Behavioral gating, categorical encoding and memory in frontal cortex mirror task-related receptive field plasticity in auditory cortex

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Abbreviated title: Behavioral gating, categorical encoding and memory in frontal cortex

Word count (excluding abstract, legends, methods, references): 5460

Figures: 7 in text, 7 supplementary

Key words: Frontal Cortex, Prefrontal Cortex, Attention, Memory, Top-down, Gating, Auditory, Ferret

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ABSTRACT

Top-down signals from frontal cortex (FC) are conjectured to play a critical role in the attentional control of sensory processing. To explore this interaction, we compared activity in FC and primary auditory cortex (A1) during behavioral tasks that required categorical discrimination between reference and target stimuli. FC responses were behaviorally-gated, categorically encoded current behavioral meaning of stimuli, were unimodal or bimodal for auditory and visual tasks, and could persist for hours following behavior. This mirrors earlier findings in A1, that attention triggered rapid, selective, persistent, task-related changes in receptive fields. Simultaneously recorded local field potentials exhibited behaviorally-gated changes in inter-areal coherence that were selectively modulated between FC and focal regions of A1 responsive to target sounds. Taken together, these results suggest that A1 and FC dynamically establish a two-way functional connection during auditory behavior that controls the flow of sensory information and maintains a memory of recent task-relevant stimulus features.

INTRODUCTION

Studies of prefrontal cortex (PFC) have provided considerable evidence for its role in high-level executive functions including stimulus categorization (Everling et al., 2002, 2006; Miller and Cohen, 2001), planning and decision-making (Gold and Shadlen, 2007), and working memory (Fuster and Alexander, 1971, Kubota and Niki, 1971, Funahashi, 2006). A fundamental component of all these functions is postulated to be the control of the flow of sensory inputs through cortex via top-down feedback from PFC (Buschman and Miller, 2007; Fritz et al., 2007a; Gregoriou et al., 2009). The effects of top-down feedback are manifested by changes in spatial or feature stimulus selectivity that occur in sensory areas when an animal engages in a behavioral task (Fritz et al., 2003; 2005; Weinberger, 2007; Womelsdorf et al., 2008; Anton-Erxleben et al., 2009; Patzwahl and Treue, 2009) and are thought to reflect its attentional focus and the task's objective and rules (Miller and Cohen, 2001). Premotor cortex (PMC) shares some common response properties with PFC such as similar attentional modulation and representation in some task conditions (Di Pelligrino and Wise, 1993; Wallis and Miller, 2003).

If frontal cortex (FC) is a source of top-down command signals that modulate sensory representations in order to optimize processing of task-relevant information, one would predict that this modulation would be contingent on behavioral state and task-dependent stimulus meaning. Another prediction would be the existence of a strong correspondence between modulation of evoked sensory responses in FC and in sensory areas. To explore and test this hypothesis, we have developed the ferret as a new animal model preparation for studying FC control of auditory and visual behavior. Recent anatomical studies have shown that the anterior

sigmoid (ASG), proreal (PRG) and orbital cortex of ferrets share common features of neuroanatomical structure and connectivity with prefrontal cortex in primates, other carnivores and rodents (Duque and McCormick, 2009; current studies by SRS) and thus are likely to play a similar role in brain function as PFC in other species.

We recorded the activity of single neurons and the local field potentials in FC (ASG and PRG) during auditory discrimination behaviors (tone detection and two-tone discrimination) that we have previously shown to drive rapid receptive field plasticity in the primary auditory cortex (A1) (Fritz et al., 2003; 2005). For example, in A1, tasks that require the identification of a pure tone target in a sequence of broadband noise distractors cause an enhancement of responses to stimuli at the frequency of the target tone (Fritz et al., 2003). We predicted activity in FC that would be consistent with an output signal that could control the frequency specific enhancement observed in A1 for attended tonal targets (Fritz et al, 2007c). In order to assess changes in local field potential (LFP) coherence between these two regions during behavior, we also recorded activity simultaneously in A1 and FC. Finally, in order to determine whether FC responses and selective target encoding were independent of modality, we compared activity in FC during both auditory and visual discrimination tasks with the same operant structure in order to dissociate sensory effects from motor and motivational cues.

RESULTS

To study the representation of task-relevant auditory stimuli during behavior we recorded the activity of 719 single units in frontal cortex (FC) of five ferrets, trained on a variety of auditory and visual discrimination tasks. Neural activity was recorded during behavior and also during passive presentations of an identical sequence of task stimuli before and after behavior. These recordings were in two adjacent frontal, dorsal areas (**Figure 1**): the proreal gyrus (PRG) dorsal to the orbital gyrus, that is similar to the primate dorsolateral prefrontal cortex, and the premotor cortex (PMC) in the rostral region of the anterior sigmoid gyrus (ASG) (Duque and McCormick, 2009; unpublished data, SRS). Responses were similar in both frontal areas, and were grouped together in all of our analyses.

All tasks shared the same basic structure, in which animals learned by conditioned avoidance (Heffner and Heffner, 1995; Fritz et al., 2003, 2007c) to lick water from a spout during the presentation of a class of "reference" stimuli, and to cease licking after the presentation of the class of "target" stimuli in order to avoid a mild shock (**Figure 2a**, **Supplementary Figure 1**). The specification and stimulus characteristics of reference and target classes depended on the task.

FC responses are contingent on behavior

The responses of many FC neurons (n=282/718, 39%) showed marked behavioral gating, in which neural spiking activity was not influenced by passive presentation of stimuli *before*

behavior but was significantly modulated by stimulus presentation *during* behavior ($p<0.05$, jackknifed *t*-test). In most cases this behavioral modulation reflected selective responses to target (90% of modulated neurons) rather than to reference sounds. Even when significant, reference responses were much weaker than target responses (**Figure 3b**). A much smaller proportion of neurons (56/718, 8%) responded to stimuli in the pre-behavioral passive state and these responses were likely the result of persistent effects from earlier behavioral sessions on the same day (see **Figure 5**). Thus, many FC neurons did not respond to sensory stimuli unless they were behaviorally salient targets in either an ongoing (or very recent) task.

Examples of two types of FC modulation patterns illustrating behavioral gating are shown in **Figures 2b-c**. In the first example (**Figure 2b**), the unit gave a strong sustained response to the target tone during a tone-detection task (middle panel). In contrast, there was no response elicited by the same sounds in the identical sequence presented passively prior to the behavior (left panel). This unit had a persistent, but much weaker response after behavior (right panel). In 54% of units responsive during behavior, the firing rate was *enhanced* by the target. In the remaining 46%, the firing rate was *suppressed* by the target stimulus during behavior, as illustrated by the second unit (**Figure 2c**). We did not observe spatial segregation of FC neurons with these two response polarities. Neighboring frontal neurons with opposite signs of modulation could be found in the same penetration, or even at the same recording site. For example, the second unit (with opposite modulation polarity **Figure 2c**) was recorded later in the same electrode penetration, only 150m away from the first unit (**Figure 2b**). During the recordings from the second unit, the animal performed a series of target detection tasks in successive behavioral blocks, each with different target stimuli. In each case, neural activity was

suppressed by the target stimulus, regardless of its acoustic structure (tone, tone-in-noise or click train). The overall time-course and pattern of responses was similar for all three target conditions (although it is possible that small differences in the post-stimulus time histograms (PSTHs) could reflect specific features of the stimuli). Trial-by-trial rasters of these neurons' activity are shown in **Supplementary Figure 2**.

The pattern of FC responses varied considerably from unit to unit. **Figure 3a** shows heat maps that summarize the target responses of 219 cells that gave significant target responses during the tone detection task and for which data was collected pre-behavior (passive), during behavior, and post-behavior. Neurons were grouped by whether their activity was enhanced or suppressed during behavior and then ordered by their response latency during behavior. The same neuronal grouping and ordering was also used for both passive epochs. A broad view of the FC responses in the heat maps illustrates several important properties: (1) Excitatory and inhibitory responses to the target tones during behavior were found in roughly equal numbers of neurons; (2) The temporal array of response latencies from different neurons formed a continuous population representation of the target event in time – including the entire stimulus duration and subsequent decision windows. Latencies ranged from as short as 20-30 ms to more than 1 sec, and were distributed nearly uniformly (see **Supplementary Figure 3**), suggesting a precise temporal representation of both target and decision periods; (3) Responses to target tones in the passive state *prior* to behavior were generally weak or absent. If present, responses tended to have the *same* polarity as those observed for the same cell during behavior (e.g., as in **Figure 2c**) and these small pre-passive responses may reflect an attenuated memory from previous behavioral blocks on the same day (see **Supplementary Figure 6**); (4) Response profiles could

be phasic, sustained, built up or ramped down during the time course of the target stimulus (see more examples below).

The average responses of FC neurons to target tones are shown segregated according to their polarity in **Figures 3b,c**. As the average PSTHs (middle panels) indicate, both excitatory and inhibitory populations maintained activity from the onset of target "recognition" ~25-250 ms after onset of the target stimulus window until the end of the shock window 1.8 sec later. The excitatory cells tended to respond with slightly shorter latency and return to baseline more rapidly after the end of the shock window.

There was a striking asymmetry in FC responses during behavior to the classes of target and reference sounds, with a high selectivity for target responses, allowing for clear categorical discrimination between the two stimulus classes. Interestingly, when present, reference responses often exhibited an opposite polarity to the target responses (compare average target and reference responses in middle panels of **Figures 3b,c**). Thus, even when reference responses occurred, the difference in response polarity would enhance discriminability between the stimulus classes.

These effects were largely independent of variability in behavioral performance and recording location in FC. We observed a slight trend in the relation of response strength to task performance (**Supplementary Figure 4**), but this effect was not significant, perhaps because data was collected only after animals were completely trained on the task. There was also a small but significant tendency for neurons from the same recording site and/or from the same penetration to be modulated similarly during behavior, suggesting some topography of these

effects in FC (see **Supplemental Figure 5**), although we observed no large-scale, systematic effects and as mentioned above, neighboring neurons could exhibit opposite polarity of modulation (**Figures, 2b, 2c**).

FC responses encode categorical meaning of stimuli

As suggested by **Figure 2c**, FC activity encoded the category (or task-related meaning) of the stimulus as a "target" during behavior, rather than the physical nature of the task stimuli. A striking example of FC responses encoding functional category independent of physical properties is shown in **Figure 4**. Data from this unit were recorded during both tone detection and tone discrimination tasks. In the initial two tone-detection task (**Figure 4a**), the target consisted of two randomly alternating tones (550 or 2200 Hz), while references were a class of thirty different broadband noises. During this task, both target tones evoked equally strong responses, whereas references evoked no response. In the second, two tone-discrimination task (**Figure 4b**), the target consisted of only the higher frequency (2200 Hz) tone, while the lower (550 Hz) tone acted as a reference. In this second task, the unit ceased responding to the 550 Hz tone as it changed its meaning from target to reference and responded only to the 2200 Hz tone, which had retained its original meaning as target in the new task.

This flexible and adaptive categorical response to changing stimulus meaning was observed across a large number of FC cells (**Figure 4c**). In the set of 66 units for which data was collected during both behaviors and which gave significant responses during at least one behavior, we contrasted the average response to the target tone in both tasks (left panel) against

the responses to the same tone when it switched meaning from target to reference (right panel). Across the population, responses to the tone that remained a target were significantly correlated (r=0.51, p<0.001), while responses to the tone that switched meaning were not (r=0.16, p>0.2).

"Tabula rasa" and Memory neurons - persistence and extinction of post-behavioral responses

The behaviorally-gated modulation of many FC neurons was accompanied by a lack of responsiveness to passively presented stimuli. Indeed, many units (231/325, 71%) had a "Tabula rasa" character, responding vigorously to acoustic target stimuli only during the task and losing responsiveness to identical stimuli presented passively immediately afterwards. However, the remaining behaviorally-modulated FC neurons (94/235, 29%) manifested persistent post-behavioral responses to target stimuli. The modulated responses of these memory neurons persisted after task performance, generally decreased in amplitude, and finally extinguished, over a variable time course of minutes to an hour or more (e.g., **Figure 5a**). Thus the "behavioral gate" for these neurons opened rapidly after task onset but then closed slowly with a variable half-life after task offset. For a small number of FC neurons, these post-behavior memory responses were exceptionally long lasting. The neuron shown in **Figure 5b** continued responding to the target sound more than two hours after behavior was completed (fourth panel). The slightly diminished response (after two hours) was potentiated and restored to a much stronger response when the behavior was repeated (fifth panel). We note that these memory effects cannot be attributed to persistent motor or licking behavior since the ferrets showed virtually no licking whatsoever during passive epochs.

An overall view of memory cell responses in FC can be discerned by examining the average target response magnitude as a function of trial number following the onset of the recording epochs over the entire population of cells that responded to task stimuli during behavior and with data available before and after behavior (n=219) (**Figure 5c**). There was a clear enhancement of the responsiveness to the post-passive stimulus presentation (blue line) following task performance (red line) relative to the pre-behavioral passive epoch (green line). After the task was concluded, population responses gradually weakened over about 20 trials (~200 seconds), although individual neurons could maintain a significantly elevated response for extended durations (as shown in the examples above). The extent of persistence varied substantially across the FC population, as illustrated by the prolonged enhancement seen in a subset of 94 units that showed significant modulation after behavior (dashed blue). **Figure 5d** provides another global view of this post-task enhancement on a cell-by-cell basis through a scatter plot of the amplitude of modulation by target stimuli during behavior *versus* during post-behavioral passive stimulus presentation. The two measures are well correlated ($r=0.55$, $p<0.001$), indicating that persistent effects tended to resemble responses during the task.

Given the long-term persistence of memory responses, some of the modulation observed during passive stimulation before behavior may reflect persistence from earlier behaviors. Pre-behavior modulation was generally weaker if only the first behavioral session of the day was considered (**Supplementary Figure 6**). This result suggests that memory for the last task-salient target lasted for hours, rather than days.

Category-specific changes in local field potentials within and across FC and A1

In order to better understand the mechanisms giving rise to the behavioral gating of spiking responses in FC, we extracted LFPs by low pass filtering the recordings made during auditory tasks. **Figure 6a** shows the average evoked LFP following the onset of a reference (left panel) or a target sound (right panel) under active and pre-passive conditions. Surprisingly, despite the absence of substantial spiking responses during pre-passive stimulus presentation (**Figures 2-3**), we observed significant evoked responses to both reference and target stimuli during pre-passive stimulus presentation. Then, during behavior, the response evoked by the reference sounds was attenuated, while the response evoked by the target tone showed an overall response amplification including an increased early depolarization and later hyperpolarization. This pattern of evoked potentials suggests that, in the passive state, auditory signals arrive at the FC, but the gating mechanism prevents spiking activity for either reference or target stimuli. Subsequently, during behavior, the input to FC was selectively attenuated for reference responses while target inputs were selectively enhanced by increasing input gain. With the gate now open during behavior, these target inputs elicited spiking activity in FC.

In order to study dynamic changes in functional connectivity between auditory and frontal areas, we recorded LFPs simultaneously from A1 and FC during tone detection tasks. When we measured coherence between these areas (Mitra and Bokhil, 2008), we observed a strong depression of synchronous activity in the range of 10-20 Hz during behavior, compared to passive pre-task presentation of the same stimuli (**Figure 6b**, left panel). Moreover, this coherence change was strongly selective for the particular regions of A1 “near” the target

frequency (i.e., regions where cells responded preferentially to target sounds, **Figure 6c**). The change in inter-areal coherence was much smaller when measured only for A1 sites "far" from the attended target frequency. Thus the behaviorally-driven change in LFP coherence was highly selective for the region of the A1 tonotopic map that encoded the target sound, suggesting an attentional spotlight in the frequency domain in A1.

We also observed that the decrease in coherence did not reverse immediately after behavior was complete. When we analyzed only the first ten passive trials after behavior (~100 sec), we observed only a persistent change, marked by only partial return to baseline coherence (**Figure 6b**, right panel). This persistent post-task change in LFP coherence mirrors the post-task persistence observed in FC spiking responses (**Figure 5c**) and the post-task persistence of behaviorally-triggered tuning changes in A1 (Fritz et al., 2003, Elhilali et al., 2007).

Overlapping FC responses to auditory and visual target stimuli

Two ferrets were trained on a visual task that followed the same behavioral paradigm as the auditory tasks (**Figure 2a**). In the visual task, reference signals consisted of a sustained light, and target signals of a flashing light. Both auditory and visual stimuli evoked vigorous responses in some FC neurons during task performance (93/149 neurons modulated during either one or the other behavior). Activity during behavior in both modalities was qualitatively similar, in that it was gated by behavior and responses were much larger to the target stimulus.

Figure 7a illustrates simultaneous recordings from 2 FC neurons during a sequence of two tasks—auditory (tone detection) and visual (flashing-light detection). An auditory "tabula rasa" neuron (top row) responded significantly to target only during the auditory task, and displayed no response to the target in the visual task. By contrast, a bimodal (i.e., auditory and visual) unit (second row) responded to both auditory and visual targets (although post-behavioral persistence was stronger after the visual task). When neurons responded to stimuli of both modalities, the responses were often similar (**Figure 7b**, r=0.30, p<0.01). Of the FC neurons studied during both visual and auditory tasks, about 1/3 of the 93 responsive cells were bimodal, showing a common representation of target stimuli irrespective of sensory modality (**Figure 7c**). The remaining units studied in the two task conditions were fairly equally divided between unimodal auditory and visual responses.

Although the majority of task-modulated FC cells (206/326, 63%) appeared to multiplex target-recognition and motor behavioral responses (see **Supplementary Figure 7**), it is important to emphasize an important feature of this blend of sensory modality-independent and modality-dependent target coding. We note that the target recognition signal in many FC neurons is not equivalent to motor output. In both the auditory and visual tasks, animals performed virtually the same motor behavior, namely to lick during reference stimuli and cease licking after target presentation, and showed comparable behavioral performance in both tasks. If these cells had encoded strictly motor-related decisions or commands (i.e., inhibition of licking), their responses would have been identical in both auditory and visual tasks, but this was not true of unimodal cells (**Figure 7c**).

DISCUSSION

We have developed the ferret as a new animal model to study the neural basis of auditory attention and top-down control of auditory processing during goal-directed behavior (Fritz et al., 2007b,c). Our previous work on task-related receptive field plasticity (Fritz et al., 2003, 2005, 2007a; Atiani et al., 2009) and other studies in A1 (Polley et al., 2006; Keuroghlian and Knudsen, 2007) suggested the hypothesis that top-down signals trigger changes in the receptive field properties of A1 neurons that optimize signal processing for salient features of target sounds. We initiated recordings in ferret frontal cortex (FC) because it is a promising source of top-down control (Miller and Cohen, 2001). Ferret FC has recently been neuroanatomically characterized (Duque and McCormick, 2009; unpublished data SRS), and evidence exists for multiple pathways between FC and AC, including indirect polysynaptic connections as well as direct reciprocal projections (unpublished work of SRS, A. Duque, and J. Bizley, V.Bajo, F. Nodal and A. King). The present study is the first to explore neuronal responses in the FC (specifically PRG and rostral ASG) of the behaving ferret and interactions between FC and AC during auditory attention, based upon simultaneous recordings from the two regions.

Several new results have arisen from our observations, including: (1) behavioral gating of acoustic inputs to FC; (2) flexible, selective, categorical representation of the functional class of target stimuli in FC, independent of physical properties of the target or even the modality of the stimulus (visual or auditory); (3) an array of multiple response latencies in FC that establish a fine-grained temporal representation of behavioral event; (4) post-behavioral persistence, or

memory, of attention-driven modulation in FC; (5) feature-selective changes in LFP coherence between FC and A1 during behavior.

Behavioral gating of FC responses

Responses in FC, with the exception of just a few cells, were behaviorally gated and highly selective for target stimuli (**Figures 2, 3**), with equal numbers of neurons showing enhancement or suppression during behavior (**Figure 3**). Responsiveness to targets persisted for 5-100 minutes or more following task performance (**Figures 3a, 5**). The striking behavioral gating of FC responses likely reflects target recognition or a cognitive decision process, rather than purely pre-motor or motor-related output because modulation of spiking activity that could be explained by motor activity was removed before we measured modulation by sensory stimuli (**Supplementary Figure 7**). Two other observations support this view: we observed the same FC modulation in memory neurons to stimuli presented in passive blocks following behavior, when ferrets displayed no licking. Moreover, in successive auditory and visual tasks, we found that many unimodal cells showed modulation selectively for only the visual or the auditory target, despite similar motor behavior in both behavioral paradigms.

These results are in agreement with previous reports of selective gating in monkey prefrontal cortex (PFC) (unpublished data in conference proceedings from D. Freedman, M. Riesenhuber, T.Poggio, E. Miller, 2002 and C. Hussar and T. Pasternak, 2008). However, our findings emphasize the value of comparing passive and behavioral states as a window of insight into mechanisms of encoding salient events and stimuli in FC. The pre-passive control provided

a critical "low-attention" baseline to measure the effects of behavioral modulation and gating. Recordings post-behavior also demonstrated the long-lasting effects triggered by attention, a form of memory in FC that could persist for hours following task performance. Moreover, in the pre-behavior passive condition, LFP measurements exhibited significant responses to *both* reference and target stimuli. By contrast, during behavior, LFP responses became categorical, being selectively suppressed for reference stimuli and enhanced for target stimuli. Clearly, thre are also invaluable insights to be gained from a comparison between FC responses under different behavioral task conditions (see **Figure 4**). However, by comparing neural activity during passive listening and behavior, we may gain particular insight into the mechanisms that underlie behavioral gating, which are known to be impaired in schizophrenia (Mayer et al. 2009).

Categorical Representation of Target Stimuli

The ability to change behavioral and neural responses to identical sensory stimuli, depending upon the current task and context, is an essential component of flexible, goal-directed behavior (Duncan, 2001). Neurons in frontal cortex are likely to contribute to this adaptive ability because of their extraordinary flexibility, responding differently to identical stimuli depending upon the task requirements and behavioral contexts (Sakagami and Niki, 1994; Rainer et al., 1998; White and Wise, 1999; Wallis and Miller, 2003; Duncan, 2001; Miller and Cohen, 2001; Everling et al., 2002, 2006). Our results are consistent with these findings, as demonstrated by the rapid, adaptive change in coding between tone detection and discrimination tasks. When task conditions changed, the strong responses to the acoustic target stimulus during tone

detection disappeared when the same sound became a reference stimulus during two-tone discrimination (**Figure 4**).

Rule or strategy-guided behavior is shown when identical stimuli elicit different responses (as shown above) or when two different stimuli elicit the same neural response (**Figure 2c**). A dramatic illustration of a common representation of target stimuli, independent of sensory modality, was our comparison of modulation during auditory and visual behaviors (**Figure 6**). Many FC neurons were activated similarly during both auditory and visual tasks, irrespective of target stimulus modality, suggesting that some FC responses form supramodal representations. However, our results also revealed neighboring neurons that were modality-specific, being selectively modulated only during auditory or a visual task performance. Therefore, as in the primate PFC, there may be not only a modular "domain-specific" organization of the ferret frontal cortex (Levy and Goldman-Rakic, 2000; Romanski, 2004) but also areas for integration of auditory and visual information (Sugihara et al., 2006).

Categorical representation of visual stimuli has been shown to occur in primate dlPFC (Freedman et al., 2001) where neurons respond to just those categories that are currently relevant. In more recent studies (Russ et al., 2007, 2008), recordings were obtained in an auditory categorization task from primate vlPFC. Similar to our results, FC neurons in vlPFC generally encoded the categorical percept, rather than the acoustical features of the stimulus. However, unlike previous studies in primate PFC our results show that modulation in ferret dorsal FC was often highly specific for the category of "target" sounds, and showed only weak responses to the "reference" stimuli (**Figures 2-4**). This category asymmetry may reflect

differences in task structure between the conditioned avoidance paradigm used here (Heffner and Heffner, 1995; Fritz et al., 2003) and the 2AFC paradigms used in other studies (Freedman et al., 2001; Russ et al., 2008; Kusunoki, 2009a,b; Romo et al., 2004; Lemus et al., 2009), highlighting an important direction for future studies to resolve these differences.

Variable response latencies - representation of behavioral event time in FC

The time at which neurons in a given cortical area respond to sensory stimuli can provide insight into the level the neurons occupy in the sensory processing to decision-making (bi-directional) hierarchy. In FC, latencies ranged from early ultra-fast (20 ms) up to over 1.5 second, forming a continuous representation of time during target presentation and behavioral response (**Figure 3** and **Supplementary Figure 3**). We observed a modal peak in neural latency of 50-150 ms, which may reveal the timing of the early target recognition response. This neural latency preceded the behavioral latency (withholding of licking) during task performance (~200 ms, see examples in **Supplementary Figures 1, 2**).

The temporal structure of responses also varied in shape from phasic to showing prolonged tonic firing up to 1500 ms, throughout and beyond the post-stimulus behavioral decision point (**Figure 3** and **Supplementary Figure 3**). The long-latency responses may arise from gating through a multisynaptic, feedforward circuit that could serve as a neural representation of time and behavioral state during working memory or other cognitive tasks (Goldman, 2009). This type of continuous representation has also been observed in primate dlPFC (Zaksas and Pasternak, 2006) and in rat medial PFC (Fujisawa et al., 2007). Our results

are also compatible with recent studies of dynamic population coding of category information in PFC (Meyers et al., 2008), supplementary motor cortex and basal ganglia (Mushiake and Strick, 1995; Shima and Tanji, 2000; Tanji, 2001; Salinas, 2009).

The lasting imprint of attention – short and long-term memory responses in A1 and FC

In performing tasks with delayed response, it is necessary to hold information in temporary storage to guide subsequent action (Baddeley and Hitch, 1974; Baddeley, 1986, 2000). Working memory is the system used for this temporary representation of information just experienced or just retrieved from long-term memory. These active representations are usually short-lived (on the order of seconds), but can be maintained for longer periods of time through active rehearsal strategies. Although there is debate as to the centrality of working memory as an explanatory concept for understanding the function of FC (Goldman-Rakic 1987, 1998; Petrides, 2000) neurophysiological recordings from FC (PFC and premotor cortex) often show persistent, sustained levels of neuronal firing during the retention interval of auditory, visual and somatosensory delayed response tasks (Bodner et al., 1996; Romo et al., 2004; Funahashi, 2008; Lemus et al., 2009) and even in untrained animals (Meyer et al., 2007). This sustained activity is thought to provide a bridge between the stimulus cue and its contingent response. The physiological evidence for working memory in our study is limited because of our task design, in which there is only a short (400 ms) retention interval between target stimulus offset and the onset of the shock period. Nevertheless, previous working memory results in monkey dorsolateral PFC (Bodner et al., 1996) resemble those reported here: (1) neuronal responses to the tone had latencies as short as 20-60 msec, (2) the same percentage of cells (25%) were

activated or inhibited during the delay period, (3) and the trend of discharge decreased in some cells and increased in others (i.e. firing rate ramping up or down).

In the present study we demonstrate that the effects of attending to task-relevant stimuli in FC could persist over time well beyond the time-course of classic working memory (**Figure 5**). We observed persistent modulation on multiple timescales. While "Tabula rasa" (blank slate) neurons "forgot" the selective target responses right away and showed no post-behavioral persistence, whereas "memory" cells maintained target responses for up to 2 hours or longer. The population profile of forgetting on multiple timescales that we have observed in FC is compatible with the model of Fusi and colleagues (2007) and suggests that that the memory of the last salient behavioral stimulus can persist in FC for a considerable time before fading away. Such persistence in our study occurs only if the animal is not faced with new task condition in which the behavioral meaning of the original target stimulus is changed (as in a switch between tone detection and discrimination tasks – **Figure 4**).

FC has been shown to play a key role in directing attention to behaviorally relevant sensory signals and in making decisions concerning these signals. We conjecture that FC participates in a mechanism that "tags" the representation of a target sound (Morris, 2006; Frey and Frey, 2008). Prefrontal cell assemblies representing new information would then maintain responsiveness to behaviorally relevant, salient signals. The persistent effects that we observe suggest that FC plays a role in longer-term memory and information storage, in addition to its role in behavioral control and executive function (Knudsen, 2007; Fuster, 2008; Jung et al., 2008). Since we have previously demonstrated persistent changes in receptive field shape for

salient acoustic features in A1 (Fritz et al. 2003, 2005, 2007a; Elhilali et al., 2007) our current study offers an intriguing link between the time course of memory in FC and A1.

Inter-areal LFP coherence – evidence for gating and for top-down sensitivity control

As we have shown, ferret dorsal FC selectively encodes task-salient stimuli and may be a source of top-down signals that selectively modify A1 receptive fields during goal-directed behavior. Our simultaneous measurements of LFP in the two cortical regions revealed coherence patterns that changed significantly depending on the behavioral state of the animals, as well as the specific behavioral meaning of the stimuli (**Figure 6**). Changes in inter-areal coherence were strongest in the alpha and beta frequency range (10-20 Hz), showing a striking decrease during performance of a tone detection task. Moreover, the marked decrease was selective for A1 sites that responded to the target frequency ("near" cells) whereas only a small change was observed when the frequency of the target was far from the best frequency of the A1 site ("far" cells). Thus LFP coherence between FC and A1 is selectively shaped by a spotlight focused on the attended target frequency zone along the frequency (tonotopic) axis in A1. We also observed that the change in LFP coherence persisted in the post-behavioral epoch, mirroring post-task persistence of FC spiking responses and of behaviorally-triggered receptive field changes in A1 (Fritz et al., 2003, 2005, 2007a).

Neuropsychological studies suggest that prefrontal damage disrupts normal inhibitory modulation of inputs to primary auditory cortex (Knight et al., 1999, Ehlis et al., 2009). We speculate that in the conditioned avoidance behavioral paradigm, in which the target triggers the

cessation of an ongoing (licking) behavior, FC may play a similar inhibitory role. In this light, the target-frequency selective decrease in coherence we have observed during behavior reflects the selective removal of inhibition near target frequency, and hence, enhancement of processing for the salient target frequency during behavior (Fritz et al., 2003). Such inhibition could be mediated by a direct inhibitory influence of the frontal cortex on the primary auditory cortex, or indirectly by a frontal-thalamic (FC to thalamic reticular nucleus to MGB to A1) gating system or by another indirect, subcortical route (such as FC to nucleus basalis to A1). A testable prediction, based on neuropsychological evidence (Knight et al., 1999), is that FC may exert a differential excitatory effect on auditory association areas. Although a causal link remains to be tested in future studies, all of our observations are consistent with the hypothesis that the FC can shape the responses of specific sensory areas during behavior by changing cortical sensory filter properties.

Summary

Our physiological study of frontal cortex in the behaving ferret provides a new animal model, in addition to primate and rodent, for studying the role of the frontal cortex in attention. Overall, our results agree with the conclusions of earlier studies in the monkey (Everling et al. 2002, 2006; Kusunoki et al., 2009a,b) that a central role of FC cells is to signal the occurrence of a particular, task-relevant event, usually by responding more strongly to targets than to non-targets. In ferret dorsal FC, as in monkey dorsolateral PFC, neurons selectively and adaptively encode task-relevant information, are modulated by selective attention for action and contribute to the top-down control of cognitive and sensory processes that facilitate the organization of

behavior to achieve goals. The question of neuroanatomical homology is still unresolved although current neuroanatomical studies suggest that there may be a homology between ferret PRG and macaque dlPFC (Duque and McCormick, 2009; unpublished results of SRS, 2009). However, the overall functional homology of responses across species is striking, notwithstanding structural differences in cytoarchitecture, topology and neuroanatomical connectivity (Wise, 2008), and may be understood as shared neuronal solutions to the common problem confronting many animals of representing currently salient stimuli relevant to shifting behavioral goals in an ever-changing environment.

Our hypothesis, that the FC exerts dynamic and selective control over sensory filters in A1 during auditory behavior is also in accord with prevailing views of PFC as the source of top-down modulatory influence on other brain areas, particularly sensory cortices, in the service of behavioral goals (Miller and Cohen, 2001; Duncan, 2001) and executive control of memory retrieval (Tomita et al., 1999). In support of this hypothesis, we observe a rich tapestry of response patterns in FC that share key properties of task-related receptive field plasticity in A1, specifically a behavioral contingency and a time course of persistence that mirrors the time-course of A1 plasticity. Moreover, FC responses categorically distinguished task-salient stimuli regardless of their physical nature or modality, and exhibited LFPs that were consistent with it being a source of frequency-specific top-down signals to A1. These findings highlight the challenge for future work in integrating the interplay of attention and memory (Woodman et al., 2007; Messinger et al., 2009) and the role of FC in top-down signaling that triggers adaptive, task-related plasticity in auditory cortex.

ACKNOWLEDGEMENTS

We would like to thank Alvaro Duque, Jenny Bizley, Vicky Bajo and Fernando Nodal for discussion and for generously sharing their unpublished neuroanatomical data on the ferret frontal cortex, Nima Mesgarani for assistance with customized MATLAB software development, Mounya Elhilali for discussion and for analysis of memory effects in A1, Steve Gotts for discussion of LFP coherence, Catherine Carr for cheerfully sharing lab resources for neuroanatomical studies, and Alexandra Israelson, Alexandra Rubin, Kevin Donaldson, David Levitt for assistance in training the ferrets in this study. This research was funded by grants from the National Institutes of Health, R03DC005938 (JBF), R01DC005779 (SAS, JBF), and F32DC008453 (SVD).

AUTHOR CONTRIBUTIONS

JBF and SAS designed and JBF conducted all behavioral physiological experiments; SVD analyzed data; JBF, SAS, SVD evaluated results; JBF and PBY made neuroanatomical injections; SRS processed all histological tissue, analyzed neuroanatomical results and made one figure; SVD made all other figures; JBF, SAS and SVD wrote paper.

METHODS

All experimental procedures were approved by the University of Maryland Animal Care and Use Committee and were in accord with National Institutes of Health Guidelines.

Behavior

All five adult female ferrets in the study were trained on a variety of auditory tasks, and two of the ferrets were also trained on the visual task. These task variants were based on the conditioned avoidance behavioral paradigm (Heffner and Heffner, 1995; Fritz et al., 2003). **Figure 2** illustrates the basic structure of trials in four task variants (three auditory and one visual task) and the acoustic and visual stimuli used (**Figure 2A**). Each behavioral trial consisted of a sequence of reference stimuli (randomly ranging from 1-6) followed by a target (except on catch trials in which 7 reference stimuli were presented with no target). Ferrets licked water from a spout during presentation of the reference stimuli but were required to stop licking upon presentation of a target stimulus in order to avoid a mild shock (shock window, 400-800 ms after target offset). All stimulus presentation and behavior control were performed by custom software (Matlab).

Ferrets were trained once a day (for 50-200 trials to satiation) in a sound-attenuated test box until they reached criterion, defined as consistent performance for two sessions with >80% hit rate accuracy and >80% safe rate for a discrimination rate of >0.65. Initial training to criterion on the simplest task (tone detection – detecting a tone in the presence of noise) in the free-running test

box took ~6 weeks for each ferret. Ferrets were subsequently trained on the auditory and visual task variants and could readily switch from one to another (responses of a frontal neuron when the animal engaged in three different task variants are shown in **Figure 2C**).

After the initial training was completed, surgery was conducted to implant a stainless steel headpost for head restraint during physiological recording (see below). After recovery from surgery, ferrets were retrained on the task, while restrained in a lucite holder, with their head fixed to enable stable recordings. Animals were used in behavioral physiology experiments no more than 1-2 times per week, for 6-8 hours per experiment. During experiments, each task condition block contained ~40-80 trials, and animals worked for reward in 1-6 task blocks on each recording day. Overall performance of the ferrets on two of the auditory task variants, single tone detection and two-tone discrimination tasks (Fritz et al., 2003, 2005) is shown in **Supplementary Figures 1B,1C**. Comparable performance was achieved for the other task variants.

Surgery

In order to secure stability for electrophysiological recording, a stainless steel headpost was surgically implanted on the skull. Ferrets were initially anesthetized with ketamine/xylazine, then intubated and maintained in deep anesthesia with isoflurane throughout the surgery. Using sterile procedure, overlying tissue was removed to expose the dorsal and lateral skull surface. The headpost was mounted on the midline with Durelon cement, and secured to a headcap fastened to the skull with titanuium screws and Zimmer bone cement, leaving clear access to primary

auditory cortex and frontal cortex in both hemispheres. Antibiotics and analgesics were administered as needed before, during and after surgery.

Neurophysiological recording

Experiments were conducted in a double-walled sound attenuation chamber (IAC). Small craniotomies (1-2 mm in diameter) were made over primary auditory cortex (A1) and/or frontal cortex (FC) prior to 6-8 hour recording sessions. We recorded simultaneously from both loci using multiple independent electrode drives (Alpha-Omega) to independently direct up to four electrodes in each cortical area. The electrodes were configured in a square pattern with ~800 microns between electrodes. Responses were recorded with tungsten microelectrodes (3-10 M□, FHC) and then stored for analysis off-line. A typical recording yielded 1-2 isolated single units from each electrode.

The A1 and FC regions were initially located with approximate stereotactic coordinates, and then further identified physiologically. Physiological indicators of ferret A1 have been widely used in the past (**Figure 1C**). They include a tonotopic map, short latency (~20 ms), vigorous sustained or transient responses to tones and noise, and spectrotemporal receptive fields (STRFs) with clearly defined excitatory and inhibitory response areas along both the spectral and temporal axes (Shamma et al., 1993; Bizley et al., 2005). We also recorded from the frontal cortex of the ferret (in the proreal gyrus and rostral anterior sigmoid gyrus). To clarify neuroanatomical nomenclature, we note that these two cortical areas are both classified as prefrontal cortex according to the criterion of strong reciprocal connections with the mediodorsal nucleus of the

thalamus in the study of Duque and McCormick (2009). Based upon our further cytoarchitectonic studies, we prefer to refer to these areas as part of frontal cortex, although we agree with Duque and McCormick that the dorsal portion of their orbital gyrus, which we call the proreal gyrus, is probably homologous with primate dorsolateral prefrontal cortex. Further neuroanatomical work will be necessary to settle this issue. Physiological localization of the PRG and PMC was particularly difficult because of the absence of a stereotaxic atlas for the ferret brain and was made even more challenging for a variety of reasons, including low neuronal firing rates, and poorly defined external landmarks. However we were guided by a few reports of the anatomical and physiological properties of the ferret FC (Lockard, 1985; Haider et al., 2006; Duque and McCormick, 2009). The challenge from an experimental point of view was finding responsive neurons in FC, given the commonly weak or absent response in the passive (non-behaving) animal. Consequently, it was often necessary to engage the animal in a preliminary behavioral task in order to evoke responses on one or more of the electrodes in the FC and identify neurons suitable for recording.

Neuroanatomical localization of frontal cortex recording sites

Following extensive recording in the frontal cortex, two of the ferrets in the study were injected with tracer (at each injection site 1 ml of green retrobeads – Lumafluor Inc) with a Hamilton syringe in order to mark the recording area, and explore its neuroanatomical connections. Two weeks following the injections, the ferrets were euthanized with an overdose of sodium pentobarbital (65 mg/kg ip) and transcardially perfused with saline followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for subsequent histological study. The

brains were removed, postfixed overnight in 4% paraformaldehyde, followed by a series of 15% and 30% sucrose until sunk. The brain was cut in 50 mm coronal sections in a freezing microtome and collected in 0.1 M phosphate buffer. Some sections (1:2) were stained with cresyl violet for Nissl in order to view cytoarchitectural structure.

Stimuli

During physiological recording, digital stimuli were converted to analog signals, amplified, and delivered through inserted earphones (Etymotic) that were calibrated *in situ* at the beginning of each experiment to ensure uniform, equalized gain across a 5-octave frequency range from 500 Hz to 16 kHz. Visual stimuli were delivered through two LEDs positioned just to the left and right of the midline, 15 cm in front of the animal.

All auditory stimuli were presented at ~70 dB SPL, and references and targets were consistently of the same duration (either 1 or 1.5 sec) for a given behavioral block in an experimental session. For the tone detection task, references consisted of 30 different broad-band (500-16000 Hz) temporally orthogonal ripple combinations (TORCs, Klein et al., 2000) and targets consisted of pure tones. Although the target tone frequency in tone detection tasks varied randomly during training and behavioral physiology experiments, in any single block of recordings usually only one or two tone frequencies were used. In other detection tasks with different targets (such as detecting a click sequence target, or a tone embedded in noise), the reference sounds also consisted of TORCs and the target, while fixed for a given experimental block, would vary in successive blocks. In two-tone discrimination tasks, or click rate discrimination tasks, the

reference stimuli were brief TORCs (0.5 sec) with attached tones or clicks of different frequencies (**Figure1B**). In visual discrimination experiments, a steady light (1 sec duration) served as a reference stimulus, whereas a spatially separate brighter flashing light served as the target.

All passive task measurements used precisely the same set of reference and target stimuli, in the identical order as the trial sequences in behavioral blocks, but without any behavioral contingencies. The animal was cued to the passive condition by the absence of water flow from the spout. Hence, no licking was observed during passive stimulus presentation.

Analysis

To measure single unit spiking activity, the continuous electrophysiological signal was digitized and bandpass filtered between 300 and 6000 Hz. Single units were then classified using principal components analysis and *k*-means clustering (David et al., 2009). Only clusters with 80% or greater isolation across all data files (i.e., 80% or more spikes in the cluster were likely to have been produced by a single neuron) were used for analysis. Varying the isolation threshold from 80% to 99% did not change any of the population-level effects observed in this study. The tail shock introduced a strong electrical artifact, and signals recorded during this period were discarded before processing.

Significant neural modulation by auditory and visual stimuli was determined by linear regression of time-varying spike activity (binned at 50 ms) against stimulus (target and reference) and

motor (licking) events. Neurons were classified as significantly modulated if the occurrence of a stimulus predicted a change in firing rate that could not be explained by motor activity alone ($p<0.05$, jackknifed *t*-test), preventing any artifactual stimulus modulation due to correlations in motor activity and stimulus events. Examples of neurons with activity significantly correlated with sensory or motor events appear in **Supplementary Figure S7**.

To compare responses across the neural population under different behavior conditions (passive versus active, tone detection versus discrimination, etc.), the average firing rate was measured during the 1-1.5 sec duration of stimulus and the subsequent 800 ms silent period/shock window. Similarity of population responses was then measured by the correlation coefficient between the average responses under the different conditions. Significance was determined by a randomized paired *t*-test (a bootstrapping procedure by which the probability of the measured correlation coefficient was computed directly from a distribution of correlation coefficients measured for randomly shuffled behavior conditions).

Local field potentials (LFP) were extracted by low-pass filtering the same continuous recordings below 200 Hz. A hardware notch filter removed artifacts at 60 Hz, and activity between 55 and 65 Hz was discarded to avoid artifacts.

Coherence of simultaneously recorded LFP was computed using a multi-taper method (Mitra and Bokhil, 2008). Because coherence has a fixed lower bound of zero, noise biases coherence toward positive values. Thus, when comparing coherence between behavior conditions, the number of trials included in each condition was fixed so as to keep the noise bias the same in

each condition. Significant differences in coherence between behavior conditions were determined by a jackknifed *t*-test measured across different recording sites.

FIGURE CAPTIONS

Figure 1. Sites of physiological recordings. (**a)** Schematic lateral surface view of the ferret brain indicating the location of auditory cortex and frontal cortex. The auditory cortex (AC) is located on the anterior and posterior ectosylvian gyrus (ASG and PSG), with A1 situated in the dorsal posterior AC. The proreal gyrus (PRG) and the orbital gyrus (ORB) comprise part of the ferret prefrontal cortex. The premotor cortex (PMC) spans the rostral portion of the anterior sigmoid gyrus (ASG). We recorded with a 4-electrode array from the PRG and/or PMC in the frontal cortex, and simultaneously from a second 4-electrode array in A1. The numbers (1-3) indicate the rostrocaudal position of recording sites in frontal cortex as shown below in two representative brains **1b** and corresponding coronal sections **1c**. (**b)** Dorsal view of brains of two experimental ferrets. The recording areas in frontal cortex are encircled (FC recordings were made in both hemispheres, but for simplicity, shown only in the right hemisphere). The location of A1, where simultaneous recordings were made, is also indicated. The numbered lines indicate the rostrocaudal position in FC of the coronal sections shown below **1c**. Stars indicate recording sites marked by lesions and fluorescent dye in these sections. (**c)** Coronal Nissl-stained sections from three different rostrocaudal levels of frontal cortex (as indicated in **1b**) showing recording sites. (1): section through PRG. Arrowheads indicate entrance and endpoint of penetrations marked by lesions. (2): section at the rostrocaudal level of the PRG and PMC border. The arrowhead points to the cortical depression caused by numerous electrode penetrations superficially marked by fluorescent dye (green beads) as seen in the lower inset figure. (3): section through rostrolateral PMC. Arrowheads point to two recording sites labelled with fluorescent dye (green beads), which are shown in greater detail in the corresponding upper inset.

Figure 2. Behavioral paradigm and examples of typical FC neuron responses. **(a)** Conditioned avoidance task structure. In each trial, the animal was presented with a random number (1-7) of reference sounds (*blue*), to be discriminated from a target sound (*red*) that differed along a feature dimension that defined the task. Animals freely licked water from a spout throughout the reference stimuli, and learned to refrain from licking upon hearing the target sound in order to avoid receiving a small shock during the "shock window" 400-800 ms after target offset. All of the auditory stimuli were of equal duration (consistently either 1 sec or 1.5 sec in a given behavioral block) and sound level (~70 dB). In most tasks, reference sounds consisted of spectrotemporally modulated broadband noise (Klein et al., 2000). In detection tasks, the target stimulus could vary substantially. The animals were trained to respond to a range of acoustic targets such as a pure tone (fixed or variable frequency), tone-in-noise, or click-train. In the two-tone discrimination task, *both* reference and target stimuli were hybrid sounds, consisting of modulated noise stimuli with a tone attached at the end: the two tones differed in frequency. In the visual tasks, reference stimuli consisted of a series of steady lights followed by a brighter, rapidly flashing target. (**b)** unit showing enhanced responses during a tone-detection task. The PSTH responses to the target tone (red) and the reference noise (blue) were aligned to the onset of the 1-second long stimuli (indicated by black bar at top; white bar indicates quiet 400 ms reaction interval; red bar indicates 400 ms shock window between 400-800 ms following target offset). No responses were observed during passive presentation of the acoustic stimuli prior to the task. During the task, only the target tone elicited strong responses that declined gradually after the end of the tone. Post-task, the response to the target persisted weakly. (**c)** PSTH of responses of a suppressed cell in a detection task sequence. Panels display responses of the same

FC neuron before behavior, and during a series of detection tasks (for three types of target – tone, tone-in-noise and click train). *Prior* to behavior, activity was weakly suppressed by target sounds, perhaps because of persistent effects from prior behavioral sessions. During the behavior, suppression was stronger and built up, becoming substantially stronger well past the offset of the target tone, through the 400 ms reaction window and into the 400 ms shock window.

Figure 3: FC population target responses during tone-detection behavior. **(a)** Three heat maps show population responses (*n*= 219) to target sounds during tone-detection tasks in the pre-behavior passive condition (left panel), during active behavior (middle panel), and in the post-behavior passive condition (right panel). Each horizontal line in the heat map illustrates modulation in a single neuron during 400 ms before onset, 1 sec during the stimulus and 800 ms after offset. The neurons are normalized to have the same peak modulation and are grouped by sign (enhanced versus suppressed) and ordered by onset latency of modulation during behavior. Red indicates activity enhanced from baseline and blue indicates suppressed activity. After behavior, there are some faint, persistent post-behavioral passive effects (right panel). In contrast, the little modulation present in the left panel pre-behavioral passive heat map is likely due to the imprint of previous behavioral sessions on the same recording day. (**b)** Each panel shows the average percent target and reference modulation for neurons that showed enhanced target responses, during each of the behavioral conditions in **3a**. Stimulus and shock windows are labeled as in Figure 2. During behavior, population activity increases rapidly after the target onset (red line) and is sustained through the shock window before returning rapidly to baseline. Very little modulation by references is observed (blue line). In addition to being weaker, persistent modulation after behavior tends to return to baseline soon after target offset. (**c)**

Average target and reference modulation for target-suppressed cells in **3a**. During behavior, response latency is slightly slower than for enhanced neurons, but otherwise shows similar persistence.

Figure 4. FC categorical responses to task-relevant sounds. (**a)** A single unit's response to two randomly alternating target tones in a tone detection task, plotted as in **Figure 2**. This cell responds to either of the two target tones (550 or 2200 Hz) but not to any of the 30 different reference noise stimuli. (**b)** During tone discrimination the 550 Hz tone becomes a reference sound, and the unit now stops responding to this tone, while maintaining its response to the 2200 Hz target tone. (**c)** Each point indicates the response of a neuron (n=66/118 responsive neurons) to the same target tone during tone detection (vertical axis) and discrimination (horizontal axis). Responses to the target tone are strongly correlated between behavior conditions (r=0.51, p<0.001). The regression line (blue) has a slope of 0.9, indicating that response magnitude is similar in both conditions. (**d)** Responses of the same neurons to the tone whose categorical meaning switches from target during tone detection to reference during two-tone discrimination, plotted as in **4c**. Responses are much weaker when the tone acts as a reference in the two-tone discrimination task, and the responses are not correlated between behavior conditions (r=0.16, p>0.2).

Figure 5. Persistence and extinction of FC responses following behavior. **(a)** Neuron showing persistent target modulation following tone detection behavior. Behavior condition (passive or active) and time relative to the beginning of behavior (hours:minutes) is indicated in the upper right of each panel, with PSTHs plotted as in Figure 2. The target response post-behavior is more

phasic than during behavior and fades within one hour after the task performance. (**b)** Neuron showing exceptionally persistent modulation, plotted as in **5a**. Target responses persist over two hours after the task was completed and returns rapidly to high levels during a second behavior session. (**c)** Average normalized response magnitude for the population of behaviorally activated neurons (n=219), as a function of trial number since the beginning of each epoch (i.e., before, during, after, and long after behavior). The dashed line indicates responses of the subset of 94 "memory" neurons that were significantly modulated after behavior. (**d)** Scatter plot comparing target modulation during behavior (horizontal axis) versus post-behavior (vertical axis, r=0.55, p<0.001, slope=0.31).

Figure 6. Selective top-down modulation of LFP in A1 and FC. (**a)** Average local field potential (LFP) response in FC evoked by reference (left panel) and target stimuli (right panel) during pre-behavior passive presentation (green line) of tone detection stimuli and during behavior (red line). Responses are evoked by both references and targets during passive presentation, despite the absence of spiking responses. During behavior, the magnitude of the reference response is reduced, while the target response shows stronger early depolarization and later depolarization. (**b)** Average coherence of LFPs recorded simultaneously in A1 and FC during reference phase of tone detection behavior (left panel, n=339 pairs of A1 and FC sites). During passive presentation of reference sounds (both pre-behavior passive, blue, and post-behavior passive, green), there was strong coherence in the alpha-beta frequency range (10-20 Hz). This coherence was greatly diminished during behavior (red). Average coherence measured only during the first 10 trials of each condition (~40 reference stimuli) reveals that the passive post-behavior condition was only partially restored to the original pre-behavior baseline, reflecting the persistent change in

coherence and the gradual return of intra-areal communications to the passive state. (**c)** In some recordings (n=102 site pairs), two successive tone detection blocks with different target frequencies were performed. Data from these experiments were divided into two groups, pairs where the target frequency was near the best frequency (BF) of the A1 site (left panel) or far from its BF (right panel). The behaviorally-induced decrease in 10-20 Hz modulation occurred almost exclusively for the A1 sites near the target frequency and was dramatically diminished for the far A1 sites.

Figure 7. Bimodal and unimodal sensory responses in FC. **(a)** Examples of single unit responses to successive auditory and visual tasks, plotted as in Figure 2. The cell in the top row shows modulation during behavior only to the auditory target, whereas the cell in the second row shows target modulation by both the visual and the auditory stimuli. This cell also shows persistent target responses, which are particularly strong for the visual target. (**b)** Scatter plot of auditory (horizontal axis) and visual (vertical axis) target responses shows that cells respond similarly if they are bimodal (i.e., respond to both auditory and visual stimuli, n=93, r=0.30, p<0.01). (**c)** Venn diagram reveals that about 1/3 of responsive FC neurons show bimodal response modulation by both visual and auditory targets, and 2/3 show unimodal modulation by either visual or auditory targets.

SUPPLEMENTARY FIGURE CAPTIONS

Figure S1. (**a)** Example of performance during a tone detection task. The average lick rate during the presentation of reference sounds (blue line) is flat across the duration of the sounds and the silent periods before and after, indicating that the ferret knows that is "safe" to lick to this class of stimuli. After the onset of the target tone (0.4 sec), however, the lick rate drops to nearly zero (red line), and remains low throughout the duration of the shock phase (1.8-2.2 sec). Bars at the top indicate sound, silent and shock epochs as in **Figure 2b** and other PSTH plots. Performance during each behavioral session was evaluated quantitatively by measuring discrimination rate (DR), 100 times the ratio of hit rate (percent trials when licking ceased in the shock window following targets) to safe rate (percent trials when licking did not cease in shock window following references) (Heffner and Heffner, 1995). DR for this session was 56. (**b)** Histogram of DR for all sessions for all five animals performing the tone detection task, free-running (left panel) and head-fixed (right panel). Chance performance of (DR=25) is indicated by the dashed red line. The lower average DR for head-fixed behavior (47.4 versus 61.0 free running) is explained largely by a lower safe rate (65.7 versus 85.9), reflecting an overall decrease in licking in the head-fixed condition. (**c)** Histogram of DR during the more difficult tone discrimination task, plotted as in **S1b**.

Figure S2. (**a)** Rasters of spiking activity during reference (blue) and target (red) presentation for the neuron shown in Figure 2B. Rasters are sorted from first (bottom) to last trial (top). Corresponding PSTHs are plotted below the rasters. During passive listening, this neuron was not modulated by any task stimuli. During behavior, target responses appeared within the first

two trials and persisted throughout the task. Target responses also persisted briefly during passive presentation after behavior. Average lick rates during reference and target presentation show the typical decrease in licking following the onset of the target sound. In this case the animal sometimes returned to lick briefly before the actual shock window. (**b)** Rasters, PSTHs, and lick rates for the neuron shown in **Figure 2c**.

Figure S3. Histogram of target response latency for the 325 neurons that showed significant modulation during behavior. Latency was computed as the first time bin showing a significant change in firing rate from baseline ($p<0.05$, jackknifed *t*-test). Excited neurons are plotted in red, suppressed neurons in blue. Latencies were distributed over the entire stimulus presentation period, the silent post-stimulus period and the shock period. The median latency for excited neurons (540 ms) is slightly but significantly shorter than the median latency for suppressed neurons (670 ms, $p<0.01$, jackknifed *t*-test).

Figure S4. Comparison of DR versus target modulation strength for the 325 neurons that showed significant sensory modulation during behavior. Each point plots performance during an experimental behavioral block (horizontal axis) against the percent modulation of the target response for a recorded neuron (vertical axis) during the behavioral epoch. There is a weak trend toward stronger modulation for better performance, but it is not significant.

Figure S5. (**a)** Similarity of target modulation between neurons at the same recording site. Black points plot the fraction of target modulation measured for pairs of neurons sorted from the same recording site during the same experiment. The correlation between common-site pairs ($r=0.27$)

is significantly greater than zero (p<0.001, jackknifed t-test), while there is no correlation between neurons recorded simultaneously from different electrodes ~0.4 mm apart (gray dots). (**b)** Similarity of target modulation between neurons from the same electrode penetration. Black points indicate the fraction of target modulation measured for pairs of neurons recorded from the same electrode at different depths. The correlation between common-site pairs (r=0.14) is significantly greater than zero (p<0.001, jackknifed t-test), while there is no correlation between neurons recorded from different electrodes ~0.4 mm apart (gray dots).

Figure S6. (**a)** Comparison of target modulation for each neuron recorded during behavior (horizontal axis) and during pre-passive listening before behavior (vertical axis). Modulation is generally weaker in the pre-behavior condition (slope=0.175), but it is correlated between conditions (r=0.28, p<0.001). (**b)** Comparison of target modulation during and before behavior, restricted only to the first behavior session of the day. For this subset of neurons, the passive target response is weaker, and the correlation between passive and behaving responses is not significant.

Figure S7. (**a)** Example of a neuron whose activity can be explained largely by motor activity. The PSTH (left panel) shows modulation of neural responses associated with target and reference onset during behavior. However, for this neuron, the temporal pattern of modulation parallels that of the lick plot below the PSTH. Significant sensory- and motor-related modulation was determined by regression of motor and stimulus onset events against the time-varying neural response (50 ms bins), normalizing for any bias from correlations between sensory and motor events. The results of the regression (right panel) show the relative change in the neural response

associated with reference, target, and lick events. For this neuron, all observed response modulation can be explained by lick events. (**b)** Neuron whose activity can be explained largely by stimulus activity, plotted as in **S7a**. In this case, the observed modulation is explained entirely by target onsets, rather than lick events. (**c)** Scatter plot comparing single-trial spike variance that can be explained for each neuron by motor events (horizontal axis) and sensory events (vertical axis). Neurons could be either exclusively sensory, exclusively pre-motor or a combination of both.

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Figure 1

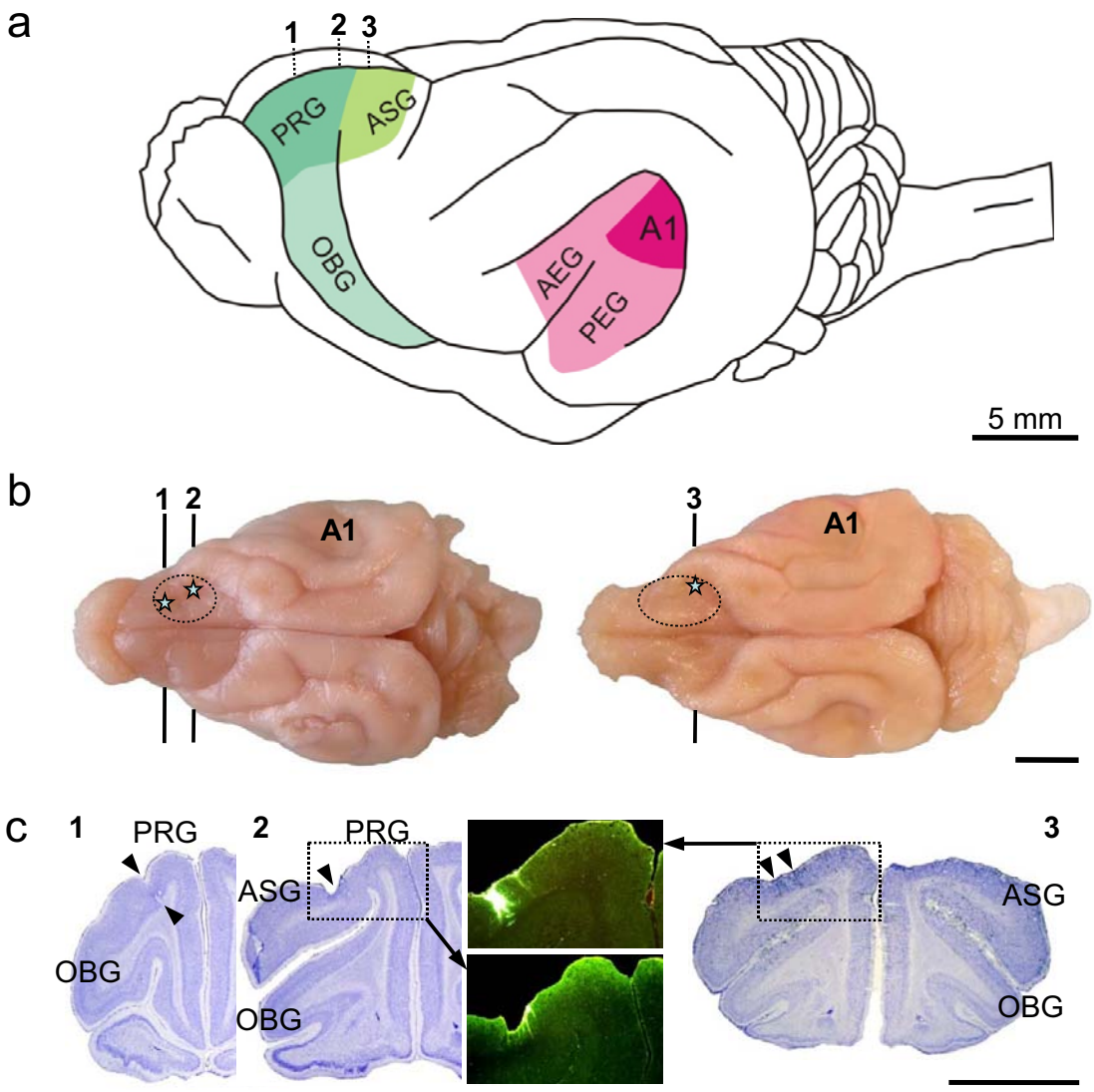

a
1
2
3
PRG
ASG
OBG
AEG
PEG
A1
5 mm
b
1
2
A1
3
A1
c
1
PRG
OBG
2
PRG
ASG
OBG
3
ASG
OBG

Figure 2

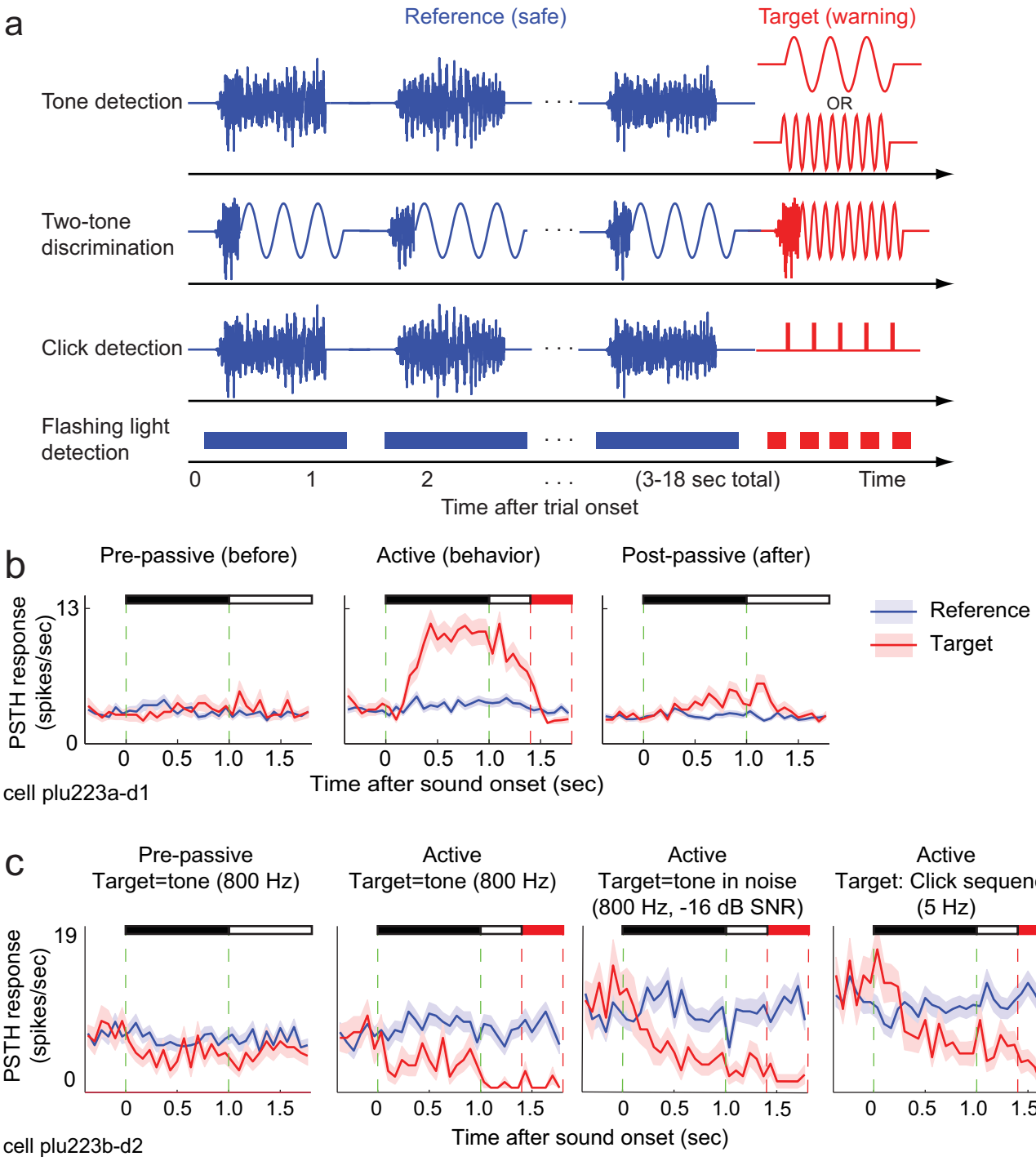

a
Reference (safe)
Target (warning)
Tone detection
OR
Two-tone discrimination
Click detection
Flashing light detection
0
1
2
(3-18 sec total)
Time
Time after trial onset
b
Pre-passive (before)
Active (behavior)
Post-passive (after)
PSTH response (spikes/sec)
13
0
0
0.5
1.0
1.5
Reference
Target
Time after sound onset (sec)
cell plu223a-d1
c
Pre-passive
Target=tone (800 Hz)
Active
Target=tone (800 Hz)
Active
Target=tone in noise
(800 Hz, -16 dB SNR)
Active
Target: Click sequence
(5 Hz)
PSTH response (spikes/sec)
19
0
Time after sound onset (sec)
cell plu223b-d2

Figure 3

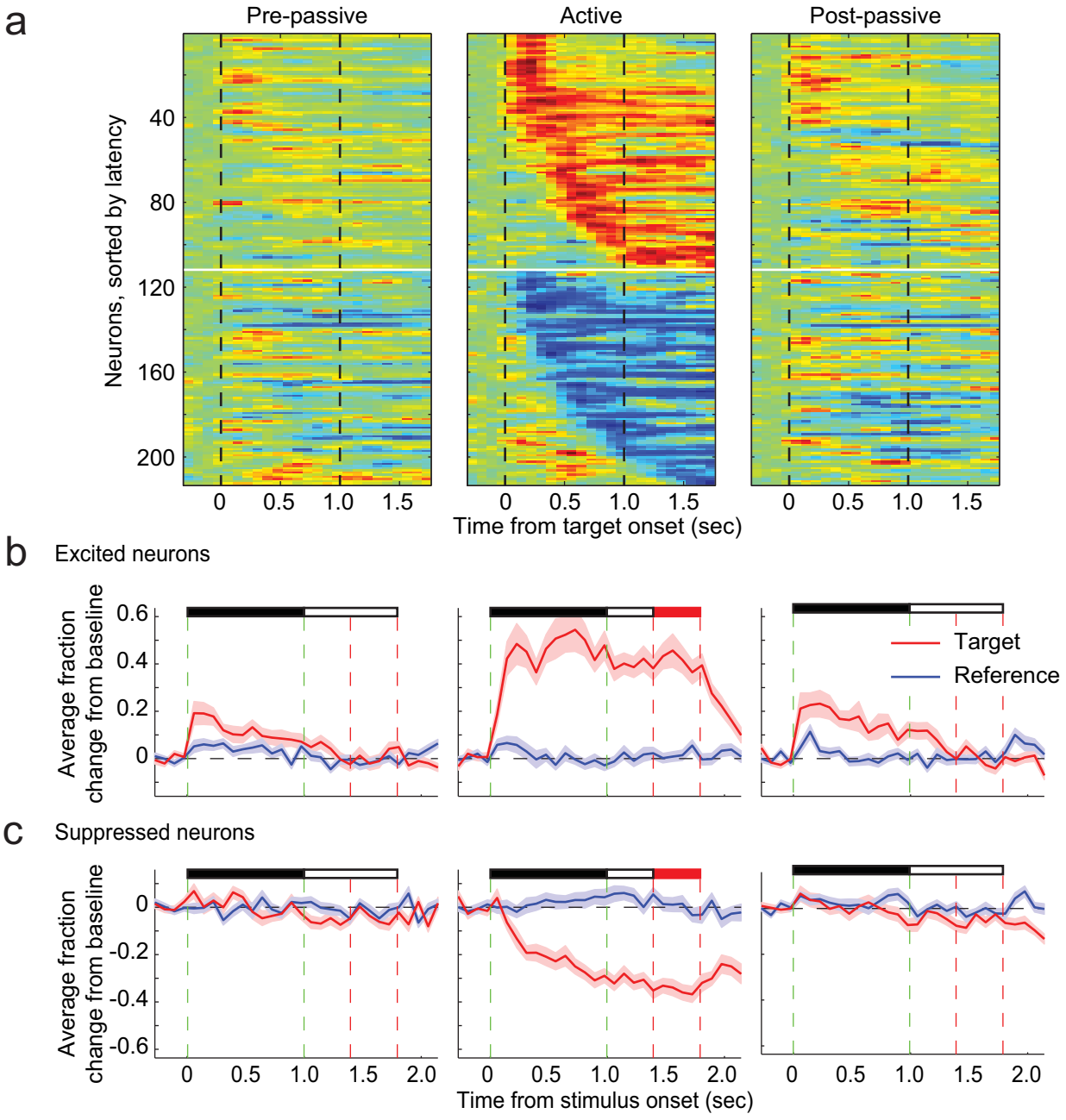

a
Pre-passive
Active
Post-passive
Neurons, sorted by latency
40
80
120
160
200
0
0.5
1.0
1.5
Time from target onset (sec)
b
Excited neurons
Average fraction change from baseline
0.6
0.4
0.2
0
Target
Reference
c
Suppressed neurons
Average fraction change from baseline
0
-0.2
-0.4
-0.6
2.0
Time from stimulus onset (sec)

Figure 4

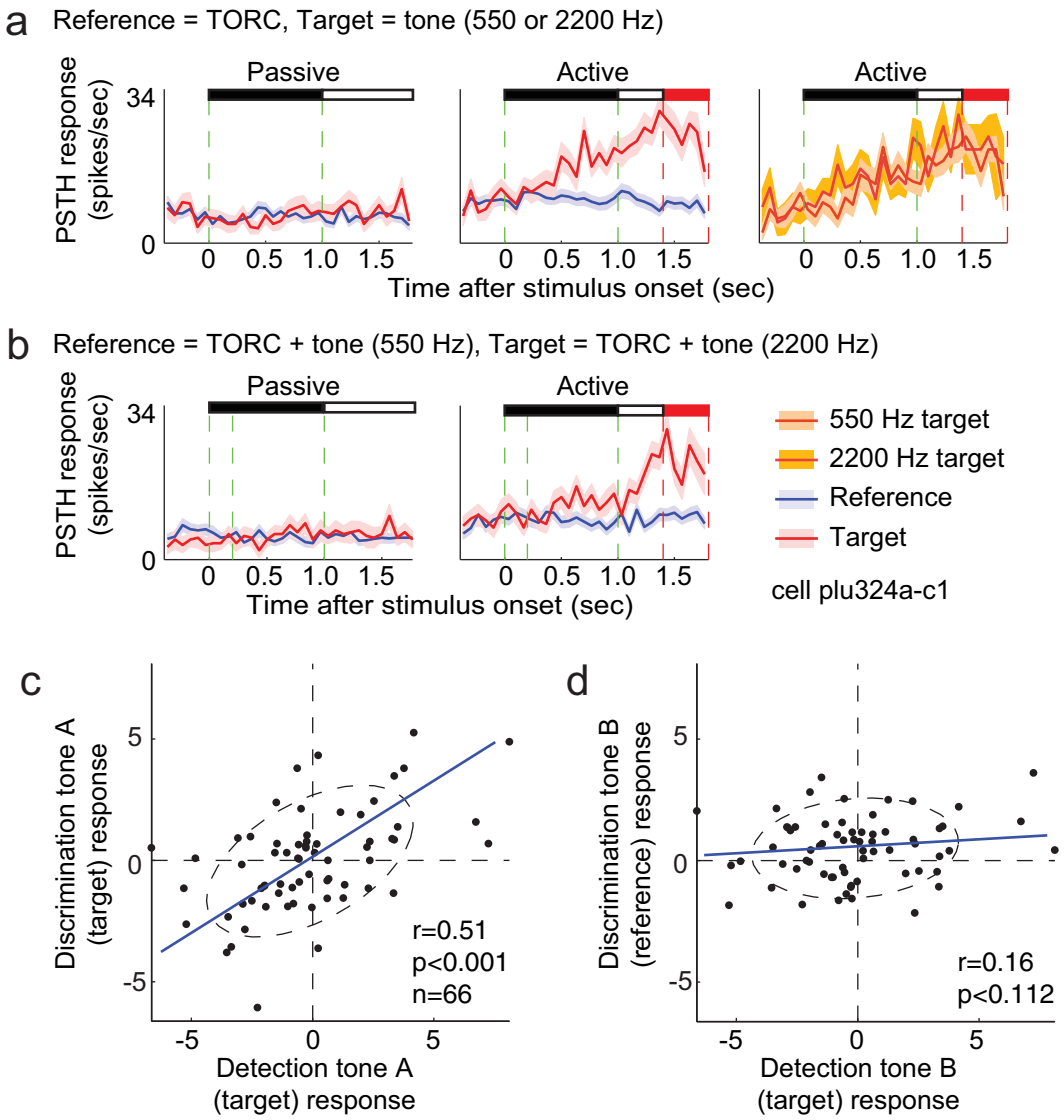

a
Reference = TORC, Target = tone (550 or 2200 Hz)
Passive
Active
Active
PSTH response (spikes/sec)
34
0
0 0.5 1.0 1.5
Time after stimulus onset (sec)
b
Reference = TORC + tone (550 Hz), Target = TORC + tone (2200 Hz)
Passive
Active
550 Hz target
2200 Hz target
Reference
Target
cell plu324a-c1
c
Discrimination tone A (target) response
5
0
-5
r=0.51
p<0.001
n=66
Detection tone A (target) response
d
Discrimination tone B (reference) response
r=0.16
p<0.112
Detection tone B (target) response

Figure 5

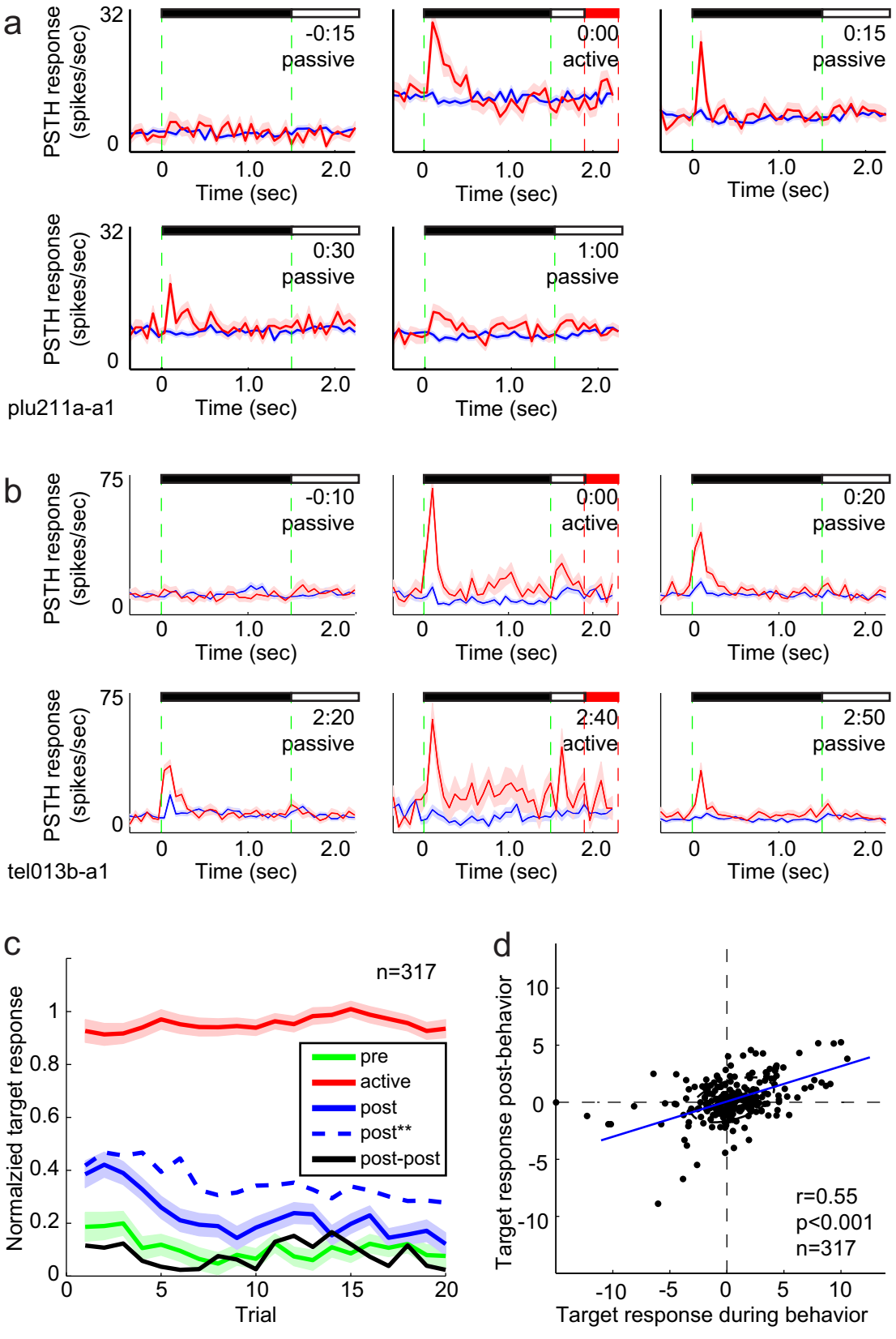

a
PSTH response (spikes/sec)
32
0
-0:15
passive
0:00
active
0:15
passive
0:30
passive
1:00
passive
0
1.0
2.0
Time (sec)
plu211a-a1
b
75
-0:10
passive
0:20
passive
2:20
passive
2:40
active
2:50
passive
tel013b-a1
c
n=317
Normalzied target response
pre
active
post
post**
post-post
Trial
d
Target response post-behavior
Target response during behavior
r=0.55
p<0.001
n=317

Figure 6

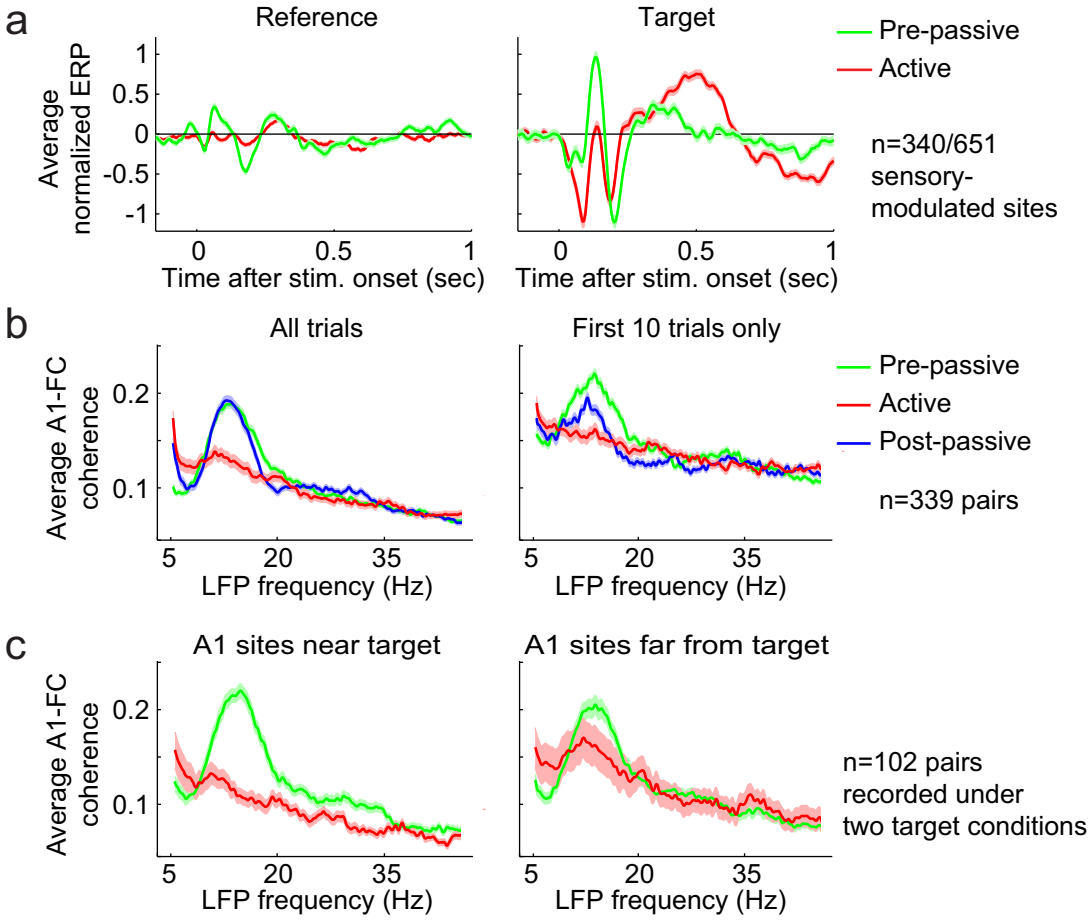
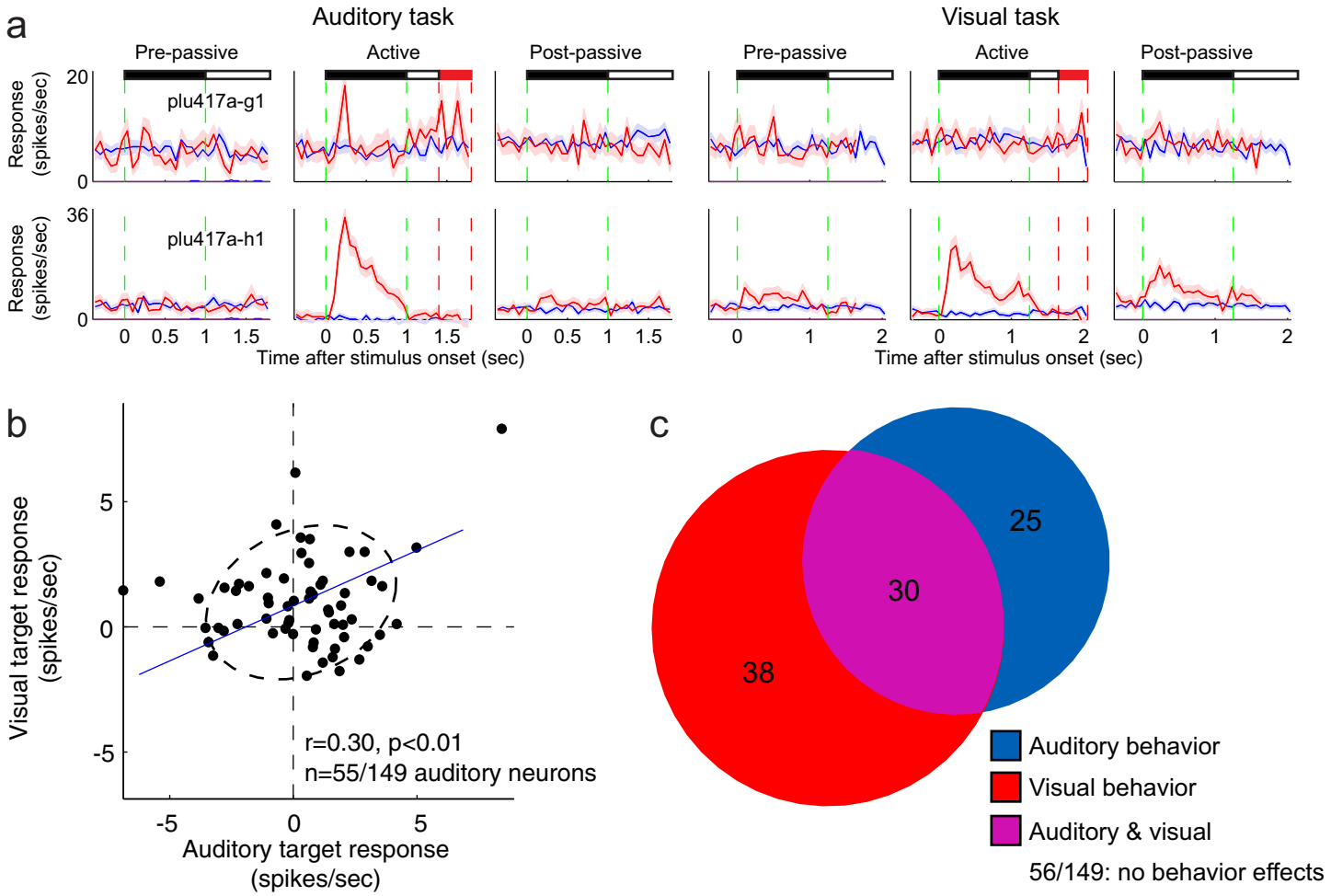

a
Reference
Target
Average normalized ERP
1
0.5
0
-0.5
-1
0
0.5
1
Time after stim. onset (sec)
Pre-passive
Active
n=340/651 sensory-modulated sites
b
All trials
First 10 trials only
Average A1-FC coherence
0.2
0.1
5
20
35
LFP frequency (Hz)
Pre-passive
Active
Post-passive
n=339 pairs
c
A1 sites near target
A1 sites far from target
n=102 pairs recorded under two target conditions

Figure 7


a
Auditory task
Visual task
Pre-passive
Active
Post-passive
Response (spikes/sec)
plu417a-g1
plu417a-h1
Time after stimulus onset (sec)
b
Visual target response (spikes/sec)
Auditory target response (spikes/sec)
r=0.30, p<0.01
n=55/149 auditory neurons
c
25
30
38
Auditory behavior
Visual behavior
Auditory & visual
56/149: no behavior effects