Title

Neural correlates of task switching in prefrontal cortex and primary auditory cortex in a novel stimulus selection task for rodents

Running title

Neural correlates of auditory stimulus selection

Author List

Chris C Rodgers, 1 - xrodgers@gmail.com

Michael R DeWeese, 1, 2 - deweese@berkeley.edu

1 - Helen Wills Neuroscience Institute, University of California - Berkeley, 132 Barker Hall; Berkeley, California 94720

2 - Department of Physics, University of California - Berkeley, 132 Barker Hall; Berkeley, California 94720

Summary

Animals can selectively respond to a target sound in the presence of simultaneous distractors, similar to the way in which humans can respond to one person's voice at a cocktail party. To investigate the underlying neural mechanisms, we recorded single-unit activity in primary auditory cortex (A1) and medial prefrontal cortex (mPFC) of rats selectively responding to a target sound from a mixture. We found that pre-stimulus activity in mPFC encoded the selection rule — the sound to which the rat would respond. Surprisingly, pre-stimulus and stimulus-evoked activity in A1 also encoded the selection rule, a cognitive variable typically considered the domain of prefrontal regions. However, stimulus tuning was not strongly affected. We suggest a model in which activation of a specific network of neurons underlies the selection of an imminent sound from a mixture, giving rise to robust and widespread rule encoding in both brain regions.

Highlights

1. Rats were trained on a novel auditory stimulus selection task
2. Pre-stimulus and stimulus-evoked activity in mPFC and A1 encoded selection rule
3. Stimulus selection did not strongly alter stimulus tuning in A1

Introduction

Humans can select and respond to one person's voice even while many others are speaking at the same time. We do this effortlessly, yet no known algorithm can solve this "cocktail party problem" in realistic settings, perhaps because we do not fully understand the relevant computations performed in the brain (Cherry, 1953; Sayers and Cherry, 1957; Ding and Simon et al., 2012; McDermott, 2009). Other social animals such as birds and rodents demonstrate a similar ability (Bee and Micheyl, 2008); for instance, mother mice respond to distinct pup calls when several are calling at once (Geissler and Ehret, 2001). Humans use selective attention, the cognitive process of selecting and responding to a single target stimulus amongst simultaneous distractors (Desimone and Duncan, 1995), to solve the cocktail party problem (Ahveninen et al., 2011). Experiments in visual selective attention reveal that prefrontal cortex sends top-down "bias signals" to sensory cortex (Miller and Cohen, 2001; Moore et al., 2003) in order to select the target stimulus and subsequently enhance its neural representation, while suppressing the representation of distractors. Similar mechanisms may be at work in the auditory cortex: electrocorticographic (Mesgarani and Chang, 2013; Zion Golumbic et al., 2013) and magnetoencephalographic (Ding and Simon, 2012) recordings show that brain activity is dominated by the attended voice. Without recordings from single neurons it is difficult to ascertain what changes on the single-neuron level give rise to these effects.

Towards this goal, we have developed a new behavioral task for rats with three key properties: first, on each behavioral trial the subject hears a pair of simultaneous sounds; second, the subject then selects and responds to a single sound from the pair; third, the subject can switch which of the sounds it selects, and does so multiple times within a session. The third property requires cognitive flexibility because, depending on which

sound the subject selects, the same pair of stimuli drives a different behavioral response ("same stimulus; different response"). We are aware of no purely auditory single-unit studies in any animal satisfying these three conditions. The analogous ability in vision — to respond to a behaviorally relevant stimulus in the presence of competing distractors — has been referred to as stimulus selection (Knudsen, 2007; Reynolds and Chelazzi, 2004; Pestilli et al., 2011) and we will refer to our task as auditory stimulus selection.

Similar visual and cross-modal tasks have been termed set shifting (Stoet and Snyder, 2004), task switching (Sasaki and Uka, 2009), and selective attention (Moran and Desimone 1985; Hocherman et al., 1976; Otazu et al., 2009). Other studies have investigated "response selection": how decisions are translated into appropriate motor actions, following stimulus selection or even in the absence of an explicit stimulus (Young and Shapiro, 2011; Turken and Swick, 1999). We also note a similarity between our task and the Wisconsin Card Sorting Task for diagnosing disorders of executive function (Monchi et al., 2001). Our behavioral paradigm shares attributes with all of these, but for consistency we will refer to our task as stimulus selection below.

Although monkeys are the traditional model organism of choice for complex cognition (Gold and Shadlen, 2007), rodents are capable of sophisticated decision-making, in some ways very similar to humans (Raposo et al., 2012; Brunton et al., 2013; Zariwala et al., 2013). Rodents also show behavioral flexibility under the control of the prefrontal cortex (Karlsson et al., 2012; Kvitsiani et al., 2013; Young and Shapiro, 2011), even though this region is not necessary for simple sensory discriminations (Pai et al., 2011). The mPFC in particular appears to be critical for task switching (Birrell and Brown, 2000; Floresco et al., 2008; Durstewitz et al., 2010; Ragozzino et al., 1999). For example, when rats learn to switch the navigational strategy they use to solve a maze, the mPFC encodes this switch and inactivating the area severely disrupts performance (Rich and Shapiro; 2009). Rodent mPFC thus appears to maintain a representation of the current task rule, analogous to the rule-encoding neurons observed in primate PFC (Wallis et al., 2001; Asaad et al., 2000; Johnston and Everling, 2007), although large parts of the monkey PFC appear to be functionally and anatomically unique to primates (Wise, 2008).

Although much is known about visual stimulus selection, we are unaware of any single-unit studies of purely auditory stimulus selection. Moreover, a rodent model of this behavior would be especially useful because of the ease and speed with which they can learn cognitively demanding tasks (Carandini and Churchland, 2013) and as a first step toward more complex behaviors, such as selective attention, which at present are typically studied only in primates. Toward these goals, we recorded from single neurons in both mPFC and the primary auditory cortex (A1) of rats performing our task. We found that the pre-stimulus, anticipatory activity of our recorded neurons in mPFC encoded which sound would be selected. Surprisingly, we also found this pre-stimulus effect in a sizable fraction of our recorded neurons in A1. Finally, stimulus-evoked activity in both brain regions was similarly modulated, although this did not appear to alter tuning properties in a way that would be obviously beneficial for responding to the selected sound.

Results

A novel behavioral task for rodents: auditory stimulus selection

We developed an auditory stimulus selection task for rats, in which the subject was trained to respond to either of two simultaneously presented sounds.

The rat initiated each trial (Figure 1A) by holding its nose in the center port of a three-port behavior box for at least 250 ms — the “hold period.” This triggered speakers on the left and right to play in stereo one of the following four equally likely stimulus pairs: LEFT+HIGH, RIGHT+HIGH, LEFT+LOW, or RIGHT+LOW (Figure 1B). Each stimulus pair was a simultaneous combination of a broadband noise burst from either the LEFT or RIGHT speaker, and either a HIGH- or LOW-pitched warble (frequency-modulated tone). After the stimulus, the rat could then choose to “go left” (poke its nose in the left port), “go right” (poke its nose in the right port), or “nogo” (not poke either side). Correct pokes into the side ports were rewarded with water; incorrect pokes were penalized with a 2-6 s timeout (Methods).

On each trial, one of the sounds in the stimulus pair (the “target”) indicated the correct response but the other sound (the “distractor”) was uninformative. To indicate which sound the rat should select, the behavioral session alternated between “localization” blocks of trials (during which the noise burst was the target) and “pitch discrimination” blocks (during which the warble was the target). Each block consisted of 80 trials each (Figure 1C), the first 20 trials of which were reserved to indicate the block change. During these 20 “cue trials,” the rat heard only a target sound without distractor.

Behavioral controls (Figures S2B, S2C) showed that the rats respond to the target sound, not to the target/distractor combination. We refer to this task as auditory stimulus selection, by which we mean that the rat selectively responds to one of two simultaneous sounds, and can be trained to select either of the two sounds on demand. (An alternative term is stimulus feature selection, since two simultaneously played sounds may be perceived as a single sound with two features.) Stimulus selection — selectively responding to a behaviorally relevant target in the presence of distractors — is one component of selective attention, a broader and more complex ability that also includes perceptual enhancement (Knudsen, 2007; Reynolds and Chelazzi, 2004; Pestilli et al., 2011). Tasks that require sustained tracking (Mitchell et al., 2007) or the enhanced detection of faint stimuli (Cohen and Maunsell, 2009) are the gold standard of selective attention research. Nonetheless, our task represents an important step forward; we are aware of no other paradigms to study stimulus selection in rodents, nor any single-unit studies of purely auditory stimulus selection in any animal.

Rats perform the task well above chance

We ensured that the rats were in fact selecting the correct target sound by verifying that their behavioral response was driven by the target sound, significantly above chance and significantly more than it was driven by the distractor sound, and also that they were not using the same stimulus/response strategy in both blocks. Some strategies allow 50% performance without using any information from the target, such as always going to the choice port for the current block even in response to a nogo target, a strategy that we commonly observed in rats before they were fully trained. For this reason we verified that performance was significantly and consistently greater than 50% in both blocks, and also that the animals were responding to the target

sound and not the distractor (Methods), before and after implanting the recording electrodes. This typically required about 40 one-hour training sessions, over about eight weeks. Our best rats' typical performance during recording sessions was approximately 85% in both blocks (Figure 2). In general, the rats performed well above chance, rapidly and correctly changing which sound they selected after each block change.

Because our task associates a different choice/reward port with each block, a different distribution of motor responses is required in localization (50% go left, 50% nogo) than in pitch discrimination (50% go right, 50% nogo). We note two consequences of this. First, this allows us to identify an interesting type of error trial on which the rat appeared to respond to the wrong sound. On such "interference" trials, the rat heard a "go" distractor and incorrectly went to the choice port associated with that distractor, instead of doing what the target sound indicated. We later analyze the neural correlates of this error. Second, it is plausible that the rat's motor plan differs between the blocks. There is a similarity in this sense between our task and some blocked visual spatial attention tasks, in which 80% of the trials require a saccade in the same direction (Cohen and Maunsell 2009). It can be difficult to tease apart response selection from stimulus selection (but see Erlich et al., 2011; Sato and Schall, 2003; Steinmetz and Moore 2012). We return to this issue later.

Anticipatory neuronal activity in mPFC encodes the selection rule

We next asked what differences in neuronal activity between blocks correlated with the selection of the target. We implanted tetrodes into A1 and/or mPFC and recorded single-unit action potentials (spikes) from multiple neurons during behavior. By analogy with the rule-encoding neurons in primate prefrontal cortex, we hypothesized that mPFC would encode the selection rule. That is, we expected that the firing rates of single mPFC neurons would differ significantly between localization and pitch discrimination trials. We first confined our analysis to the hold period, the interval before stimulus onset while the rat is holding its nose in the center port and presumably preparing to select the target sound from the imminent stimulus pair.

We found that the hold period activity of a majority of mPFC neurons robustly encoded the selection rule on correct trials. An example unit (Figure 3A) fired significantly more ($p < 0.001$, Mann-Whitney U-test) in the hold

period during localization trials (mean: 12.1 Hz) than it did during pitch discrimination trials (mean 7.2 Hz). A different but simultaneously recorded single unit in mPFC (Figure 3B) fired significantly more during pitch discrimination (mean 5.4 Hz) than during localization (mean 2.7 Hz). In both cases the effect persisted across the entire session of over 1300 trials, alternating with each block just as the behavior did. Across our recorded population of mPFC neurons, 63% (76/121) of the neurons individually and significantly encoded the selection rule during the hold period (Figure 3C). Of these, 36 neurons preferred (*i.e.*, fired more during) localization and 40 preferred pitch discrimination; neither preference was significantly more common (binomial test, $p > 0.05$).

Anticipatory neuronal activity in A1 also encodes the selection rule

Surprisingly, we also found a similar effect in A1 (Figure 4). Although encoding of selection rule was our hypothesized result in mPFC, this was unexpected in A1, especially given the absence of auditory stimulation in the pre-stimulus period. Across our recorded population, 36% (36/99) of A1 neurons encoded selection rule. As with mPFC, neither population was significantly larger (13 preferring localization, 23 pitch discrimination; binomial test, $p > 0.05$). Since A1 is known to encode many types of sounds in a sparse fashion (DeWeese et al., 2003; Hromádka et al., 2008; Carlson et al., 2012), we were not surprised to observe that only some of our recorded neurons in A1 significantly responded to our task stimuli (Supp Info). However, rule encoding was approximately equally widespread in both stimulus-responsive (14/49) and non-responsive (22/50) neurons. This finding is reminiscent of human imaging results suggesting that neurons in auditory cortex may carry top-down attention signals even in the absence of stimulus information (Ahveninen et al., 2011).

These effects were strong: among the significantly rule-encoding neurons, the median increase in firing rate during the preferred block was 74.7% in mPFC and 99.7% in A1. We controlled for the possibility that these results in either brain region could be explained by firing rate drift over the course of the session or by spike sorting errors arising from small differences in spike waveform shape between blocks (Supp Info). We did not observe clustering or any other topographic organization of neurons preferring the same block, which implies that these effects could have been obscured in multi-unit recordings. In sum, these results demonstrate widespread and robust encoding of selection rule in the pre-stimulus activity of both mPFC and A1 neurons.

Error trial analysis

In the previous sections we considered only correct trials. We next considered interference trials, during which the rat erroneously chose the port associated with the other block, suggesting that it was selecting the wrong sound from the mixture. If encoding of selection rule in the anticipatory activity is important for successful stimulus selection, then the encoding should be weaker or even reversed when the rat selected the wrong sound.

In mPFC, the encoding of selection rule was significantly weakened on interference trials, versus correct trials (Figure 3D). In A1, we observed a more extreme effect (Figure 4D): the rule encoding was actually reversed on interference trials, meaning that firing rates were greater during the non-preferred block on such trials. These observations are consistent with the idea that anticipatory activity predicts which sound the rat will respond to, even for trials on which the rat appears to respond to the distractor by going to the wrong choice port. Although the activity thus predicts a motor response to the block-irrelevant port, it does not differ between trials where the rat ultimately goes to the block-relevant port (correctly or incorrectly) or chooses the nogo response (Figures S3C, S4C).

Within-trial timescale of the encoding of the selection rule

We next asked how long before the stimulus the encoding emerged, and for how long afterwards it persisted. For each rule-encoding neuron, we compared across blocks the smoothed firing rates in every 50 ms bin before and after the stimulus onset, up to plus or minus 3 seconds from the stimulus onset. We thereby determined the largest interval of time around the hold period during which the neural activity significantly encoded the selection rule. Across the dataset, the median inter-trial interval was 4.0 s (inter-quartile range: 2.7 s to 5.3 s) and so this time range (plus or minus 3 s) will overlap with the previous and/or next trial in many cases.

The temporal dynamics of the encoding varied widely across neurons in both regions (Figures 5A, B). For some neurons, rule encoding was strictly confined to the hold period: their firing rate was modulated only in the

immediate pre-stimulus period. Other neurons showed significant encoding at all time bins tested: their firing rate was persistently elevated throughout the preferred block. We found neurons spanning this range of timescales in both brain areas. In A1, the median rule-encoding unit first developed a significant block preference 0.55 ms pre-stimulus (IQR: 0.15 s to 1.2 s); in PFC the median was 0.625 ms pre-stimulus (IQR: 0.34 to 1.0 s). That is, the majority of rule-encoding neurons developed this property well before the rat initiated a trial by center-poking.

To examine the typical dynamics within each population and to determine which brain area first encodes the selection rule, we averaged the normalized activity (mean: 0, variance: 1) of all rule-encoding neurons in both brain regions during their preferred block. On average, the population activity ramped up gradually before stimulus onset, over a timescale of several seconds, and then fell relatively quickly afterward (Figure 5C). The activity in mPFC was first significantly elevated 2.8 seconds before stimulus onset, while population activity in A1 became elevated 0.9 seconds before stimulus onset. That the effect occurs first in mPFC is consistent with its hypothesized role as the origin of top-down bias signals to sensory cortex (Miller and Cohen, 2001); however, we emphasize that the wide variability in timescale within both regions, and the fact that only a minority of our dataset was collected in simultaneous A1/PFC recordings, complicates a direct comparison between brain regions.

Changes in baseline activity correlate with similar changes in evoked activity

Given that the pre-stimulus activity encoded the selection rule, we next assessed whether the stimulus-driven activity in A1 differed between blocks. We first defined the evoked response window of each neuron as the period of time after stimulus onset during which the firing rate was significantly elevated above the pre-stimulus rate (Supp. Info). The evoked response on each trial was then defined as the number of spikes emitted during this window. We analyzed the mPFC neurons in the same way and found a population of neurons showing auditory responses to our task stimuli that were low-latency and tightly locked to stimulus onset, similar to A1 (Figure 6A, B). Such neurons were rarer in PFC than in A1, though not significantly so (PFC: 31/90, A1: 49/99; $p > 0.05$, Fisher's exact test). Evoked responses were significantly weaker in PFC (Figure S6).

Based on the previous results, in which we found that the increased firing rate during the preferred block often persisted for a period of time after stimulus onset, we expected that the evoked firing rate would also be higher during the preferred block. In both regions, this is indeed the case: an increase in pre-stimulus firing rate during one block correlates with an approximately equal increase in evoked firing rate during the same block (Figure 6C, D; exemplar: Figure 4B). 21% (9/43) of A1 neurons and 24% (4/17) of PFC neurons showed a significant elevation of evoked response during their preferred block. We note that this analysis had less power than the pre-stimulus analysis because of the additional variability introduced by the stimulus dependence (Supp. Info).

We next used an ideal decoder analysis (Methods, Figure 6E) to ask whether the recorded neurons encoded the identity of the noise burst or warble with greater fidelity in either block, either due to changes in stimulus tuning, the baseline elevation described above, or some other effect. We can decode the identity of both the noise burst and the warble from the evoked responses in A1 (n=57 neurons in 22 simultaneous ensembles) and PFC (n=25 neurons, 13 ensembles). The A1 neurons provide a significantly better source of data from which to decode the sound identity, probably due to their stronger responses and tighter stimulus selectivity. However, for both brain regions and both sounds, we cannot decode the sound any more accurately from the localization trials than from the pitch discrimination trials. Other versions of this analysis yielded generally similar results, even when we separately considered neurons preferring each block (Supp. Info, Figure S6F).

We conclude that, although neurons showing an increased pre-stimulus firing rate in one block generally showed an equivalent increase in the evoked rate during the same block, these changes in evoked rate do not obviously improve the detectability of the target sound. However, we note that our ensembles of neurons provided useful information about the identity of both sounds, and the brain has access to a pool of neurons orders of magnitude larger than our recorded population. It may be that the problem faced by cortex in this task is not to maximize the information available about the stimuli in individual neurons, but rather a wiring problem

of how to flexibly re-route the relevant stimulus information to the relevant motor neurons at every block change.

Discussion

Auditory stimulus selection: task switching between conflicting auditory discriminations

When human listeners hear two simultaneous voices they can selectively respond to either one. This is a complex ability, and our task models one part of it — selecting and responding to one of two simultaneous sounds. Our subjects can voluntarily switch which sound they select, and do so at each block change within a single recording session. The rats learned the task with less than eight weeks of training and performed many trials per session (median: 698; inter-quartile range: 507 to 912). To our knowledge this is the first published example of rodents performing such a stimulus selection task in any sensory modality.

Previous studies have identified critical roles for mPFC in behavioral flexibility in several contexts. For example, elegant work (Rich and Shapiro, 2009) established that not only does the mPFC encode the switches in navigational strategies ("go east" vs "turn right") that rats use to solve a maze, but also that inactivating this region severely and selectively impairs their ability to perform the switch. Other studies of task switching in rodents required them to switch between a sensory discrimination and a (potentially habitual) fixed response ("follow the light" vs "always go left"; Floresco et al., 2008; Durstewitz et al., 2010). Many researchers are interested in extending these results to task switching between sensory discriminations, but it is often challenging to induce the switch when it requires ignoring a previously trained stimulus. Even in cross-modal switching, where the targets and distractors come from entirely different modalities, strong cueing mechanisms violating our "same stimulus; different response" condition have been used to induce the switch: introducing novel stimuli (Birrell and Brown, 2000), deleting distractors (Otazu et al., 2009), or changing the behavioral arena completely (Haddon and Killcross, 2007). Finally, most previous studies required rats to shift no more than once per session, sometimes just once per lifetime, while our study requires multiple switches per

session. We believe our task advances the study of task switching in rodents to be much closer to the standard set by human and non-human primate studies.

Despite its clinical and computational relevance (Ding and Simon, 2012), the auditory cocktail party problem remains less studied than comparable visual tasks. Even in primates we are not aware of any single-unit studies of purely auditory stimulus selection. A multi-unit study (Lakatos et al., 2013) required monkeys to sustain attention to streams of pure tones; however, the researchers found that the monkeys were unable to ignore the distractor stream if it was within 1.5 octaves of the target stream. Human voices, even those with very different pitch, are much closer than this and actually overlap extensively in acoustic frequency (McDermott, 2009). For this reason, we believe animal models of this ability should use stimuli that, like ours, overlap at least partially in frequency and require solutions not based purely on frequency separation. In sum, we believe our task represents an important first step toward understanding the cocktail party problem in rats, paving the way toward future studies with advanced genetic and cell type-specific manipulations afforded by rodent models.

Anticipatory activity in both mPFC and A1 encodes the selection rule

We found that rodent mPFC robustly encode the subject's selection rule, analogous to the rule-encoding role of primate prefrontal cortex (Asaad et al., 2000; Wallis et al., 2001; Johnston and Everling, 2007). Rule encoding develops in our recorded mPFC population over 2.5 seconds before the stimulus onset, as the rat is planning to initiate a trial or even finishing the previous trial. The widespread nature of the encoding and the broad timescales over which it persists are perhaps surprising because only one bit of information needs to be encoded — pitch discrimination or localization — and this information is only necessary while making a decision on each trial. One possibility is that this persistent activity represents a memory trace of the selection rule (Funahashi et al., 1989), meaning that it densely and persistently encodes cognitive variables like selection rule. In fact the cortex may shift to a completely different network state (Karlsson et al., 2012) depending on which stimulus the rat plans to select.

We also observed anticipatory encoding of the selection rule in primary auditory cortex, a surprising result since encoding of selection rule in the absence of sensory stimulation has traditionally been considered the domain of prefrontal areas. However, attention is known to modulate the pre-stimulus activity of single neurons in monkey V2 and V4, although not in V1 (Luck et al., 1997; Reynolds et al., 2000). At a larger spatial scale, visual attention can produce a similar increase in pre-stimulus baseline in V1, as assessed both with fMRI in humans (Pestilli et al., 2011) and with voltage sensitive dye in monkeys (Chen and Seidemann, 2011). Higher visual cortex also shows pre-stimulus modulation by attention in humans (Pestilli et al., 2011; Kastner et al., 1999; Thut et al. 2006). More generally, single neuron activity in primary sensory cortex can anticipate reward (Shuler and Bear, 2006) or a motor response (Niwa et al., 2012), and anticipation of a visual stimulus can trigger a hemodynamic response in V1, though without a corresponding change in neural activity (Sirotin and Das, 2009). In this light, then, perhaps it is not surprising that primary sensory cortex could also encode the selection rule for an imminent stimulus. In this way both the information about the stimulus and the information about how that stimulus should be interpreted are encoded in the same neurons, providing a possible locus for the behavioral decision to be made.

We observed a surprising amount of similarity between A1 and mPFC; rule encoding appears to be a distributed process. Although we observed auditory-evoked responses in both brain areas, these were weaker and less common in mPFC. This suggests that the role of mPFC in our task is to modulate stimulus processing in sensory cortex, rather than directly encoding the stimulus.

Comparison with studies of selective attention and task-relevant plasticity

This pre-stimulus change in baseline contributed in an additive way to the strength of the sensory-evoked responses in both A1 and PFC; however, we found limited evidence for any additional modulation of sensory-evoked responses in A1. For example, the neurons did not appear to encode the target stimulus with any greater fidelity than the distractor stimulus. This is consistent with some, but not all, previous studies of auditory task switching. Although neuronal activity in A1 is robustly modulated in the aroused/engaged behavioral state versus the passive/idle state (Otazu et al., 2009; Lee and Middlebrooks, 2011), the neuronal

effects of shifting between different engaged behaviors tend to be weaker or even non-existent. For instance, switching between an auditory task and an olfactory or visual task does not change evoked spiking auditory responses in A1 (Otazu et al., 2009; Lakatos et al., 2009), and switching between temporal and spatial auditory discriminations does not significantly change spatial tuning in A1 (Lee and Middlebrooks, 2011). Moreover, although a series of pioneering experiments demonstrated task-relevant plasticity in A1 of ferrets trained to detect a tone (Fritz et al., 2003; Fritz et al., 2010), these effects were somewhat nuanced: they could induce suppression or facilitation at the task-relevant frequency, and a different reinforcement paradigm could reverse the overall direction of the effect (David et al., 2012). More studies of complex auditory behaviors, potentially with recordings from higher auditory cortex, will be necessary to determine which behavioral paradigms produce task-related modulation of evoked spiking responses in auditory cortex.

At first, the lack of evidence for tuning modulation is a surprising result, given that visual selective attention enhances target representations and suppresses distractors in V4 and other visual areas (Cohen and Maunsell, 2011; David et al., 2008; Mitchell et al., 2007; Reynolds and Heeger, 2009). However, selective attention consists of two component processes with separate behavioral measures: stimulus selection and perceptual enhancement (Knudsen, 2007; Reynolds and Chelazzi, 2004; Pestilli et al., 2011). Target-enhancing modulation of evoked responses is believed to mediate perceptual enhancement (although see Zénon and Krauzlis, 2012), as assessed behaviorally by a lower threshold or steeper psychophysical curves (Cohen and Maunsell, 2009; Moore et al., 2003). This predicts that only tasks which require perceptual enhancement will produce such effects.

In contrast, stimulus selection is often investigated with easily detectable stimuli far above threshold (Hocherman et al., 1976; Stoet and Snyder, 2004) and such studies, like ours, often find no modulation of evoked responses in sensory cortex (Sasaki and Uka, 2009; V Mante, D Sussillo, KV Shenoy, WT Newsome [in review]). It may be that stimulus selection is the dominant computational challenge in such tasks and perceptual enhancement therefore less important; similarly, the cocktail party problem is difficult because both

voices are of competing intensity, not because the target voice is barely audible. Additive, pre-stimulus baseline increases have been observed with attention in V1 and may lead to efficient stimulus selection (Chen and Seidemann 2012; Pestilli et al., 2011); our data support a similar hypothesis in A1. In summary, while the mechanisms by which selective attention mediates perceptual enhancement remain an important area of inquiry, enhancement of sensory evoked responses in A1 may not be necessary for our task or other similar stimulus selection paradigms.

Stimulus selection via activation of latent circuits for each target

Based on our results, we propose a model for stimulus selection based on task-specific activation of latent circuits, rather than task-specific adaptation of a single circuit. We found subpopulations of neurons in both A1 and mPFC — one activated during the localization block, the other during the pitch discrimination block. The signature of this activation is increased baseline activity. However, they do not change their tuning for specific stimuli. We hypothesize that the difference between the circuits is their downstream connectivity: each circuit may project to separate circuits in a downstream effector region, perhaps the striatum since the corticostriatal projection plays an important role in auditory decisions (Znamenskiy and Zador, 2013). In this model, only one circuit is activated at a time, via feedforward excitation perhaps originating in mPFC, and only this circuit has sufficient baseline activity to drive behavior.

In some ways, this model is more parsimonious than the traditional tuning change model of auditory attention, which requires that prefrontal (or other) brain regions be able to modulate the tuning of many A1 neurons as quickly as the subject shifts his attention. Although attention does produce tuning changes (David et al., 2008; Fritz et al., 2003) over minutes (which is the fastest that they can be estimated from the data), it is unclear how known synaptic mechanisms could mediate task-specific tuning changes on a sub-second timescale instead. By contrast, our model requires only circuits with essentially fixed stimulus tuning, and the selection mechanism occurs by activating one of them, rather than by changing the tuning of any of them. This reflects the challenge of the task, which does not require amplifying the neural representation of a faint stimulus but rather a discrete change in sensorimotor mapping. This is consistent with the idea that task-relevant

modulation in auditory cortex acts to increase the fidelity with which the appropriate motor action can be read out (Fritz et al., 2010; David et al., 2012).

Might the pre-stimulus effects we describe be due to a difference in motor plan? Because each block is associated with a different choice port, it is plausible that the rat plans a different response in each block (go left versus go right). Rodent frontal (Erlich et al., 2011) and primate primary auditory (Niwa et al.; 2012) cortex are both known to encode imminent motor actions. In those studies the neural encoding of motor plan was observed in a delay period between stimulus and response, and correlated with the subject's response. Our neural effects differ in a few ways. First, the baseline increase precedes the stimulus and does not differentiate between trials on which the rat ultimately gave a go or nogo response (Figures S3C, S4C); thus the activity has little information about the future action of the rat, which is inconsistent with the most straightforward meaning of motor plan. (One important exception to this is that the pre-stimulus activity does predict motion to the wrong port on the relatively rare interference trials. This could reflect a plan to select the wrong stimulus, to go the wrong port, or both.) Second, the timecourse of the neural effects was quite protracted, even persistent, in many of our recorded neurons. The baseline increase precedes the initiation of the trial, often overlapping with the previous trial. Throughout this period, the rat is engaged in various motor actions, such as reward consumption. Nevertheless, the effects we have observed may still correlate with the rat's motor plan. Our task requires remapping sensory stimuli to motor responses, and it is reasonable to expect rule encoding to reflect both the sensory and motor aspects of this remapping.

Our results establish the rat as a model organism for auditory stimulus selection, paving the way for future investigations of the cocktail party problem with emerging optical and genetic tools amenable to rodents. We have presented what we believe to be the first single-unit results in any animal performing an auditory stimulus selection task and we have found widespread and robust rule encoding in mPFC and A1, though little change in the stimulus tuning of evoked responses. We propose a simple model to explain these results: task-specific activation of latent circuits, rather than task-specific adaptation of a single circuit.

Methods

Behavior training

We used male Long-Evans rats and began training them when their body mass reached 150g-200g, approximately 45-60 days old. Rats were given restricted access to water in the day before the training session so that they would be motivated to obtain water rewards. After each session they were given ad lib access to water for one hour. We monitored body weight and other markers to ensure they remained healthy.

We used a typical “shaping” procedure to train the rats. First they learned the localization task and pitch discrimination tasks separately and without a distractor. Next they learned to alternate between the tasks. Finally they learned to respond to the mixed stimulus containing target and distractor based on the block. Human intervention was required to determine when the rats were ready to progress to the next stage of training (generally, at least 80% hit rate). Human intervention was also required to discourage certain unwanted response strategies using the following tools: 1) increasing error timeout; 2) temporarily enforcing “all GO” or “all NOGO” trials (and dropping such trials from analysis); 3) giving water rewards out of the left or right port even in the absence of good performance in order to maintain motivation or encourage a task switch. Once the rats were sufficiently well-trained that little or no human intervention was required, they were implanted with the drive. The entire training process takes about 8 weeks.

Trial timings

In three rats (CR12B, CR17B, YT6A) the hold period was drawn from a uniform distribution on 0-100ms; after pilot results indicated pre-stimulus effects, the hold period duration was increased to 250-350 ms in the other three rats. All trials with a hold period <50 ms were discarded for the analyses in Figure 3 and Figure 4. Hold period response was counted in the minimum window that applied to all trials: 50 ms for the first 3 rats and 250 ms for the rest.

The duration of the choice period differed between sessions, but was fixed within a session (or if it was changed slightly within a session, then the trials before the change were discarded from analysis). Correct entries into the choice port on go trials were rewarded with water from the same port. Incorrect entries into the choice port on nogo trials results in a 2-6 s timeout. Correct nogo responses were not explicitly rewarded with water, although the rat avoided a timeout with this response. Poking neither port on a go trial was not explicitly punished with a timeout, other than a lost opportunity for reward.

Chance performance on the task

In order for the rat to perform significantly above chance within a session, its behavior had to satisfy three criteria: 1) the rat performed significantly above 50% in each block, meaning that it must be using some information from the target sound (which is the only possible source of information on the correct response) to decide whether to go or nogo; 2) the rat is significantly more likely to do the action indicated by the target than the action indicated by the distractor; 3) the rat is not using a “fixed strategy”, that is, the same mapping from stimulus pair to behavioral response in each block. (Because the target and distractor swap roles in each block, satisfying the second criterion is sufficient to satisfy the third.)

The first criterion rules out strategies like “always go left during localization”, which was a common strategy while first learning the task. We used a binomial test to compare the proportion of hits to 0.5 in both blocks and discarded any sessions that were not significantly ($p > 0.05$) above 50% in either block. The second criterion rules out certain hypothetical strategies such as always getting the congruent-nogo stimulus (RIGHT+HIGH) correct, and otherwise guessing randomly between the correct choices for that stimulus pair in each block. This fixed strategy yields 62.5% in both blocks but it uses information equally from both target and distractor; thus, it fails the second criterion. To test this, we used a paired Mann-Whitney U-test to compare whether the action on each trial was correct for the target versus correct for the distractor. In practice, none of the rats actually adopted such a hypothetical strategy: although some sessions failed the first criterion and were discarded ($p > 0.05$ for 4/55 sessions, in some cases soon after surgical implantation of tetrodes), no sessions failed the

second criterion ($p < .005$ for all sessions). Therefore the first criterion (performance above 50% in both blocks) is actually the most relevant, and we mark the chance level on the plot as 50%.

Construction and implantation of tetrode microdrives

We constructed tetrodes by cutting lengths of 12.5 micron nichrome wire coated with partially annealed polyimide insulation (Kanthal Palm Coast), twisting them, and heating with a heat gun until the 4 individual strands melted together. The tetrodes were then routed through polyimide guide tubes and glued to the moveable plastic tab within a potentiometer. We pinned the individual wires using gold pins (Neuralynx) into a custom printed circuit board (custompcb.com, beta-layout.com) that we designed. Two complete drives were built, one for A1 and the other for PFC. These were connected with a custom-designed adapter to a 32-channel preamp/headstage (Triangle BioSystems International).

Standard surgical techniques were used (Supp. Info). Craniotomies were performed directly dorsal to the target areas (A1: 5.25 mm posterior and 6.5 mm left from bregma; prelimbic (PL) region of mPFC: 3.0 mm anterior and 1.0 mm left from bregma) and the tetrodes subsequently lowered downward into the target areas before recording.

Recording and signal processing

The electrodes were lowered by approximately 100-200 microns before most recording sessions by turning the potentiometer's screw. Before recording, we waited 30 minutes to allow the tetrodes to fully adjust. Broadband data were acquired at 30KHz and digitized and stored using a neural signal processor from Blackrock Microsystems. After the behavior, white noise bursts were presented passively to the animal to detect field and/or multi-unit auditory responses. Strong, low-latency auditory responses indicated that the electrodes were in A1 (in combination with the stereotactic coordinates used during implantation and, when possible, post-mortem histological reconstruction of electrode tracks). We only considered sessions in which we believed the electrodes to be in the correct brain regions.

We filtered the data offline to separate LFP (<200Hz) and spikes (>3 KHz). Butterworth, non-causal (temporally symmetric) filters were used to ensure that no phase distortion occurred. We extracted 0.8 ms spike waveforms using our own contributions to the open-source OpenElectrophy software suite, reduced the dimensionality with principle component analysis, clustered with KlustaKwik, and manually reclustered as necessary with Klusters (Hazan et al., 2004) while blind to the experimental variables. Single units were identified based on the existence of a refractory period and minimal cluster overlap with other putative single units or noise.

We analyzed the data with Python and the modules numpy, scipy, scikits-learn, rpy2, and pandas, as well as custom-written data analysis code. Except where otherwise noted in the text, we observed consistent results across all subjects and therefore pooled the data.

Decoder analysis

An ideal decoder was trained on the evoked rates, including baseline. We implemented this decoder using the LogisticRegression object in scikits-learn and assessed its performance by the number of trials on which the identity of the noise burst and/or warble was correctly predicted. Figure 6E shows the results on ensembles of simultaneously recorded neurons, but the results were similar for individual neurons (no effect of block).

References

Ahveninen, J., Hämäläinen, M., Jääskeläinen, I.P., Ahlfors, S.P., Huang, S., Lin, F.-H., Raij, T., Sams, M., Vasios, C.E., and Belliveau, J.W. (2011). Attention-driven auditory cortex short-term plasticity helps segregate relevant sounds from noise. Proc. Natl. Acad. Sci. U. S. A. 108, 4182–4187.

Asaad, W.F., Rainer, G., and Miller, E.K. (2000). Task-specific neural activity in the primate prefrontal cortex. J. Neurophysiol. 84, 451–459.

Bee, M.A., and Micheyl, C. (2008). The cocktail party problem: what is it? How can it be solved? And why should animal behaviorists study it? J. Comp. Psychol. 122, 235–251.

Birrell, J.M., and Brown, V.J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. J. Neurosci. 20, 4320–4324.

Brunton, B.W., Botvinick, M.M., and Brody, C.D. (2013). Rats and humans can optimally accumulate evidence for decision-making. Science 340, 95–98.

Carandini, M., and Churchland, A.K. (2013). Probing perceptual decisions in rodents. Nat. Neurosci. 16, 824–831.

Carlson, N.L., Ming, V.L., and DeWeese, M.R. (2012). Sparse codes for speech predict spectrotemporal receptive fields in the inferior colliculus. PLoS Comput. Biol. 8, e1002594.

Chen, Y., and Seidemann, E. (2012). Attentional modulations related to spatial gating but not to allocation of limited resources in primate V1. Neuron 74, 557–566.

Cherry, E.C. (1953). Some experiments on the recognition of speech, with one and with two ears. J. Acoust. Soc. Am. 25, 975–979.

Cohen, M.R., and Maunsell, J.H.R. (2009). Attention improves performance primarily by reducing interneuronal correlations. Nat. Neurosci. 12, 1594–1600.

Cohen, M.R., and Maunsell, J.H.R. (2011). Using neuronal populations to study the mechanisms underlying spatial and feature attention. Neuron 70, 1192–1204.

David, S. V, Hayden, B.Y., Mazer, J. a, and Gallant, J.L. (2008). Attention to stimulus features shifts spectral tuning of V4 neurons during natural vision. Neuron 59, 509–521.

David, S. V, Fritz, J.B., and Shamma, S.A. (2012). Task reward structure shapes rapid receptive field plasticity in auditory cortex. Proc. Natl. Acad. Sci. U. S. A. 109, 2144–2149.

Desimone, R., and Duncan, J. (1995). Neural mechanisms of selective visual attention. Annu. Rev. Neurosci. 18, 193–222.

DeWeese, M.R., Wehr, M., and Zador, A.M. (2003). Binary spiking in auditory cortex. J. Neurosci. 23, 7940–7949.

Ding, N., and Simon, J.Z. (2012). Emergence of neural encoding of auditory objects while listening to competing speakers. Proc. Natl. Acad. Sci. U. S. A. 2012, 5–10.

Durstewitz, D., Vittoz, N.M., Floresco, S.B., and Seamans, J.K. (2010). Abrupt transitions between prefrontal neural ensemble states accompany behavioral transitions during rule learning. Neuron 66, 438–448.

Erlich, J.C., Bialek, M., and Brody, C.D. (2011). A cortical substrate for memory-guided orienting in the rat. Neuron 72, 330–343.

Floresco, S.B., Block, A.E., and Tse, M.T.L. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behav. Brain. Res. 190, 85–96.

Fritz, J., Shamma, S., Elhilali, M., and Klein, D. (2003). Rapid task-related plasticity of spectrotemporal receptive fields in primary auditory cortex. Nat. Neuro. 6, 1216–1223.

Fritz, J.B., David, S. V, Radtke-Schuller, S., Yin, P., and Shamma, S.A. (2010). Adaptive, behaviorally gated, persistent encoding of task-relevant auditory information in ferret frontal cortex. Nat. Neurosci. 13, 1011–1019.

Funahashi, S., Bruce, C.J., and Goldman-Rakic, P.S. (1989). Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J. Neurophysiol. 61, 331–349.

Geissler, D.B., and Ehret, G. (2002). Time-critical integration of formants for perception of communication calls in mice. Proc. Natl. Acad. Sci. U. S. A. 99, 9021–9025.

Gold, J.I., and Shadlen, M.N. (2007). The neural basis of decision making. Annu. Rev. Neurosci. 30, 535–574.

Haddon, J.E., and Killcross, S. (2007). Contextual control of choice performance: behavioral, neurobiological, and neurochemical influences. Ann. N. Y. Acad. Sci. 1104, 250–269.

Hazan, L., Zugaro, M., and Buzsáki, G. (2006). Klusters, NeuroScope, NDManager: a free software suite for neurophysiological data processing and visualization. J. Neurosci. Meth. 155, 207–216.

Hocherman, S., Benson, D.A., Goldstein, M.H., Heffner, H.E., and Hienz, R.D. (1976). Evoked unit activity in auditory cortex of monkeys performing a selective attention task. Brain Res. 117, 51–68.

Hromádka, T., DeWeese, M.R., and Zador, A.M. (2008). Sparse Representation of Sounds in the Unanesthetized Auditory Cortex. PLoS Biol. 6, 14.

Johnston, K., Levin, H.M., Koval, M.J., and Everling, S. (2007). Top-down control-signal dynamics in anterior cingulate and prefrontal cortex neurons following task switching. Neuron 53, 453–462.

Karlsson, M.P., Tervo, D.G.R., and Karpova, A.Y. (2012). Network resets in medial prefrontal cortex mark the onset of behavioral uncertainty. Science 338, 135–139.

Kastner, S., Pinsk, M. a, De Weerd, P., Desimone, R., and Ungerleider, L.G. (1999). Increased activity in human visual cortex during directed attention in the absence of visual stimulation. Neuron 22, 751–761.

Knudsen, E.I. (2007). Fundamental components of attention. Annu. Rev. Neurosci. 30, 57–78.

Kvitsiani, D., Ranade, S., Hangya, B., Taniguchi, H., Huang, J.Z., and Kepecs, A. (2013). Distinct behavioural and network correlates of two interneuron types in prefrontal cortex. Nature.

Lakatos, P., O'Connell, M.N., Barczak, A., Mills, A., Javitt, D.C., and Schroeder, C.E. (2009). The leading sense: supramodal control of neurophysiological context by attention. Neuron 64, 419–430.

Lakatos, P., Musacchia, G., O'Connel, M.N., Falchier, A.Y., Javitt, D.C., and Schroeder, C.E. (2013). The spectrotemporal filter mechanism of auditory selective attention. Neuron 77, 750–761.

Lee, C.-C., and Middlebrooks, J.C. (2011). Auditory cortex spatial sensitivity sharpens during task performance. Nat. Neuro. 14, 108–114.

Luck, S.J., Chelazzi, L., Hillyard, S.A., and Desimone, R. (1997). Neural mechanisms of spatial selective attention in areas V1, V2, and V4 of macaque visual cortex. J. Neurophysiol. 77, 24–42.

McDermott, J.H. (2009). The cocktail party problem. Current Biology : CB 19, R1024–7.

Mesgarani, N., and Chang, E.F. (2012). Selective cortical representation of attended speaker in multi-talker speech perception. Nature 485, 233–236.

Miller, E.K., and Cohen, J.D. (2001). An integrative theory of prefrontal cortex function. Annu. Rev. Neurosci. 24, 167–202.

Mitchell, J.F., Sundberg, K.A., and Reynolds, J.H. (2007). Differential attention-dependent response modulation across cell classes in macaque visual area V4. Neuron 55, 131–141.

Monchi, O., Petrides, M., Petre, V., Worsley, K., and Dagher, a (2001). Wisconsin Card Sorting revisited: distinct neural circuits participating in different stages of the task identified by event-related functional magnetic resonance imaging. J. Neurosci. 21, 7733–7741.

Moore, T., Armstrong, K.M., and Fallah, M. (2003). Visuomotor origins of covert spatial attention. Neuron 40, 671–683.

Moran, J., and Desimone, R. (1985). Selective attention gates visual processing in the extrastriate cortex. Science 229, 782–784.

Niwa, M., Johnson, J.S., O'Connor, K.N., and Sutter, M.L. (2012). Activity related to perceptual judgment and action in primary auditory cortex. J. Neurosci. 32, 3193–3210.

Otazu, G.H., Tai, L.-H., Yang, Y., and Zador, A.M. (2009). Engaging in an auditory task suppresses responses in auditory cortex. Nat. Neurosci. 12, 646–654.

Pai, S., Erlich, J.C., Kopec, C., and Brody, C.D. (2011). Minimal impairment in a rat model of duration discrimination following excitotoxic lesions of primary auditory and prefrontal cortices. Front. Syst. Neurosci. 5, 74.

Pestilli, F., Carrasco, M., Heeger, D.J., and Gardner, J.L. (2011). Attentional enhancement via selection and pooling of early sensory responses in human visual cortex. Neuron 72, 832–846.

Ragozzino, M.E., Detrick, S., and Kesner, R.P. (1999). Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. J. Neurosci. 19, 4585–4594.

Raposo, D., Sheppard, J.P., Schrater, P.R., and Churchland, A.K. (2012). Multisensory Decision-Making in Rats and Humans. J. Neurosci. 32, 3726–3735.

Reynolds, J.H., and Chelazzi, L. (2004). Attentional modulation of visual processing. Annu. Rev. Neurosci. 27, 611–647.

Reynolds, J.H., and Heeger, D.J. (2009). The normalization model of attention. Neuron 61, 168–185.

Reynolds, J.H., Pasternak, T., and Desimone, R. (2000). Attention increases sensitivity of V4 neurons. Neuron 26, 703–714.

Rich, E.L., and Shapiro, M. (2009). Rat prefrontal cortical neurons selectively code strategy switches. J. Neurosci. 29, 7208–7219.

Sasaki, R., and Uka, T. (2009). Dynamic readout of behaviorally relevant signals from area MT during task switching. Neuron 62, 147–157.

Sayers, B.M., and Cherry, E.C. (1957). Mechanism of binaural fusion in the hearing of speech. J. Acoust. Soc. Am. 29, 973–987.

Shuler, M.G., and Bear, M.F. (2006). Reward timing in the primary visual cortex. Science 311, 1606–1609.

Sirotin, Y.B., and Das, A. (2009). Anticipatory haemodynamic signals in sensory cortex not predicted by local neuronal activity. Nature 457, 475–479.

Steinmetz, N.A., and Moore, T. (2012). Lumping and splitting the neural circuitry of visual attention. Neuron 73, 410–412.

Stoet, G., and Snyder, L.H. (2004). Single neurons in posterior parietal cortex of monkeys encode cognitive set. Neuron 42, 1003–1012.

Thut, G., Nietzel, A., Brandt, S., and Pascual-Leone, A. (2006). Alpha-band electroencephalographic activity over occipital cortex indexes visuospatial attention bias and predicts visual target detection. J. Neurosci. 26, 9494–9502.

Turken, A.U., and Swick, D. (1999). Response selection in the human anterior cingulate cortex. Nat. Neuro. 2, 920–924.

Wallis, J.D., Anderson, K.C., and Miller, E.K. (2001). Single neurons in prefrontal cortex encode abstract rules. Nature 411, 953–956.

Wise, S.P. (2008). Forward frontal fields: phylogeny and fundamental function. Trends Neurosci 31, 599–608.

Young, J.J., and Shapiro, M.L. (2011). The orbitofrontal cortex and response selection. Ann. N. Y. Acad. Sci. 1239, 25–32.

Zariwala, H.A., Kepecs, A., Uchida, N., Hirokawa, J., and Mainen, Z.F. (2013). The limits of deliberation in a perceptual decision task. Neuron 78, 339–351.

Zénon, A., and Krauzlis, R.J. (2012). Attention deficits without cortical neuronal deficits. Nature 489, 434–437.

Zion Golumbic, E.M., Ding, N., Bickel, S., Lakatos, P., Schevon, C.A., McKhann, G.M., Goodman, R.R., Emerson, R., Mehta, A.D., Simon, J.Z., et al. (2013). Mechanisms underlying selective neuronal tracking of attended speech at a “cocktail party”. Neuron 77, 980–991.

Znamenskiy, P., and Zador, A.M. (2013). Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination. Nature 497, 482–485.

Figure Captions

Figure 1. Behavioral paradigm.

A) Left: a schematic of the behavioral arena with left (L), center (C), and right (R) ports (or nose-pokes), and left and right speakers. Right: timeline of each trial. The rat initiates a trial by nose-poking the center port, in the position shown on the left. After a hold period, an auditory stimulus plays in stereo. Following this, the rat may choose to go to the left port (blue arrow), go to the right port (red arrow), or do neither of those (a “nogo” response).

B) Task stimuli (left: description; right: spectrogram of the auditory waveform). On each trial, the rat hears one of four possible auditory stimulus pairs: LEFT+HIGH, RIGHT+HIGH, LEFT+LOW, or RIGHT+LOW. Each is a simultaneous combination of a broadband noise burst played from either the left or right speaker, and a low-pitched or high-pitched warble. The warble is always played with equal intensity from both speakers.

C) Task rules. The session consists of alternating localization and pitch discrimination blocks of 80 trials each. Left: In localization blocks, the rat must go left for sounds containing LEFT and it must nogo for sounds containing RIGHT; the low- or high-pitched warble is an irrelevant distractor. Right: In pitch discrimination blocks, the rat must go right if the stimulus pair contains LOW and it must nogo if the stimulus pair contains HIGH; the localized noise burst is an irrelevant distractor. Good performance depends on selecting and responding to the target sound, not the distractor sound.

Figure 2. Trained rats select and respond to the target sound, not the distractor

A) Behavior performance during recording sessions. Each hash mark is the performance during localization (blue) and pitch discrimination (red) in a single recording session. Performance is well above chance (black dotted line, see Methods).

B) Distribution of behavioral responses to an example stimulus pair (RIGHT+LOW) over the course of an average session. We averaged across all sessions from a single rat (CR21A) and binned the trials into groups of 10 to smooth the traces. The x-axis shows both trial number and block type. The correct response to this stimulus pair is to go right during pitch discrimination and to nogo during localization (see Figure 1C). Each trace shows the probability that the rat will go right (red), nogo (gray), or go left (blue); black open squares mark the correct response for that block. The rat responds correctly most of the time, even though the required action changes abruptly at the block boundaries. Cue trials, during which this stimulus pair does not occur, begin each block and are shaded in cyan and pink throughout this figure.

C) Combined performance, similar to (B) but averaged over all sessions, rats, and stimuli. Correct responses (black trace) are the most common outcome. Performance is consistently high throughout, except immediately after a block change. The orange trace shows the probability of an “interference” trial (see text).

D) Analysis of performance immediately after block changes. All localization blocks from (C) are averaged together as are all pitch discrimination blocks. (In order to emphasize block transitions, the x-axis repeats itself after trial 160; the block structure is cyclical and so the cyan shaded areas are identical.) Immediately after the beginning of a new block (cyan and pink areas), performance decreases briefly but recovers within a few trials.

Figure 3. Pre-stimulus activity in mPFC encodes the selection rule

A) Left: An example mPFC single unit that fires more during the hold period for localization (blue bars throughout this figure) than for pitch discrimination (red bars); error bars SEM. Inset: Extracellular waveforms (mean plus or minus standard deviation) on each channel of the tetrode, duration 0.8 ms. The waveforms are colored red and blue based on the block in which they were recorded, but are almost entirely overlapping (purple). Right: peri-stimulus time histogram (PSTH) of the same unit, averaged over all correct trials from each block. The firing rate is significantly higher ($p < 0.001$) during the hold period (gray shading) for localization (mean 12.1 Hz, n=483 trials) vs. pitch discrimination (mean 7.2 Hz, n=295 trials). We assessed significance for all neurons with the Mann-Whitney U-test and controlled for multiple comparisons with the Benjamini-Hochberg false discovery rate.

B) Another example mPFC single unit, this one preferring pitch discrimination. This neuron's firing rate is persistently elevated at all plotted timepoints. The hold period firing rate is significantly higher ($p < 0.001$) during pitch discrimination (mean 5.4 Hz) vs. localization (mean 2.7 Hz). Trial counts are the same as the simultaneously recorded unit in (A).

C) Stacked histogram of the ratio of hold period firing rate (pitch discrimination over localization) for all mPFC neurons. Red and blue bars are significantly modulated neurons.

D) Rule encoding during the hold period is diminished on interference trials. We averaged together the firing rates in the preferred and non-preferred blocks of each rule encoding neuron, after normalizing by subtracting the firing rate on correct trials in the non-preferred block. Error bars: SEM; orange bars: interference trials; white bars: correct trials. The population response on interference trials is significantly decreased during the preferred block and increased during the non-preferred block. Thus, the encoding of selection rule is diminished on trials on which the rat may be selecting the wrong sound. Significance was assessed with a paired Mann-Whitney test (n=57 neurons) which is invariant to the subtractive normalization performed.

Figure 4. Pre-stimulus activity in A1 also encodes the selection rule

A) An example neuron recorded in primary auditory cortex (A1). This neuron responds significantly more ($p < 0.001$) during localization (8.0 Hz, n=312 trials; blue throughout this figure) than during pitch discrimination (4.8 Hz, n=253; red). Note the peak following stimulus onset, which was used to analyze the evoked response (Figure 6). Throughout this figure, we use the same conventions and statistical procedures as in Figure 3.

B) Another simultaneously recorded example A1 neuron that encoded the selection rule. This neuron significantly ($p < 0.001$) prefers pitch discrimination (10.1 Hz, n=312 trials) over localization (2.0 Hz, n=253).

C) Stacked histogram of the ratio of hold period firing rate (pitch discrimination over localization) for all A1 neurons.

D) Rule encoding during the hold period is inverted on interference trials for A1 neurons. Same conventions as Figure 3D, but the effect is stronger here. The population response on interference trials (orange bars) is significantly greater during the non-preferred block than during the preferred block ($p < 0.05$, n=16 neurons, paired Mann-Whitney U-test), opposite to the encoding on correct trials (white bars).

Figure 5. Within-trial timescale of the encoding of selection rule

A) PSTHs from example rule-encoding mPFC neurons in each block (blue: localization, red: pitch discrimination). Note that the timescale is much broader than in previous figures. Firing rates are smoothed with a 50ms Gaussian kernel, normalized to equal variance, and locked to stimulus onset at time 0 ms. The time interval containing the hold period during which the traces significantly diverge is shaded gray. Although these neurons were identified based on a difference in firing rate during the hold period, the traces often diverge for much longer than that. We observed a wide variety of timescales and dynamics in the block-specific anticipatory modulation. The first neuron effectively fires persistently more in one block. The third and fourth neurons demonstrate that the firing rate can either rise during the preferred block, or, less commonly, drop during the non-preferred block. The fifth neuron shows that the anticipatory effect can be limited to just the hold period alone.

B) Example neurons from A1, following the conventions of (A). Again, the neurons exhibit a wide variety of dynamics, from essentially persistent block-specific activation for over three seconds preceding the stimulus (first neuron), to very brief activation well under 1 second (last neuron). The third neuron shows increased baseline firing and increased stimulus-evoked firing (peak immediately after time zero) in the same block. This was typical of our dataset (see Figure 6).

C) Population timecourse: the curves represent the average response during the preferred block across all rule-encoding neurons in mPFC (purple) and A1 (green). The firing rates of all rule-encoding neurons were normalized (mean: 0, variance: 1) and then averaged together. Traces are mean response, plus or minus SEM across neurons. Thick mean trace: timepoints during which the population response significantly exceeds zero, the mean firing rate ($p < 0.05$, one-sample t-test across neurons). In both populations, the firing rate in the preferred block shows a gradual increase, peaking around the time of stimulus onset, and then decreases more quickly back to baseline. The PFC population increases its response earlier (first significantly activated

2.8 s before stimulus onset, n=76) than the A1 population (first significantly activated 0.92 s before stimulus onset, n=36), consistent with the hypothesized role of PFC as the source of top-down modulation.

Figure 6. Limited evidence for modulation of stimulus-evoked activity

A) An example A1 neuron exhibiting a preference for some acoustic stimuli (LEFT+HIGH, LEFT+LOW) over others (RIGHT+HIGH, RIGHT+LOW), but no change in this tuning with block (localization: blue; pitch discrimination: red). Black triangle: stimulus onset; shaded area: response window for this neuron.

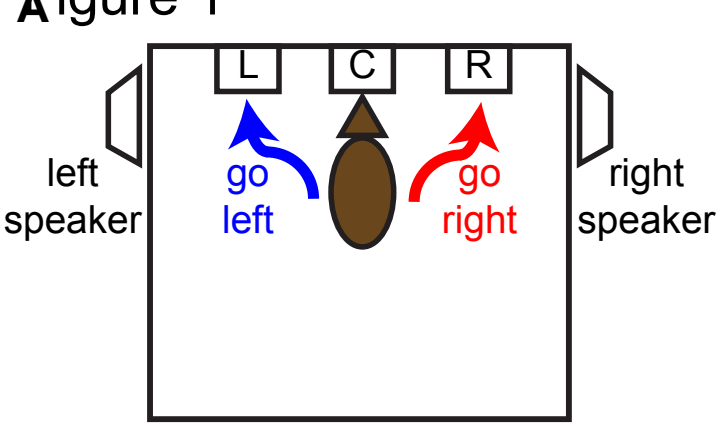
B) An example mPFC neuron that responds to the task stimuli with a low-latency response. Auditory responses were weaker in mPFC neurons compared with A1 neurons (Figure S6).

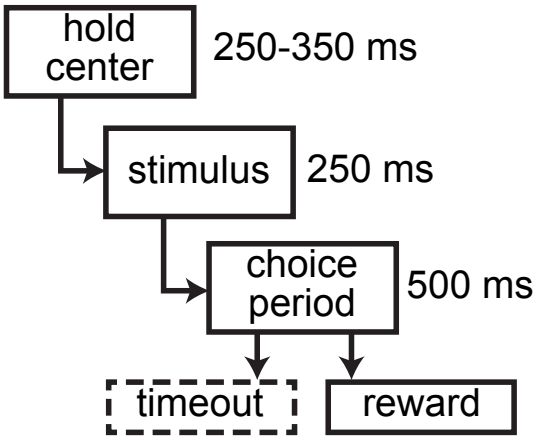
C) For A1 neurons, increase in hold period activity during one block correlates with increased evoked response during that block. For each neuron, the change in evoked response (driven spikes in pitch discrimination vs. localization) is plotted against the change in hold period firing rate (anticipatory spikes in pitch discrimination vs. localization). The trend line (n=43 neurons, r=0.52) has a slope of 0.98, suggesting that much of the modulation of evoked strength is due to anticipatory modulation (example: Figure 4B).

D) Following the conventions of (C), but for auditory-responsive PFC neurons. Again, a change in baseline activity correlates closely with a change in evoked activity (n=17 neurons, slope=1.46, r=0.85).

E) No evidence for tuning changes that increase the decodability of the target sound. The identity of the noise burst (LEFT or RIGHT) or the warble (LOW or HIGH) can be decoded from the trial-by-trial responses of simultaneously recorded ensembles of auditory-responsive cells in either A1 or PFC. It can be decoded significantly better ($p < 0.001$) from A1 cells (n=22 ensembles of 57 neurons total) than from mPFC cells (n=13 ensembles of 25 neurons total), but it cannot be decoded significantly better during either block. The chance decoding level, attainable by a neuron with no information about the stimulus, is 0.5. The mean and SEM over the ensembles is shown. Significance was assessed with a 3-way ANOVA on brain region, target sound, and block.

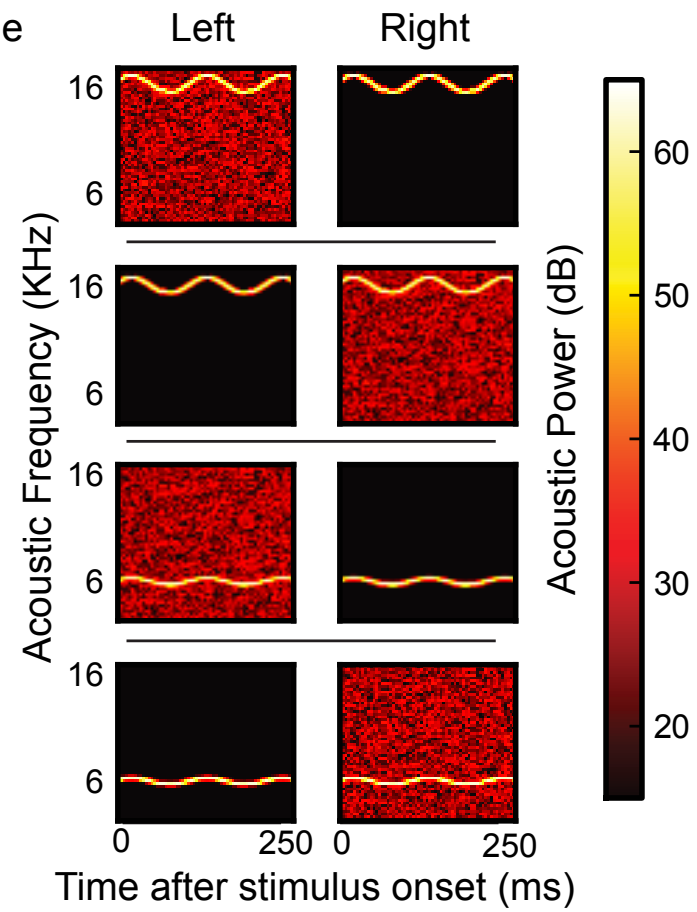
Figure 1





B

Stimulus Pair	Abbrev. Name
left noise burst + **high** warble	LEFT + HIGH
right noise burst + **high** warble	RIGHT + HIGH
left noise burst + **low** warble	LEFT + LOW
right noise burst + **low** warble	RIGHT + LOW



C

Localization

LEFT + HIGH	RIGHT + HIGH
LEFT + LOW	RIGHT + LOW

Correct Response

go left

nogo

go right

Pitch Discrimination

LEFT + HIGH	RIGHT + HIGH
LEFT + LOW	RIGHT + LOW

Figure 2

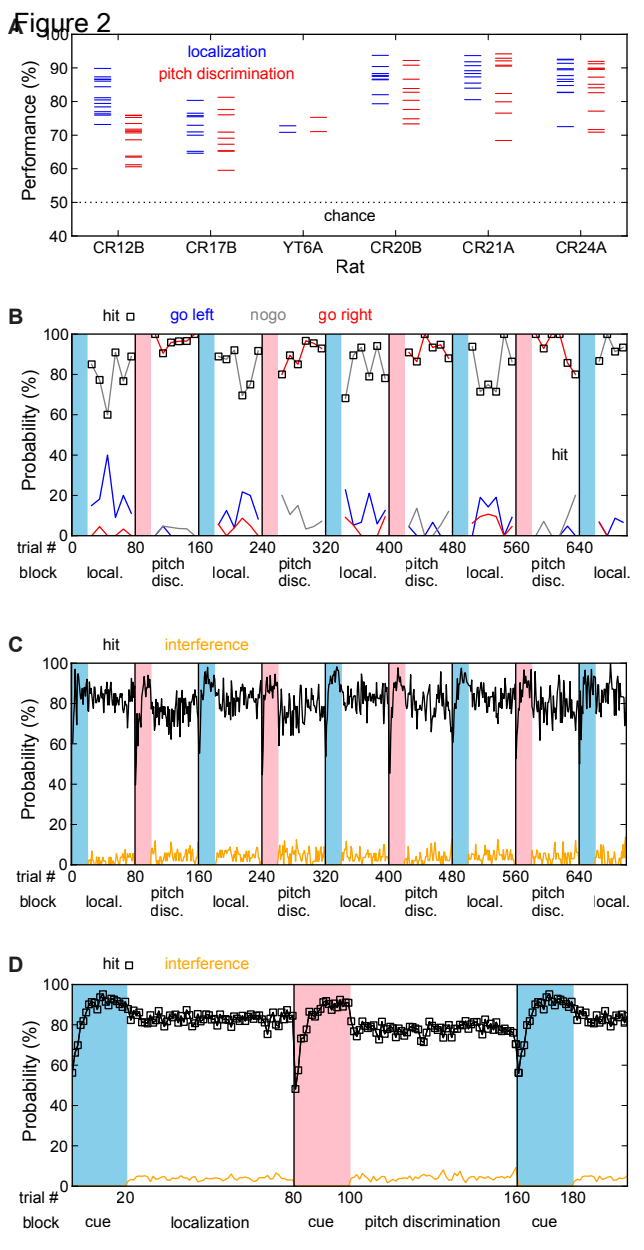

A
localization
pitch discrimination
Performance (%)
chance
CR12B
CR17B
YT6A
CR20B
CR21A
CR24A
Rat
B
hit
go left
nogo
go right
Probability (%)
hit
trial #
block
local.
pitch disc.
C
hit
interference
Probability (%)
D
hit
interference
Probability (%)
cue
localization
pitch discrimination

Figure 3 throughout: localization pitch discrimination

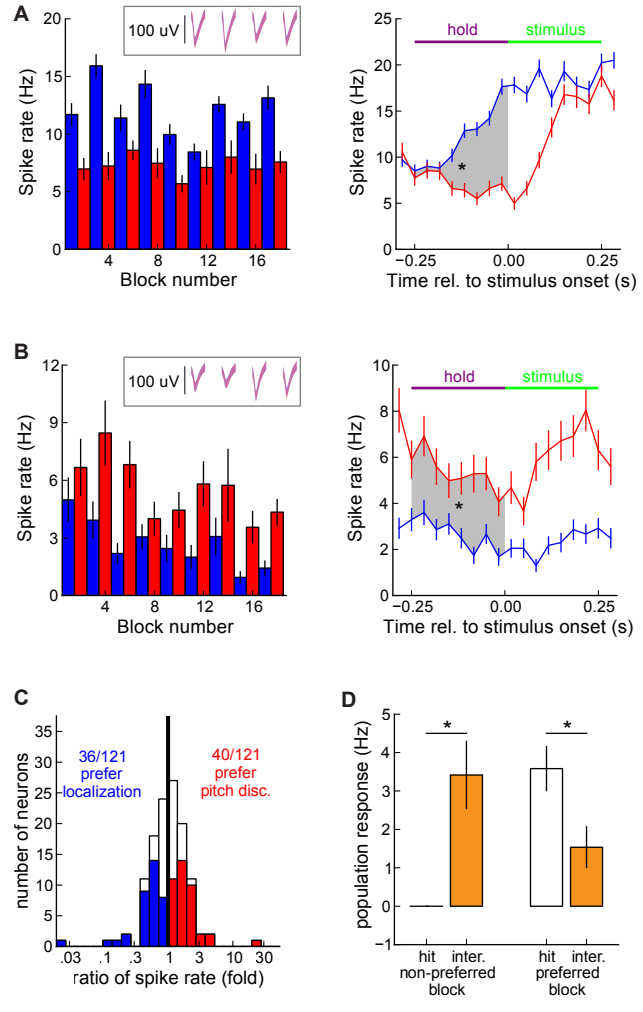


Figure 4 throughout: localization pitch discrimination

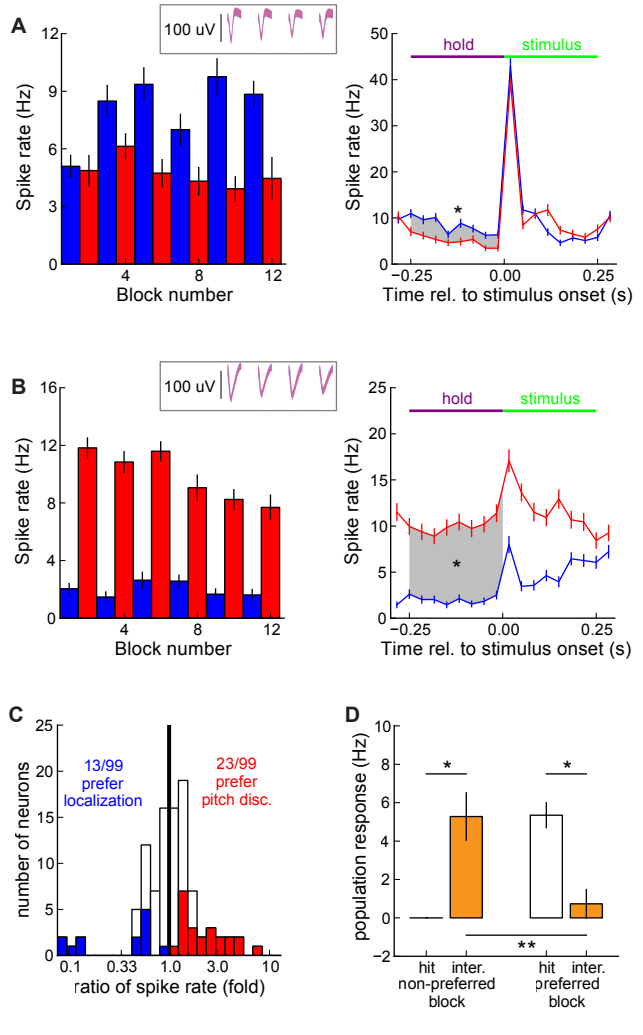


Figure 5

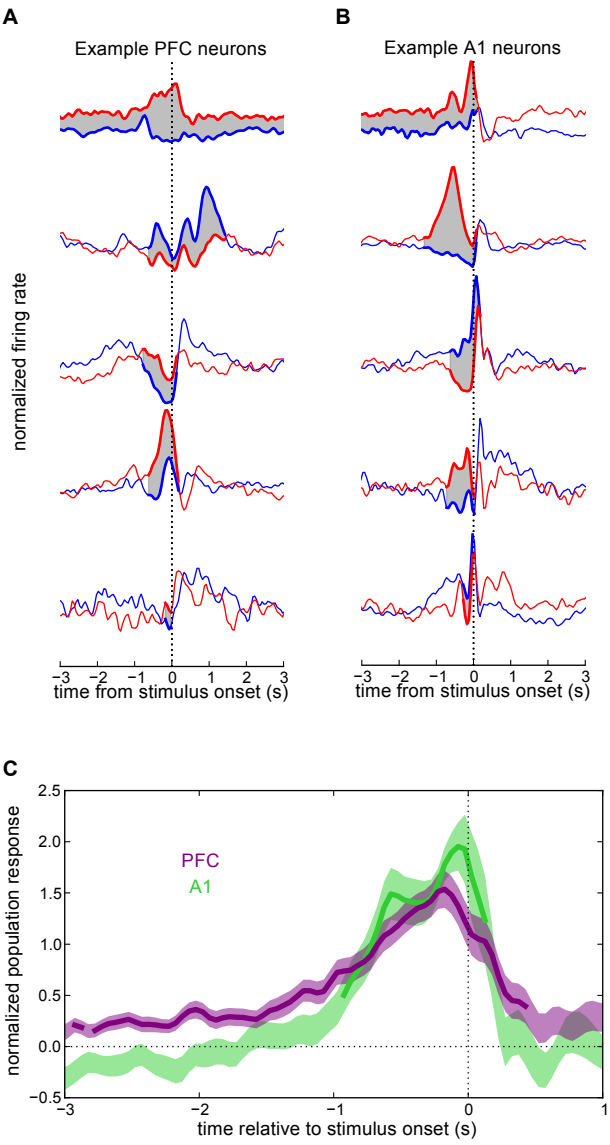
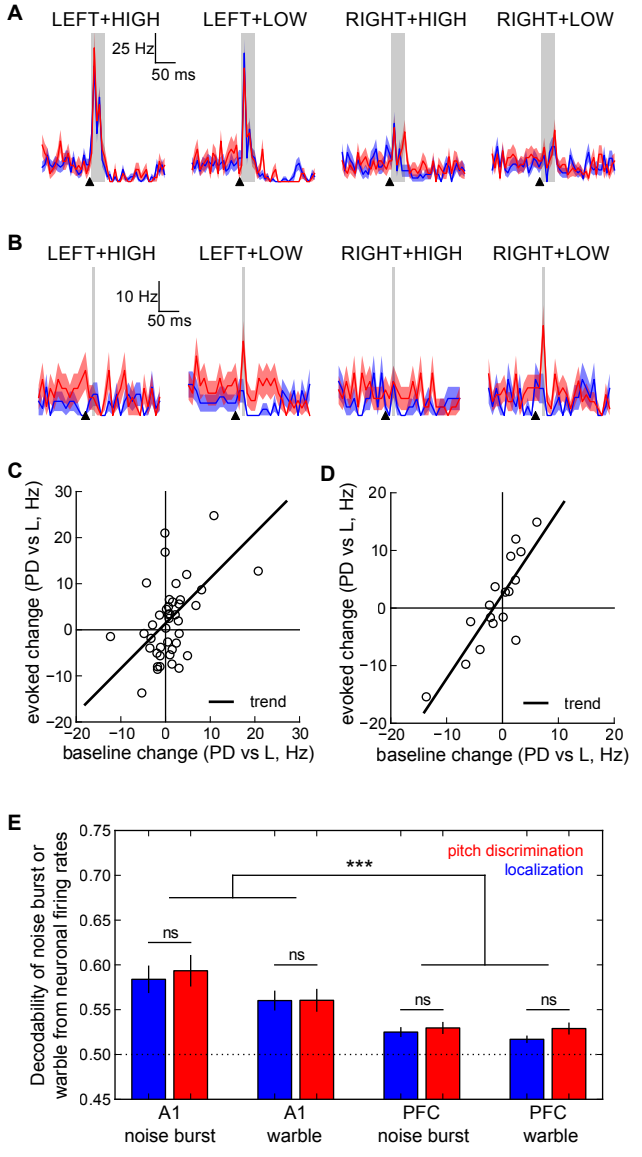

localization
pitch discrimination
A
Example PFC neurons
B
Example A1 neurons
normalized firing rate
−3 −2 −1 0 1 2 3
time from stimulus onset (s)
−3 −2 −1 0 1 2 3
time from stimulus onset (s)
C
normalized population response
2.5
2.0
1.5
1.0
0.5
0.0
−0.5
PFC
A1
−3
−2
−1
0
1
time relative to stimulus onset (s)

Figure 6 throughout: localization pitch discrimination



Inventory of Supplemental Information

Rodgers and DeWeese [manuscript]

Supplemental Data

Supplementary Experimental Procedures

Supplemental Data

Each figure is linked to the main figure with the same number. Not all main figures have associated supplementary figures.

Figure S2. Behavioral performance, related to Figure 2

A) Performance of each rat in greater detail, with go and nogo trials separately considered in each block. Rats generally did better on GO than on NOGO trials (first and third columns above second and fourth). Some rats did better on localization than on pitch discrimination (first and second columns above third and fourth). Error bars show SEM across sessions.

B, C) Performance of two rats who performed a slightly modified “catch trial” task on the last day of recordings. This task was designed to probe whether the rats learn to respond to a unified stimulus pair, or whether they learn to respond just to the target sound regardless of the identity of the distractor. On a small proportion (15%) of trials, we replaced the distractor with a neutral sound, to which the rat had never been trained. For example, on catch trials during localization the rat heard the same target as always (LEFT or RIGHT) with a novel mid-range warble of no behavioral relevance. If the rats had memorized each of the four possible stimulus pairs and were unable to generalize, they should perform at chance on these novel stimulus pairs. The performance on catch trials (red) and standard trials (white) for each trial type is shown, with 95% bootstrapped confidence intervals.The rats perform just as well on catch trials as on standard trials (unpaired Mann-Whitney U-test on the outcome of each trial, $p > 0.05$ in all cases). This suggests that the rats are selecting the target stimulus, not memorizing a fixed set of four stimulus pairs.

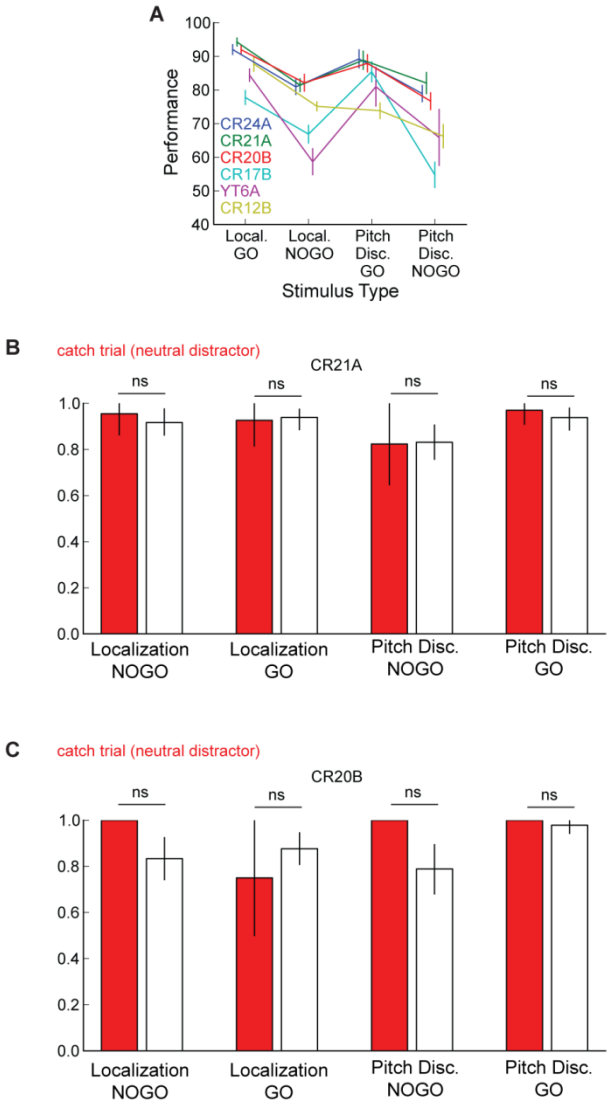

A
Performance
100
90
80
70
60
50
40
CR24A
CR21A
CR20B
CR17B
YT6A
CR12B
Local. GO
Local. NOGO
Pitch Disc. GO
Pitch Disc. NOGO
Stimulus Type
B
catch trial (neutral distractor)
CR21A
ns
1.0
0.8
0.6
0.4
0.2
0.0
Localization NOGO
Localization GO
Pitch Disc. NOGO
Pitch Disc. GO
C
catch trial (neutral distractor)
CR20B
ns
1.0
0.8
0.6
0.4
0.2
0.0
Localization NOGO
Localization GO
Pitch Disc. NOGO
Pitch Disc. GO

FIGURE S2

Figure S3. PFC hold period, related to Figure 3

A) Stacked histogram of the difference, rather than ratio, of hold period firing rate between blocks for all mPFC neurons.

B) Alternative presentation of the hold period effect across mPFC neurons. The hold period firing rate in each block is shown as the x- and y-coordinate of each point (red and blue: significant block preference; gray: not significant). Note the logarithmic scaling, necessary to avoid crowding the points with low firing rate. Error bars are 95% confidence intervals obtained by bootstrapping and were truncated at the edge of the plot. Significance was assessed with a Mann-Whitney U-test as described in the text.

C) Analysis of the hold period effect in mPFC on various types of correct and error trials. The trials are grouped by the meaning of the target sound, the rat's response, and the meaning of the distractor sound. Neurons are grouped by their preferred block. White bars represent correct trials, on which the rat's response matches the target sound. The gray bar represents "go-on-nogo" error trials when the target meant nogo but the rat went to the choice port anyway. (The opposite error, nogo-on-go, was too rare to include in this analysis. We only analyzed neurons from sessions with at least 3 trials of each type.) The orange bar represents "interference" trials on which the rat heard a distractor sound meaning go and went to the choice port associated with that distractor (WP, or wrong port). To aid in visualization, firing rates were normalized by subtracting the mean response on correct trials during the non-preferred block, and then averaged across neurons. There is no significant difference in the hold period activity between correct go, correct nogo, and incorrect go-on-nogo trials. Thus, if the hold period activity represents a motor plan, it is not tuned for go versus nogo, or it is subject to change after the stimulus. However there is a significant difference between interference trials and all other trial types in that block. That is, when the rat gives the response that would be appropriate in the other block, the anticipatory activity is higher in the non-preferred block and lower in the preferred block — *i.e.*, the block modulation is attenuated, trending toward reversed. Significance between each pair of bars was assessed with

a paired Mann-Whitney U-test across neurons, which is invariant under the subtractive normalization performed, and the p-values were Bonferroni corrected.

D) Histogram of the number of neurons preferring pitch discrimination (red), localization (blue), and neither (black). In some rats, no mPFC neurons were recorded.

E) Proportion of rule-encoding neurons in mPFC is consistent across rats. The data from (D) are now expressed a percentage of total neurons. No percentages are plotted for rats with fewer than 8 neurons total recorded in mPFC.

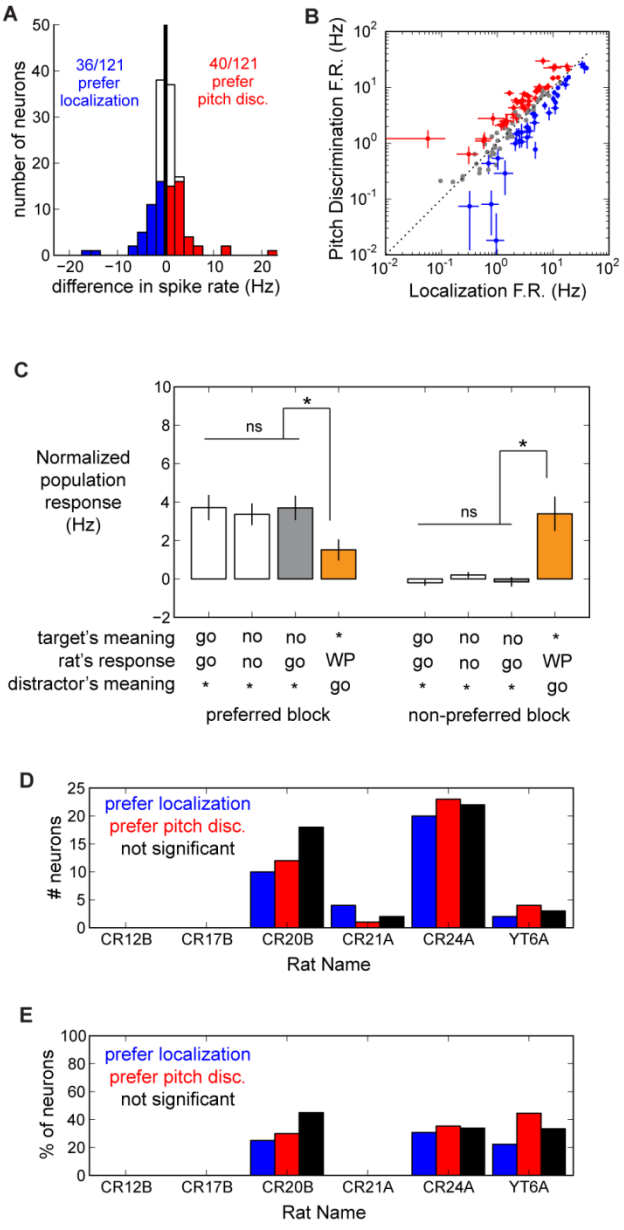

A
number of neurons
36/121
prefer
localization
40/121
prefer
pitch disc.
difference in spike rate (Hz)
B
Pitch Discrimination F.R. (Hz)
Localization F.R. (Hz)
C
Normalized
population
response
(Hz)
ns
*
ns
*
target's meaning
rat's response
distractor's meaning
preferred block
non-preferred block
D
neurons
prefer localization
prefer pitch disc.
not significant
CR12B
CR17B
CR20B
CR21A
CR24A
YT6A
Rat Name
E
% of neurons
prefer localization
prefer pitch disc.
not significant
CR12B
CR17B
CR20B
CR21A
CR24A
YT6A
Rat Name

FIGURE S3

Figure S4. A1 hold period, related to Figure 4

A, B) Following the conventions of Figure S3, but now for A1 neurons instead of mPFC neurons.

C) Following the conventions of Figure S3C (correct trials: white; go-on-nogo errors: gray; interference trials: orange). The effects are similar to those for mPFC, but the effect on interference trials is stronger. Now the direction of hold period modulation is significantly reversed -- the firing rate is higher during such trials in the non-preferred block than in the preferred block. However, there is no difference between the other trial types, regardless of whether the rat performed a go or nogo response. Thus, if the hold period activity represents a motor plan, it is not selective for the major distinction of go versus nogo.

D) Histogram of the number of neurons preferring pitch discrimination (red), localization (blue), and neither (black). In one rat, no A1 neurons were recorded.

E) Proportion of rule-encoding neurons in A1 is consistent across rats. The data from (D) are now expressed a percentage of total neurons. No percentages are plotted for rats with fewer than 8 neurons total recorded in A1.

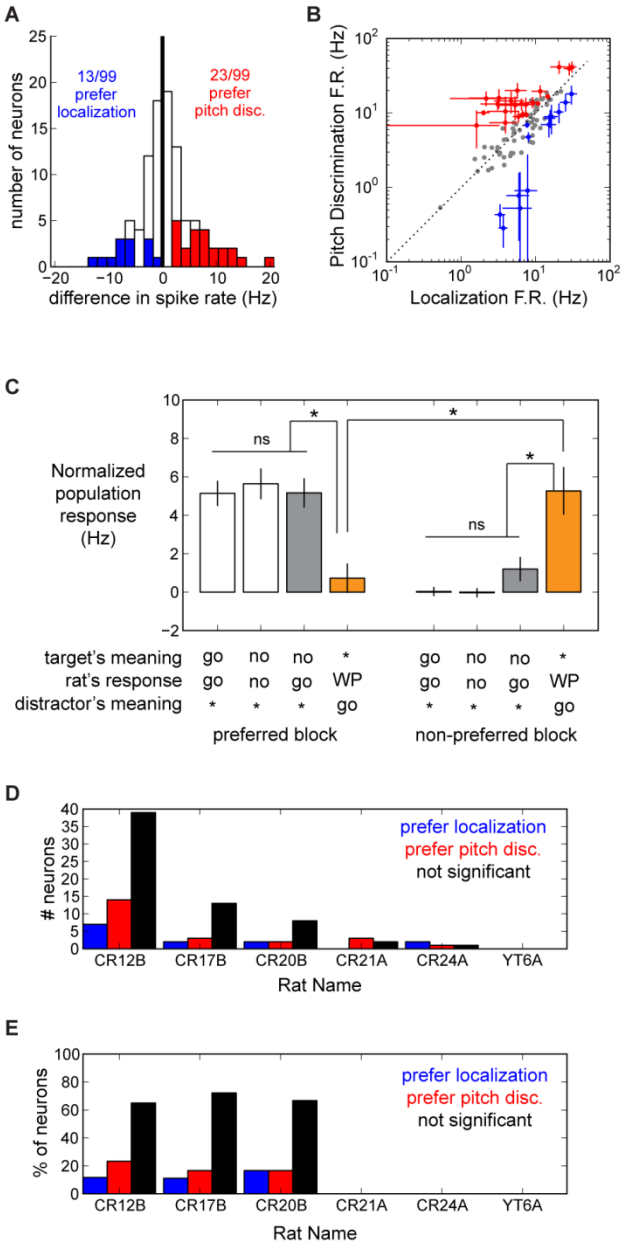

A
number of neurons
13/99 prefer localization
23/99 prefer pitch disc.
difference in spike rate (Hz)
B
Pitch Discrimination F.R. (Hz)
Localization F.R. (Hz)
C
Normalized population response (Hz)
ns
*
target's meaning
rat's response
distractor's meaning
preferred block
non-preferred block
D
neurons
prefer localization
prefer pitch disc.
not significant
CR12B
CR17B
CR20B
CR21A
CR24A
YT6A
Rat Name
E
% of neurons
Rat Name

FIGURE S4

Figure S6. More information on evoked activity, related to Figure 6

A) PSTH of a typical A1 neuron, to illustrate the notion of "evoked response strength." This neuron's response strength is near the median of the A1 population. All stimuli and trials are included in this PSTH. Note that the response to the onset of the sound is rapid, short-latency, tightly stimulus-locked, and brief. The onset window is shaded. This was defined as the continuous set of time bins post-onset over which the firing rate was significantly greater than the rate preceding the stimulus. Some mPFC units also showed similar (though weaker) stimulus-driven responses.

B) An example A1 neuron showing one of the strongest and most sustained recorded responses.

C) Distribution of onset response strengths across across n=49 auditory-responsive A1 neurons (blue) and n=31 auditory-responsive PFC neurons (orange). The response strength is expressed as the average number of additional spikes (over baseline) during the onset response window for that neuron. All trials and stimuli are included, and the baseline rate is calculated over the 50 ms preceding the stimulus onset. The responses are significantly stronger in A1 neurons (median: 0.11 spikes) than in PFC neurons (median: 0.02 spikes), $p < 0.05$, unpaired Mann-Whitney U-test. Note the long tail of the distribution: a small subpopulation fires much more strongly than the median.

D) Alternative presentation of onset response strength. The data are the same as in (C), but now expressed as percentage of baseline firing rate. Because the response window is so brief, a small number of additional spikes over baseline typically represents a large (many-fold) increase in rate. Again the responses are stronger in A1 neurons (median: 209% of baseline) than in PFC neurons (median: 171% of baseline), $p < 0.001$, unpaired Mann-Whitney U-test.

E) Distribution of latencies to center of onset response window across the same populations as (C) and (D). The PFC latencies are significantly longer (median: 19.75ms) than the A1 latencies (median: 16.75ms). $p < 0.05$, unpaired Mann-Whitney U-test.

F) A non-significant trend in the direction predicted by the tuning change hypothesis: neurons preferring pitch discrimination increase their tuning for the warble during pitch discrimination. Each neuron is a data point, with x-coordinate equal to performance of warble decoder during localization and y-coordinate the same during pitch discrimination. Thus, points above the line represent neurons that increase their tuning for the warble during pitch discrimination. We considered that our decoder analysis (Figure 6E in the main text) might be averaging together opposite effects in the localization-preferring and pitch discrimination-preferring neurons. When we include only pitch discrimination-preferring, auditory-responsive neurons in both brain regions (n=12 in A1, n=8 in PFC), there is a trend toward increased decodability for the target sound (warble) during pitch discrimination. However the effect is not significant (A1: p=0.146, PFC: p=0.070, binomial test). Moreover we did not observe a similar effect for localization-preferring neurons (data not shown).

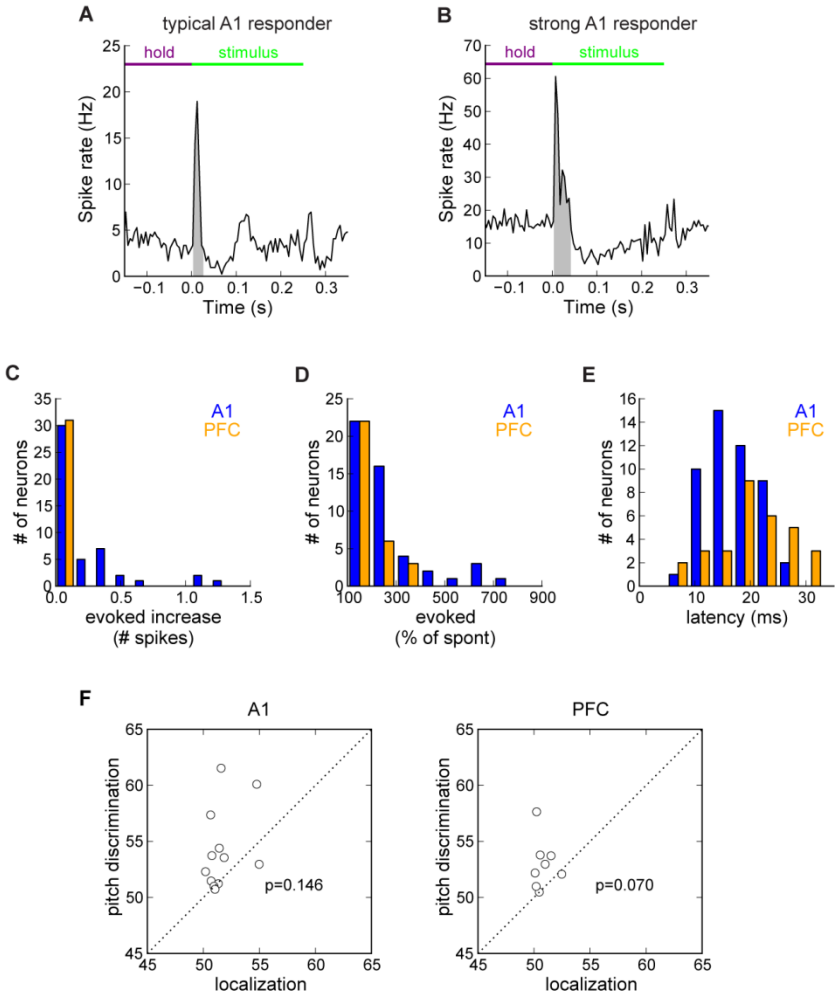

A
typical A1 responder
hold
stimulus
Spike rate (Hz)
Time (s)
B
strong A1 responder
hold
stimulus
Spike rate (Hz)
Time (s)
C
A1
PFC
# of neurons
evoked increase
(# spikes)
D
A1
PFC
# of neurons
evoked
(% of spont)
E
A1
PFC
# of neurons
latency (ms)
F
A1
pitch discrimination
localization
p=0.146
PFC
pitch discrimination
localization
p=0.070

FIGURE S6

Supplementary Experimental Procedures

Surgical implantation

Rats were anesthetized using ketamine/xylazine and isoflurane as necessary to maintain a deep anesthesia, assessed using toe pinch. Skin and fascia were resected from the midline and the skull cleaned. Titanium screws (Small Parts) were inserted into each cranial plate. Two stainless steel screws with a wire soldered onto the head were inserted into the occipital plate above the cerebellum. These were later soldered onto the reference and ground inputs on the microdrive.

Craniotomies were performed directly dorsal to the target areas (A1: 5.25 mm posterior and 6.5 mm left from bregma; PL: 3.0 mm anterior and 1.0 mm left from bregma). The dura was removed and the tetrodes gradually inserted into each region. The craniotomy was filled with agar which surrounded and protected the tetrodes. Methyl methacrylate (Teet's, Henry Schein) was used to affix the entire drive to the skull and screws.

Finally the tissue was flushed thoroughly with sterile saline and sutures were used if necessary to seal the skin around the implant. Aseptic technique was maintained throughout and the tetrodes themselves were disinfected before implantation. Post-operatively, the rat was given buprenorphine and/or meloxicam to provide analgesia and its health and weight monitored twice daily. Once the rat was fully recovered, the behavioral task was resumed, now concurrent with electrophysiological recording.

Analysis of possible confounds: waveform variation and firing rate drift

In addition to the standard spike-sorting procedure for identifying stable units, we also used an extra, stricter check on the quality of our data to ensure that the hold period effect could not be due to sorting errors arising from small variations in waveform between blocks. For each neuron, we identified the sub-cluster of sorted spikes occurring just during the localization hold period, and calculated the Mahalanobis distance (in the first four PCA feature dimensions) between this subcluster and the entire cluster of spikes from this unit. We

assessed the significance of this distance by randomly permuting the labels on the subcluster and the full cluster 2000 times, and calculating the probability of observing a distance less than or equal to the true distance. We repeated this analysis for the other block (pitch discrimination). We discarded the neuron from analysis entirely if the subcluster in either block was significantly more separated from the full cluster than the permuted subclusters were ($p < 0.05$, permutation test). We also repeated the analysis with simulated Gaussian sub-clusters of the same size as the actual sub-clusters and derived the distribution of mean-squared distance from the cluster center, again rejecting any neuron whose sub-cluster that exceeded the 95th percentile of this distribution in either block.

Additionally, we also considered the possibility that a spurious hold period effect could arise from a slow increase or decrease in firing rate over the entire session, perhaps due to drift or motivation, even though the multiple switches between blocks within each session made such a possibility unlikely. We reasoned that, if this were true, then when taking block number into account the difference between block types should no longer be significant. We fit a linear model to the square root of the spike count in the hold period on each trial, using both block type (localization or pitch discrimination) and block number (1, 2, 3, …) as predictors. (Square root is a variance-stabilizing transform for Poisson counts.) We assessed the significance of each predictor with ANOVA. Any neuron that showed a hold period effect according to the analysis described in the text, but that failed to show a significant effect of block type or failed the overall F-test ($p > 0.05$) was discarded from the analysis. 8/231 neurons (combined across brain regions) were discarded for this reason.

Power analysis

We analyzed the statistical power of our methods (unpaired Mann-Whitney U-test on the spike counts across blocks) on simulated Poisson counts. We determined the total spike count had to be at least 20 spikes to detect a change between blocks; therefore neurons with fewer total spikes than this in the hold period of all trials combined were discarded from analysis. For the typical trial counts in our dataset, we would not be able to detect any less than a doubling of firing rate for a neuron with this minimum firing rate (though the method becomes much more sensitive at higher firing rates). For this reason, selection rule encoding could be even

more common than we have shown. Also, due to the fact that some of our A1 data was collected in earlier animals for which the hold periods were shorter and the trial counts lower, we have less statistical power in that portion of the dataset.

Calculation of evoked responses

For each neuron, the spike times on each trial were smoothed with a Gaussian kernel with 1 ms standard deviation. For every 0.5 ms time bin after stimulus onset, the distribution of smoothed spike counts was compared to the combined distribution of all 0.5ms time bins in the 50 ms preceding stimulus onset with a Mann-Whitney U test. The first window of contiguous time bins that were all significantly greater than the spontaneous rates was defined as the onset response window. Windows of less than 1 ms were discarded because these neurons emitted far too few spikes to analyze statistically. A few neurons showing atypical auditory onset responses (*e.g.*, 4/108 showed a slow build rather than a short-latency peak) were discarded because we were concerned that their activity might be driven by the decision rather than the stimulus.

For the average evoked response plotted on the y-axis of Figures 6C and 6D, we used a bootstrap procedure to draw equally from each stimulus, separately for each block. This procedure accounts for differences in the proportion of each stimulus type across blocks, arising from random chance or from better performance on some stimuli than others (*e.g.*, better performance on go than on nogo, Figure S2A) since only correct trials were included for this analysis.

Changes in evoked response not explained by changes in baseline

We asked whether there were any additional changes in evoked response, above and beyond what could be explained by pre-stimulus effects, by subtracting the block-specific baseline firing rate from the evoked response on each trial and then repeating the bootstrap procedure described immediately above.We found that a small population (6/43, or 14%) of neurons increased their evoked response significantly ($p < 0.05$ from the overlap of the bootstrapped distributions in each block), above and beyond any baseline changes. (Another analysis in which we directly compared across blocks the number of spikes emitted in response to each

stimulus individually yielded similar results, as did a stimulus*block ANOVA on each neuron.) However, unlike the other results in the paper, these neurons were largely (4/6) observed in a single animal (CR12B), the rat which had the most difficulty with pitch discrimination trials, and in these neurons the firing rate was higher during pitch discrimination. One possibility is that the greater difficulty this rat had with one block led to this block-specific increased in evoked rate; our other rats were more evenly matched in performance between blocks.

Acknowledgements

The authors would like to thank Mohammad Dastjerdi and Christian Fernandez for developing the initial rodent behaviors in the lab and managing behavioral training; Alfonso Apicella for helping set up the lab; Tony Zador, Uri Livneh, Erin Rich, Nicholas Steinmetz, Anita Devineni, and Aaron Koralek for comments on the manuscript; Sarah Kochik, and Vuong Vu for managing behavioral training; Trevor Dolinajec, Lily Lin, Danielle Jobe, and Yassi Sabahi for electrode fabrication; Yuliy Tsank, Sharon Lin, Ambika Rustagi, Amish Shah, Carol Pham, Francine Foo, Helene Moorman, Jason Murphy, Jason Zhang, Josh Chong, Kelly Clancy, Tanling Hsu, Pooja Shah, and Tommy Li for help with rat behavior; and Vivek Ayer for technical assistance.