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Learning Strategy Trumps Motivational Level In Determining Learning-Induced Auditory Cortical Plasticity

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Abstract

Associative memory for auditory-cued events involves specific plasticity in the primary auditory cortex (A1) that facilitates responses to tones which gain behavioral significance, by modifying representational parameters of sensory coding. Learning strategy, rather than the amount or content of learning, can determine this learning-induced cortical (high order) associative representational plasticity (HARP). Thus, tone-contingent learning with signaled errors can be accomplished either by (1) responding only during tone duration ("tone-duration" strategy, T-Dur), or (2) responding from tone onset until receiving an error signal for responses made immediately after tone offset ("tone-onset-to-error", TOTE). While rats using both strategies achieve the same high level of performance, only those using the TOTE strategy develop HARP, *viz*., frequency-specific decreased threshold (increased sensitivity) and decreased bandwidth (increased selectivity) (Berlau and Weinberger, 2008). The present study challenged the generality of learning strategy by determining if high motivation dominates in the formation of HARP. Two groups of adult male rats were trained to bar-press during a 5.0 kHz (10s, 70 dB) tone for a water reward under either high (HiMot) or moderate (ModMot) levels of motivation. The HiMot group achieved a higher level of correct performance. However, terminal mapping of A1 showed that only the ModMot group developed HARP, *i.e*., increased sensitivity and selectivity in the signal frequency band. Behavioral analysis revealed that the ModMot group used the TOTE strategy while HiMot subjects used the T-Dur strategy. Thus, type of learning strategy, not level of learning or motivation, is dominant for the formation of cortical plasticity.

Keywords

instrumental conditioning; memory; neurophysiology; primary auditory cortex; representation

1. INTRODUCTION

The cerebral cortex is acknowledged to be a major site of memory storage. A deeply ingrained and generally implicit assumption is that learning-related cortical plasticity reflects the amount of learning: the greater the learning, the greater the plasticity. However, to the best of our knowledge, this assumption has never been evaluated empirically. The current study directly speaks to this issue by training two groups of rats to solve an auditory-

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cued task to different degrees of correct performance and evaluating the amount of learning-related plasticity in the primary auditory cortex (A1). Different amounts of motivation were used to control the levels of asymptotic performance so that a highly-motivated group (HiMot) was expected to exhibit significantly better performance than a moderately-motivated (ModMot) group. The assumption would be supported by finding greater physiological plasticity in A1 in the HiMot group than in the ModMot group. However, the reverse finding would both challenge the assumption and require a novel explanation of factors that are critical for the magnitude of the formation of cortical plasticity during learning.

The metric of cortical plasticity employed in the present study is referred to as “high order associative representational plasticity” (HARP). It transcends measures of the development of plasticity (usually increased neural response) to signal sensory stimuli, such as conditioned stimuli, which can provide information only about the changes in neural responses for cue stimuli themselves (see Weinberger, 2007). As memories comprise information about specific events, *i.e*., they have *content*, an alternative approach is needed to determine the extent to which the plasticity constitutes the representation of specific acquired information.

A solution has been to use a hybrid experimental design in which sensory physiology methods are combined with standard behavioral training protocols. For example, it is possible to determine the effects of a learning experience on the receptive fields or the topographic organization of sensory cortex of the signal cue (reviewed in Weinberger, 2007). This approach provides such information by revealing the effects of a learning experience upon the *sensory dimension of the cue*. For example, in the case of a tone, the hybrid design would reveal the extent to which behavioral learning is accompanied by a systematic change in the representation of acoustic frequency, including the frequency of *e.g*., the conditioned stimulus (CS). An increase in response to all frequencies would indicate that learning has simply enhanced the processing of tones in general; there would be no change in the *representation of the frequency dimension*. In contrast, plasticity that is specific to the signal frequency would reveal a selective modification of the representation of frequency, in which the processing and representation of the signal cue have become favored. Associatively induced changes in the representation of a stimulus dimension would constitute HARP. In short, findings of HARP in A1 would show that sensory processing and representation, even at the early stage of primary sensory cortex, are systematically modified; in short, the conceptual distinction between sensory processing and learning/memory does not apply to primary sensory cortex.

Extensive studies of A1 during the past 15-20 years have characterized HARP. It was first demonstrated that conditioning with a tone produces a systematic shift in the frequency receptive fields of neurons, including a shift in tuning such that the frequency of a tonal CS becomes the new best frequency (BF) (Bakin and Weinberger, 1990). This type of finding, replicated in many laboratories, has revealed that associative learning is accompanied by a specific facilitation of the processing and representation of sounds that have gained behavioral relevance (reviewed in Edeline, 1999; Ohl and Scheich, 2005; Suga and Ma, 2003; Weinberger, 2007).

Further studies showed that HARP is a better candidate for a substrate of memory than far more commonly reported cortical neurophysiological correlates of learning because it possesses all of the major features of associative memory. In addition to being associative (Bakin and Weinberger, 1990), it is highly specific (within a fraction of an octave of the reinforced frequency: Edeline and Weinberger 1993), rapidly-acquired (within 5 trials: Edeline, Pham and Weinberger, 1993), consolidates (become stronger over hours and days:

Galvan and Weinberger, 2002) and exhibits long-term retention (tracked for up to 2 months: Weinberger, Javid and Lepan, 1993). Additionally, HARP develops in all studied tasks, including one-tone and two-tone discriminative classical conditioning and instrumental conditioning (Bakin, South and Weinberger, 1996; Blake, Strata, Churchland and Merzenich, 2002; Edeline and Weinberger, 1993) as well as specialized tasks (Fritz, Shamma and Elhiliali, 2005a; Polley, Steinberg and Merzenich, 2006), across motivational valence (*e.g.*, Bakin and Weinberger, 1990; Hui, Wong, Chavez, Leon and Weinberger, 2008; Kisley and Gerstein, 2001). Moreover, in addition to acoustic frequency, HARP develops for all acoustic parameters that have been used as cues (frequency, see above, stimulus level: Polley, Heiser, Blake, Schreiner and Merzenich, 2004; rate of tone pulse: Bao, Chang, Woods and Merzenich, 2004; envelope frequency of FM modulated tones: Beitel, Schreiner, Cheung, Wang and Merzenich, 2003; tone sequence: Kilgard and Merzenich, 2002; and auditory localization: Kacelnik, Nodal, Parsons and King, 2006). Furthermore, HARP develops during associative learning in all investigated taxa: big brown bat [*Eptesicus fuscus*] (Gao and Suga 1998; 2000), cat [*Felis catus*] (Diamond and Weinberger, 1986), guinea pig [*Cavia porcellus*] (Bakin and Weinberger, 1990), owl monkey [*Aotus trivirgatus boliviensis*] (Recanzone, Schreiner and Merzenich, 1993), rat [*Rattus rattus*] (Kisley and Gerstein 2001; Hui et al., 2008) and also in humans (Molchan, Sunderland, McIntosh, Herscovitch, and Schreurs, 1994; Morris, Friston and Dolan, 1998; Schreurs, McIntosh, Bahro, Herscovitch, Sunderland, and Molchan, 1997).

In addition to seeking HARP in the HiMot and ModMot groups trained with different levels of motivation, we also assessed the *learning strategies* that the animals used to solve the task at hand; they were required to bar-press (BP) in the presence of a tone but to withhold responses during silence. The rationale for also taking into account learning strategy is based on recent findings in which groups of rats performed the same task but exhibited differential plasticity in A1 that could be attributed to the use of different learning strategies (Berlau and Weinberger, 2008). These learning strategies are governed not by the acoustic parameter that is explicitly paired with reward (*e.g.*, frequency), but by the cue-features of the steady-state pure tone: its onset, plateau and offset (Figure 1, *top*). Subjects may employ tone onsets or offsets in learning situations (Kehoe and Weidman, 1999; Levis, 1966; 1971). Two equally successful strategies for this task make different use of these tone components. The first strategy relies on multiple components: (1) Bar-press during tone duration, using the tone onset as a cue to initiate responding and offset as a cue to stop responding ("tone-duration", T-Dur). A second strategy relies on only one component of the tone, its onset: (2) Bar-press from tone onset until receiving an error signal for BPs after tone offset, ignoring the tone offset cue ("tone-onset-to-error", TOTE) (Figure 1A & 1B).

Although both groups of animals achieved comparable high levels of asymptotic performance, only one group developed HARP in A1, that which used the TOTE strategy. These animals alone developed cue-specific decreases in frequency threshold (increased sensitivity) and bandwidth (increased frequency selectivity) (Berlau and Weinberger, 2008).

These findings suggest that learning strategy can be important in the formation of HARP in the primary auditory cortex. In the prior study, both groups learned the task to the same high level. Therefore, it was not possible to assess the relationship between the level of learning and the amount of plasticity. The current study does so by strongly biasing the level of learning by training the two groups at different motivational levels.

2. METHODS

2.1. Subjects

Male Sprague–Dawley rats (300-325 g) from Charles River Laboratories (Wilmington, MA) were housed in individual cages in a temperature controlled (22° C) vivarium and maintained on a 12/12 light/dark cycle (lights on at 7:00 am) with *ad libitum* access to food and water before the onset of training. They were handled daily and retained in the vivarium for a minimum of one week prior to any treatments. All procedures were performed in accordance with the University of California Irvine Animal Research Committee and the NIH Animal Welfare guidelines.

The goal of this experiment was to determine the influence of motivational level on the degree of learning-related plasticity in A1 after animals attained asymptotic performance, while monitoring learning strategy. Rats were placed on water restriction to maintain their weight either at ~85% (moderately motivated group; ModMot, n = 8), or at ~70% (highly motivated group, HiMot, n = 6) compared to unrestricted litter controls. Home-cage water supplements were given when necessary to maintain their weight targets. The ModMot group had the same water restriction, weight range and training as a group used in our previous study (Berlau and Weinberger, 2008) and therefore constituted a replication group. All subjects had *ad lib* access to food throughout the training period. In addition, cortical mapping data were available from a group of naïve rats of the same age and size (n = 9), as previously reported (Berlau and Weinberger, 2008).

2.2. Behavioral Training and Determination of Learning Strategy

The training apparatus has been described previously (Berlau and Weinberger, 2008). Briefly, training was conducted in an instrumental conditioning chamber (H10-11R, Coulbourn Instruments, Whitehall, PA) contained within a sound-attenuating enclosure (H10-24A, Coulbourn). The chamber contained a bar (2 cm above floor, 2 cm from right wall), a water cup attached to a lever (H14-05R, Coulbourn) that could deliver 0.1 ml of water to an opening 9 cm to the left of the bar (H21-03R, Coulbourn), a speaker (H12-01R, Coulbourn) 13 cm above this opening, and an overhead house light (H11-01R, Coulbourn). The speaker was calibrated for three locations at animal head height in the mid-line front (nearest the water dipper), center and rear of the training chamber.

All rats were shaped to bar-press (BP) for water reward during three daily 60 min sessions on a free operant schedule (1:1). The water cup was available for 5 s. Tones were not presented during shaping. During training, rewards were given for BPs made during the presence of a 10 s pure tone signal stimulus (CS = 5.0 kHz, 70 dB SPL). Thus, a maximum of two water rewards could be delivered per training trial. A BP made during silent inter-trial intervals were errors (ITI, first 4 days, 4–12 s; after 4 days, 5-25 s random schedule) and resulted in a time-out, *i.e*., lengthening of time to the next trial of 3 s for first 4 days, or 7 s after 4 days. The duration of the time-out period was signaled by a flashing (200 ms on/off pulse rate) house light. BPs during a time-out period initiated another time-out until BPs were withheld for the duration of at least one complete timeout period (Figure 1A).

As noted, a single tone burst can be decomposed into at distinct acoustic cues that may act independently to control behavior (Figure 1, *top*). While the tone-onset and subsequent presence of the tone can be used as a cue for responding, either the acoustic tone-offset cue or the error signal (flashing light) could have been used to terminate responding. Subjects whose BP responses begin at tone onset and continue until a BP results in an error signal use a learning strategy without regard to tone offset: a “tone-onset-to-error” learning strategy (TOTE). In contrast, subjects that bar-press from tone onset to tone offset (*i.e*., stop

responding at tone offset before receiving an error signal) use a "tone-duration" strategy (T-Dur) (Figure 1B).

To determine which learning strategy was employed, we included a 2 s post-tone "catch period", hereafter termed a "post-tone grace period (PTG)", starting at the tone offset. Bar presses during PTGs were neither rewarded with water nor penalized with error signals and time-outs. This permitted determination of whether animals used tone-offset *vs*. error signal cues to stop bar-pressing. Lack of BPs during the PTGs indicated use of tone offset, and thus use of the T-Dur strategy. In contrast, high levels of bar-pressing during the PTGs followed by cessation of BPs after an error signal, indicated failure to use the tone offset cue, while using the error cue, and thus use of the TOTE strategy.

2.3. Duration of Training

HiMot subjects were trained to asymptote. We defined asymptotic performance as four consecutive days during which the performance coefficient of variation (CV) across days was ≤ 0.10, where CV = standard deviation / mean. The number of training days to asymptote varied in the HiMot group (range: 11-23 days). It was possible to distinguish two HiMot subgroups (n=3, < 15 days and n=3, > 15 days) based upon the duration of training required to reach asymptote (subgroups mean training durations: 12.3 ± 1.5 days and 21.3 ± 0.6 days, respectively).

Subjects in the ModMot group were trained for 11 days. This short-term training was chosen because we have previously found HARP for moderately motivated subjects trained in the same task but for longer training durations (~20 days, Berlau and Weinberger, 2008). However, the development of CS-specific plasticity for moderately motivated animals after shorter training durations was unknown. This 11-day period matched the shortest duration of training that was required for HiMot subjects to reach asymptote. Thus, the current ModMot group served two purposes: (1) to determine the influence of the duration of training for HARP in general, and (2) to provide an appropriate comparison group for HiMot subjects that were also trained for short periods. If a shorter training period was not sufficient to induce the development of specific plasticity in A1, then an absence of HARP would have been evident in the current ModMot group (11 days of training). Any effect of training duration on A1 plasticity would have been revealed between short- and long-training duration, moderately-motivated groups, so the same difference could be expected between the two current HiMot subgroups (< 15 days vs. > 15 days of training). Therefore, from this and our prior study (Berlau and Weinberger, 2008), we had available a two-by-two between-groups comparison for motivation and duration of training. The issue of duration of training is addressed in the Discussion.

2.4. Recording and Analysis of Behavior

All stimuli and responses were recorded using Graphic State II (Coulbourn Instruments) software and subsequently analyzed using custom MATLAB vR2008a© software. Performance level was calculated as # CS BPs / Total # BPs. The total number of BPs for performance calculations was defined as the sum of all BPs made during CS presentations (correct response) and ITIs (incorrect response) during a session. The performance calculation did not include BPs during the PTG period as this was a "catch" period, and were analyzed separately. The levels of responding during the PTG were normalized to all BPs made during a session (*i.e*., PTG response = # PTG BPs / [# CS BPs + # ITI BPs + # PTG BPs]).

2.5. Frequency Generalization Tests

The frequency-specificity of learning was determined for both groups using frequency generalization gradients (FGG) during a single extinction session after training, before electrophysiological study. Six different frequencies were tested including the signal tone (2.8, 5.0 (CS), 7.5, 12.9, 15.8, and 21.7 kHz, 70 dB SPL). The generalization session began with ten CS trials identical to that of training to ensure that performance levels were stable relative to the previous days of training, *i.e.*, BPs made to the CS were rewarded. Subsequent test-trials were unrewarded regardless of frequency in order to prevent further tone-reward associative learning (Mackintosh, 1974). Test frequencies were presented in a pseudo-random order to yield 25 trials for each frequency (150 trials total).

Responses were calculated for each test frequency as the proportion of test trials with at least one BP response (*i.e.*, "BP" vs. "no-BP" response) relative to the total number of trials with a "BP" response within a generalization session (% of Total Responses = # Trials with response at test frequency / Total # of trials with response). Group mean frequency generalization gradients were constructed to compare the specificity of frequency-generalization gradients between groups.

2.6. Neurophysiological Mapping of A1

Complete mapping of A1 was performed after training and testing in a terminal session to obtain a comprehensive analysis of potential cortical plasticity in the functional properties and organization of A1. An additional group of untrained naïve animals (n=9) was mapped as a comparison group to determine the effects of training on A1 responses and organization. Detailed mapping methods were the same as those standard in the field (*e.g.*, Polley, Read, Storace and Merzenich, 2007; Recanzone et al., 1993; Sally and Kelly, 1988) and in our laboratory (*e.g.*, Berlau and Weinberger, 2008; Hui et al., 2009; Rutkowski et al., 2005). Rats were anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) with supplemental doses (15 mg/kg, i.p.) administered as needed to maintain suppression of reflexes. Bronchial secretions were minimized by treatment with atropine sulfate (0.4 mg/kg, i.m.) and core body temperature maintained at 37° C via a feedback heating blanket and rectal probe. Each animal was placed in a stereotaxic frame inside a double-walled sound attenuated room (Industrial Acoustics) and the skull fixed to a support via spacers embedded in a pedestal previously made using dental cement, leaving the ear canals unobstructed. A craniotomy was performed and the cisternae magnum drained of cerebrospinal fluid. After reflection of the dura, warmed saline was applied to the cortical surface intermittently throughout the mapping procedure to prevent desiccation. Calibrated photographs of the cortical surface were taken with a digital camera to record the position of each microelectrode penetration. These images were later super-imposed to create a plot map of relative penetration locations across the cortical surface.

Acoustic stimuli were delivered to the contralateral ear in an open-field with the speaker placed 2-3 cm from the ear canal. The stimuli consisted of broadband noise (bandwidth = 1 kHz–50 kHz, 0–80 dB SPL in 10 dB increments, 20 repetitions) and pure tone bursts (50 ms, cosine-squared gate with rise/fall time [10–90%] of 7 ms, 0.5–54.0 kHz in quarter-octave steps, 0–80 dB SPL in 10 dB increments). Stimuli were presented once every 700 ms with noise- or frequency-level combinations pseudo-randomized by TDT System 3 software. Frequency response areas (FRA) were obtained at each cortical locus using 10 repetitions of the frequency/level-stimulus set (252 stimuli in total).

Extracellular recordings of multi-unit clusters were made with a linear array of 4 parylene-coated microelectrodes (1–4 MΩ, FHC) that were lowered to layers III–IV, perpendicular to the surface of the cortex (400-600 μm deep) via a stepping microdrive (Burleigh Inchworm).

Neural activity was amplified (1,000x), band-pass filtered (0.3–3.0 kHz, TDT RA16 Medusa Base Station) and monitored on a computer screen and loudspeaker system (Grass AM8). Only discharges having ≥2:1 ratio were included in analyses. Responses to noise bursts were recorded before tone stimuli were presented. Responses to noise were later compared with responses to tones at each site as evidence for the borders of A1. A1 was physiologically defined as having a caudal–rostral, low–high frequency tonotopic organization with thresholds for pure tones being lower than for noise (Sally and Kelly, 1988). Complete mapping of A1 generally required 60–80 penetrations over a period of 8-12 hours.

2.7. Analysis of Responses in A1 to Sound

Frequency response areas (FRAs) were constructed offline for evoked spike-timing data using custom MATLAB R2008a© software. Tone-evoked discharges during a selected response-onset time window (6–40 ms time window after tone onset) were defined for each stimulus presentation as a spike rate that was greater than the spontaneous rate during the 50 ms immediately preceding the presentation of a tone.

FRAs were constructed for all recording sites. The FRAs yielded cardinal parameters of frequency response: minimal threshold, characteristic frequency (CF) (*i.e.*, the frequency at threshold) and tuning bandwidth (BW) (*i.e.*, breadth of tuning 20 dB above threshold). We also calculated the cortical area of representation for each CF band (see below). Threshold and BW measures were pooled across animals in a training group and averaged within CF bands to determine group-by-CF bin averages. CF bands for all analyses were an octave wide and were centered relative to the CS-frequency (*CF octave bins*: 1.77-3.54, 3.54-7.07 (CS-bin), 7.07-14.14, 14.14-28.28, and 28.28-54.0 kHz). We added in the highest CF octave bin sites with CFs slightly higher than the one octave bound to include recordings obtained from sites with CFs at the highest frequencies tested during electrophysiological recording (up to 54.0 kHz).

The CF of a responsive site was defined as the stimulus frequency having the lowest threshold (CF threshold) for an evoked response (*i.e.*, highest sensitivity). BW relative to CF threshold was calculated as the octave distance between the low- and high-frequency edges of the FRA at 10, and 20 dB SPL above threshold (see Figure 6A). CFs for each site were determined to construct CF distribution maps in Voronoi tessellations to represent the areal distribution of CF octave bands (same as above: 1.77-3.54, 3.54-7.07 (CS), 7.07-14.14, 14.14-28.28, and 28.28-54.0 kHz). The percentage of the total area of A1 that each band occupied was calculated for each animal before determining a group average by CF octave bin.

Outliers for threshold and BW measures were defined as data points outside of 1.5 times the inter-quartile range (IQR) around the CF-band median and were excluded from subsequent analyses. To avoid decreasing the number of data points in HiMot subgroup analyses, a Winsorization procedure was used to include outliers in a truncated mean limited by the 10-90th percentile of values for each CF octave band.

2.8. Statistics

All behavioral and neuronal response parameter values between relevant groups were compared using ANOVA ($\alpha = 0.05$) and *post hoc* Fisher PLSD. One-tailed analyses tested the prediction of HARP in the form of CS-specific decreases in A1 tuning threshold and bandwidth if animals use a TOTE learning strategy. Where appropriate for group and subgroup comparisons of A1 responses or behavior, two-tailed t-tests ($\alpha = 0.05$) were performed as indicated. The Bonferroni procedure was used when necessary to correct for multiple comparisons (*i.e.*, across CF octave bands), as in the past (Berlau and Weinberger,

2008; Rutkowski et al., 2005). Analyses were executed using MATLAB R2008a© software statistical packages.

3. RESULTS

3.1. Behavior

Motivational level was controlled by differential water restriction between two groups learning to bar-press to tones for water rewards. This produced significant differences in the percent of *ad lib* body weights compared to untrained littermates: ModMot = 86.6 ± 2.3%, HiMot = 68.8 ± 2.5% ($F_{(1,13)}$=197.89; $p < 0.0001$) (Figure 2A). The latency of the first bar-press after tone onset was significantly shorter in the HiMot group, consistent with a higher level of motivation with increased water restriction ($F_{(1,28)}$=24.16; $p < 0.0001$, Figure 2B).

Both groups acquired the task. However, they attained different levels of performance. Although initial performance was similar, the HiMot group began to exceed the ModMot group on day 7 (day 1-6: $F_{(1,83)}$=0.02; $p > 0.10$). At asymptote (each subject's last 4 days of training), the difference in performance was significant: HiMot = 89.8 ± 4.6%; ModMot = 71.3 ± 4.3% ($F_{(3,48)}$=13.13; $p < 0.001$) (Figure 3A). This difference in performance reflects the fact that the HiMot group bar-pressed twice during each tone, and thus received the two rewards available during the 10 s tone. In contrast, the ModMot group often exhibited "errors of omission", *i.e*, failed to BP twice during a trial ($F_{(1, 13)}$=26.62; $p < 0.001$). The groups did not differ in learning to withhold BPs during intertrial intervals ($F_{(1, 13)}$=2.54; $p > 0.10$) (Figure 3B).

Most importantly, differences in motivational level had a marked effect on *learning strategy*. As explained previously, behavior during the 2 s immediately after tone offset (PTG period) can distinguish between a T-Dur strategy (stop BPs at tone offset) and a TOTE strategy (BP until receiving an error signal). Analysis of PTG behavior indicated that, while both groups exhibited decreased PTG responses over training, the HiMot group responded significantly less than the ModMot group (between groups across first 11 days, $F_{(1,146)}$= 51.38; $p < 0.0001$) (Figure 4). Moreover, at asymptote, the HiMot group response rate was four-fold lower than the ModMot group (HiMot = 0.006 ± 0.002; ModMot = 0.027 ± 0.003 PTG/Total BPs; $F_{(3, 48)}$=7.86; $p < 0.001$) (Figure 4, inset). Thus, HiMot subjects tended to use a T-DUR strategy (fewer BPs after tone offset) while the ModMot group favored the TOTE strategy (more BPs after offset). The difference in learning strategy between groups is apparent even during the first six days of learning ($F_{(1,83)}$=33.13; $p < 0.0001$), when their performance is the same (see above).

Frequency generalization tests followed the last day of training to determine the frequency-specificity of learning. Both groups showed some specificity for the training tone frequency, by exhibiting peak responses in FGGs either at this frequency (5.0 kHz) or at the test frequency immediately higher (7.5 kHz). However, the groups did not differ in the frequency-specificity of behavior ($F_{(1,83)}$=2.85; $p > 0.05$). Therefore, motivational level did not affect the frequency-specificity of learning (Figure 5).

3.2. Mapping of Auditory Cortex

To determine if the groups differed in the processing and representation of acoustic frequency in A1, both the HiMot and ModMot groups were compared to a group of naïve rats (n = 9). The ModMot group developed HARP whereas the HiMot group did not. Furthermore, the ModMot group plasticity was specific to the CS-frequency band. Specifically, this group exhibited a significant decrease in response threshold at CF compared to the HiMot and naïve groups that was limited to the signal frequency (CS Octave Band: $F_{(2,106)}$=5.37, $p < 0.05$; between groups *posthoc*: ModMot vs. naïve, $p < 0.05$;

HiMot vs. ModMot, $p < 0.05$). The HiMot group was not significantly different from untrained naives (between groups *posthoc*: HiMot vs. naïve, $p > 0.05$) (Figure 6A).

In addition to its CS-specific increase in *sensitivity*, the ModMot group also developed a concordant CS-specific increase in *selectivity*, as indexed by a decrease in BW. This effect was significant at BW10 (CS Octave Band: $F_{(2,109)}=4.12$, $p < 0.05$; between groups *posthoc*: ModMot vs. naïve, $p < 0.05$; HiMot vs. ModMot, $p < 0.05$). The HiMot group did not develop HARP for tuning selectivity and were not significantly different from untrained naïves (between groups *posthoc*: HiMot vs. naïve, $p > 0.05$). (Figure 6B).

There was also a non-signal difference between the HiMot and both the ModMot and naïve groups. The HiMot group exhibited a decrease in BW10 for high CF frequencies (28.28-54.0 kHz Octave Band: $F_{(2,117)}=4.59$, $p < 0.05$; between groups *posthoc*: ModMot vs. naïve, $p > 0.05$; HiMot vs. naïve, $p < 0.05$; HiMot vs. ModMot, $p < 0.05$). However, this difference was not evident in BW20 (28.28-54.0 kHz Octave Band: $F^{(2,118)}=1.05$, $p > 0.05$), so its reliability and significance remain to be investigated.

Group averages for the distribution of CFs across cortical area in A1 were determined to assess whether learning had changed the tonotopic organization of A1. There were no statistically significant differences in cortical area at each CF octave between untrained naives and either ModMot or HiMot trained groups ($F_{(2,131)}=0.47$; $p > 0.6$) (Figure 7).

Despite the presence of plasticity in threshold and bandwidth for short training durations in the ModMot group, it was possible that high motivation conditions prevents the development of signal-specific plasticity in the short term. Therefore, we performed an identical comparison of A1 responses between the complete group of ModMot and the subgroup of HiMot animals (n=3) trained for < 15 days. Even when comparing groups of animals with the same short duration of training, the HiMot subgroup again did not yield any evidence of plasticity like that observed in the ModMot group. Threshold was decreased only in ModMot subjects and only in the signal frequency band (CS Octave Band: $F_{(2,89)}=8.05$, $p < 0.01$; between groups *posthoc*: ModMot vs. naïve, $p < 0.05$; HiMot vs. naïve, $p > 0.05$; HiMot vs. ModMot, $p < 0.05$), as was bandwidth at BW10 (CS Octave Band: $F_{(2,91)}=4.62$, $p < 0.05$; between groups *posthoc*: ModMot vs. naïve, $p < 0.05$; HiMot vs. naïve, $p > 0.05$; HiMot vs. ModMot, $p < 0.05$). Furthermore, the two HiMot subgroups (*i.e.*, < 15 days vs. > 15 days of training) did not differ from each other (each CF octave band, CF threshold: $p \geq 0.10$; BW10 and BW20: $p \geq 0.20$).

4. DISCUSSION

4.1. Summary of Findings

This study addressed the largely ignored issue of the relationship between the strength of learning and the amount of cortical plasticity. As noted in the Introduction, it is generally assumed that they have a direct, increasing relationship, *viz*., the stronger the learning, the greater the plasticity. We addressed this issue by using different motivation levels to achieve different levels of learning and then performed terminal neurophysiological analysis the primary auditory cortex to determine if stronger learning was correlated with greater plasticity. We found the opposite relationship.

There are three main findings. First, the HiMot group achieved a high level of learning to bar-press during presentation of a tone, but it failed to develop auditory cortical plasticity. Second, the ModMot group achieved a significantly lower level of learning, yet did develop HARP, in the form of tone-cue specific increased sensitivity (decreased threshold) and increased selectivity (decreased bandwidth). Third, the groups employed different learning

strategies - the HiMot group relied on the T-Dur strategy, *i.e*., bar-pressing from tone onset to tone offset, while the ModMot group relied on the TOTE strategy, bar-pressing from tone onset until receiving an error signal after tone offset. We interpret these findings to indicate that learning strategy can be a critical factor in the development of learning-related cortical plasticity. Indeed, strategy can be more important than the level of learning. We first consider the validity of the findings before considering their implications.

4.2. Validity of Findings

The HiMot and ModMot groups were treated differentially in two respects, motivation level and length of training. Therefore, any differences in either or both their behavior and auditory cortical plasticity might be attributed to differences in motivational level, length of training or some combination thereof. However, it seems more likely that the difference in motivational level was the critical factor.

As explained in the Methods, the HiMot group was trained to asymptote, which required 11-23 days. Two subgroups were discernable: trained less than 15 days and more than 15 days. Neither the entire HiMot group, or either subgroup developed CS-specific HARP in A1. In contrast, the ModMot group was trained for 11 days, a length comparable to the shortest duration of training in the HiMot group. As we had previously found the same HARP in a group of moderately-motivated animals trained for ~ 20 days (Berlau and Weinberger, 2008) as in the current ModMot group, the present findings revealed that such extended training is not necessary for the formation of A1 plasticity. Overall, the findings indicate that the HiMot animals both failed to exhibit HARP in A1 and used the T-Dur strategy whether they received long or shorter period of training. In contrast, animals trained under a moderate level of motivation, whether in the present study (for 11 days) or previously (for ~20 days), did develop specific decreased threshold and bandwidth and used the TOTE strategy. Therefore, the duration of training cannot account for the group differences in behavioral learning strategy or in the development of HARP in the primary auditory cortex.

The groups also differed in their attained *level of learning*. But the HiMot group achieved significantly better learning than the ModMot group, not *vice versa* (Figure 3). Therefore, if the differences in level of learning are responsible for HARP, then increased learning would have a negative effect on the formation of cortical plasticity, which seems highly unlikely.

Potential differences in the *content of learning* might be thought to account for group differences in either or both learning strategy and cortical plasticity. This factor refers to the degree to which subjects learned about acoustic frequency. That is, they could have learned simply to respond to sound *per se*, without any regard for the frequency of the tone signal, which was 5.0 kHz. However, frequency generalization gradients showed no significant difference between the HiMot and ModMot groups. Both groups exhibited generalization peaks at 7.5 kHz and the signal frequency of 5.0 kHz (Figure 5). Although sharper gradients would probably be obtained in a two-tone discrimination task (Mackintosh, 1974), the absence of a group difference in the current generalization test indicates that the groups had acquired comparable information about acoustic frequency.

In summary, several factors might account for the type of learning strategy employed, or the formation of HARP, or both: length of training, level of learning and degree of frequency specificity of learning. However, none of these parameters can explain the differences either in behavioral strategy or cortical plasticity. Therefore, we conclude that differences in motivational level affected learning strategy and the development of specific plasticity in the primary auditory cortex.

4.3. Learning Strategy and Motivation Level

That *learning strategy* can be more dominant than *motivational level* for the development of learning-induced specific associative plasticity is counterintuitive, and thus can be especially informative. At the outset, we should bear in mind that the counterintuitive nature of findings reflects current knowledge and assumptions rather than necessarily invalid results. The lack of knowledge about learning strategy and neurophysiological plasticity forces reliance on "intuition". Although it may be difficult to imagine, if learning strategy had been intensively studied while motivation had been largely ignored, then the current findings might not seem to be so surprising. However, as history cannot be replayed along a new trajectory, we turn to the current results. The question at hand is *"Why might the HiMot group have employed the T-Dur strategy, rather than the TOTE strategy?"*

HiMot animals might have better learned to cease BPs after tone offset because of effectively receiving greater "punishment" for non-rewarded BPs than the ModMot group, despite the absence of an error signal for responses during the post-tone period in both groups. That is, as the HiMot subjects were in greater need of water reward, a BP without reward could have constituted a greater difference between their expectation of reward and the actual outcome, *i.e*., greater "disappointment" (Amsel, 1958; Bell, 1985; Stellar and Stellar, 1985). This increased functional punishment would have more effectively reduced unrewarded PTG BPs in the HiMot group than the ModMot group. In so doing, tonal offset became a more salient cue for the former as it was immediately contingent and predictive of the greater punishment relative to the latter group. This in turn constituted use of the T-Dur strategy in the HiMot group. The "differential non-rewarded punishment" hypothesis also could account for the use of the TOTE strategy by the less thirsty ModMot group; the difference between a rewarded and a non-rewarded BP after tone offset was smaller, and thus less negative, so was less capable of punishing responses during the post-tone period.

Although the HiMot group did not develop detectable plasticity in A1, this failure does not imply that high motivation cannot induce HARP in other circumstances. Rutkowski and Weinberger (2005) trained rats in a related bar-pressing task for water, varying motivation level by different amounts of water restriction. They found that the greater the level of motivation, the higher the level of performance, as expected. Of greater relevance, terminal mapping of A1 revealed a signal-specific increase in area of representation within the tonotopic map. Most importantly, the amount of gain in area of representation was proportional to the magnitude of the signal's behavioral importance, as indexed by asymptotic performance level that reflected level of motivation. However, this effect of motivation level on HARP is not necessarily contradictory to the current findings of lack of plasticity with high motivation. The training protocol used by Rutkowski and Weinberger differed substantially from the regimen employed in the present study. They trained the subjects using three phases involving progressive decrease in duration of the tone signal over several weeks of training from 30 s to 10 s. Although learning strategy was not assessed, because this study predated discovery of the effects of this factor (Berlau and Weinberger, 2008), their protocol may have encouraged use of the TOTE strategy. Each phase of training presented the subjects with a new problem to solve using a new set of rules. An efficient way for each subject to ensure continued high levels of performance would be to adopt a consistent, effective learning strategy. The most common stimulus cue was tone onset, as tone durations (and therefore the expected time of offsets) changed across phases. Thus, use of a TOTE strategy ("bar press from tone onset until receiving an error signal) would have been most effective. If these subjects had used the TOTE strategy, they would be expected to have developed HARP, so that in this case, higher motivation level would have promoted greater gains in cortical area. While these considerations may reconcile the two studies of motivation, direct experimental tests are necessary, *e.g*., to determine learning strategy in a replication of the Rutkowski and Weinberger progressive

training protocol. In any event, seemingly insignificant changes in training regimens may have major effects on behavior, learning strategy and thus, cortical plasticity.

4.4. Learning Without Detectable A1 Plasticity

The HiMot group exhibited plasticity only in the form of a non-signal specific decrease in BW10 relative to both naïve and ModMot groups in the highest CF octave band measured (Figure 6B). As HARP is associative and specific, this change cannot be explained by associative processes related to learning about the signal frequency. It is possible that there is an additional and, as yet, unidentified element to the HiMot group's learning strategy that induced the bandwidth change for high-energy acoustic stimuli (*i.e*., high-frequency and high intensity). However the decrease did not appear for BW20 and is not evident in CF threshold. Thus, a possible alternative explanation is that the factors that underlie this change are distinct from those that cause differences in these tuning parameters between the ModMot and HiMot in the signal-frequency band. Further investigations will be necessary to describe other elements of learning strategies or other factors that could contribute to non-specific plasticity in bandwidth.

Nonetheless, the HiMot group succeeded to learn the bar-pressing task without exhibiting any signal-specific plasticity in A1. As plasticity is assumed to underlie learning and memory, there are several possible explanations for our failure to detect it in this study: the preparation, the elements recorded, the field of recording and the parameters analyzed.

We used a preparation under general anesthesia to be able to map the entire area of A1. While this is the only state under which complete maps can be obtained, and although anesthesia did not prevent the expression of HARP in the ModMot group, it is possible that plasticity involved in learning using a T-Dur strategy not only differs in type from that involved in using a TOTE strategy but is more sensitive to anesthesia. This possibility can be tested by recording in chronic, waking animals repeatedly over training days. This approach would both eliminate the problem of anesthesia, although at the expense of obtaining full maps, but might reveal the temporal dynamics of the formation of HARP during learning.

The current recordings obtained during terminal mapping consisted of multiple-unit discharges that exhibit good frequency tuning to tone onsets. This biases recordings to the larger cells within layers III and IV. Thus, plasticity in the HiMot group might be better expressed and detectable in single cells in these layers, or neuronal discharges in other layers, or both.

Recordings in this study were confined to the primary auditory field. It is possible that one or more of the other several auditory cortical fields might have developed plasticity in the HiMot group, and perhaps the ModMot group as well. Another possibility is that relevant plasticity might have developed in the subcortical auditory system, such as the magnocellular medial geniculate nucleus which has been implicated in associative learning in scores of reports (reviewed in Weinberger, 1982; 2008).

Alternatively, it could be that the HiMot group developed plasticity in A1, but not in the form that we measured in the current study, *i.e*., changes in frequency tuning response parameters to tone onsets. For example, as this group used tone offset to stop responding, perhaps those cells which have strong discharges to offsets developed plasticity. This possibility does not affect our current conclusion about learning strategy because there is a significant difference between groups in both correlated brain and behavior. Thus, the current findings support the conclusion that learning strategy can be a major determinant of

plasticity, and even dominates the factor of motivation, in processing within A1. However, it does not claim an actual absence of plasticity with learning in the HiMot group.

4.5. Why Does a TOTE Learning Strategy Lead to Specific Plasticity in A1?

The ModMot group relied on the TOTE strategy and formed HARP in A1 whereas the HiMot group relied on the T-Dur strategy and did not. What aspects of the TOTE strategy might be responsible for the development of specific plasticity? We suggest that reliance on signal onset, to the exclusion of signal offset, might account for this distinctive effect.

The ModMot group's TOTE pattern of behavior indicates marked utilization of the tone onset cue but little or no use of the tone offset cue. Previously, we hypothesized that the probability of forming plasticity in A1 using the TOTE strategy is linked to the dominant ability of onset transients to elicit responses in A1 cells (Berlau and Weinberger, 2008). Thus, A1 cells could develop specific plasticity during learning because their proclivity to respond to acoustic onset transients corresponds to the use of the signal onset cue, *i.e.*, use of the TOTE learning strategy.

This hypothesis is concordant with prior formulations. For example, natural sounds are often very brief (*i.e.*, transient) so some regions of the auditory system are likely to be somewhat specialized to extract information from onset transients (Masterton, 1993). The primary auditory cortex appears to be such a specialized region because its cells are particularly responsive to onset transients (Phillips and Heining, 2002), and in fact are more sensitive to onset transients than are cells in other auditory cortical fields (Heil and Irvine, 1998a).

In contrast to using a strategy dependent on tone onsets, the HiMot group relied more on the T-Dur strategy, indicating that these animals utilized tone offset, as well as tone onset, cues. Insofar as A1 cells do not appear to be equally responsive to both onset and offset transients (Heil, 1997; Lu, Williamson and Kaufman, 1992; Phillips, 1993; see Qin, Chimoto, Sakai, Wang and Sato, 2007), it is possible that the same signal tone was less effective at eliciting discharges in A1 that corresponded to the tone components which formed the basis of the learning strategy in the HiMot/T-Dur group. It is of course possible that plasticity formed elsewhere than A1 for cells having both onset and offset responses to tones, or in populations that receive convergent onset and offset responses.

5. FUTURE DIRECTIONS

The counterintuitive finding that higher motivation and the resultant higher level of learning actually results in less (actually no detectable) HARP in primary auditory cortex, is explicable on the basis of the particular learning strategy employed. In the present case, replicating and extending Berlau & Weinberger (2008), use of the TOTE strategy produced HARP despite a lower level of learning in the ModMot group. The critical features of the TOTE strategy need to be understood and the domain of possible types of strategies needs to be enlarged and studied. More generally, the present findings underscore the critical importance of determining the *factors* which influence or determine the formation of learning-related cortical plasticity, particularly those forms of plasticity which render them strong candidates for subserving memory traces. HARP is a strong candidate because (as summarized in the Introduction) it has all of the major attributes of associative memory. The relationship between the level of learning and the amount of cortical plasticity remains an important issue, but can now be seen within the framework of a particular learning strategy. Thus, even greater use of the TOTE strategy can be predicted to produce even greater plasticity, *e.g.*, beyond reduced threshold and bandwidth, so more TOTE could produce an actual gain in the area of representation of the signal frequency (see also Rutkowski and Weinberger, 2005). Also, strategy may not be the only factor that can determine cortical

plasticity in learning. Thus, the domain of potential factors needs to be studied. Finally, beyond factors, the actual behavioral *functions* of cortical plasticity remain to be delineated. Other counterintuitive findings may be awaiting discovery.

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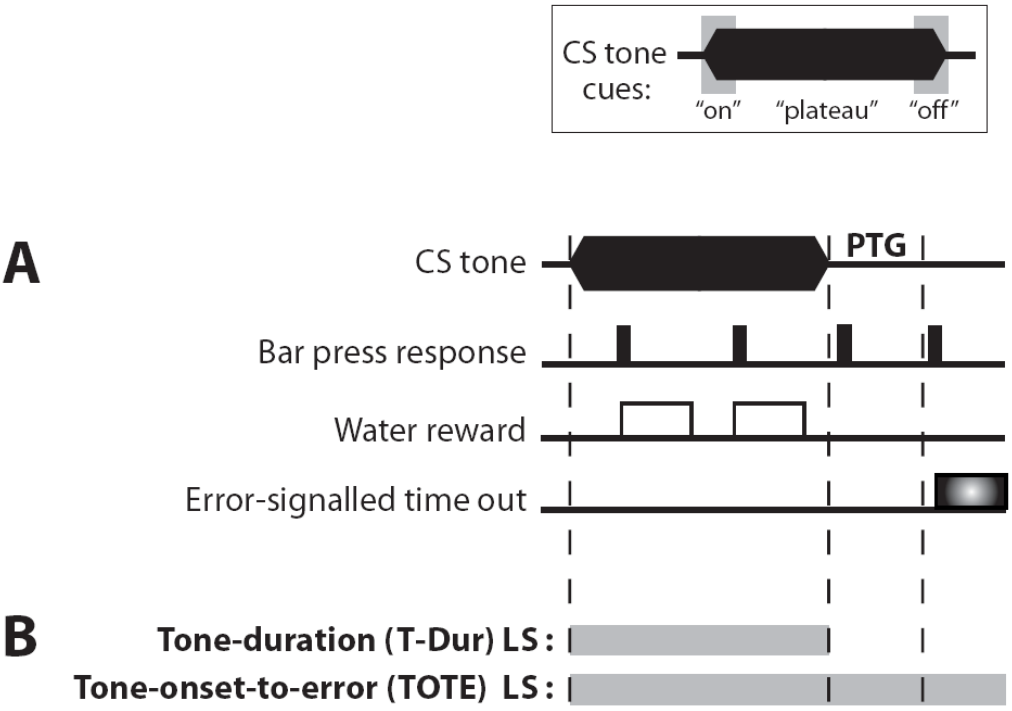

Figure 1.

Training protocol includes a post-tone grace period (PTG, 2 s) to determine learning strategy. **A**, Each subject was required to bar-press (BP) to a 10 s, 5.0 kHz (70 dB SPL) pure tone to receive water reward. BPs made during inter-trial-intervals (ITI) were signaled as errors with a flashing overhead light for the duration of a time-out penalty period. BPs made during the PTG were neither rewarded nor penalized. **B**, The absence or presence of responses during the PTG reveal different learning strategies. If BPs are absent, both tone onset is used to initiate and tone offset to terminate responses. This comprises a "tone-duration" strategy (T-Dur). If BPs are present during the PTG, the only acoustic cue used is the tone onset to initiate reponses. The tone offset is ignored and instead the presentation of an error signal cues the termination of responding. This defines a "tone-onset-to-error" learning strategy (TOTE). Gray bars demarcate the distribution of BPs across the duration of a training trial in animals using either T-Dur or TOTE learning strategies. Note that animals using a TOTE strategy continue BPs until receiving an error signal.

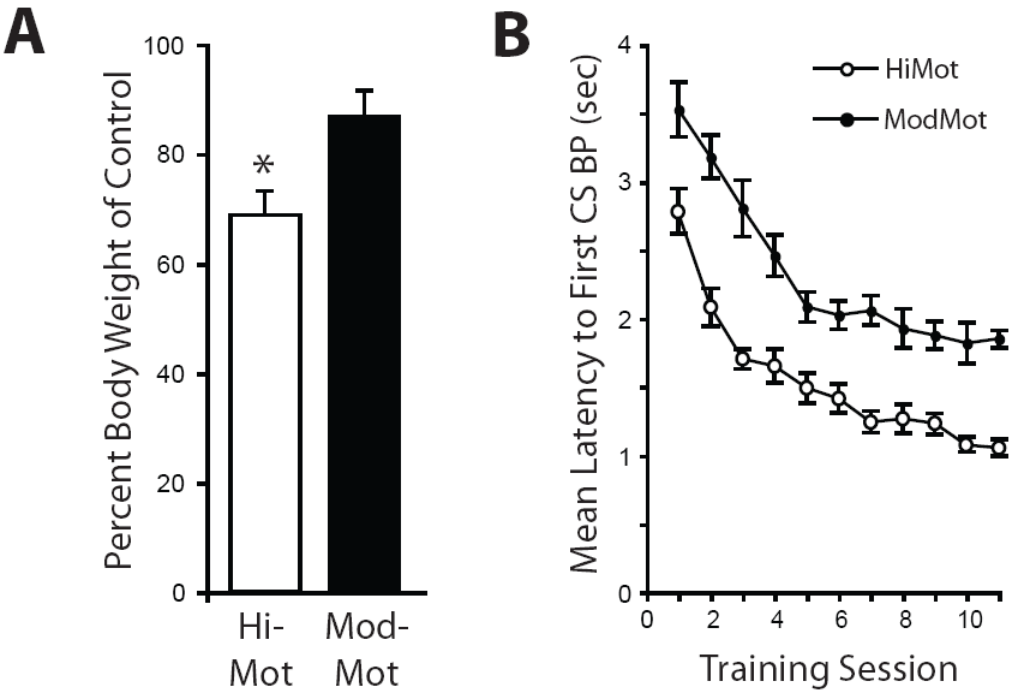

Figure 2.

Groups were trained in the same protocol under two different motivational levels. One group was highly motivated (HiMot, n=6) while the other group was moderately motivated (ModMot, n=8). **A**, Each group was water deprived to maintain body weight at ~70% of *ad lib* control rats for the HiMot group, or ~85% for the ModMot group. Values represent significantly different mean percent body weight for each group across all days of training (*$p < 0.0001$). **B**, An immediate effect of motivation level was evident in the latency to bar-press to tone presentations. Highly motivated animals were consistently faster to respond than moderately motivated animals beginning on the first day of training ($p < 0.001$). Only the first 11 sessions are shown to contrast the immediate difference between groups early in training.

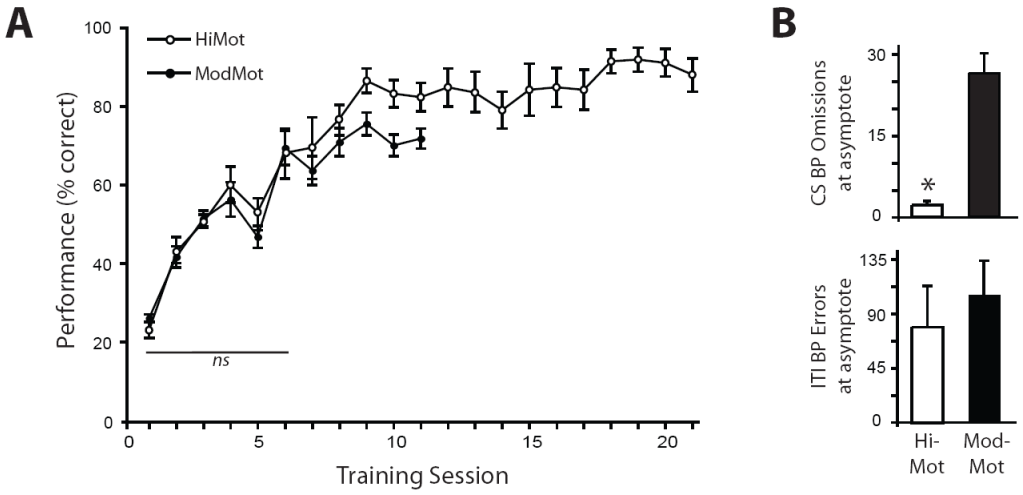

Figure 3.

Performance curves for ModMot and HiMot groups. **A**, Performance was calculated as the number of BPs during CS tones divided by the total number of tone or ITI BPs during the session ((# CS BPs / [# CS BPs + # ITI BPs])*100). Note that BPs made during the PTG period during which responses are neither rewarded nor penalized are not taken into account in the measure for performance. Initial learning was the same between groups across the first 6 days of training ($p > 0.10$), however asymptotic levels of performance were greater in the HiMot group ($p < 0.001$). The number of subjects in each group (HiMot/ModMot) by day (d) are as follows: d1(6/8), d2(6/8), d3(6/8), d4(6/8), d5(6/8), d6(6/8), d7(6/8), d8(6/8), d9(6/8), d10(6/8), d11(6/4), d12(5/0), d13(4/0), d14(4/0), d15(3/0), d16(3/0), d17(3/0), d18(3/0), d19(3/0), d20(3/0), d21(3/0), d22 (1/0, not shown in group mean). **B**, The difference in asymptotic performance level is accounted for by fewer errors of omission in the HiMot group than in the ModMot group (*top*, *$p < 0.001$). There was no difference in errors of commission between groups (*bottom*, $p > 0.10$). Values shown represent the mean number of errors during a session at asymptote.

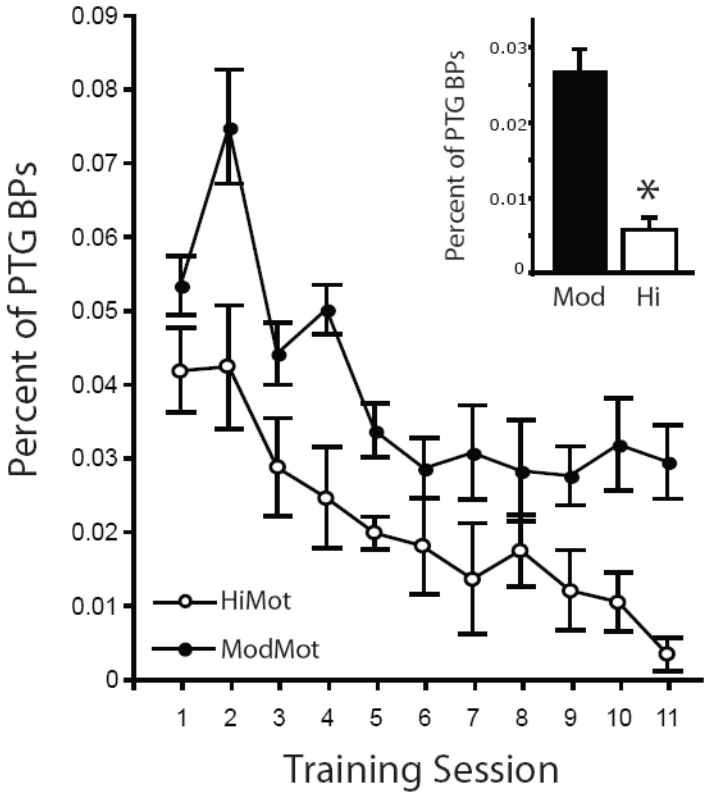

Figure 4.

Post-tone grace period (PTG) bar-press responses are diagnostic of learning strategy. Highly motivated subjects (HiMot) have fewer responses during the PTG than moderately motivated subjects (ModMot). PTG responses are normalized to the total number of BPs made during a session: Percent of PTG response = PTG BPs / [CS BPs + ITI BPs + PTG BPs]. HiMot responses decrease towards zero throughout training unlike ModMot responses which initially increase and later stabilize at an elevated level. Reduced numbers of PTG BPs indicate that HiMot group uses the tone offset to cue the termination of responses in a tone duration (T-Dur) learning strategy. Elevated PTG BPs in the ModMot group indicate that the tone offset is ignored and instead the presence of an error signal is used to cue response termination. Thus, ModMot subjects use a tone-onset-to-error (TOTE) strategy. Only the first 11 sessions are shown, however HiMot subjects that were trained for longer periods continued to show near-zero PTG BPs. ***Inset***, PTG BPs at asymptote. HiMot subjects respond significantly less during the PTG than ModMot subjects who exhibit PTG BPs even at asymptote.

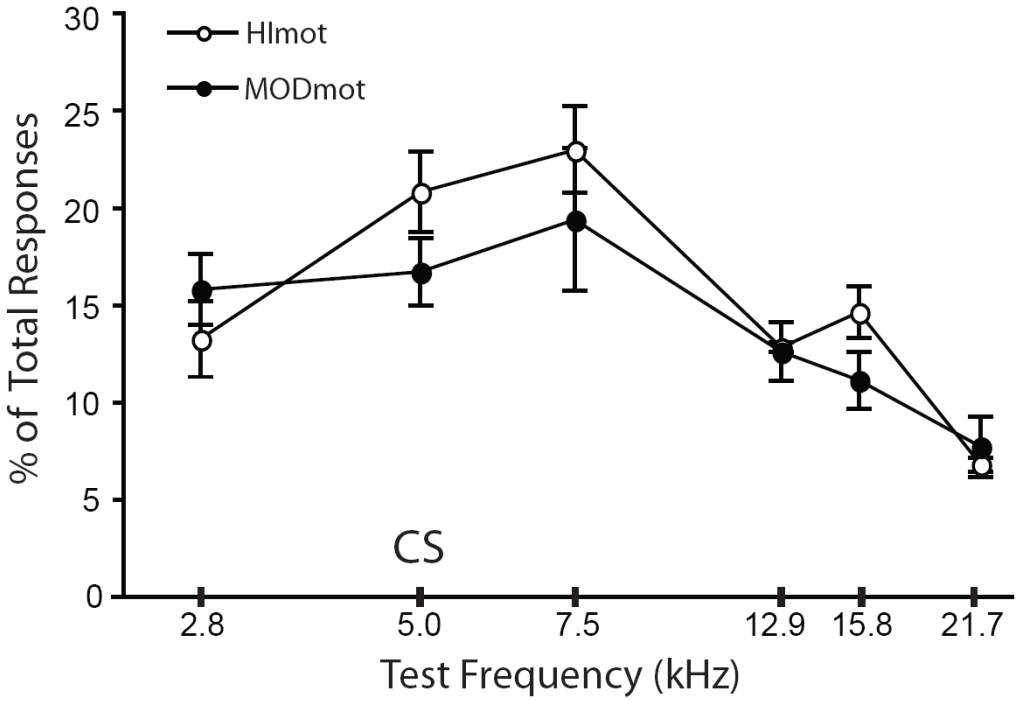

Figure 5.

Frequency-specificity of bar-pressing behavior. Stimulus-frequency generalization tests were conducted twenty-four hours immediately after the last day of training. There were no significant differences between mean group frequency generalization gradients. Values represent the proportion of all “BP” vs. “no-BP” responses during the test session made in response to each test frequency. Both groups exhibited peak responses at (5.0 kHz) or near (7.5 kHz) the training frequency.

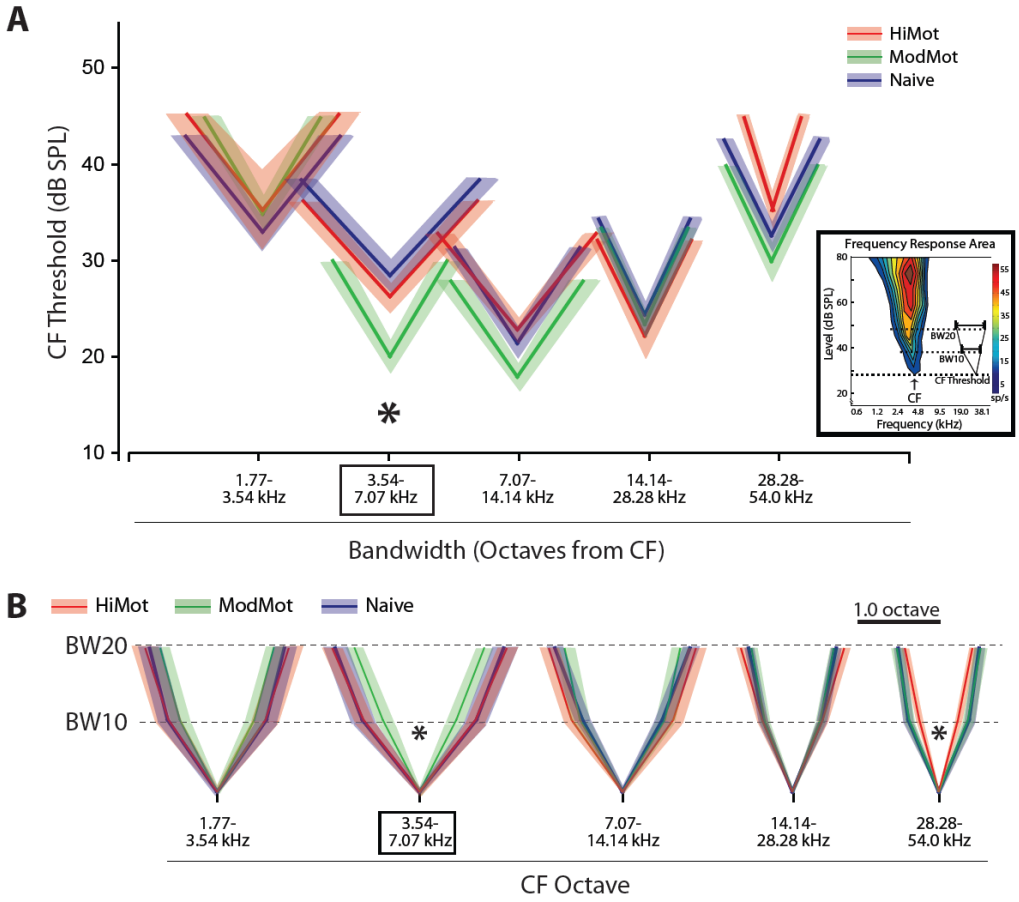

Figure 6.

Learning does not induce A1 plasticity in highly motivated subjects. Instead, only subjects that use a TOTE learning strategy exhibit CS-specific plasticity in minimum threshold and tuning bandwidth. **A**, FRAs were constructed for each recording site in A1 to determine response threshold and tuning bandwidth *(right)*, and pooled according to CF octave bands for each group. Each "V" represents the tip of the average group tuning curve in each CF octave showing response threshold (*y*-axis) and bandwidth 10 dB SPL above threshold (BW10, *x*-axis). Solid lines surrounded by shaded areas represent group means ± SE, respectively. Only the ModMot group has significant decreases in both threshold and BW10 that are specific to the signal-tone frequency band (CF threshold and BW10, ANOVA: $p < 0.05$; *marked with asterix**). CF threshold is not significantly different from naives in any frequency band in the HiMot group. The decrease in threshold for the ModMot group in the octave band immediately above that of the CS-frequency is significant only before correction for multiple comparisons (*before Bonferroni correction*: CS Octave Band: $F_{(2,111)}=2.39$, $p < 0.05$) and the difference is only between the two trained groups (between groups *posthoc*: ModMot vs. naïve, $p > 0.05$; HiMot vs. naïve, $p > 0.05$; HiMot vs. ModMot, $p < 0.05$). **B**, Decreases in bandwidth 10 dB SPL above threshold (BW10) were only present in the ModMot group. For BW20, the decrease in tuning bandwidth was only significant prior to correction for multiple comparisons (*before Bonferroni correction:* CS Octave Band: $F_{(2,111)}=2.39$, $p < 0.05$) and the difference was only between the two trained groups (between groups *posthoc*: ModMot vs. naïve, $p > 0.05$; HiMot vs. naïve, $p > 0.05$; HiMot vs. ModMot, $p < 0.05$). The HiMot group is not significantly different from naives in

any frequency band except in a non-specific decrease in BW10 the highest CF band (BW10, ANOVA: $p < 0.05$; *marked with asterix**). *See text for detailed statistics*.

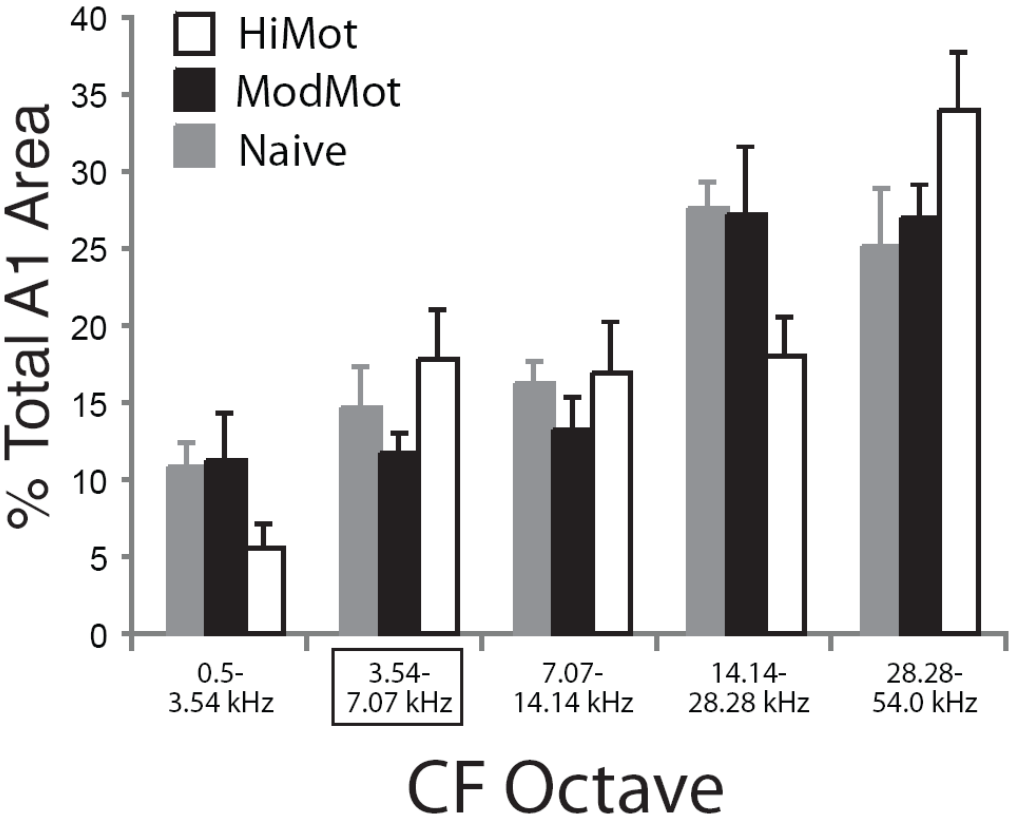

Figure 7.

Learning does not change cortical area in either HiMot or ModMot groups. The amount of cortical area representing the CS and all other CF octave bands were equivalent among the trained and naïve groups. Therefore, learning did not induce areal reorganization in A1 either instead of (in the case of the HiMot group) or in addition to (for the ModMot group) FRA changes in threshold and bandwidth. Each bar represents the mean (+SE) percentage of the total area of A1 within each CF octave band.