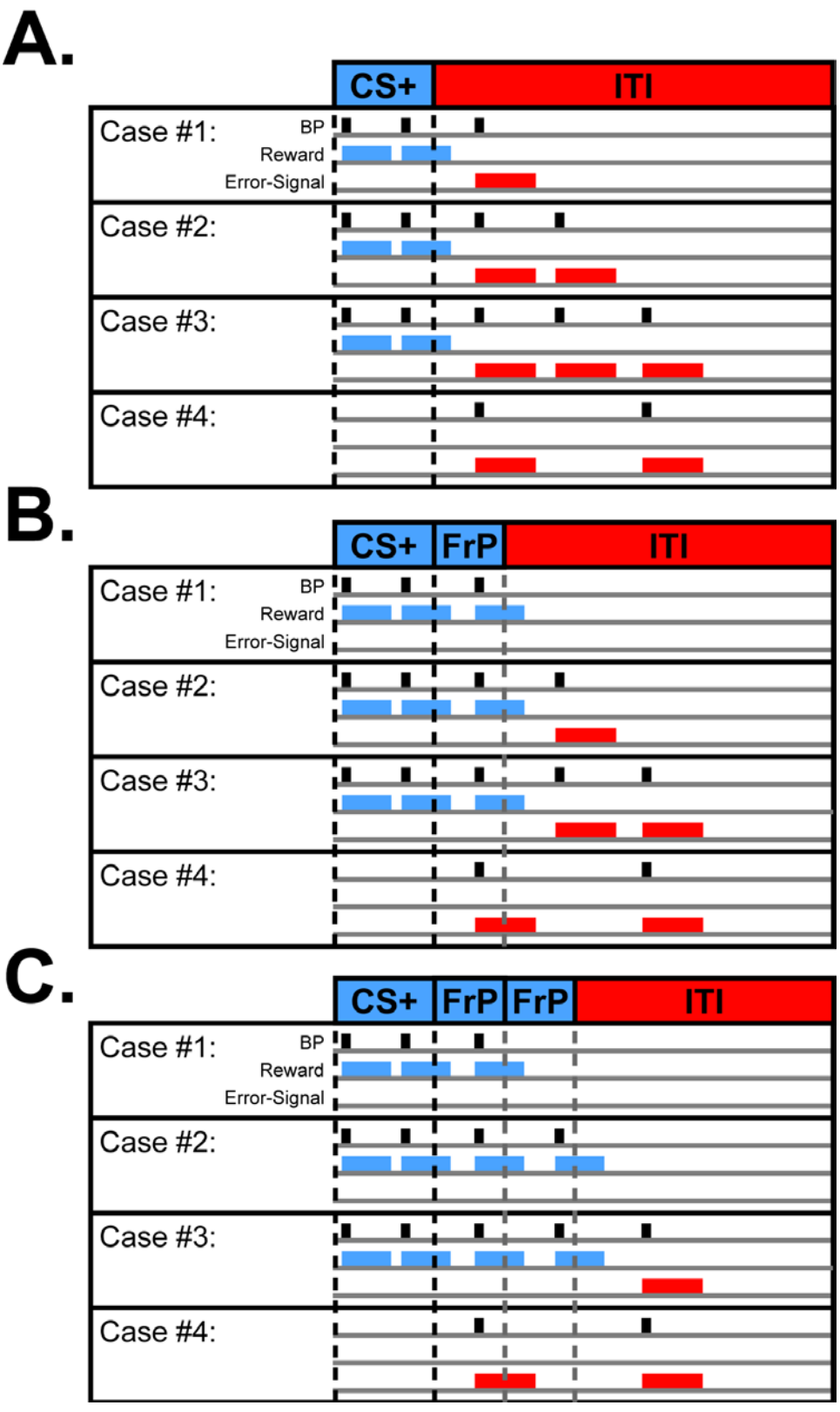
with the University of California, Irvine, Animal Research Committee and the NIH Animal Welfare guidelines.

Behavioral training

The training apparatus and stimuli were identical to those used in Experiments #1 and #2. Behavioral training and analysis methods were similar to those used in Experiments #1 and #2 except for the differences outlined below. Subjects in the Rescue (RSC) group were overtrained on the three tone discrimination task (TTD) in the same way as group OT from Experiment #1 (Figure 4.1). After they reached behavioral criterion on the TTD task, (three consecutive sessions during which the coefficient of variation was ≤ 0.10, where CV = standard deviation/mean of daily performance) RSC animals received extended training for at least two additional weeks. At the end of overtraining they then underwent a combined extinction/generalization session to determine the frequency specificity of their learning at that point.

The following day they began training on the variable free period (VFrP) task. Figure 4.2 depicts the different free period conditions and shows examples of different potential patterns of behavior during the VFrP task.. The VFrP task was designed to reinstate use of the TOTE strategy. It was identical to the TTD task with one key exception: in order to reinstate TOTE use, the VFrP task varied the number of rewards available during the free period following CS+ presentation. The animal was still required to barpress at least once during CS+ presentation in

Figure 4.2 (following page): The VFrP task was designed to encourage animals to again come to rely on triggering the error signal as a cue to stop responding during a trial. It accomplished this by changing the free period reward contingencies so that on any given CS+ trial, the free period could have 0, 1, or 2 rewards available. As there was no indication how many rewards were available on any given trial, the error signal was the only reliable indication that the animal had acquired all possible rewards on that trial. A-C shows the three different free period conditions (A) when none area available, (B) one and (C) two. The same four cases are used in A-C.


A.
CS+
ITI
Case #1:
BP
Reward
Error-Signal
Case #2:
Case #3:
Case #4:
B.
CS+
FrP
ITI
Case #1:
BP
Reward
Error-Signal
Case #2:
Case #3:
Case #4:
C.
CS+
FrP
FrP
ITI
Case #1:
BP
Reward
Error-Signal
Case #2:
Case #3:
Case #4:

order to trigger the free period, however the number of rewards available during the free period was varied from trial to trial during VFrP training. In the TTD task only one water reward was available during the free period while in the VFrP task, one water reward was available on average during the free period. The number of rewards was either 0, 1, or 2 on any given CS+ trial. All barpresses made after the final free period reward triggered the flashing light error signal and reset the ITI timer. No cues were presented to indicate how many rewards were possible during the free period, thus the only reliable indication that no further rewards were available was the error signal. This encouraged the animal to once again become reliant upon triggering an error signal to indicate when it should stop responding during a trial.

Each VFrP session was limited to 90 trials in order to keep the amount of tone exposure and reward rate roughly consistent between the VFrP and TTD protocols. The CS+ and CS- stimuli were the same as those used for in TTD (10s duration; CS+ = 5.0 kHz, Low CS- = 2.1 kHz, High CS- = 11.9 kHz; all at 70 dB). Each session consisted of 63 CS+ trials and 27 CS- in order to keep ratio of CS+ to CS- trials consistent at 70%. Trial order was randomized during each session with the only restriction that each free period condition (0, 1, or 2 rewards) occurred 21 times during a session.

VFrP training consisted of five daily sessions. The day after the fifth session each animal underwent a second generalization test to determine if VFrP had altered the frequency specificity of their learning.

Behavioral analysis

Performance and strategy measures were identical to those used in Experiment #1. Comparisons were made between the RSC group in the present experiment and the standard

trained (ST) and overtrained (OT) groups from Experiment #1 in order to infer whether renormalization had taken place in the RSC group before beginning VFrP training.

Neurophysiological recordings

Complete mapping of A1 was performed 2-3 days following the second extinction generalization session using the same methods outlined in Experiment #1. Identification of the primary auditory cortex and calculation of representational area were done using the same procedures and criteria as those used in Experiment #1.

Statistics

All behavioral and CF area measures were analyzed using ANOVA ($\alpha = 0.05$) and post hoc analyses were performed using t-tests with bonferroni α correction for multiple comparisons. Brain–behavior relationships were assessed using Pearson's correlations.

Results

Behavior leading up to VFrP protocol training

In order to determine whether reinstatement of TOTE behavior was sufficient to "rescue" A1 plasticity after map renormalization, we first had to determine whether renormalization had taken place. In experiment #1 we showed that animals trained for a standard amount of time (group ST) had RP but those who were overtrained (group OT) did not. With that in mind the RSC group underwent a similar amount of overtraining on the TTD protocol (RSC: 22.67 ± 2.27 sessions; OT: 26.7 ±1.27 sessions; $t_{(14)}=1.81$, $p=0.09$). A number of significant behavioral changes were observed to result from overtraining. To determine whether the RSC group had

undergone these changes as well, we compared their behavior during their final TTD session with the behavior of the ST and OT groups during their final TTD sessions.

We compared the three groups using a number of different behavioral measures (Figure 4.3). The groups were compared using 1-way ANOVAs and with post hoc t-tests where applicable. A significant group effect was found for task performance ($F_{(2,24)}$=20.03, p<0.005; Figure 4.3A), a reflection of a subject's efficiency at acquiring reward. Post hoc analysis

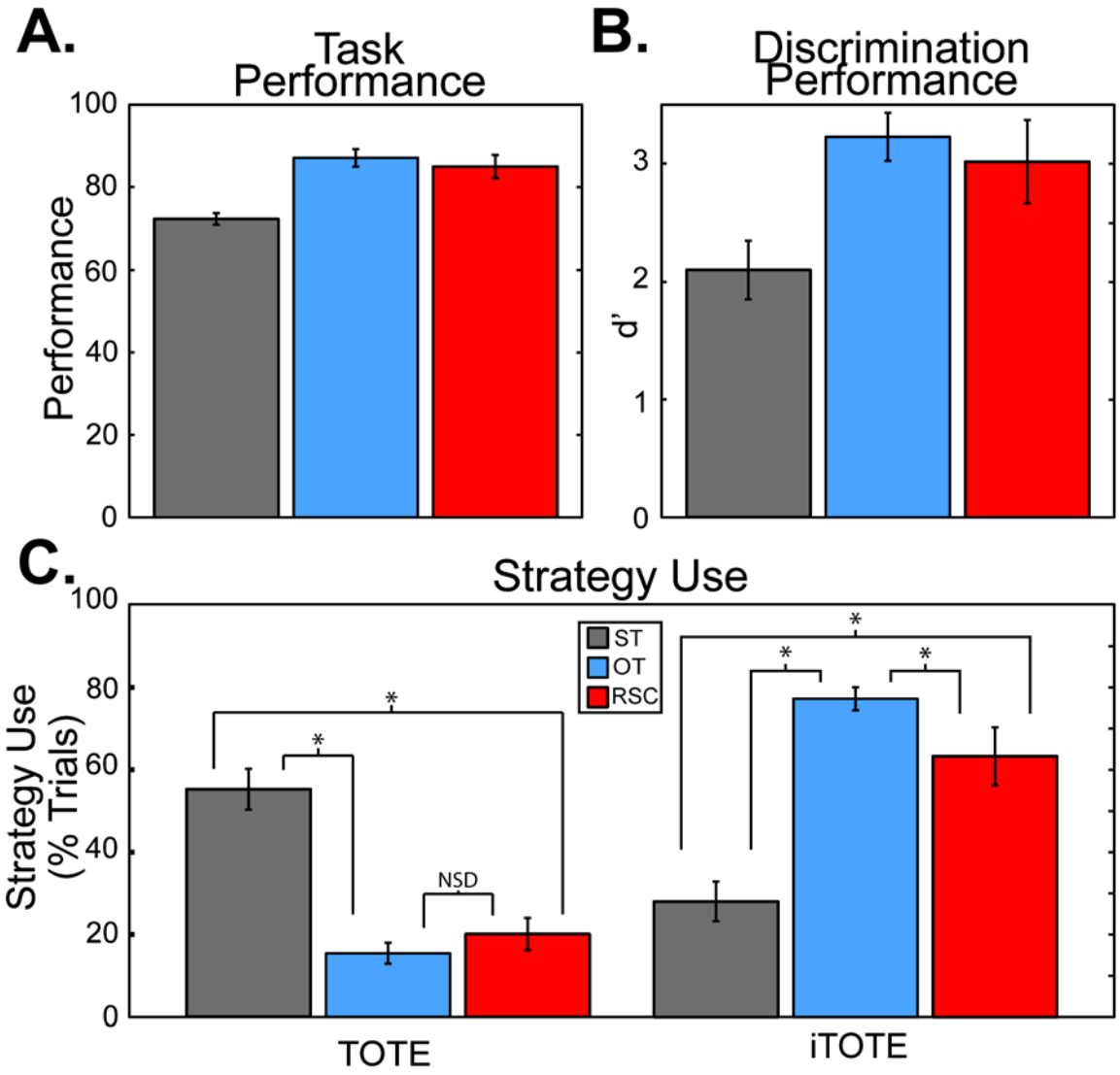


Figure 4.3: Comparison of A) task performance, B) discrimination performance and C) strategy use between the RSC, ST and OT groups during their respective final training sessions on TTD. The RSC animals were highly similar to the previous group OT. While the RSC group used iTOTE less frequently than the OT animals they had learned to replace TOTE use with iTOTE by the end of overtraining. Error bars = SE; * = p<0.05

revealed that performance was higher in the RSC ($t_{(15)}$=4.83, p<0.001) and OT ($t_{(19)}$=6.34, p<0.001) groups compared to the ST group while the RSC and OT groups were not significantly different from each other ($t_{(14)}$=0.64, p=0.53). Discrimination performance showed a similar result: a significant main effect of group was observed ($F_{(2,24)}$=6.92, p<0.01; Figure 4.3B) and post hoc analysis indicated that the RSC ($t_{(15)}$=2.29, p<0.05) and OT ($t_{(19)}$=3.63, p<0.01) had better discrimination performance that the ST group. Again, the RSC and OT groups were not significantly different ($t_{(14)}$ = 0.6, p=0.56). This shows that the RSC group were just as proficient as the OT group at the end of TTD training and both groups performed better than the ST group.

While the RSC group and the OT group were equally good at solving the TTD task, we also examined whether they solved the task in the same way, i.e. what strategy they employed. Significant group effects were found for TOTE ($F_{(2,24)}$=9.87, p<0.001; Figure 4.3C) and iTOTE use ($F_{(2,24)}$=3.41, p<0.001; Figure 4.3C). Post hoc analysis found that the ST group used the TOTE strategy much more frequently than the RSC ($t_{(15)}$=5.05, p<0.001) and OT ($t_{(19)}$=7.28, p<0.001) groups while the RSC and OT groups used iTOTE much more frequently that the ST group (iTOTE; RSC vs ST: $t_{(15)}$=4.51, p<0.001; OT vs ST: $t_{(19)}$ = 9.05, p<0.001). While the RSC and OT groups used TOTE at similar levels ($t_{(14)}$=1.11, p=0.28), the OT group used iTOTE more frequently than the RSC group did ($t_{(14)}$=2.34, p<0.05).

Even though the RSC group used iTOTE somewhat less frequently than the OT group did (RSC iTOTE = 63%±7% vs OT iTOTE = 77%±3% ; mean ±SEM), iTOTE was the dominant strategy used by the RSC group at the end of TTD training. During their final TTD training session, RSC animals used iTOTE significantly more frequently than they used TOTE. The high similarities between the RSC and OT group in terms of performance and strategy use indicates that the two groups were equally proficient at solving the TTD task and they relied on the same

strategy, i.e., iTOTE, to solve it. This indicates that by the end of TTD training the RSC group had undergone strategy refinement and most likely, map renormalization.

Behavior following VFrP protocol training

To determine how successful the VFrP task was at reinstating TOTE use, we compared the RSC group's strategy use during the final TTD training session with strategy use during the final VFrP session. For our measures of strategy use during the final VFrP session we focused on the CS+ trials where one free period reward was available. These trials serve as the best indication of how the VFrP protocol influenced strategy use as these trials are essentially identical to the TTD CS+ trials. Between the final TTD session and the final VFrP session TOTE use increased significantly ($t_{(10)} = 7.73$, $p<0.001$; Figure 4.4) while iTOTE use decreased ($t_{(10)}=6.39$, $p<0.001$; Figure 4.4). This demonstrates that the VFrP protocol successfully reinstated TOTE use for the RSC animals.

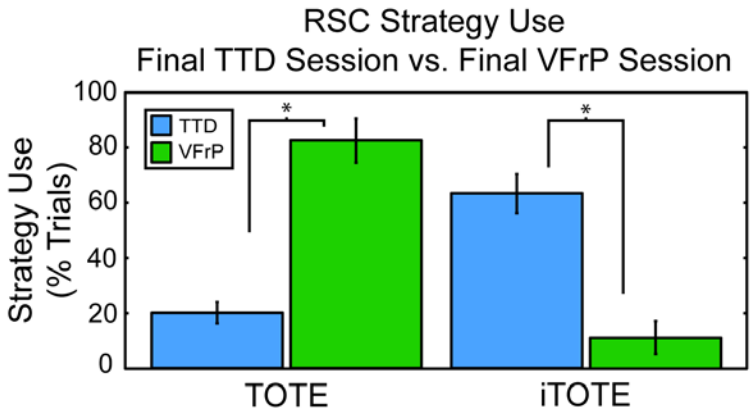


Figure 4.4: The VFrP protocol was highly successful at reinstating TOTE use. After only five training sessions the RSC group once again came to favor use of the TOTE strategy.

An example animal's behavior during VFrP training is shown in Figure 4.5. Each line represents a trial and an asterisk indicates when an animal barpressed during the trial. The trials are organized based on their free period reward condition and are arranged temporally. Note that while the trials are arranged relative to each other, the exact order of the trials was randomized during the training session meaning that adjacent lines do not

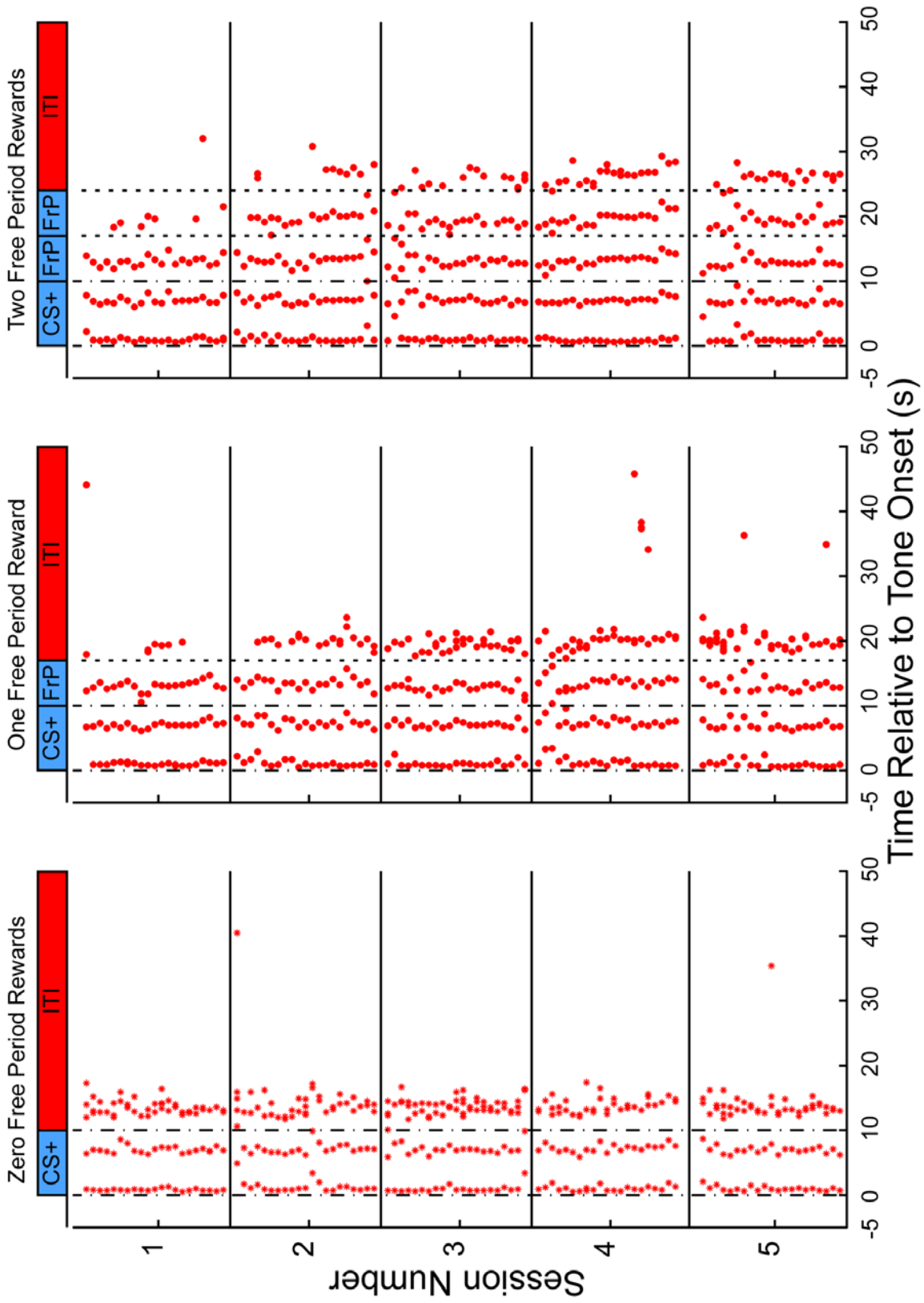


Figure 4.5: Example rasters of an RSC animal's behavior during VFrP training.

necessarily reflect antecedent or subsequent trials. The timing of CS onset and offset are marked by broken lines and the max duration of the free period reward windows (FrP-1: 17s; FrP-2: 17s and 24s) are marked with dashed lines. For this particular animal, substantial TOTE use was clearly evident as of the second VFrP session, as evidenced by the increased consistency with which a barpress occurred just after the end of the free period. Towards the end of the second session the animal begins to trigger the error signal during the 2 free period reward condition with consistency as well.

To determine how effective the VFrP protocol was at reinstating TOTE, we compared levels of strategy use in the RSC animals during their final VFrP session with strategy use of the ST and OT animals during their final TTD training sessions. One way ANOVAs revealed significant group effects for TOTE ($F_{(2,24)} = 48.13$, $p<0.001$) and iTOTE ($F_{(2,24)} = 67.75$, $p<0.001$) use. Post hoc analysis found that the three groups all displayed significantly different amounts of TOTE use. Both RSC ($t_{(14)}=10.59$, $p<0.001$) and ST ($t_{(19)}=7.28$, $p<0.001$) animals used TOTE more than OT animals. In fact, RSC animals used TOTE even more frequently than ST animals did ($t_{(15)} = 3.278$, $p<0.01$). The arrangement was reversed for iTOTE use. The highest levels of iTOTE use were found for OT animals, who had significantly greater iTOTE strategy use compared with ST ($t_{(19)}=9.4$, $p<0.001$) and RSC ($t_{(14)}=13.207$, $p<0.001$) animals. RSC animals had the lowest levels of iTOTE, even lower than the ST animals ($t_{(15)}=2.28$, $p<0.05$). These results indicate that the VFrP protocol very effective at reinstating TOTE use.

Frequency specificity of learning

The RSC animals underwent two stimulus generalization sessions to examine the frequency specificity of their learning. Comparison of the RSC group's generalization gradients

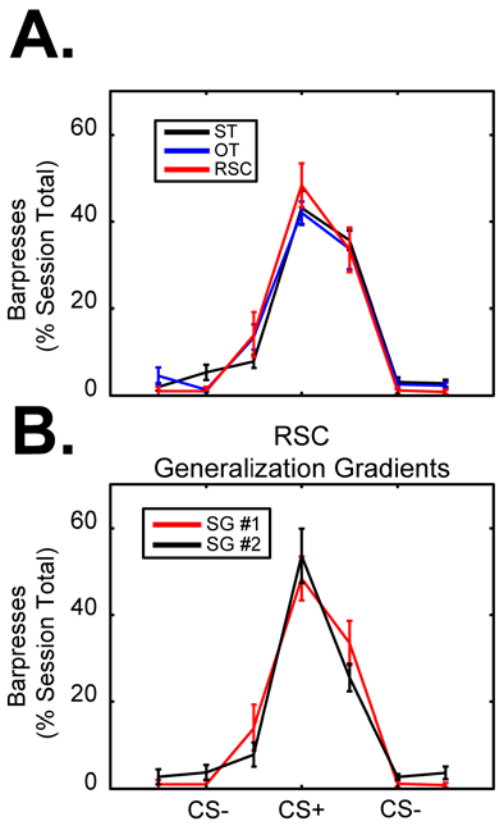


Figure 4.6: The RSC group learned the same amount about frequency as the ST and OT groups. (A) After TTD training the ST, OT and RSC groups had identical generalization gradients. (B) VFrP training did not change the RSC group's generalization gradient.

from the first generalization session with the ST and OT groups indicated that there were no differences between the three groups in the specificity of their learning about frequency (Frequency X Group: $F_{(12,168)}$, p = 0.54; Figure 4.6A). We also compared the RSC group's first and second generalization gradient to see if the VFrP training influenced the group's learning about frequency. There was no significant interaction term (Frequency X Group: $F_{(6,70)}$, p = 0.33; Figure 4.6B) indicating that the VFrP protocol did not influence the RSC group's learning about frequency.

Neural analysis

All RSC animals underwent physiological mapping of A1 2-3 days after their final stimulus generalization session. We used a two-way ANOVA (Frequency X Group) to compare the RSC animals' representational area for the stimulus generalization frequencies with the ST, OT and Naïve groups to determine whether reinstating TOTE use simultaneously reinstated RP in A1. The main effect of Group was not significant ($F_{(3,224)}$=0.67, p=0.57) but Frequency ($F_{(2,224)}$=23.21, p<0.001) and the Group X Frequency interaction ($F_{(18,224)}$=2.64, p<0.001) were significant (Figure 4.7). To determine what drove the interaction effect we ran one-way ANOVAs for each frequency to determine how the groups differed. This revealed significant group effects for only the CS+ ($F_{(3,32)}$=4.74, p<0.01) and the highest stimulus generalization frequency, 18.3 kHz, ($F_{(3,32)}$=6.23, p<0.01; all other

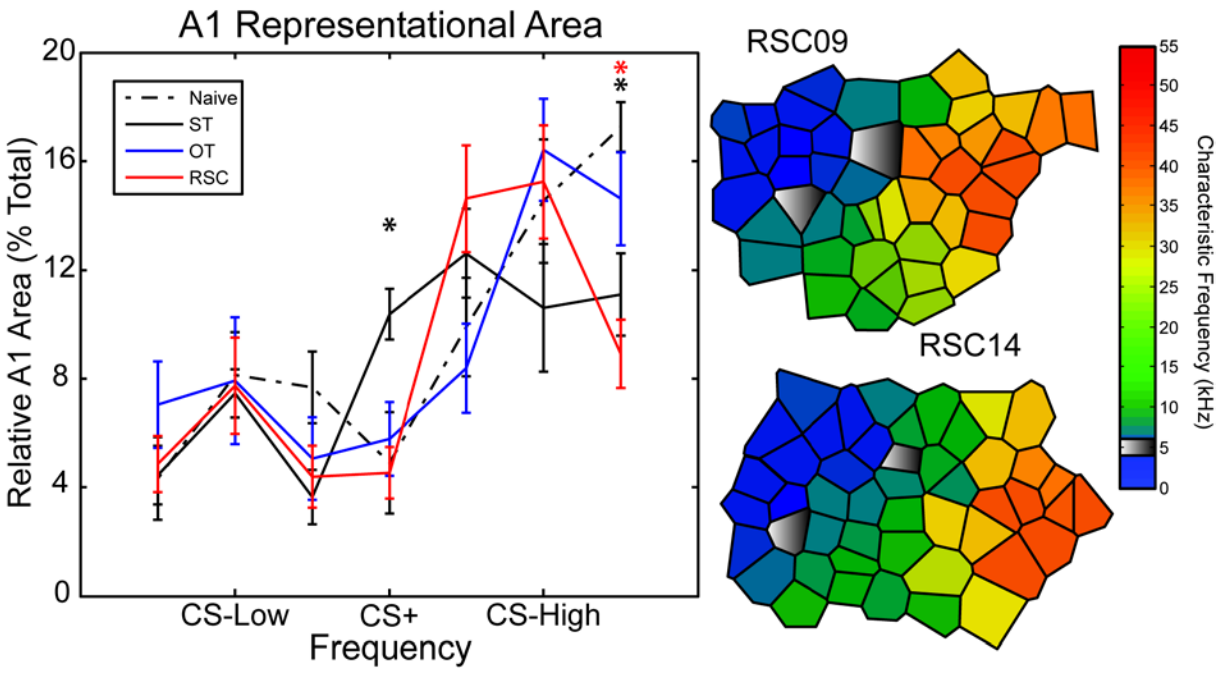


Figure 4.7: Group RSC representational area and example maps. The left plot depicts relative A1 area for the RSC, Naïve, ST, and OT groups. The RSC group did not have representational plasticity. The were no different from naive for all frequencies examined except the 18.3 kHz frequency which was significantly decreased compared to naive. The ST group also had a reduction at 18.3 kHz but they also had an increase at the CS+, a change that was not found in the RSC group. Example maps from two RSC animals are shown on the right.

frequencies $p>0.05$). Figure 4.7 depicts the mean representational area for each of the stimulus generalization frequencies for all of the groups alongside two example RSC group maps.

Post hoc analysis revealed that the CS+ group effect was driven by an increase in CS+ representational area for the ST group. The ST group had significantly greater CS+ area compared with Naïve ($t_{(18)}=2.94$, $p<0.01$), OT ($t_{(19)}=2.98$, $p<0.01$), as well as the RSC ($t_{(15)} = 4.26$, $p<0.001$) groups. Neither the OT ($t_{(17)}=0.41$, $p=0.69$) nor the RSC ($t_{(13)}=0.16$, $p=0.88$) groups were significantly different from Naïve animals, nor were they different from each other ($t_{(14)}=0.69$, $p=0.50$). This shows that the RSC group did not develop an increased area for the CS+ frequency, even though they used TOTE at even higher levels than the ST animals did.

Post hoc analysis for the 18.3 kHz frequency revealed that the effect was driven by changes in ST and RSC groups compared with the Naïve group. Both the ST ($t_{(18)} = 3.45$, $p<0.01$) and RSC ($t_{(13)} = 5.92$, $p<0.001$) groups had significantly smaller 18.3 kHz area compared to naïve. The OT group did not significantly differ from Naïve ($t_{(17)} = 1.39$, $p=0.18$). On average the ST group had less 18.3 kHz area than OT, and more than RSC but neither of these comparisons were significant (vs OT: $t_{(19)} = 1.62$, $p=0.12$; vs RSC: $t_{(15)} = 1.02$, $p=0.33$). The RSC group did have significantly less 18.3 kHz area than the OT group ($t_{(14)} = 1.49$, $p<0.05$). While the change at 18.3 kHz was common between the ST and RSC groups, the lack of a concurrent, specific expansion of CS+ representational area demonstrates that TOTE use is not sufficient to induce representational plasticity.

Even though reinstatement of TOTE did not reproduce an expansion of CS+ representational area, CS+ representational area was still correlated with strategy use. We correlated each animal's levels of TOTE and iTOTE use during their final VFrP training session with their relative A1 areas for each of the stimulus generalization frequencies. Across all the representational areas examined, the only significant correlations were between TOTE/iTOTE use and CS+ area (all other correlations $p>0.05$). In the first experiment, CS+ area was correlated with TOTE and iTOTE use as well (see Chapter 2). Note that in this case, the directions of the relationships are opposite what was found before. For the RSC animals, CS+ representational area was positively correlated with iTOTE use ($r=0.82$, $p<0.05$; Figure 4.8B) and negatively correlated with TOTE use ($r=-0.86$, $p<0.05$; Figure 4.8A).

These findings support the hypothesis that the previously observed relationship between TOTE and A1 plasticity was observed because TOTE use occurs during auditory problem solving. To further explore this idea we combined the RSC, ST and OT groups to determine

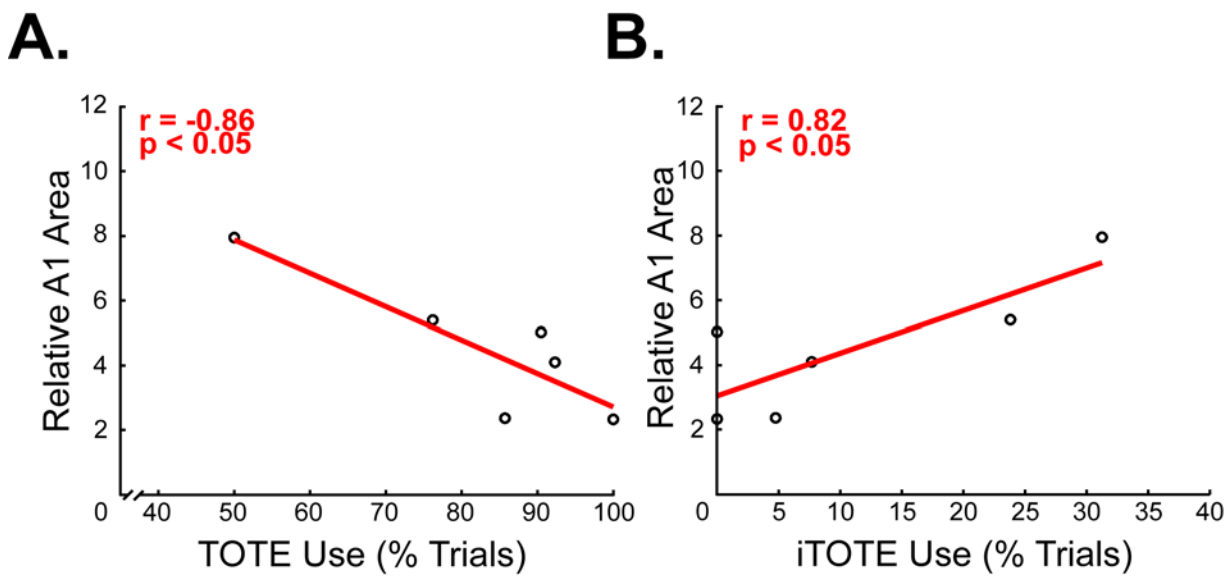


Figure 4.8: While the RSC group did not have representational plasticity in the form of a CS+ area expanion, their observed levels of CS+ area were correlated with their use of the TOTE (A) and iTOTE (B) strategies during their final VFrP session. Note that here the directions of the relationships are reversed from what was observed in Chapter 2. Here, TOTE use is found to be negatively correlated with CS+ area and iTOTE use positively so.

whether a common correlation could be found for all groups. We organized the group data in two different ways. First, we examined whether levels of TOTE and iTOTE use were correlated with CS+ representational area across all the groups. When the groups were combined in this way neither TOTE ($r = 0.003$, $p = 0.99$; Figure 4.9A) nor iTOTE ($r = -0.16$, $p = 0.42$; Figure 4.9B) was found to be significantly correlated with CS+ area.

We also combined the groups based on whether the strategy they used reflected active problem solving or not. For this arrangement we use the term Learning Strategy to refer to a strategy that emerges during learning and whose use promotes further learning. In contrast, a Response Strategy emerges in response to learning but does not or cannot promote further learning. During TTD, TOTE is a Learning Strategy while iTOTE is a Response Strategy. During VFrP however, iTOTE is a Learning Strategy while TOTE is a Response Strategy. When we organized the groups based on their levels of Learning Strategy use (TOTE for the ST and OT groups; iTOTE for the RSC group) and Response Strategy use (iTOTE for the ST and OT

groups; TOTE for the RSC group) we found that CS+ area was significantly correlated with both (Figure 4.9 C&D). Learning Strategy use was positively correlated with CS+ area ($r = 0.59$, $p<0.005$; Figure 4.9C) while Response Strategy use was negatively correlated with CS+ area ($r = -0.66$, $p<0.001$; Figure 4.9D). As Learning Strategy use is high when animals are engaged in

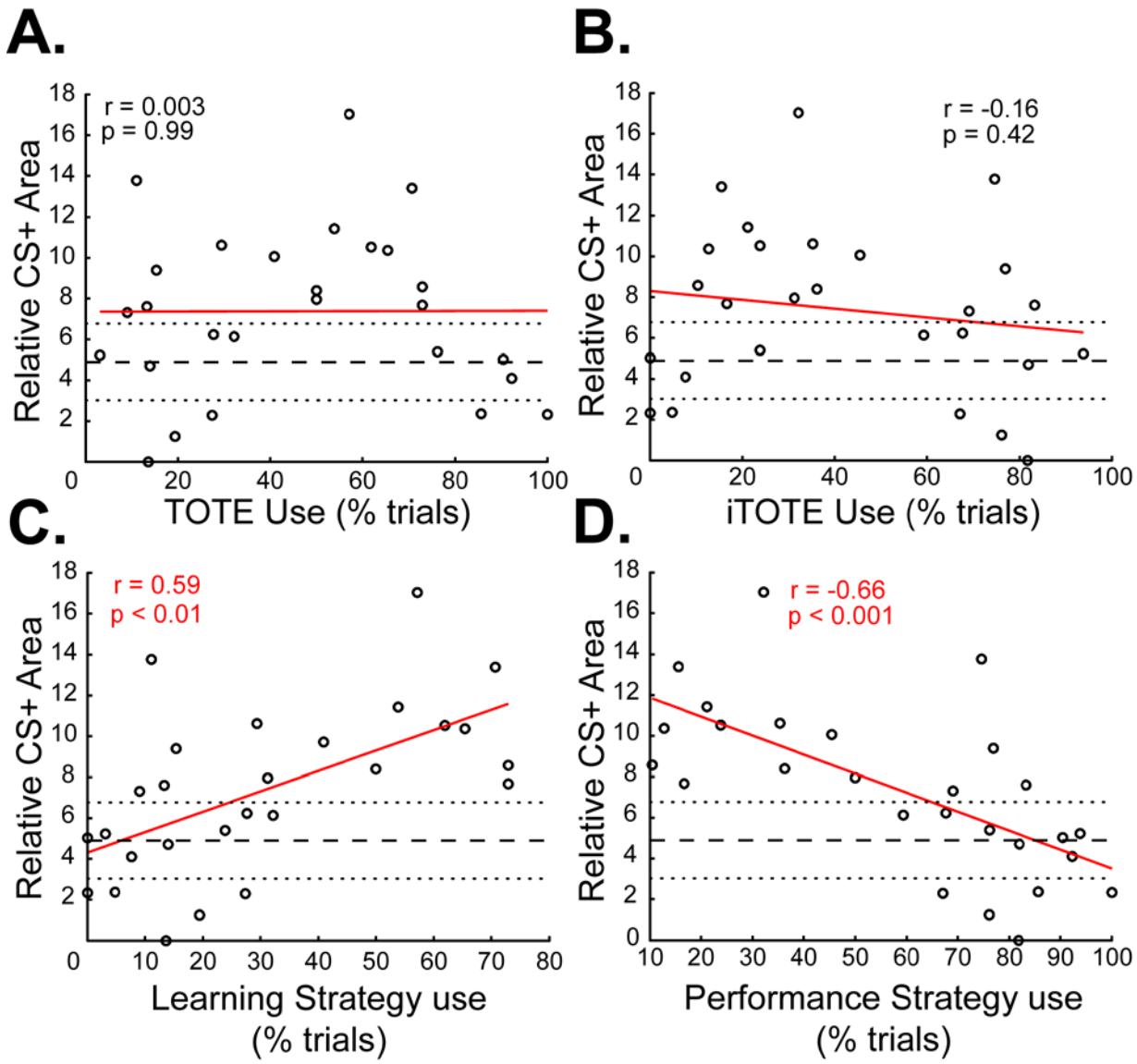


Figure 4.9: To further examine the relationship between strategy use and representational plasticity we grouped the RSC, ST and OT groups. When the groups' data was organized in terms of their use of the TOTE (A) and iTOTE (B) strategies, CS+ area was not correlated with strategy use. However, when we organized the groups' data based on whether the strategy the animal used reflected a Learning Strategy (C) or a Performance Strategy (D) we found that use of a Learning Strategy is positively correlated with CS+ area while use of a Performance Strategy negatively so.

problem solving, this supports the conclusion that RP in A1 is related to problem solving, not use of the TOTE strategy.

Discussion

The present experiment sought to better understand the relationship between the TOTE strategy and RP in A1. Previous studies have observed a relationship between TOTE use and A1 plasticity (Berlau & Weinberger, 2008; Bieszczad & Weinberger, 2010a,c). This relationship was also observed in experiment #1 (chapter 2). Animals trained on the three tone discrimination task (TTD) initially learned to solve it using the TOTE strategy and developed RP in the process. With overtraining, animals replaced the TOTE strategy with a more refined version of it, iTOTE, and map renormalization occurred. If renormalization occurred simply because animals stopped using TOTE then reinstatement of TOTE should rescue plasticity after renormalization. We overtrained a group of rats on the TTD task and then trained them on the variable free period (VFrP) task, a modified version of TTD designed to reinstate TOTE use. After five VFrP training sessions, TOTE use was successfully reinstated, however physiological mapping revealed that RP was not present. Map renormalization could not have occurred simply because animals stopped using the TOTE strategy, then, because TOTE use was still prevalent. Furthermore, it shows that TOTE use is not a sufficient condition for the induction of RP in A1.

While the RSC group did not have CS+ plasticity, we found that significant correlations between CS+ area and levels of TOTE and iTOTE use during their final VFrP training session were still present. Similar relationships were found in Experiment #1 except the direction of the relationships was reversed. In Experiment #1 TOTE use was positively correlated with CS+ area while iTOTE was negatively correlated with CS+ area. The reverse was found in the RSC group:

for them iTOTE was positively correlated with CS+ area while TOTE use was negatively correlated. To further explore this we combined the RSC group with the standard trained (ST) and overtrained (OT) groups from experiment #1. When these animals were grouped based on the levels of TOTE and iTOTE use they displayed during their final training sessions neither strategy was significantly correlated with CS+ area. We then organized the groups based on whether the strategy they used was a Learning Strategy or a Response Strategy. A Learning Strategy is a strategy adopted in the course of learning whose use promotes further learning. For the TTD task, TOTE is a Learning Strategy while in VFrP, iTOTE is a Learning Strategy. In contrast, a Response Strategy is one developed in response to learning whose use does not or cannot promote further learning. For TTD, iTOTE is a Response Strategy while in VFrP, TOTE is a Response Strategy. When the groups were organized along these lines we found that Learning Strategy use was positively correlated with CS+ area while use of a Response Strategy was negatively correlated with CS+ area.

Taken together, these results indicate that the previously observed relationship between A1 plasticity and the TOTE strategy was found because in those instances TOTE was a Learning Strategy. The two other tasks used in previous investigations of strategy use and A1 plasticity were the grace period (GRC) and free period (FrP) protocols. Both promoted TOTE use by discouraging the animals from using the tone offset as a cue to stop barpressing. The GRC protocol (Berlau & Weinberger, 2008; Bieszczad & Weinberger, 2010a) promoted TOTE use by making the period immediately after tone offset ambiguous. Barpresses during a 2s period immediately after tone offset triggered neither water reward nor error signal presentation. The FrP protocol (Bieszczad & Weinberger, 2010c) promoted TOTE use by providing animals the opportunity to barpress for one additional water reward after tone offset. For both protocols,

TOTE was most likely a Learning Strategy because the arrangement of reward and error signal contingencies should have resulted in strategy refinement had training been extended.

The FrP protocol used by Bieszczad and Weinberger (2010c) used the same free period reward contingencies as those used in the TTD task described in Experiments #1-3. Furthermore, the duration of FrP training was determined using the same behavioral criterion as the one used in the present set of experiments. Therefore, if training had been extended, it is reasonable to expect that animals trained on the FrP protocol would have adopted iTOTE just as the overtrained group (OT) from Experiment #1 did and most likely would have undergone map renormalization just like the OT group as well.

It is also reasonable to assume that strategy refinement would have also occurred if training had been extended for animals on the GRC protocol as well. While strategy use was not quantified with a microanalysis method such as we have in the current set of experiments, the macro level analyses employed by Berlau and Weinberger (2008) do suggest that strategy refinement did occur for animals trained on the GRC protocol. Animals trained on the GRC protocol significantly decreased their average number of ITI barpresses over the course of training. By the time animals were half-way through training on the grace period protocol they were performing less than one ITI barpress per trial on average (Berlau &Weinberger, 2008, Figure 3d). This means that on some trials, those animals avoided error signal generation entirely which would suggest they were already in the process of refining the TOTE strategy.

This new perspective on strategy use might help explain why plasticity was not found in animals previously described as relying on a tone duration (t-Dur) strategy. The t-Dur strategy is characterized by use of tone onset as a cue to start barpressing and the tone offset as a cue to stop barpressing. An animal who uses t-Dur only barpresses during the duration of the tone, as the

name implies. The t-Dur strategy was initially described in Berlau and Weinberger (2008) as the strategy adopted by animals trained on a standard instrumental protocol in which barpresses during the tone triggered reward and all barpresses during the ITI triggered the error signal. The other instance of the t-Dur strategy was described by Bieszczad and Weinberger (2010a) who found that highly-motivated animals trained on the GRC protocol adopted the t-Dur strategy, not TOTE. In both instances animals that used t-Dur did not have RP in A1. For both tasks, since water rewards are only available during the tone duration, t-Dur use would produce the most efficient pattern of responding during a trial. In this way it would not encourage any further learning and therefore would be considered a Response Strategy. Therefore, the absence of plasticity for animals that used the t-Dur strategy was not because A1 had failed to develop plasticity, but rather that A1 had undergone renormalization by the time animals were investigated for RP.

The demonstration that RP is related to use of a Learning Strategy indicates that such plasticity facilitates learning, but what kind of learning? There is a general tendency to assume that plasticity in sensory systems reflects perceptual learning. This viewpoint holds that plasticity in sensory systems functions to fine tune the neural processes required for stimulus detection and discrimination. This would account for the cycle of map expansion and renormalization that occurs initially during TTD training since animals have to learn about auditory stimuli and their meaning during this period. However, it has trouble explaining why CS+ area was still correlated after the RSC group underwent VFrP training.

The finding that CS+ area was still correlated with Learning Strategy use after VFrP training suggests that a second expansion-renormalization cycle occurred during VFrP training. This suggests that RP is not a reflection of perceptual learning, because the content of the

learning in the VFrP task is decidedly non-auditory. The only change from TTD to VFrP was free period reward contingency. No new cues were introduced and all the old cues and contingencies were identical, except those associated with free period reward. The animal is not learning new auditory information, so why should non-auditory learning affect plasticity in A1? The most likely explanation is that even though the learning is not explicitly auditory, the animal must determine how to best utilize the auditory information it already acquired in light of the change in reward contingency. Plasticity does not simply reflect the animal learning about a sensory cue, it reflects the animal learning about the sensory cue in relation to other sensory cues in its environment.

Chapter 5: Conclusions, General Discussion and Future Directions

Summary of Experiments: Strategy Refinement and Map Renormalization

Recent studies of representational plasticity in the primary auditory cortex (A1) identified a novel behavioral factor, learning strategy, which is intimately related with the induction of representational plasticity in A1 (Berlau &Weinberger, 2008). The present experiments sought to develop a better understanding of this relationship by investigating the role of learning strategy in the maintenance of representational plasticity.

Multiple reports have demonstrated that representational plasticity (RP) induced during learning dissipates with extended training (Reed et al., 2011; Takahashi et al, 2010; Yotsumoto, Watanbe & Sasaki, 2008; Ma et al., 2010, Tennant et al., 2012). In Experiment #1 we hypothesized that the loss of RP was caused by a change in strategy during overtraining. To test this we trained two groups of rats on the three tone discrimination task (TTD) which was designed to promote RP in A1. It did this in two ways; the inclusion of two CS- frequencies increased the specificity of learning about frequency, and the reward contingencies were arranged so as to encourage use of the TOTE strategy which has previously been associated with RP in A1 (Berlau & Weinberger, 2008; Bieszczad & Weinberger, 2010a,c). One group received a standard amount of training (group ST), i.e., their training stopped once they reached our behavioral criterion defined by the stability of their task performance. The other group was overtrained (group OT), i.e. their training continued for an additional 2-3 weeks after they reached criterion. On the day following their final TTD session, each animal underwent stimulus generalization to determine the specificity of their learning about frequency. They then underwent physiological mapping, 2-3 days after the generalization test, to determine whether RP was present. We also

developed a novel method of behavioral analysis in order to examine and quantify learning strategy use. By examining an animal's pattern of responses during a trial we were able to identify what cues the animals relied on during the trial and by extension what strategy they used.

When they reached criterion, animals in the ST and OT groups both favored the tone-onset to error (TOTE) strategy. The TOTE strategy is characterized by the animal's use of the tone onset as a cue to start responding and its reliance upon triggering an error signal as an indication of when to stop. During overtraining, TOTE use diminished and was replaced by a new, more refined strategy. The internalized TOTE strategy (iTOTE) was similar to TOTE in that it relied on tone onset as a cue to start responding, but it is distinguished from TOTE in that the animal relies upon an internal cue to stop responding before it triggers an error signal. During their final training sessions the ST group heavily favored TOTE while the OT group favored iTOTE. The stimulus generalization test given to every animal at the end of training revealed that overtraining did not influence the specificity of the two groups' learning about frequency. Therefore the main behavioral effect of overtraining on the OT group's behavior was that they replaced the TOTE strategy with the iTOTE strategy.

Physiological mapping of A1 revealed that animals in the ST group had RP in the form of an expanded CS+ representation in A1 while animals in the OT group did not. This indicates that map renormalization occurred during overtraining. The observation that strategy refinement also occurred during overtraining suggested that the two were related. When we examined whether changes in CS+ area were related to strategy use we found that area changes were positively correlated with TOTE use and negatively correlated with iTOTE use. This suggests

that map renormalization during overtraining resulted from the change in strategy use (or vice versa).

However, another possibility is that the plasticity dissipated simply due to the passage of time. In at least one instance, map renormalization was observed after training was discontinued for a period of time without any adverse effect on the animals' performance (Molina-Luna et al., 2008). Such a finding suggests that RP may only be stable for a set period of time, after which point it begins to decay. Experiment #2 addressed this possibility directly. A group of rats were trained in the same manner as the ST group from Experiment #1. Once they reached criterion, their training was discontinued for a period of 2-3 weeks, a duration comparable to the length of the overtraining in Experiment #1. If the loss of plasticity following overtraining was simply due to the passage of time, the long term maintenance (LTM) group should also show a comparable loss of RP. However, if the LTM group still had plasticity after their time off, that would support the idea that the renormalization was related to the change in strategy use that occurred during overtraining. Physiological mapping of the LTM group revealed that they indeed had RP, even with multiple weeks between their final two training sessions. The combined results from the first two experiments provide further evidence that TOTE is a critical factor influencing RP in A1, which is consistent with previous studies highlighting TOTE use as a key factor regulating the induction of plasticity in A1 (Berlau & Weinberger, 2008; Bieszczad & Weinberger 2010a). The present findings extend this relationship by demonstrating a role for TOTE in the maintenance of A1 plasticity as well.

Experiment #3 was designed to better determine why TOTE use is so closely tied to RP in A1. Bieszczad and Weinberger (2010c) proposed that TOTE use promoted plasticity in A1 because it caused animals to rely on tone onset and ignore tone offset. In doing so, use of the

TOTE strategy would preferentially strengthen synapses in A1 related to onset tuning while synapses related to offset tuning would weaken due to homeostatic mechanisms or simply disuse. Alternatively, TOTE and A1 plasticity may both be related to auditory problem solving. Experiment #1 found that TOTE is replaced with iTOTE during overtraining which indicates that an animal using TOTE is still learning about their environment. This raises the possibility that the relationship between TOTE and A1 plasticity has been observed because they both occur while animals are engaged in learning and problem solving. To test between these two alternatives, we overtrained a group of rats on the TTD task and then trained them on a task designed to reinstate TOTE use. If use of the TOTE strategy is sufficient to produce plasticity, reinstating TOTE use should rescue A1 plasticity after map renormalization.

The rescue (RSC) group was overtrained on the TTD task in a similar manner as the OT group from Experiment #1. After overtraining was finished, RSC animals then underwent five training sessions in the variable free period task (VFrP). This task was identical to TTD except that the number of free period rewards varied from trial to trial between 0-2. This change encouraged animals to use TOTE again because it made the error signal the most reliable indication that no more rewards were available during a trial. The variable free period (VFrP) protocol was highly successful at reinstating TOTE use. However, after VFrP training the RSC group did not have RP in the form of increased CS+ representational area. This demonstrates that TOTE use is not sufficient to induce RP after map renormalization.

While the amount of CS+ representational area in the RSC group was not significantly greater than Naïve levels, it was still correlated with TOTE and iTOTE use during the RSC group's final VFrP training session. In Experiment #1 we observed that CS+ area was positively correlated with TOTE use and negatively correlated with iTOTE use. In the RSC group, the

direction of the relationship was reversed. CS+ area for the RSC group was positively correlated with iTOTE use and negatively correlated with TOTE use. While these results appear to be contradictory, they are in fact revelatory.

We propose that learning strategies—as determined by the specific collection of cues an animal uses to guide its behavior—can be classified into two categories depending on the training context in which those strategies are used. Strategies that are used during learning that promote further learning through their use are classified as Learning Strategies. In contrast, strategies adopted in response to learning that do not or cannot promote further learning are classified as Response Strategies. During TTD training, TOTE is a Learning Strategy while iTOTE is a Response Strategy. In VFrP, iTOTE is a Learning Strategy while TOTE is a Response Strategy. Therefore, the RSC group had a positive correlation between CS+ area and iTOTE use because iTOTE is a Learning Strategy and the correlation between CS+ area and TOTE was negative because TOTE is a Response Strategy in VFrP.

To further explore this relationship we combined the RSC with the ST and OT groups from Experiment #1. When the groups were combined based on their levels of iTOTE and TOTE use during their final training session neither TOTE nor iTOTE was significantly correlated with CS+ area. When the groups were instead combined based on Learning Strategy use and Response Strategy use, we found that CS+ area was positively correlated with Learning Strategy use and negatively correlated with Response Strategy use. This supports the hypothesis that the relationship between TOTE and A1 plasticity resulted from their common relationship with learning and problem solving. Taken together, these experiments provide new insight into RP.

Renormalization and Plasticity

A common conclusion drawn from reports of map renormalization is that they indicate that map plasticity is involved in learning but not memory (Kilgard, 2012). This conclusion, however, ignores numerous findings that demonstrate a relationship between RP and memory. Artificial induction of plasticity not only influences learning, it can also produce behavioral memory (Bieszczad, Miasnikov & Weinberger, 2013; Weinberger et al., 2013). Map plasticity is also correlated with memory strength as measured by resistance to extinction (Rutkowski & Weinberger, 2005; Bieszczad & Weinberger, 2010a). The observation that map plasticity can dissipate without producing a catastrophic drop in performance demonstrates that map plasticity is not the substrate underlying the performance of that task. It does not mean that map plasticity does not serve as a substrate for memory; it simply rules out one possibility. There are multiple functional benefits to neural processing and representation that would arise from an increased neural population activated by CS presentation. It would increase the number of neurons synchronously activated by the CS, which would increase A1's ability to drive downstream targets. Such facilitation may in turn may promote further plasticity downstream from A1's initial target region. This kind of polysynaptic plasticity would allow A1 to influence regions or networks it otherwise would not have access to. In a computational sense, an increased number of cells activated by the CS would enable higher fidelity information transfer to other regions because the increase in neurons would provide A1 with a higher bandwidth to communicate with other regions. Similarly, the population increases would allow for a greater amount of information to be represented within the population itself. As the population of cells responsive to the CS increases, the number of potential activity patterns and/or network states that population could produce increases exponentially.

The only model that explicitly addresses the issue of map renormalization is the Expansion-Renormalization model proposed by Kilgard (2012; see also Reed et al., 2011). Kilgard proposes that the function of the expansion-renormalization cycle is to select and strengthen the best circuit to perform a task. He likens the process to Darwinian evolution. Expansions increase the pool of potential circuits is that could be used to solve the task after the best circuit is selected, the expansion dissipates and the best circuit is strengthened. Unfortunately, Kilgard fails to provide testable predictions of how his model will behave under different circumstances.

Lingering plasticity: failures of detection and expression

All examinations of A1 map renormalization have examined plasticity in anesthetized animals outside of the training context. This leaves open the possibility that RP might still be present if investigated in awake, behaving animals. If this were the case, map renormalization would reflect a change in the factors regulating the expression of plasticity rather than a reduction in plasticity itself. This kind of change would dramatically reduce the increased metabolic costs associated with a map expansion by restricting it to instances where such caloric expenditures are beneficial to the animal. This would suggest that the degree of neuronal contextualization depends upon how contextually specific the learning is that results in plasticity.

Selfless plasticity: promoting plasticity elsewhere

The general idea that map plasticity is involved in learning but not the storage of mnemonic changes suggests that it may function to direct plasticity in other regions. To consider how such a process might work, consider two target regions downstream of A1: in this case, the ventral striatum (VS) and the secondary auditory cortex (A2). A recent study by Znamenskiy

and Zador (2013) found that manipulation of the auditory corticostriatal pathway was sufficient to bias an animal's decision on an auditory discrimination task. The VS is the main target of the corticostriatal projections originating from auditory cortex and receives projections from both A1 and A2 (McGeorge & Faull, 1989). A2 has been proposed to be a storage site for long term memories based on the finding that lesions produce impaired remote, but not recent memories (Sacco & Sacchetti, 2010).

Putting aside the substantial differences in training procedures (Znamenskiy & Zador, 2013: instrumental auditory discrimination; Sacco & Sacchetti, 2010: auditory classical fear conditioning), the simple network described above provides a plausible substrate to describe how A1 may influence the storage of long term behavioral memories while not being a critical site of storage. In this example the function of A1 plasticity is to strengthen the corticostriatal projections originating in A2. To this end, an expansion of representational area within A1 could accomplish this in multiple ways. One possibility is that A1 expansions would allow A1 to induce the plasticity through polysynaptic facilitation. By directly activating A2 and VS, synaptic plasticity would develop at the A2-VS synapse, provided the relative timing of A2 and VS is right. Since A1 was coincidentally active it would also develop synaptic plasticity just as A2 does. However, since the plasticity induced at the A1-VS synapses was induced using cells acquired from an expansion, once A1's tonotopic axis renormalizes A1 would no longer be able to activate the A1-VS synapses in the same way. Once plasticity dissipates in A1, the strengthened A2-VS pathway would not be affected.

A1 may not need to use brute force to strengthen the connection between the other structures; in theory it could manipulate the connection in more subtle ways. Increased tonic activation would raise a target cell's membrane potential such that subthreshold synaptic activity

would become suprathreshold. Alternatively, if A1 activated inhibitory cells it could modulate the output of particular principal cells. This list is by no means exhaustive but rather is intended to offer some possible methods by which activity—and, by extension, plasticity—could be modulated by the activities of a third party.

Renormalization and pathology

While the above treatments of plasticity focus on potential benefits, plasticity is not always beneficial. Map plasticity has also been linked with clinical pathologies such as focal dystonia (Elbert et al., 1998), phantom limb syndrome (Flor et al., 1995) and tinnitus (Mühlnickel et al., 1998). These pathological conditions may result from a failure in the normal process of expansion and renormalization.

Future Directions

The following experiments all aim to further refine understanding of the relationship between Learning Strategy use and RP. Using the broad categories of Learning and Response Strategies as a foundation, the following experiments aim to further develop this conceptual distinction and better understand the functions of representational plasticity.

Primary vs. secondary expansion-renormalization cycles

The observation that the CS+ area was correlated with strategy use in the rescue (RSC) group from Experiment #3 suggests that the RSC group underwent a secondary expansion-renormalization cycle when they first began VFrP training. This possibility merits follow-up investigation because of the potential implications of such a finding. Demonstration that an expansion is present shortly after the animals begin VFrP training would strongly support the

proposal that RP is related to use of a Learning Strategy. Furthermore, it would suggest that RP is induced when previously acquired information needs to be used in light of new learning, even when the content of the new learning is of a different sensory modality.

It should be noted here that iTOTE is only a Learning Strategy in the VFrP task when it is in the process of being refined into TOTE. Before that point, the status of iTOTE is unclear. Use of iTOTE during VFrP should promote learning because use of iTOTE during the CS+ trial where no free period reward is available should violate the animal's expectations by triggering an error signal of unexpected water reward. If RP is engaged during Learning Strategy use then an expansion should be observed in animals when they first start transitioning from iTOTE into TOTE during VFrP training.

The objectivity of Response Strategies

Response Strategies are defined as strategies that do not or cannot produce learning. Inherently, this definition implies that there are two sub-categories of Response Strategies: objective Response Strategies that cannot produce new learning, and subjective Response Strategies which do not produce learning. The Response Strategies discussed up until this point, e.g. iTOTE during TTD, TOTE during VFrP, and t-Dur during standard and grace protocols, are examples of objective Response Strategies. Each of these strategies is highly refined and optimized for the particular protocol in which they are developed and in that way cannot produce learning.

A subjective Response Strategy is a strategy that does not produce new learning. To put it another way, a subjective Response Strategy is a strategy that should be a Learning Strategy but for some reason fails to promote learning. Instead of refining the strategy into another Learning

Strategy or an objective Response Strategy, animals using a subjective Response Strategy perseverate and continue to use the same strategy over and over. As all of the Response Strategies observed in the current set of experiments are objective Response Strategies, it is unclear whether use of a subjective Response Strategy would also lead to map renormalization or if that is only a characteristic of objective Response Strategies.

Possible alternative explanations of renormalization

Future studies of map renormalization should address alternative possible causes of renormalization. The most obvious possibility is that map renormalization reflects a failure in the expression of plasticity under anesthesia. Another possibility is that the expression of plasticity becomes contextually gated with extended training.

Typically, the tonotopic map of A1 is investigated in the middle cortical layers, III & IV. This opens up the possibility that map plasticity is expressed differentially within the cortical layers. As the different cortical layers have distinctive patterns of afferent and efferent projections, it is possible that the other cortical layers are differentially sensitive to the induction and renormalization of plasticity.

Neuromodulatory regulation

Future studies should investigate the potential roles for acetylcholine (ACh) and dopamine (DA) in the induction and maintenance of RP. Tone presentation paired with either nucleus basalis stimulation (Bakin & Weinberger, 1996; Bieszczad, Miasnikov & Weinberger, 2013), or ventral tegmental area stimulation (Bao, Chan & Merzenich, 2001) is sufficient to induce specific plasticity for the paired tone in A1. Dopamine (Stark & Scheich, 1997) and ACh levels (Butt et al., 2009) increase in the auditory cortex as animals are engaged in auditory

learning. Indirect evidence suggests that DA (Schultz, Apicella & Ljungberg, 1993; Ljungberg, Apicella & Schultz, 1992) and ACh (Orsetti, Casamenti & Pepeu, 1996) levels return back to baseline after extensive training. The dynamics of DA and ACh suggest they may serve as the mechanism underlying the induction and maintenance of representational plasticity.

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