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Muscarinic receptors regulate auditory and prefrontal cortical communication during auditory processing

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Abstract

Much of our understanding about how acetylcholine modulates prefrontal cortical (PFC) networks comes from behavioral experiments that examine cortical dynamics during highly attentive states. However, much less is known about how PFC is recruited during passive sensory processing and how acetylcholine may regulate connectivity between cortical areas outside of task performance. To investigate the involvement of PFC and cholinergic neuromodulation in passive auditory processing, we performed simultaneous recordings in the auditory cortex (AC) and PFC in awake head fixed mice presented with a white noise auditory stimulus in the presence or absence of local cholinergic antagonists in AC. We found that a subset of PFC neurons were strongly driven by auditory stimuli even when the stimulus had no associative meaning, suggesting PFC monitors stimuli under passive conditions. We also found that cholinergic signaling in AC shapes the strength of auditory driven responses in PFC, by modulating the intra-cortical sensory response through muscarinic interactions in AC. Taken together, these findings provide novel evidence that cholinergic mechanisms have a continuous role in cortical gating through muscarinic receptors during passive processing and expand traditional views of prefrontal cortical function and the contributions of cholinergic modulation in cortical communication.

Keywords

Sensory processing; Auditory cortex; Prefrontal cortex; Acetylcholine; Cortical gating

1. Introduction

The prefrontal cortex (PFC) in mammals has been associated with various cognitive functions, such as stimulus categorization and integration, decision making, and executive control (Dalley et al., 2004; Miller and Cohen, 2001; Roy et al., 2014). PFC neurons exhibit

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Author contributions

N.M.J. and H.J.G. performed all experiments. N.M.J. processed and analyzed the data. K.S. and N.K. edited the manuscript, and K.S. provided technical support. X.H. supervised the study. N.M.J., H.J.G., and X.H. wrote the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neuropharm.2018.10.027>.

a high degree of selectivity for conditioned sensory stimuli and can be differentially regulated by contextual associations or task demands (Chang et al., 2010; Euston et al., 2012; Hyman et al., 2012; Maren and Quirk, 2004; Moorman and Aston-Jones, 2015). Interestingly, prefrontal regions are also responsive to conditioned auditory stimuli that had been previously learned, when re-tested under anesthesia, suggesting that learned sensory information can reach PFC in the absence of behavior (Pirch et al., 1985a, 1985b). While the responsivity of PFC within the context of well-learned behavioral tasks is well studied, the intrinsic responsiveness of PFC subregions to non-behaviorally relevant sensory stimuli is less well established. In particular, we wanted to know if sensory information is conveyed across multiple prefrontal regions during awake passive states, and if there is an underlying mechanism that gates sensory signals into PFC under these conditions?

One candidate for gating sensory input into executive regions is the neurotransmitter acetylcholine, which innervates both sensory and association cortices through projections from the basal forebrain cholinergic system. The cholinergic system is well suited to coordinate neural activity across large brain areas, as it provides diffuse and global innervation of the cortex (Zaborszky et al., 2012). The extensive reach of these projections allow for coincident cholinergic release in disparate regions, which could coordinate networks during stimulus processing, or task engagement by enhancing neural synchrony. Synchrony among distal neural networks is proposed to facilitate information transfer across interconnected areas (Buschman and Miller, 2007; Fries et al., 2001; Siegel et al., 2012).

Much of our understanding of cholinergic influences on stimulus processing comes from task performing animals where cholinergic agonists generally improve performance or sensory discrimination (Boix-Trelis et al., 2007; Herrero et al., 2008; Liang et al., 2008; Rogers and Kesner, 2004). Studies in which basal forebrain stimulation is paired with concomitant sensory stimuli have demonstrated that activation of cholinergic networks enhances the response reliability of cortical neurons, and increases the correlated temporal activity of individual neurons within the networks they serve (Froemke et al., 2007; Goard and Dan, 2009; Kilgard and Merzenich, 2002; Letzkus et al., 2011; Pinto et al., 2013). While these studies suggest that the cholinergic system has an active role in modulating neural networks during active behavioral states, the contributions of the cholinergic system to cortical dynamics and cross-regional connectivity in the absence of well-defined behavioral associations is not well studied.

Previous work has shown that the cholinergic projections densely innervate primary and secondary auditory regions affording them the potential to regulate auditory processing across multiple levels (Chavez and Zaborszky, 2017). Cholinergic modulation of cortical processing occurs via nicotinic (nAChRs) and muscarinic (mAChRs) receptor activation; both of which can facilitate sensory processing in complementary ways. In auditory cortex (AC), nAChRs located on thalamic projection terminals have been shown to augment the strength of presynaptic inputs (Gil et al., 1997; Kawai et al., 2007; Lambe et al., 2003). There is also evidence that post-synaptically located nAChRs can increase excitability by increasing the frequency, magnitude, and duration of the postsynaptic response (Chu et al., 2000; Levy and Aoki, 2002; Roerig et al., 1997). In parallel, acetylcholine has been shown to enhance how the visual cortex responds to stimuli presented within an attended visual

receptive field by acting at metabotropic mAChRs (Herrero et al., 2008). mAChRs are ubiquitously expressed across almost all major cortical inhibitory neuron subtypes (Disney and Aoki, 2008), where activation leads to overall enhanced cortical excitability primarily through reducing peri-somatic inhibition of layer V pyramidal neurons (Kruglikov and Rudy, 2008; Nunez et al., 2012). Acetylcholinesterase inhibitors, locally applied, generally mimic the effects of individual agonists on auditory cortex, enhancing frequency specific responses (Ashe et al., 1989). Given their diverse functionality, both nAChRs and mAChRs could coincidentally promote cortical representations of sensory inputs through increasing the signal to noise ratio of sensory information (Hasselmo and Sarter, 2011). While several studies have identified a role for cholinergic signaling in modulating cortical representations in auditory cortex during awake or anesthetized conditions (Ashe et al., 1989; Bandrowski et al., 2001; Chu et al., 2000; McKenna et al., 1988, 1989; Metherate et al., 1990, 1992, 2012; Metherate and Weinberger, 1989, 1990), the contributions of cholinergic signaling in conveying sensory representations from auditory to association areas, and the potential contributions of each receptor subtype to this process, has not been explored.

To investigate if PFC monitors sensory stimuli during passive sound processing, and if the cholinergic system has an ongoing role in this process, we combined electrophysiological, pharmacological and optogenetic techniques in awake head-fixed mice, as they were presented with auditory stimuli passively. We discovered that PFC responds to sensory input under these conditions, and that cholinergic signaling in the AC plays an important role in modulating the strength of PFC responses through muscarinic receptor activation. These findings suggest that cholinergic interactions contribute to passive sound processing and play a role in cortical communication within and across brain structures.

2. Materials and methods

All procedures involving animals were approved by the Boston University Institutional Animal Care and Use Committee (IACUC). A total of 38 transgenic mice were used in this study (3–6 months-old, on the day of recording). 32 Chat-ChR2 transgenic mice were obtained by crossing Chat-Cre mice (B6;129S6-Chat^{tm1(cre)}Lowl/J) with Ai32 mice (B6;129S-Gt(ROSA)26Sor^{tm32(CAG-COP4*H134R/EYFP)}Hze/J (both were obtained from Jackson Laboratory, Maine). 6 control Ai32 animals served as controls for optogenetic experiments.

2.1. Surgical procedures

Mice were surgically implanted with a head-plate as described previously (McCarthy et al., 2011). Briefly, under isoflurane anesthesia, a custom head-plate designed to allow access to PFC and AC was anchored to the skull with 3 stainless steel screws. A fourth screw was connected to a metal pin and placed in the skull above contralateral parietal cortex to serve as the ground.

2.2. In vivo electrophysiology

Upon complete recovery from surgery, typically one week, mice were first habituated to the head-fixed condition in the recording apparatus, enclosed in a custom sound attenuation

chamber (Industrial Acoustics Company Inc., Bronx, NY) the day before the recording experiment was performed. During the recording and habituation sessions, mice were head-fixed to a custom holder that was anchored to the recording table, and loosely wrapped in a breathable mesh (Butler Schein, Dublin, OH; Vet-Flex EZTear). On the recording day, electrode probes were slowly lowered through two small craniotomies into the PFC (AP +2.0, ML +0.4, DV -2.75) and the AC (AP -2.3 to +3.6, ML + 4.0 to +4.5, DV -1.0) using motorized micromanipulators (Siskiyou, Grants Pass, OR) at 100–200 $\mu\text{m}/\text{min}$. A 16 contact linear probe (Neuronexus, Ann Arbor, MI; model: A1 $\times$ 16–10 mm-100–177-A16) with 100 μm spacing between electrode contacts was inserted dorsal-ventrally into the PFC. A 32 channel probe (4 shanks, 8 sites per shank with 100 μm spacing between electrode contacts and 400 μm spacing between shanks, Neuronexus, Ann Arbor, MI; model: A4 $\times$ 8–5 mm-100–400-177-A32) was positioned into the AC, perpendicular to the cortical surface. Because of the curvature of the AC cortical surface, not all four shanks could be placed at precisely the same depth during each experiment. Probes were advanced until all electrode contacts were within the cortical tissue and shanks were positioned along the rostro-caudal axis of the AC. An optical fiber, 200 μm in diameter, was lowered through a third craniotomy using a microdrive (Siskiyou, Grants Pass, OR) into the nucleus basalis (NB; AP +0.5, ML +1.5, DV -4.5) for optogenetic stimulation.

Extracellular recordings were made with a TDT multichannel RZ2 recording system (Tucker Davis Technologies, Alachua, FL). To record spikes, signals were digitized at 24414 Hz and band pass filtered between 300 and 5000 Hz. Spikes were identified by threshold crossings, and the thresholds were manually set at the beginning of each recording session.

Timestamps and waveform snippets (1.3 ms long) were stored for later offline processing. Local field potentials (LFPs) were low pass filtered at 1000 Hz, and digitized at 3051.8Hz.

2.3. Pharmacology

The 32 channel recording probe in AC was also coupled to an infusion pipette under the control of a picospritzer for local drug infusion (Fig. 1B). The infusion pipette was a pulled glass pipet with an opening of approximately 5 μm in diameter, and was placed so that the tip was halfway between the two innermost shanks and terminated halfway between the deepest and most superficial electrode contacts. After a baseline recording session, a picospritzer was used to deliver 500 nL of drug or vehicle into the AC over 5–10 min. Post-drug recording sessions were performed 20–30 min after the completion of drug infusion to allow for diffusion. We infused the muscarinic antagonist scopolamine at a concentration of 10 $\mu\text{g}/\mu\text{L}$ (5 μg : low dose) or 100 $\mu\text{g}/\mu\text{L}$ (50 μg : high dose), and the nicotinic antagonist mecamylamine at a dose of $\mu\text{g}/\mu\text{L}$ (0.5 μg). All drugs were dissolved in artificial cerebro-spinal fluid (ACSF, Tocris Bioscience, Bristol, UK). ACSF was used as control for infusion conditions.

2.4. Auditory stimulus

White noise auditory stimuli were generated with a RZ2 Bioamp processor and RP2.1 real time processor (Tucker Davis Technologies), and digitized at a frequency of 48828 kHz. Stimuli were played with a multi-field magnetic speaker (Tucker Davis Technologies, model: MF1), positioned at 20 cm from the animal's head, and calibrated to be 70 dB sound

pressure level (SPL) with a conditioning amplifier and microphone (Brüel and Kjær, Naerum, Denmark; amplifier: model: type 2690—0S2; microphone: type 4939-A-011). The stimuli consisted of 500 ms white noise bursts (5 ms linear ramp to 90% of peak amplitude).

2.5. Optogenetic stimulation

We positioned an optical fiber in the nucleus basalis (NB) in order to optogenetically stimulate cortically projecting cholinergic neurons in Chat-ChR2 transgenic mice that express ChR2 selectively in cholinergic neurons, while simultaneously recording in AC and PFC (Fig. 1D, condition 1). Laser light for optogenetic stimulation of NB was delivered through a multimode fiber, 200 μm in diameter, optically shielded (Thorlabs, Newton NJ; model: BFH48–200), and coupled to a 473 nm DPSS laser (Shanghai Laser Ltd., Shanghai, China; model: BL473T5–200FC). Laser power was calibrated to 15 mW at the fiber tip prior to insertion. 40 Hz square light pulses (20% duty cycle) lasting 750 ms were delivered via TTL trigger from the RZ2 recording system.

2.6. Recording session

The recording protocol consisted of combinations of optogenetic and auditory stimuli. Two trial types were analyzed: one consisted of a 500 ms long white noise burst alone, and the other consisted of a 750 ms pulse of light (40Hz) delivered alone. The inter-trial interval (ITI) between a single burst of white noise, or a light pulse, was a fixed 10 s and these stimuli were intermixed randomly. During each experiment, animals first received a combined 100 white noise stimuli trials and 100 light alone trials before drug infusion. We then performed drug or vehicle infusion. Approximately 30 min after drug infusion, animals received another 100 white noise stimuli trials and 100 light alone trials (Fig. 1D). Thus, during each experimental session, each animal received 200 white noise stimuli trials and 200 light alone trials.

2.7. Histology

At the end of the experiments, all mice were anesthetized and transcardially perfused for histological verification of electrode placement using cresyl acetate (C-1893; SigmaAldrich, Natick MA). Mice were perfused with 30 mL buffered saline, followed by 30 mL 4% paraformaldehyde. Brains were carefully removed and post-fixed overnight in 4% paraformaldehyde before being transferred to a 30% sucrose solution, and then sectioned coronally in 40 μm slices with a freezing microtome (CM, 2000R; Leica). Tissue sections were collected throughout the basal forebrain, AC, and PFC. Alternate sections were then rinsed with 0.05M PBS buffer and mounted on gelatin-coated slides and allowed to dry overnight. Sections were then rehydrated by a descending series of alcohol rinses (2 min each in 100%, 95%, 90%, 70%, and 50%) before being placed in deionized water for 5 min. Following rehydration, sections were incubated in 0.1% cresyl acetate for 5 min, followed by dehydration in an ascending series of alcohol rinses (50%, 70%, 90%, 95%, 100% (2X) for 3 min each and cleared with xylene (534056; SigmaAldrich, Natick MA) for 15 min. Slides were then coverslipped using DPX (06522; SigmaAldrich, Natick MA) mounting medium and analyzed.

To validate ChR2 expression patterns, we also performed immunohistochemistry with antibodies directed against ChAT (Choline acetyltransferase is selectively expressed in cholinergic neurons) as described previously (Deckmann et al., 2014). Briefly, sections were rinsed with 0.05 M Tris-HCL-buffer (Tris, pH = 7.6), followed by 60 min rinse in blocking buffer containing 5% serum (Jackson ImmunoResearch, 017-000-121) and 0.2% Triton. Sections were incubated for 24 h with goat anti-ChAT antibody (AB144P, Millipore; Temecula, CA) diluted at 1:500. Sections were then rinsed 3X at 10 min each in Tris-HCL and then incubated with the secondary antibody Alexa Fluor 594 (donkey-anti-goat; Life Technologies A11058; Grand Island, NY) at 1:200 for 2h. Sections were rinsed again and then mounted on gelatin-coated slides using anti-fade mounting media Vectashield (H-1400, Burlingame, CA).

2.8. Statistical analysis

All statistical analysis was performed with custom Matlab functions (MathWorks, Natick, MA). Friedman's test or ANOVAs, were used for comparisons between post-infusion conditions; Wilcoxon signed-rank tests, repeated measures ANOVA, and paired t-tests were used for comparisons between pre and post-infusion measurements; Pearson's linear correlation to compute correlation between response magnitude and recording depth. In some instances the logarithm of the data values were used for statistical analysis. All tests were two-tailed except for chi-squared tests, which were one-tailed with respect to the chi-squared distribution. Post-hoc tests included corrections when multiple comparisons were made. This would include instance where multiple time windows, multiple frequency bands, multiple cortical layers, or combinations of these terms were analyzed. P-values were corrected using the standard Bonferroni procedure.

All drug comparisons compared the effects of each drug infusion separately to its own baseline period, prior to drug infusion, within the same animals. An ACSF condition was included as a comparison for the effects of intracranial infusion alone.

2.9. LFP spectral analysis

LFPs were first bandpass filtered between 1 Hz and 150 Hz, and then down-sampled by a factor of 8 (381 Hz) prior to further analysis. In awake, head fixed conditions, we occasionally found motion induced artifact. To remove trials contaminated with motion artifact from subsequent analysis, we took the root mean square (RMS) in 100 ms bins for each trial, and rejected trials if the averaged RMS exceeded 5 standard deviations at any time throughout a trial.

We used the open source software package Chronux (www.chronux.org) to compute multi-taper spectrograms, using three tapers and a 500 ms sliding window with 5 ms step. Single trial spectrograms were first calculated, and then averaged across trials. To examine the effects of optogenetic stimulation, we calculated normalized spectrograms by dividing the average power during each time window by the mean power during the baseline period, defined as 5 s window immediately preceding optogenetic stimulation. Time-frequency windows of interest were quantified by summing all normalized power within given frequency and time ranges.

To quantify the influence of pharmacological agents on baseline LFP power for comparison between the baseline and post-drug infusion condition, we calculated multi-tapered power spectra during the 4–9 s window of the ITI. Note, the ITI is 10 s long (Fig. 1). We calculated the log(power) at 10–100 Hz and 1–10 Hz frequency bands, and compared the log(power) before and after drug infusion. We also computed cortical desynchrony by taking the ratio of power at 10–100 Hz and power at 1–10 Hz. To examine the effect of optogenetic stimulation of NB on cortical desynchrony, we compared cortical desynchrony during the 750 ms light stimulation window to the 750 ms time period immediately before optogenetic light illumination onset as the baseline.

2.10. Current source density (CSD) analysis

CSD analysis estimates the second spatial derivative of LFP signals to determine the relative current across the cortical laminar depth. CSDs were calculated using LFPs recorded from the four laminar probes inserted in AC, as described previously (Mitzdorf, 1985; Nicholson and Freeman, 1975). We averaged LFPs recorded from each electrode contact across all trials prior to CSD calculation.

First, we applied spatial smoothing across the eight electrode contacts within the same shank as described in (Sakata and Harris, 2009).

$$\Phi(z) = \frac{\Phi(z + \Delta z) + 2\Phi(z) + \Phi(z - \Delta z)}{4}$$

Where z is the depth perpendicular to the cortical surface, Δz is the electrode spacing, and Φ is the potential. Then we estimated the CSD as described in (Mitzdorf, 1985; Nicholson and Freeman, 1975).

$$-CSD(z) \approx \frac{\Phi(z + \Delta z) - 2\Phi(z) + \Phi(z - \Delta z)}{\Delta z^2}$$

To quantify the total current across cortical lamina during temporal windows of interest, we averaged the root mean square (RMS) of CSD curves at all depths. Additionally, for population analysis of CSDs between drug-treatments, we separately averaged CSD RMS values from either the two inner shanks or the two outer shanks, to reflect the spatial symmetrical orientation of the probe shanks with respect to the infusion pipette (Fig. 1B, right, 4A). For display purposes, CSDs were interpolated linearly and plotted as pseudocolor images.

We analyzed the CSD latencies for both the current sink onset and the current source peak across drug conditions to determine the effects of drug infusion on AC sound evoked responses. We first identified the electrode contact on each laminar shank with the largest current sink, calculated as the average amplitude within 50 ms of sound onset. This electrode contact was used to quantify CSD onset latency. Sound onset latency was chosen as the point at which the CSD crossed 3 S.D. from baseline. The end of the sink was calculated by taking the time point at which the rising phase of the CSD crossed 3 S.D. from baseline.

CSD profiles were not used to estimate current in PFC, because the probe was not oriented perpendicular to the cortical lamina.

CSD analysis was also used to determine the granular layer on each shank in AC. Along each shank, the channel that had the earliest sink was considered to be in the granular layer. This layer identification was used to select granular layer channels for ERP latency, and used for anatomical classification of laminar CSD magnitudes and MUA firing rates. For laminar analysis of CSD curves, peak-trough values were taken from the mean supragranular and infragranular channels, as well as granular channels, for each mouse. Additionally, CSD traces were used for spectral analysis of the baseline LFP. When used for spectral analysis—in which averaging occurs in the spectral domain—spatial smoothing was not applied to the CSD.

2.11. Event related potentials (ERPs) analysis

To quantify auditory ERPs in PFC, we averaged LFPs recorded across all trials, and all electrode contacts of the 16 channel linear probe in PFC. ERP magnitude was calculated by subtracting the minimum from the maximum value of the ERP within 100 ms following stimulus onset. In PFC, ERPs at various depths were either averaged together, or analyzed separately to examine depth-dependence. ERP latencies were chosen as the point at which the ERP exceeded 3 S.D. from baseline. We limited our analysis of latency to trial-averaged ERPs that exceeded 3 S.D. of the baseline activity within 100 ms post sound onset in PFC (28 of 32 recordings).

To facilitate latency comparisons between PFC and AC, we also calculated AC latencies using ERPs. ERPs were taken from the electrode contact centered on the granular layers and ERPs that did not exceed 3 S.D. in this window were excluded. Latency values were then calculated from AC granular layer ERPs in the same manner as PFC latencies. In instances where a latency values were less than 10 ms, those events were not included (recordings from 2 out of 128 total shanks) as it is physiologically improbable to be a result of auditory input and more likely to be a result of random deviation in the baseline LFP.

2.12. Spike analysis

Spike snippets were detected with manual thresholds during recording sessions and analyzed offline. Multi-unit (MU) activity was reported consistent with other auditory studies using laminar electrodes (Guo et al., 2012; Steinschneider et al., 2008). This was done because of generally lower signal amplitude obtained with laminar probes and the inability for each electrode contact to be independently positioned to best capture nearby single neuron activity leading to inconsistencies in single-unit isolation (Bragin et al., 2000).

We calculated the width of spike waveforms at half of their peak-trough maximum. Spike events were only included if their width at half-maximum was between 0.1 and 0.4 ms. Additionally, coincident events indicative of motion induced artifact across multiple contacts on the same probe were removed if they occurred within two samples of any other spike (83 μ sec).

MUA peri-stimulus time histograms (PSTHs) were calculated using 2 ms time bins for population and statistical analysis. AC MUAs were considered sound responsive if the mean z-score exceeded an absolute value of 2 at early onset (10–30 ms) or sustained an average z-score of 1 throughout the duration of the 500 ms auditory stimulus. Similarly, PFC MUAs were considered responsive if their mean z-score exceeded an absolute value of 2 during the 10–60 ms window after sound onset or exceeded 1 throughout the duration of the stimulus. Firing rate curves were binned with a 16 ms moving average for display purposes. In AC, in order to measure the firing rate (FR) decline following sound onset, we measured the integrated firing rate difference from baseline for each MU within the early (0–72 ms) and late (72–190ms) windows from stimulus onset (Fig. 6A and C).

The peak evoked PFC activity was calculated as the maximum of the mean population firing rate across all MUAs. Peak PFC firing rates were calculated for each population defined by anatomy and drug group individually. Cross-correlograms for AC MUAs within the duration of the sound window (0–500 ms) were calculated between pairs of electrode shanks and across contacts within each shank. Cross-correlograms were normalized by the mean number of spike events per bin multiplied by the length of the cross-correlogram. To compare latencies of MUAs in PFC and AC, MUs with early burst-type response patterns were selected and the latencies to the half-maximum of their firing rate curves were used to estimate response latencies. Significantly more early bursttype MUs were present in AC (492 MUs) than in PFC (40 MUs).

3. Results

3.1. Prefrontal cortex (PFC) shows subregional variations in auditory evoked responses that lag responses in auditory cortex (AC) during passive sound processing

While it has been well established that neurons in PFC respond robustly to auditory stimuli that are relevant to behavioral tasks (Chang et al., 2010; Euston et al., 2012; Hyman et al., 2012; Maren and Quirk, 2004; Moorman and Aston-Jones, 2015), few studies have explored the extent to which subregions of PFC are responsive to the passive presentation of sound in awake rodents. A small number of studies have previously identified PFC neural activity in response to auditory stimuli: CS presentations before fear conditioning for example (Burgos-Robles et al., 2009; Chang et al., 2010), but none to our knowledge, have compared the response properties between AC and multiple regions of PFC simultaneously in awake animals. In order to better understand how sound is coincidentally represented in AC and PFC during passive sound processing, we recorded from both structures using multi-contact electrode probes (Fig. 1A-C) in awake head-fixed mice. Animals underwent acclimation and habituation to the recording configuration and sound booth prior to the recording session. This experimental design is similar to other experiments where naturalistic stimuli were delivered to habituated head fixed animals (Hromadka et al., 2008; Lee et al., 2012; Pinto et al., 2013). Acoustic white noise was used as a stimulus to activate as much of the AC tonotopy as possible, in order to evaluate the strength of interactions across regions. To reduce potential for stimuli to have an aversive quality, we presented the stimulus at an intensity of 70 dB sound pressure level (SPL). Our goal was to capture excitatory and

inhibitory interactions across multiple tonotopic regions during a single stimulus so that we could test the effects of cholinergic antagonists on auditory responses.

We first quantified the multi-unit activity (MUA) recorded in PFC, and found that the 12.0% of multi-units (MUs) significantly increased their firing rate during sound presentation, while 0.2% decreased ($n = 502$ MUs recorded in 32 mice; Fig. 2A, C). These findings are similar to a number of studies that assessed responsive neurons (~ 11 –25%) in prefrontal areas for conditioned auditory stimuli prior to fear conditioning (Burgos-Robles et al., 2009; Chang et al., 2010). We also observed that MUs exhibited complex temporal dynamics, showing a combination of monophasic and ramped firing responses upon sound presentation. Responses were generally followed by a slight reduction after sound offset that lasted for ~ 500 ms before returning to baseline (Fig. 2C). The observed response profile as well as the mean latency of the responses were similar to those noted in ventral orbital cortex in head restrained mice using a white noise auditory stimulus (Winkowski et al., 2018). Because the mouse PFC is layered medial-laterally and is parallel to the dorsal-ventral axis of electrode insertion (Fig. 1B, Figure S7), we could not sample across the cortical layers as we could with AC. Instead, the PFC linear electrode targeted the middle cortical layers (deeper layer three and superficial layer five) of a number of anatomically dissociable PFC sub-regions, including the cingulate (Cg), prelimbic (PrL), infralimbic (IL), and medial orbital (MO) cortices. Supplemental Figure S7 shows the electrode penetrations, in relationship to the anatomical boundaries of PFC. We found that while all PFC sub-regions were responsive to the auditory stimulus, they differ in their response magnitude, with IL being less responsive than the more superficial Cg, PrL, or deeper MO subregions ($\chi^2 = 6.43$, $p = 0.01$; Fig. 2E).

In contrast, the majority of AC MUs showed sound modulation: 52% of MUs increased their firing rate during sound presentation, and 0.5% decreased ($n = 980$ MUs recorded in 32 mice; Fig. 2D, right). The neurons with suppressed firing rates showed higher baseline firing rates when compared to neurons that increased their firing rate (baseline: 42.24 ± 8.65 Hz; mean $\pm$ SEM: suppressed group. vs. 35.97 ± 6.92 Hz; mean $\pm$ SEM activated group). These findings are consistent with other studies demonstrating that broadband auditory stimuli evoke a relatively sparse, yet tonotopically extensive response in awake, un-anesthetized animals (Evans and Whitfield, 1964; Hromadka et al., 2008). MUs typically exhibited a large transient increase immediately following sound onset, but some channels also showed low levels of sustained activation throughout the duration of the stimulus (Fig. 2B, left). We found that MUs granular layers (based on current source density (CSD) profiles -described below), showed the greatest increases in firing rates at sound onset, while the remaining sites showed more gradual responses that were broader in duration (Fig. 2F). MU activity also occurred significantly earlier in AC compared to prefrontal cortex ($t(5,30) = -2.132$; $p = 0.034$; Fig. 2F).

We further examined the auditory stimulus evoked local field potentials (LFPs) recorded in both AC and PFC and found large event related potentials (ERPs) on all recording sites in PFC and AC (Fig. 3A). PFC ERPs had an average onset latency of 28.48 ± 7.39 ms (mean $\pm$ S.D., $n = 32$ mice, Fig. 3Bi-3Di). Interestingly, we detected a significant correlation between ERP amplitude and dorsal-ventral depth ($R = -0.154$, $p = 5e-4$, $n = 512$ recording sites, 32

mice), with the largest ERPs observed on the most superficial subregions (Cg and PrL) where the firing rate increases were also greatest (Fig. 2E). We also detected a systematic increase in latency with depth, with the deepest recording sites lagging the most superficial recording sites by ~ 5 ms (Fig. 3Ci). The changes in ERP amplitude and latency along the dorsolateral axis of PFC suggest that mouse PFC subregions receive differing levels of auditory input, with the superficial subregions (i.e., Cg and PrL) receiving auditory input earlier and of greater magnitude than deeper subregions (i.e. IL and MO).

In AC, robust ERPs were evoked by auditory input at most recording sites, with some sites exhibiting larger ERPs than others, presumably because some sites were within regions of the AC tonotopy that were outside the frequency range of the band-limited white noise auditory stimulus used (sampled at 48.8 kHz). Across the cortical depth, we found that the largest ERPs were in the middle layers, usually the two middle electrode sites, ~ 300–500 μ m from the surface of the cortex (Fig. 3Aii). AC ERPs paralleled the evoked MUAs, with the most robust responses observed immediately after sound onset (Fig. 3Bii, 2D), consistent with previous studies investigating AC (AI, AAF, or AII subregions) (Joachimsthaler et al., 2014; Stiebler et al., 1997). The mean onset latency for ERPs in AC granular layers (defined by current source density analysis; see methods) was 17.89 ± 2.95 ms (mean $\pm$ S.D., $n = 126$ shanks, 32 mice; Fig. 3Cii, Dii). When we compared the ERP response latencies, we found that activity also occurred significantly earlier in AC compared to prefrontal cortex (Wilcoxon rank-sum test, $p = 1.8e-11$), consistent with the MU latencies described earlier. The difference of ~ 10 ms in activation between PFC and AC is consistent with that observed in ketamine anesthetized rats where an 8–18 ms delay was observed between A1 and PFC (Martin-Cortecero and Nunez, 2016). Taken together, these results demonstrate that mouse PFC is activated by auditory stimuli during passive sound processing, and superficial PFC sub-regions receive stronger and earlier auditory input.

3.2. Local muscarinic receptor antagonism disrupts cortical desynchrony in AC by altering intracortical connectivity

We configured our AC recording probe with a microinfusion pipette that was positioned between the two inner shanks of the four shank electrode in AC for targeted drug delivery (Figs. 1B and 4A). The nicotinic blocker, mecamylamine (0.5 μ g), was delivered at a dosage shown to be effective in altering sound evoked response latency and characteristic frequency thresholds when locally delivered into AI in rodents (Liang et al., 2006; Metherate, 2004). However, we could not find similar studies in which the muscarinic blocker scopolamine was delivered intracortically into AC during sound processing. Therefore we chose a high dose (50 μ g) and a low dose (5 μ g) that border the broad range of effective doses cited in the literature as influencing behavior in active and passive experimental paradigms, as well as disrupting the influence of cholinergic activation in cortical circuits (Boix-Trelis et al., 2007; Herrero et al., 2008; Ingles et al., 1993; Maruki et al., 2003; Rogers and Kesner, 2004; Santucci and Shaw, 2003; Zhou et al., 2011).

As systemic application of cholinergic antagonists have been shown to modulate the cortical LFP power spectrum (Kalmbach and Waters, 2014), we first examined the effects of cholinergic antagonism on LFP power spectra during the inter-trial interval (ITI) period

relative to the pre-infusion ITI period (Fig. 4B). Neither ACSF, mecamylamine, nor low dose scopolamine altered the LFP power spectrum, on inner or outer shank locations following drug infusion (Wilcoxon signed-rank test, $p > 0.05$). However, infusion of high dose scopolamine produced a moderate reduction in LFP power at higher frequencies (10–100Hz) on the inner electrode shanks closest to the infusion site (Wilcoxon signed-rank test, Bonferroni-corrected p -value = 0.039, $n = 9$ mice; Fig. 4B), but not on the outer shanks distal to the infusion site (Wilcoxon signed-rank test, $p > 0.05$). This effect confirms that 500 nL scopolamine delivery was largely isolated to the immediate vicinity of the infusion site. We found no effect of scopolamine infusion on low frequency power on either the inner or outer shanks (1–10 Hz: all Wilcoxon signed-rank test, $p > 0.05$, $n = 9$ mice; Fig. 4B). Because field potentials can express volume conduction, particularly at low frequencies, we wanted to be certain that scopolamine did not induce reductions in LFP power that may have been masked by volume conductance into our recording area from adjacent regions away from the infusion site. Therefore, we further analyzed CSD waveforms, the second spatial derivatives of LFPs that estimate local current, and found no significant change in power in the 1–10Hz range on inner or outer shanks (all Wilcoxon signed-rank test, $p > 0.05$, $n = 9$ mice; Figure S10). Analysis of the CSD waveform also revealed that only the inner shanks in the 10–100Hz frequency range showed significant reductions following scopolamine infusion (Wilcoxon signed-rank test, Bonferroni-corrected p -value = 0.039, for frequency bin, $n = 9$ mice), recapitulating the only significant effect revealed by the LFP power analysis.

We then examined the efficacy of cholinergic antagonism on cortical desynchrony, defined as the ratio of LFP power at high frequency (10–100 Hz) to low frequency (1–10 Hz). Cortical desynchrony is a widely observed phenomenon that coincides with cholinergic activation of sensory cortex, and is characterized by a simultaneous decrease of LFP power at low frequencies (< 10 Hz) and an increase in power at high frequencies (10–100 Hz) (Goard and Dan, 2009; Kalmbach et al., 2012; Kalmbach and Waters, 2014; Metherate and Ashe, 1993; Pinto et al., 2013). We induced cortical desynchrony through optogenetic stimulation of nucleus basalis (NB) in Chat-ChR2 transgenic mice selectively expressing ChR2 in NB cholinergic neurons (Fig. 1C). Prior to drug infusion, we found that optogenetic stimulation of NB cholinergic neurons induced cortical desynchrony in both AC and PFC (Wilcoxon signed rank test, $p = 1.405e-6$ for PFC; $p = 6.48e-7$ for AC, $n = 32$ mice; Fig. 4C). Cortical desynchrony in AC and PFC was short lasting, limited to the light stimulation window, and was not detectable in control Ai32 transgenic mice (see Figure S1). Infusion of high dose scopolamine reduced cortical desynchrony (chi-square = 8.49, $p = 0.0036$, both inner and outer shanks). This effect was manifest by selectively blocking the decreases in low frequency power in AC (Wilcoxon signed-rank test Bonferroni-corrected p -value = 0.008), but not the increases in high frequency power (Wilcoxon signed-rank test, $p > 0.05$), suggesting that only the low frequency component of cortical desynchrony depends on mAChR activation. Neither low frequency nor high frequency power was impacted by ACSF vehicle, mecamylamine, or low dose scopolamine infusion (Fig. 4E; Wilcoxon signed-rank test, all $p > 0.05$). Disruption of low frequency components of desynchronization is consistent with previous studies (Kalmbach and Waters, 2014; Metherate et al., 1992), and

confirmed that high dose scopolamine was effective in blocking muscarinic receptors and neutralizing the influence of intrinsic cholinergic activation.

It is interesting that the muscarinic blocker scopolamine reduced cholinergically induced cortical desynchrony across broad cortical areas, recorded on both the outer and the inner recording shank locations (Fig. 4E). Since scopolamine only altered LFP power during the ITI at inner shank locations, and not outer shanks locations (Fig. 4B), the observed effects of scopolamine on cortical desynchrony on the outer shanks cannot be explained by direct antagonism of muscarinic receptors at the outer shanks.

Optogenetic activation of NB also produced robust cortical desynchrony in PFC that was not blocked by vehicle or any of the antagonists infused into AC (see Figure S5; all Wilcoxon signed-rank test, $p > 0.05$). Many previous studies have linked cholinergic activation to cortical desynchrony in sensory or motor cortices. Our observation that cortical desynchrony is also present in PFC, highlights the broad projection patterns of basal forebrain cholinergic neurons (Zaborszky et al., 2012). While high dose scopolamine infusion in AC altered cortical desynchrony in AC, it failed to alter cortical desynchrony in the PFC suggesting the effects produced by local scopolamine infusion in AC were confined to AC, and not relayed to PFC.

3.3. Optogenetic stimulation did not alter sound evoked amplitude

Because previous work has demonstrated that behavioral training or basal forebrain (BF) stimulation can significantly reorganize receptive fields or temporal response profiles in AC (Fritz et al., 2007; Froemke et al., 2007; Kilgard and Merzenich, 1998a; b; Polley et al., 2006), we wanted to confirm that our optogenetic stimulation parameters did not promote plasticity changes during recording. Traditionally this plasticity occurs over days or weeks and often involves stimulus-response based learning with coincident activity, although rapid, within session plasticity has been reported (Froemke et al., 2007). Plasticity measures often involve changes in the tuning of AC neurons modulated by context with behaviorally relevant auditory stimuli gaining a larger cortical representation (Rutkowski and Weinberger, 2005). In order to be certain that our optogenetic stimulation parameters did not induce plasticity that would confound interpretations of post-infusion data, we calculated the RMS values of sound-evoked CSD in AC. We examined the mean RMS of the CSD recorded from all channels on each shank, within 180 ms of sound onset where sound evoked significant CSD changes (Fig. 5B). We analyzed the RMS values from the first 20 trials and the last 20 trials of the 100 trial pre-infusion session for all Chat-ChR2 transgenic mice and Ai32 control mice. If optogenetic stimulation induces plasticity effects, where the same sound stimulus will have a greater cortical representation, we would expect to see greater RMS values in sound-evoked CSDs. However, we found across both groups that there was no difference in the RMS values between control ($n = 6$) and Chat-ChR2 animals ($n = 32$) at the beginning (Wilcoxon rank sum test, $p = 0.707$) or the end of the recording sessions (Wilcoxon rank sum test, $p = 0.701$). These results suggest there were no plasticity effects on sound evoked auditory responses within our recording sessions as a result of optogenetic trials.

3.4. Muscarinic receptors contribute to intracortical signaling whereas nicotinic receptors alter the timing of thalamocortical input

To examine the effects of cholinergic receptor blockade on sound evoked AC responses across cortical layers, we characterized sound evoked CSD response profiles. In AC, CSD has been shown to allow for the dissociation of thalamic and cortical afferent pathways (Happel et al., 2010). The short latency phase of sensory-evoked CSD responses in AC consists of a current sink in the granular layers flanked by current sources in the infragranular and supragranular layers. This profile has been attributed to strong feedforward activation from the thalamus (thalamocortical), although within-column (intra-columnar) recurrent excitation in AC contributes to the overall amplitude of the early phase sink/source (Happel et al., 2010). Furthermore, the latent phase of the response consists of an inversion of the initial source-sink-source triplet, with a strong source in the granular layers and sinks in the supragranular and infragranular layers, primarily reflecting connections from adjacent regions of AC (intracortical). Similarly, in our dataset, we found that the auditory stimulus produced a large current sink in the middle layers ~300–500 μm from the cortical surface (Fig. 5A, blue color), followed by a strong source that emerged ~75 ms after auditory stimulus onset (Fig. 5A, red color).

We computed CSD profiles for each recording shank, and determined the granular layers by identifying the channel with the earliest CSD sink. We then computed CSD profiles for each recording shank and used the largest CSD sink to characterize the time course of the sink source transition. The use of eight site probes, where sites were separated by 100 μm , allowed us to sample the majority of cortical depth, although infragranular locations were more sparsely sampled (see Figure S9 for quantifications of all electrode placements relative to the granular location). The population CSD from this channel, across animals, exhibited an early current sink (0–72 ms) when the current was below zero, followed by a late current source (72–180 ms) when the current was above zero (Fig. 5B). We further determined the threshold of the early sink to estimate the response properties of AC to sound presentation. Across animals, the latency for current sinks, defined as significantly deviated from baseline, was 16.70 ± 0.25 ms from stimulus onset, and the current sink returned to baseline at 59.07 ± 3.73 ms (mean $\pm$ s.e.m., $n = 128$ shanks, 32 mice). These response profiles are consistent with general findings reported in AC using CSD analysis previously (Happel et al., 2010; Intskirveli and Metherate, 2012; Metherate et al., 2012; Szymanski et al., 2009, 2011).

To characterize the influence of cholinergic antagonists, we combined CSDs recorded from the two inner shanks, closer to the infusion pipette, and the two outer shanks, distal to the infusion pipette (Fig. 4A). After administering high dose scopolamine, the magnitude of the CSD sinks and sources responses showed a reduction on the inner shanks that was not present on the outer shanks (Fig. 5E).

We further investigated the effect of high dose scopolamine across all cortical layers by quantifying net laminar current, calculated as the mean RMS of the CSD recorded from all channels on each shank, during the two temporal windows described above: the early window (0–72 ms), and the late window (72–180 ms after noise onset; Fig. 5B). We utilized a non-parametric Friedman test to quantify the change in net current for each drug condition combining shanks (inner/outer) and time windows (early:sink/late:source). This analysis

revealed that only high dose scopolamine had a significant effect on net current (chi-square = 5.9, $p = 0.0195$). Scopolamine produced moderate reductions in overall net laminar current flow on the inner shanks during the early window (Wilcoxon signed-rank test, Bonferroni-corrected p -value = 0.11, $n = 9$ mice) and robust effects during the late window (Wilcoxon signed-rank test, Bonferroni-corrected p -value = 0.039, $n = 9$ mice; Fig. 5D and F). On the outer shanks, high dose scopolamine did not alter the net laminar current (Wilcoxon signed-rank test, $p > 0.05$ for early and late windows, $n = 9$ mice; Fig. 5D and F). Neither effect was present on outer shanks in any of the other shank, layer and drug condition combinations analyzed. This finding suggests that mAChR inactivation not only can reduce thalamocortical signaling in the granular layer, but also cortico-cortical signaling in supergranular layers. In comparison, neither ACSF, nor the nAChR blocker mecamylamine, changed net laminar current on the inner or outer shanks during the two windows analyzed (Wilcoxon signed-rank test, $p > 0.05$).

Finally, we examined the effects of the different cholinergic receptor antagonists on thalamocortical activation by analyzing the evoked CSD sink onset latency. Latency to sink onset (< 20 ms) is thought to be a more direct measure of thalamocortical activation as sink amplitude involves both contributions from thalamic input and intra-columnar recurrent excitation (Happel et al., 2010; Szymanski et al., 2011). Mecamylamine significantly delayed evoked response latency on the inner shanks, but not on the outer shanks (Wilcoxon signed-rank test, Bonferroni-corrected p -value = 0.008, $n = 5$ mice; Figure S2A-S2B, S2D), confirming that the dosage of mecamylamine was efficacious in blocking nAChRs, and consistent with delays reported previously at this concentration (Liang et al., 2006; Metharate, 2004). Given the strong influences of scopolamine on intra-columnar activity which contributes to sink amplitude described above (Fig. 5E), we could not perform a statistical quantification of sink onset latency changes for high dose scopolamine. Because of this, we cannot rule out the possibility of contributions of mAChR to thalamocortical latency, although we found no differences in onset latency for low dose scopolamine or vehicle conditions on the inner or outer shanks (Wilcoxon signed-rank test, $p > 0.05$).

3.5. Scopolamine enhances the duration of sound evoked responses in AC outside of the infusion area

To better understand how scopolamine effects on current flow contribute to neural output, we analyzed the time-course of the evoked MUAs in AC. Granular layer MUs showed the strongest onset responses, which returned to baseline within ~ 75 ms. Omnibus analysis revealed significant interactions only for high dose scopolamine between time window (early/late as stated above) and the presence of drug (pre-infusion/post-infusion: Repeated Measures ANOVA, $F = 32.01$, $p = 4.8e-4$) and between presence of drug and shank position (inner/outer: Repeated Measures ANOVA, $F = 8.49$, $p = 0.019$; Fig. 6A-D).

During the early window (0–72 ms), high dose scopolamine reduced the amplitude of MU responses in the granular layer; $t(8) = -4.05$; Bonferroni-corrected p -value = 0.016 (Fig. 6B: left) and extragranular layers: $t(8) = -4.69$; Bonferroni-corrected p -value = 0.008 (Fig. 6D: left), only on the inner shanks but not on the outer shanks (paired t -test, all layers $p > 0.05$). Analysis of the late window (72–180 ms) revealed elevated firing rates in granular layers on

outer shanks; $t(8) = 3.71$; Bonferroni-corrected p -value = 0.024 (Fig. 6B: right) but not on the inner shanks ($p > 0.05$). Likewise, for extragranular layers we saw significant elevation only on outer shanks: $t(8) = 3.86$; Bonferroni-corrected p -value = 0.02 (Fig. 6D: right) and not on inner shanks ($p > 0.05$). This slow decay of the firing rate during the late window suggests a possible reduction in lateral inhibition during auditory responses that temporally aligns with the elongated current sink noted in the CSD (Fig. 5E). This prolonged elevation in firing rate is accompanied by an increase in the cross correlation of MUA between and within shanks at lags of 0–500 ms (Figure S3A). We further analyzed the MUs from individual channels across all layers of AC to determine if any of the channels lost responsivity following high dose scopolamine infusion. While the population peak firing rate was maintained at sound onset when all channels were considered (Fig. 6A–C), a greater number MUs recorded from channels nearest the infusion cannula showed a reduction in evoked firing rate compared to the baseline (see methods; Figure S8A). In addition, following scopolamine infusion, approximately half of the MUs recorded in the supragranular layers on the outer shanks also showed a decrease in evoked responses, whereas MUs recorded in the granular or infragranular layers showed little change from the baseline condition.

3.6 Endogenous muscarinic activation in AC promotes evoked auditory responses in PFC

To determine how cholinergic receptors in AC influence sound evoked PFC responses, we characterized the effects of different antagonists on sound evoked ERPs in PFC. We found that a small local injection of high dose scopolamine into AC was sufficient to significantly reduce the strength of the mean ERP magnitude across all PFC recording sites (i.e., CG to MO subregions; Wilcoxon signed-rank test, $p = 0.008$, $n = 9$ mice; Fig. 7Aii, 7B). We observed no correlations between ERP amplitude reduction and recording depth ($R = 0.07$, $p = 0.44$, $n = 144$ recording sites, 9 mice), suggesting that scopolamine broadly influences sound responses across all PFC subregions measured. Low dose scopolamine infusion produced a moderate, but non-significant decrease in PFC ERP magnitude (Wilcoxon signed-rank test, $p = 0.22$, $n = 7$ mice, Fig. 7B). Neither ACSF nor mecamylamine had any effect on PFC ERP responses (Wilcoxon signed-rank test, $p > 0.05$, Fig. 7Ai, 7B, S4A).

Next, we examined the effects of drug infusion on PFC MU activity. Both high-dose and low-dose scopolamine infusion in AC robustly attenuated the sound evoked MU responses in PFC, with high dose scopolamine producing a reduction twice that seen with low-dose scopolamine (Fig. 7C–D, $t(111) = 2.26$; $p = 0.026$, high dose: $t(137) = 3.709$; $p = 3.02e-4$). Furthermore, this reduction was present across all PFC recording sites with no significant differences between PFC subregions (Chi-square = 0.144, $p = 0.487$, Fig. 7E and F). Neither ACSF nor mecamylamine infusion had any influence on PFC MU activity ($p > 0.05$, Fig. 7D). Together, these results demonstrate that auditory responses in PFC are dependent upon activation of AC, and that endogenous muscarinic activation in AC is conducive to subsequent activation of PFC.

4. Discussion

This study aimed to determine if auditory signals are conveyed to multiple PFC regions during passive sound processing and whether cholinergic receptors in AC contribute to modulating auditory representations under these conditions. We performed simultaneous recordings in both structures while awake head-fixed mice were exposed to repeated delivery a white-noise stimulus in the presence and absence of cholinergic antagonists applied at concentrations shown to be effective in blocking cholinergic activation.

We found that PFC responds robustly to sensory input during passive presentation, exhibiting strong ERPs, and activating a significant proportion of the neurons across all medial PFC subregions recorded. We further found that responses in PFC were delayed by ~ 10 ms from the onset of activation in AC, demonstrating that auditory information is represented in AC before being relayed to PFC through cortical or subcortical connections. These findings are similar to a recent study showing that another frontal region, orbitofrontal cortex, is also responsive to auditory input under passive presentation (Winkowski et al., 2018). The number of PFC units (~ 12%) responsive to the white noise auditory stimuli in the passive condition in PFC is significantly less than what has been reported for prefrontal regions in aversive tasks such as fear learning where (25–75%) prefrontal neurons showed high sensitivity for the auditory conditioned stimulus following fear conditioning (Baeg et al., 2001; Burgos-Robles et al., 2009). Frontal cortex neurons can also be gated by task performance, becoming more sensitive to the same auditory stimuli when animals are introduced to the training context (Fritz et al., 2010). Our findings add to these bodies of work, by finding that a subset of prefrontal neurons track auditory stimuli during passive presentation in addition to being recruited, or gated, by cognitive tasks. The number of responsive neurons were also strikingly similar to the number of cells shown to be responsive for non-specific auditory activity in non-human primates where approximately 11% of single units showed broadband sensitivity to auditory input (Romanski et al., 2005).

We do not know the pathway by which auditory information is conveyed to PFC in our paradigm, however, connections between auditory association areas and prefrontal regions have been shown in both primates (Barbas, 2007; Romanski et al., 1999; Romanski and Goldman-Rakic, 2002) and rodents (Budinger et al., 2008; Martin-Cortecero and Nunez, 2016). We cannot rule out the possibility that auditory input is conveyed through subcortical structures (Hoover and Vertes, 2007), although the strong effect of local scopolamine infusion into AC reduced ERP responses in PFC by ~70%, suggesting a primary auditory origin. This is consistent, with a recent study in anesthetized animals, where small local infusion of lidocaine into A1 effectively blocked PFC responses to auditory input (Martin-Cortecero and Nunez, 2016). In addition, that study found a delay of 8–18 ms between AC and PFC, and is consistent with our findings of ~ 10.5 s in this study and suggests the pathways active during anesthesia are likely the same pathways carrying sound information in awake animals under passive presentation. While it is possible that a 70 dB SPL stimulus could be aversive and sound information could be conveyed via limbic or fear circuits, in fear conditioning studies where latency was examined, the robust increase in responsivity of prefrontal neurons for the auditory stimulus occurs ~ 100 ms after sound onset (Burgos-

Robles et al.,2009). Presumably, if auditory information was conveyed via this circuitry, it might be expected to have a delayed presence in PFC relative to our findings.

On a related note, although we cannot directly assess the behavioral state of the animal in these studies, cortical desynchrony was robustly elevated by optogenetic stimulation of the NB, suggesting higher states of cortical desynchrony were achievable from the baseline condition. Interestingly, despite evidence that states of high cortical desynchrony can enhance processing in sensory cortices (Goard and Dan, 2009; Kalmbach and Waters, 2014; Metherate and Ashe, 1993; Pinto et al.,2013), we found no significant effect of cortical desynchrony levels on the magnitude of the PFC sound evoked response in our animals (see supplemental results and Figure S6).

With regards to information processing in AC, we found significant effects of cholinergic antagonism on local processing. Since auditory stimuli are first relayed to AC and sound processing depends on interactions between thalamocortical inputs and cortico-cortical connections that are reflected in the laminar response profile (Happel et al., 2010), we also examined the effects of cholinergic antagonisms on CSDs in AC. Our study revealed that nAChR antagonism solely influenced the timing of the onset response to thalamic input into AC, delaying the sound evoked CSD response on the recording sites nearest the drug infusion. This demonstrates that we delivered a regionally localized effective dose and it produced an effect consistent with other local infusion mecamlamine studies in AC (Liang et al., 2006). Our finding that mecamlamine alters response latency provides evidence for an ongoing role of nicotinic signaling in sensory processing under basal conditions consistent with previous findings (Metherate, 2004). Based on previous studies (Intskirveli and Metherate, 2012; Kawai et al., 2007), we expected that mecamlamine may attenuate the auditory evoked ERP at short time scales but we found no evidence of this during passive processing of sound. It is likely that the influence of nicotinic activation on sound processing is altered by attentional demand and the strength of cholinergic tone. For example, acetylcholine release can improve sensory encoding by enhancing responses to relevant stimuli during sensory discrimination (Liang et al., 2008). It is interesting that in the Liang et al. study, the nAChR agonist nicotine lowered response thresholds for characteristic frequency stimuli and increased ERP amplitude only in rats performing at high levels in a behavioral task. In intermediate and poorly performing animals, nicotine only influenced the onset latency. Our results in naïve non-performing animals were similar; the only observable effect of mecamlamine was to delay onset latency. These findings further provide evidence that basal levels of acetylcholine contribute to signal processing in passive sound processing, although the strength of regulation is most likely dynamic and heavily influenced by cognitive state.

In contrast, mAChR antagonism strongly influenced intracortical signaling within AC. The mAChR antagonist scopolamine had a strong attenuating effect on the magnitude of the late phase CSD response, and the amplitude of the early phase response, but not on the thalamocortical sink onset latency at doses where latency could be measured. This suggests that mAChRs play a prominent role in modulating AC responses that are modulated through cortical interactions. We found that the reduction in net laminar current was coincident with sustained periods of evoked MUA on shanks distal to infusion, as well as a delayed sink-

source transition following sound onset. The mechanism by which mAChRs produce this effect is still unknown, but could involve contributions of enhanced excitability of pyramidal networks or reduced inhibition of interneurons. One possible explanation is that input originating from adjacent auditory frequency response areas that would be inhibited, is attenuated in the presence of scopolamine, consistent with a mechanism where reduced lateral inhibition alters the shape of the time-course of sensory responses in AC (Wehr and Zador, 2003; Wu et al., 2008). This is consistent with the more general idea that broadly tuned cortical interneurons enhance the contrast of the auditory representations through horizontal interactions (Li et al., 2014). In our study, it is possible that scopolamine alters efferent inhibitory signaling from the region of the infusion and that the remaining inhibitory current takes longer to balance out excitatory drive from the thalamus, leading to a longer duration current sink.

While we did not test this mechanism in this study, cortical frequency tuning is thought to depend on lateral inhibition, which enhances the contrast of frequency representation in AC. This process is critically dependent on the activity of L2/3 PV and SOM interneurons (Kato et al., 2017; Li et al., 2014), which can be strongly regulated by acetylcholine (Chen et al., 2015). Lateral inhibition has been shown to be modulated by attention in humans (Engell et al., 2016), suggesting cholinergic signaling could play a pivotal role in shaping sensory response profiles across the AC tonotopy.

Our finding that scopolamine infusion into AC strongly reduced responsivity in PFC also suggests that mAChR activation contributes to circuit dynamics that are permissive to cortico-cortical gating and the elevation of sensory information into prefrontal networks. One potential mechanism by which acetylcholine could influence the strength of information flow, is by effectively lowering the threshold for excitation and promoting feedforward excitation by inactivating m-currents in layer II/III low threshold spiking interneurons. These neurons have been proposed to gate intracortical signaling by inhibiting fast spiking interneurons (Chen et al., 2015; Lee et al., 2015). This interpretation is supported in part by data showing a longer delay to the sink-source transition and prolonged time-course of MU responses. A similar phenomenon has been reported in humans where the presence of scopolamine or other M1 antagonists, delay key components of the auditory evoked potential. For example, Kilkenberg et al. found a delay at the N100 and P200 latencies in the EEGs from healthy adults (2013), while Pekkonen et al., discovered delays in earlier pre-attentive windows including the P50 and N100 responses in elderly subjects (2005). While the mechanism by which this delay occurs is unknown in humans, our data suggests muscarinic blockade reduces the recurrent interactions at superficial layers of AC that attenuate the late phase source magnitude (Figure S8B). This reduction in superficial current magnitude combined with a reduction in MU responses specifically in these layers supports a model where auditory output is supported by superficial layer efferents that ultimately contribute to the strength of the signal that reaches the PFC.

Previous studies have demonstrated that during high attentive states, regions of the auditory cortical tonotopy interact intracortically to enhance attended, and suppress unattended auditory stimuli (O'Connell et al., 2014). Interestingly, our results reveal that mAChRs play a prominent role in intracortical signaling within AC and long range cortico-cortical

signaling between AC and PFC during passive sound presentation, which is complementary to those functions previously reported during attention tasks. Our findings suggest acetylcholine signaling enhances sensory processing indiscriminately, allowing bottom-up inputs to be refined and relayed to higher cortical areas for additional processing. This finding is consistent with a model in which PFC monitors stimuli that may later become important (i.e., behaviorally relevant cues used to guide behavior). Taken together, our data provide direct evidence that cholinergic mechanisms play a role in ascending auditory processing and are complementary to the roles ascribed for acetylcholine in facilitating top-down mediated attentional performance (Guillem et al., 2011; Hasselmo and Sarter, 2011; Sarter et al., 2005a).

This previously unidentified requirement for acetylcholine in regulating long-range cortical signaling during passive sound processing also expands canonical views of the role of prefrontal cortex in sensory processing and cholinergic function. Our findings suggest that the contributions of cholinergic signaling particularly through muscarinic receptors may play a prominent role in stimulus detection under normal conditions. Given that recent evidence from PET imaging reveals a reduction in muscarinic receptors in otherwise healthy and un-medicated schizophrenia patients (Raedler et al., 2003), it is interesting to consider how reductions in cortical communication through M1 receptors may contribute to dysfunctional conditions such as schizophrenia. It has previously been posited that a key feature of this condition could involve both reductions in cholinergic muscarinic receptors combined with aberrant cortical cholinergic activity (Sarter et al., 2005b). Taken together, this suggests that treatment interventions to increase the presence of cholinergic signaling through M1 receptors may offer some benefit towards alleviating the cognitive symptoms of schizophrenia (Barak, 2009). Acetylcholine may promote long-range network communication by enhancing intrinsic oscillatory patterns during stimulus processing (Roopun et al., 2010). In this regard, cortical projecting cholinergic systems may contribute to signal processing by gating information flow between interconnected cortical regions, in addition to strengthening thalamo-cortical signaling. It is interesting that scopolamine blocked the reduction in low frequency power (1–10 Hz) during optogenetically evoked cortical desynchrony without altering high frequency power (10–100 Hz). Combined with the fact that scopolamine also reduced intracortical signaling, it is possible that a role of muscarinic activity is to reduce low frequency power during periods where intracortical signaling is prioritized.

It is not known how mAChRs contribute to intracortical signaling or low frequency desynchronization during cholinergic release. More work will be necessary to determine if these two phenomena might be related, but cholinergic signaling of interneuron sub-types potentially contribute to both processes. For example, parvalbumin positive interneurons have been shown to contribute to feedforward inhibition within cortical circuits that mediate lateral suppression during auditory stimulation (Li et al., 2014), and a recent study suggested that superficial somatostatin positive interneurons may play an active role in cholinergic modulation of cortical desynchrony and neuronal decorrelation associated by shifting intracortical interactions (Chen et al., 2015). Future experiments will be necessary to determine how this process is accomplished and how distinct cholinergic receptor activity

contributes to signal processing and cortico-cortical communication differently depending on the demands on attention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Ashe JH, McKenna TM, Weinberger NM, 1989 Cholinergic modulation of frequency receptive fields in auditory cortex: II. Frequency-specific effects of anticholinesterases provide evidence for a modulatory action of endogenous ACh. *Synapse* 4, 44–54. [PubMed: 2772838]
- Baeg EH, Kim YB, Jang J, Kim HT, Mook-Jung I, Jung MW, 2001 Fast spiking and regular spiking neural correlates of fear conditioning in the medial prefrontal cortex of the rat. *Cerebr. Cortex* 11, 441–451.
- Bandrowski AE, Moore SL, Ashe JH, 2001 Cholinergic synaptic potentials in the supragranular layers of auditory cortex. *Synapse* 41, 118–130. [PubMed: 11400178]
- Barak S, 2009 Modeling cholinergic aspects of schizophrenia: focus on the anti-muscarinic syndrome. *Behav. Brain Res.* 204, 335–351. [PubMed: 19376161]
- Barbas H, 2007 Specialized elements of orbitofrontal cortex in primates. *Ann. N. Y. Acad. Sci.* 1121, 10–32. [PubMed: 17698996]
- Boix-Trelis N, Vale-Martinez A, Guillazo-Blanch G, Marti-Nicolovius M, 2007 Muscarinic cholinergic receptor blockade in the rat prelimbic cortex impairs the social transmission of food preference. *Neurobiol. Learn. Mem.* 87, 659–668. [PubMed: 17223581]
- Bragin A, Hetke J, Wilson CL, Anderson DJ, Engel J, Jr., Buzsaki G, 2000 Multiple site silicon-based probes for chronic recordings in freely moving rats: implantation, recording and histological verification. *J. Neurosci. Methods* 98, 77–82. [PubMed: 10837874]
- Budinger E, Laszcz A, Lison H, Scheich H, Ohl FW, 2008 Non-sensory cortical and subcortical connections of the primary auditory cortex in Mongolian gerbils: bottom-up and top-down processing of neuronal information via field AI. *Brain Res.* 1220, 2–32. [PubMed: 17964556]
- Burgos-Robles A, Vidal-Gonzalez I, Quirk GJ, 2009 Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. *J. Neurosci.* 29, 8474–8482. [PubMed: 19571138]
- Buschman TJ, Miller EK, 2007 Top-down versus bottom-up control of attention in the prefrontal and posterior parietal cortices. *Science* 315, 1860–1862. [PubMed: 17395832]
- Chang CH, Berke JD, Maren S, 2010 Single-unit activity in the medial prefrontal cortex during immediate and delayed extinction of fear in rats. *PloS One* 5, e11971. [PubMed: 20700483]
- Chavez C, Zaborszky L, 2017 Basal forebrain cholinergic-auditory cortical network: primary versus nonprimary auditory cortical areas. *Cerebr. Cortex* 27, 2335–2347.
- Chen N, Sugihara H, Sur M, 2015 An acetylcholine-activated microcircuit drives temporal dynamics of cortical activity. *Nat. Neurosci.* 18, 892–902. [PubMed: 25915477]

- Chu ZG, Zhou FM, Hablitz JJ, 2000 Nicotinic acetylcholine receptor-mediated synaptic potentials in rat neocortex. *Brain Res.* 887, 399–405. [PubMed: 11134630]
- Dalley JW, Cardinal RN, Robbins TW, 2004 Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci. Biobehav. Rev.* 28, 771–784. [PubMed: 15555683]
- Deckmann K, Filipowski K, Krasteva-Christ G, Fronius M, Althaus M, Rafiq A, Papadakis T, Renno L, Jurastow I, Wessels L, Wolff M, Schutz B, Weihe E, Chubakov V, Gudermann T, Klein J, Bschleipfer T, Kummer W, 2014 Bitter triggers acetylcholine release from polymodal urethral chemosensory cells and bladder reflexes. *Proc. Natl. Acad. Sci. U. S. A.* 111, 8287–8292. [PubMed: 24843119]
- Disney AA, Aoki C, 2008 Muscarinic acetylcholine receptors in macaque V1 are most frequently expressed by parvalbumin-immunoreactive neurons. *J. Comp. Neurol.* 507, 1748–1762. [PubMed: 18265004]
- Engell A, Junghofer M, Stein A, Lau P, Wunderlich R, Wollbrink A, Pantev C, 2016 Modulatory effects of attention on lateral inhibition in the human auditory cortex. *PLoS One* 11, e0149933. [PubMed: 26901149]
- Euston DR, Gruber AJ, McNaughton BL, 2012 The role of medial prefrontal cortex in memory and decision making. *Neuron* 76, 1057–1070. [PubMed: 23259943]
- Evans EF, Whitfield IC, 1964 Classification of unit responses in the auditory cortex of the unanaesthetized and unrestrained cat. *J. Physiol.* 171, 476–493. [PubMed: 14193935]
- Fries P, Reynolds JH, Rorie AE, Desimone R, 2001 Modulation of oscillatory neuronal synchronization by selective visual attention. *Science* 291, 1560–1563. [PubMed: 11222864]
- Fritz JB, David SV, Radtke-Schuller S, Yin P, Shamma SA, 2010 Adaptive, behaviorally gated, persistent encoding of task-relevant auditory information in ferret frontal cortex. *Nat. Neurosci.* 13, 1011–1019. [PubMed: 20622871]
- Fritz JB, Elhilali M, Shamma SA, 2007 Adaptive changes in cortical receptive fields induced by attention to complex sounds. *J. Neurophysiol.* 98, 2337–2346. [PubMed: 17699691]
- Fromme RC, Merzenich MM, Schreiner CE, 2007 A synaptic memory trace for cortical receptive field plasticity. *Nature* 450, 425–429. [PubMed: 18004384]
- Gil Z, Connors BW, Amitai Y, 1997 Differential regulation of neocortical synapses by neuromodulators and activity. *Neuron* 19, 679–686. [PubMed: 9331357]
- Goard M, Dan Y, 2009 Basal forebrain activation enhances cortical coding of natural scenes. *Nat. Neurosci.* 12, 1444–1449. [PubMed: 19801988]
- Guillemin K, Bloem B, Poorthuis RB, Loos M, Smit AB, Maskos U, Spijker S, Mansvelder HD, 2011 Nicotinic acetylcholine receptor $\beta 2$ subunits in the medial prefrontal cortex control attention. *Science* 333, 888–891. [PubMed: 21836018]
- Guo W, Chambers AR, Darrow KN, Hancock KE, Shinn-Cunningham BG, Polley DB, 2012 Robustness of cortical topography across fields, laminae, anesthetic states, and neurophysiological signal types. *J. Neurosci.* 32, 9159–9172. [PubMed: 22764225]
- Happel MF, Jeschke M, Ohl FW, 2010 Spectral integration in primary auditory cortex attributable to temporally precise convergence of thalamocortical and intracortical input. *J. Neurosci.* 30, 11114–11127. [PubMed: 20720119]
- Hasselmo ME, Sarter M, 2011 Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology* 36, 52–73. [PubMed: 20668433]
- Herrero JL, Roberts MJ, Delicato LS, Gieselmann MA, Dayan P, Thiele A, 2008 Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature* 454, 1110–1114. [PubMed: 18633352]
- Hoover WB, Vertes RP, 2007 Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Struct. Funct.* 212, 149–179. [PubMed: 17717690]
- Hromádka T, Deweese MR, Zador AM, 2008 Sparse representation of sounds in the unanesthetized auditory cortex. *PLoS Biol.* 6, e16. [PubMed: 18232737]
- Hyman JM, Ma L, Balaguer-Ballester E, Durstewitz D, Seamans JK, 2012 Contextual encoding by ensembles of medial prefrontal cortex neurons. *Proc. Natl. Acad. Sci. U. S. A.* 109, 5086–5091. [PubMed: 22421138]

- Ingles JL, Beninger RJ, Jhamandas K, Boegman RJ, 1993 Scopolamine injected into the rat amygdala impairs working memory in the double Y-maze. *Brain Res. Bull.* 32, 339–344. [PubMed: 8221123]
- Intskirveli I, Metherate R, 2012 Nicotinic neuromodulation in auditory cortex requires MAPK activation in thalamocortical and intracortical circuits. *J. Neurophysiol.* 107, 2782–2793. [PubMed: 22357798]
- Joachimsthaler B, Uhlmann M, Miller F, Ehret G, Kurt S, 2014 Quantitative analysis of neuronal response properties in primary and higher-order auditory cortical fields of awake house mice (*Mus musculus*). *Eur. J. Neurosci.* 39, 904–918. [PubMed: 24506843]
- Kalmbach A, Hedrick T, Waters J, 2012 Selective optogenetic stimulation of cholinergic axons in neocortex. *J. Neurophysiol.* 107, 2008–2019. [PubMed: 22236708]
- Kalmbach A, Waters J, 2014 Modulation of high-and low-frequency components of the cortical local field potential via nicotinic and muscarinic acetylcholine receptors in anesthetized mice. *J. Neurophysiol.* 111, 258–272. [PubMed: 24155009]
- Kato HK, Asinof SK, Isaacson JS, 2017 Network-level control of frequency tuning in auditory cortex. *Neuron* 95, 412–423 e414. [PubMed: 28689982]
- Kawai H, Lazar R, Metherate R, 2007 Nicotinic control of axon excitability regulates thalamocortical transmission. *Nat. Neurosci.* 10, 1168–1175. [PubMed: 17704774]
- Kilgard MP, Merzenich MM, 1998a Cortical map reorganization enabled by nucleus basalis activity. *Science* 279, 1714–1718. [PubMed: 9497289]
- Kilgard MP, Merzenich MM, 1998b Plasticity of temporal information processing in the primary auditory cortex. *Nat. Neurosci.* 1, 727–731. [PubMed: 10196590]
- Kilgard MP, Merzenich MM, 2002 Order-sensitive plasticity in adult primary auditory cortex. *Proc. Natl. Acad. Sci. U. S. A.* 99, 3205–3209. [PubMed: 11880653]
- Klinkenberg I, Blokland A, Riedel WJ, Sambeth A, 2013 Cholinergic modulation of auditory processing, sensory gating and novelty detection in human participants. *Psychopharmacology* 225, 903–921. [PubMed: 23052568]
- Kruglikov I, Rudy B, 2008 Perisomatic GABA release and thalamocortical integration onto neocortical excitatory cells are regulated by neuromodulators. *Neuron* 58, 911–924. [PubMed: 18579081]
- Lambe EK, Picciotto MR, Aghajanian GK, 2003 Nicotine induces glutamate release from thalamocortical terminals in prefrontal cortex. *Neuropsychopharmacology* 28, 216–225. [PubMed: 12589374]
- Lee JH, Whittington MA, Kopell NJ, 2015 Potential mechanisms underlying intercortical signal regulation via cholinergic neuromodulators. *J. Neurosci.* 35, 15000–15014. [PubMed: 26558772]
- Lee SH, Kwan AC, Zhang S, Phoumthippavong V, Flannery JG, Masmanidis SC, Taniguchi H, Huang ZJ, Zhang F, Boyden ES, Deisseroth K, Dan Y, 2012 Activation of specific interneurons improves V1 feature selectivity and visual perception. *Nature* 488, 379–383. [PubMed: 22878719]
- Letzkus JJ, Wolff SB, Meyer EM, Tovote P, Courtin J, Herry C, Luthi A, 2011 A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature* 480, 331–335. [PubMed: 22158104]
- Levy RB, Aoki C, 2002 Alpha7 nicotinic acetylcholine receptors occur at postsynaptic densities of AMPA receptor-positive and -negative excitatory synapses in rat sensory cortex. *J. Neurosci.* 22, 5001–5015. [PubMed: 12077196]
- Li LY, Ji XY, Liang F, Li YT, Xiao Z, Tao HW, Zhang LI, 2014 A feedforward inhibitory circuit mediates lateral refinement of sensory representation in upper layer 2/3 of mouse primary auditory cortex. *J. Neurosci.* 34, 13670–13683. [PubMed: 25297094]
- Liang K, Poytress BS, Chen Y, Leslie FM, Weinberger NM, Metherate R, 2006 Neonatal nicotine exposure impairs nicotinic enhancement of central auditory processing and auditory learning in adult rats. *Eur. J. Neurosci.* 24, 857–866. [PubMed: 16848798]
- Liang K, Poytress BS, Weinberger NM, Metherate R, 2008 Nicotinic modulation of tone-evoked responses in auditory cortex reflects the strength of prior auditory learning. *Neurobiol. Learn. Mem.* 90, 138–146. [PubMed: 18378471]
- Maren S, Quirk GJ, 2004 Neuronal signalling of fear memory. *Nat. Rev. Neurosci.* 5, 844–852. [PubMed: 15496862]

- Martin-Cortecero J, Nunez A, 2016 Sensory responses in the medial prefrontal cortex of anesthetized rats. Implications for sensory processing. *Neuroscience* 339, 109–123. [PubMed: 27702646]
- Maruki K, Izaki Y, Akema T, Nomura M, 2003 Effects of acetylcholine antagonist injection into the prefrontal cortex on the progress of lever-press extinction in rats. *Neurosci. Lett.* 351, 95–98. [PubMed: 14583390]
- McCarthy MM, Moore-Kochlacs C, Gu X, Boyden ES, Han X, Kopell N, 2011 Striatal origin of the pathologic beta oscillations in Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* 108, 11620–11625. [PubMed: 21697509]
- McKenna TM, Ashe JH, Hui GK, Weinberger NM, 1988 Muscarinic agonists modulate spontaneous and evoked unit discharge in auditory cortex of cat. *Synapse* 2, 54–68. [PubMed: 3420531]
- McKenna TM, Ashe JH, Weinberger NM, 1989 Cholinergic modulation of frequency receptive fields in auditory cortex: I. Frequency-specific effects of muscarinic agonists. *Synapse* 4, 30–43. [PubMed: 2672402]
- Metherate R, 2004 Nicotinic acetylcholine receptors in sensory cortex. *Learn. Mem.* 11, 50–59. [PubMed: 14747517]
- Metherate R, Ashe JH, 1993 Nucleus basalis stimulation facilitates thalamocortical synaptic transmission in the rat auditory cortex. *Synapse* 14, 132–143. [PubMed: 8392756]
- Metherate R, Ashe JH, Weinberger NM, 1990 Acetylcholine modifies neuronal acoustic rate-level functions in Guinea pig auditory cortex by an action at muscarinic receptors. *Synapse* 6, 364–368. [PubMed: 2287993]
- Metherate R, Cox CL, Ashe JH, 1992 Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J. Neurosci.* 12, 4701–4711. [PubMed: 1361197]
- Metherate R, Intskirveli I, Kawai HD, 2012 Nicotinic filtering of sensory processing in auditory cortex. *Front. Behav. Neurosci.* 6, 44. [PubMed: 22833720]
- Metherate R, Weinberger NM, 1989 Acetylcholine produces stimulus-specific receptive field alterations in cat auditory cortex. *Brain Res.* 480, 372–377. [PubMed: 2713663]
- Metherate R, Weinberger NM, 1990 Cholinergic modulation of responses to single tones produces tone-specific receptive field alterations in cat auditory cortex. *Synapse* 6, 133–145. [PubMed: 2237776]
- Miller EK, Cohen JD, 2001 An integrative theory of prefrontal cortex function. *Annu. Rev. Neurosci.* 24, 167–202. [PubMed: 11283309]
- Mitzdorf U, 1985 Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. *Physiol. Rev.* 65, 37–100. [PubMed: 3880898]
- Moorman DE, Aston-Jones G, 2015 Prefrontal neurons encode context-based response execution and inhibition in reward seeking and extinction. *Proc. Natl. Acad. Sci. U. S. A.* 112, 9472–9477. [PubMed: 26170333]
- Nicholson C, Freeman JA, 1975 Theory of current source-density analysis and determination of conductivity tensor for anuran cerebellum. *J. Neurophysiol.* 38, 356–368. [PubMed: 805215]
- Nunez A, Dominguez S, Buno W, Fernandez de Sevilla D, 2012 Cholinergic-mediated response enhancement in barrel cortex layer V pyramidal neurons. *J. Neurophysiol.* 108, 1656–1668. [PubMed: 22723675]
- O'Connell MN, Barczak A, Schroeder CE, Lakatos P, 2014 Layer specific sharpening of frequency tuning by selective attention in primary auditory cortex. *J. Neurosci.* 34, 16496–16508. [PubMed: 25471586]
- Pekkonen E, Jaaskelainen IP, Kaakkola S, Ahveninen J, 2005 Cholinergic modulation of preattentive auditory processing in aging. *Neuroimage* 27, 387–392. [PubMed: 15921933]
- Pinto L, Goard MJ, Estandian D, Xu M, Kwan AC, Lee SH, Harrison TC, Feng G, Dan Y, 2013 Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat. Neurosci.* 16, 1857–1863. [PubMed: 24162654]
- Pirch JH, Corbus MJ, Ebenezer I, 1985a Conditioned cortical slow potential responses in urethane anesthetized rats. *Int. J. Neurosci.* 25, 207–218. [PubMed: 3872286]
- Pirch JH, Corbus MJ, Rigdon GC, 1985b Conditioning-related single unit activity in the frontal cortex of urethane anesthetized rats. *Int. J. Neurosci.* 25, 263–271. [PubMed: 3872287]

- Polley DB, Steinberg EE, Merzenich MM, 2006 Perceptual learning directs auditory cortical map reorganization through top-down influences. *J. Neurosci.* 26, 4970–4982. [PubMed: 16672673]
- Raedler TJ, Knable MB, Jones DW, Urbina RA, Gorey JG, Lee KS, Egan MF, Coppola R, Weinberger DR, 2003 In vivo determination of muscarinic acetylcholine receptor availability in schizophrenia. *Am. J. Psychiatry* 160, 118–127. [PubMed: 12505810]
- Roerig B, Nelson DA, Katz LC, 1997 Fast synaptic signaling by nicotinic acetylcholine and serotonin 5-HT₃ receptors in developing visual cortex. *J. Neurosci.* 17, 8353–8362. [PubMed: 9334409]
- Rogers JL, Kesner RP, 2004 Cholinergic modulation of the hippocampus during encoding and retrieval of tone/shock-induced fear conditioning. *Learn. Mem.* 11, 102–107. [PubMed: 14747523]
- Romanski LM, Averbach BB, Diltz M, 2005 Neural representation of vocalizations in the primate ventrolateral prefrontal cortex. *J. Neurophysiol.* 93, 734–747. [PubMed: 15371495]
- Romanski LM, Bates JF, Goldman-Rakic PS, 1999 Auditory belt and parabelt projections to the prefrontal cortex in the rhesus monkey. *J. Comp. Neurol.* 403, 141–157. [PubMed: 9886040]
- Romanski LM, Goldman-Rakic PS, 2002 An auditory domain in primate prefrontal cortex. *Nat. Neurosci.* 5, 15–16. [PubMed: 11753413]
- Roopun AK, Lebeau FE, Rammell J, Cunningham MO, Traub RD, Whittington MA, 2010 Cholinergic neuromodulation controls directed temporal communication in neocortex in vitro. *Front. Neural Circ.* 4, 8.
- Roy JE, Buschman TJ, Miller EK, 2014 PFC neurons reflect categorical decisions about ambiguous stimuli. *J. Cognit. Neurosci.* 26, 1283–1291. [PubMed: 24405188]
- Rutkowski RG, Weinberger NM, 2005 Encoding of learned importance of sound by magnitude of representational area in primary auditory cortex. *Proc. Natl. Acad. Sci. U. S. A.* 102, 13664–13669. [PubMed: 16174754]
- Sakata S, Harris KD, 2009 Laminar structure of spontaneous and sensory-evoked population activity in auditory cortex. *Neuron* 64, 404–418. [PubMed: 19914188]
- Santucci AC, Shaw C, 2003 Peripheral 8-OH-DPAT and scopolamine infused into the frontal cortex produce passive avoidance retention impairments in rats. *Neurobiol. Learn. Mem.* 79, 136–141. [PubMed: 12591222]
- Sarter M, Hasselmo ME, Bruno JP, Givens B, 2005a. Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Brain Res Rev* 48, 98–111. [PubMed: 15708630]
- Sarter M, Nelson CL, Bruno JP, 2005b Cortical cholinergic transmission and cortical information processing in schizophrenia. *Schizophr. Bull.* 31, 117–138. [PubMed: 15888431]
- Siegel M, Donner TH, Engel AK, 2012 Spectral fingerprints of large-scale neuronal interactions. *Nat. Rev. Neurosci.* 13, 121–134. [PubMed: 22233726]
- Steinschneider M, Fishman YI, Arezzo JC, 2008 Spectrotemporal analysis of evoked and induced electroencephalographic responses in primary auditory cortex (A1) of the awake monkey. *Cerebr. Cortex* 18, 610–625.
- Stiebler I, Neulist R, Fichtel I, Ehret G, 1997 The auditory cortex of the house mouse: left-right differences, tonotopic organization and quantitative analysis of frequency representation. *J. Comp. Physiol.* 181, 559–571. [PubMed: 9449817]
- Szymanski FD, Garcia-Lazaro JA, Schnupp JW, 2009 Current source density profiles of stimulus-specific adaptation in rat auditory cortex. *J. Neurophysiol.* 102, 1483–1490. [PubMed: 19571199]
- Szymanski FD, Rabinowitz NC, Magri C, Panzeri S, Schnupp JW, 2011 The laminar and temporal structure of stimulus information in the phase of field potentials of auditory cortex. *J. Neurosci.* 31, 15787–15801. [PubMed: 22049422]
- Wehr M, Zador AM, 2003 Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. *Nature* 426, 442–446. [PubMed: 14647382]
- Winkowski DE, Nagode DA, Donaldson KJ, Yin P, Shamma SA, Fritz JB, Kanold PO, 2018 3 1 Orbitofrontal cortex neurons respond to sound and activate primary auditory cortex neurons. *Cerebr. Cortex* 28 (3), 868–879.
- Wu GK, Arbuckle R, Liu BH, Tao HW, Zhang LI, 2008 Lateral sharpening of cortical frequency tuning by approximately balanced inhibition. *Neuron* 58, 132–143. [PubMed: 18400169]

- Zaborszky L, van den Pol A, Gyengesi E, 2012 The basal forebrain cholinergic projection system in mice. *Mouse Nervous System* 684–718.
- Zhou X, Qi XL, Douglas K, Palaninathan K, Kang HS, Buccafusco JJ, Blake DT, Constantinidis C, 2011 Cholinergic modulation of working memory activity in primate prefrontal cortex. *J. Neurophysiol.* 106, 2180–2188. [PubMed: 21795623]

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HIGHLIGHTS

- Prefrontal cortex actively monitors non-associative stimuli under passive conditions.
- Acetylcholine facilitates cortical signaling even outside of attentional contexts.
- Local scopolamine infusion reduced intracortical signaling and impaired cortical communication.
- MACHR have an ongoing role in sound processing.

Fig. 1. Experimental paradigm.

A: Head fixed mouse preparation with electrode and optical fiber placements during recording. One 16-channel electrode was positioned in PFC, an infusion pipette positioned between the two inner shanks of a 32 channel, 4 shank recording electrode in AC, and a shielded optical fiber in nucleus basalis (NB). **B:** Anatomical location of recording electrodes and infusion pipette represented in a frontal (PFC) and horizontal section (AC). PFC single shank penetration occurred along the dorsal-ventral axis with sites facing medially. For the AC array, insertion was at a 30° angle with all recording sites facing medially while the shanks and infuser were situated along the rostral-caudal axis. **C (i):** Cartoon depiction of electrode and fiber placement for histology sections shown to the right.

(ii): Section from AC showing electrode placement for this recording area. (iii): Histology image showing ChR2-GFP expression in tissue co-labeled with antibodies directed against choline acetyl transferase (Chat). Note, ChR2-GFP expression is limited to cells also expressing Chat (red). (iv): Nissl stain showing optical fiber track and placement in NB. (v): Section from frontal cortex demonstrating electrode placement from a representative animal. **D:** During the preinfusion recording period (block 1), mice were presented with 100 trials of optical stimulation alone and 100 trials of auditory stimulation alone, with the order of trials shuffled throughout the block. A second recording session was performed 30min after drug infusion (block 2). **Abbreviations:** Cg; Cingulate Cortex, Prl; Prelimbic cortex, IL; Infralimbic Cortex, LSI; CPu; Caudate Putamen, MO; Medial Orbital Cortex, LO; Lateral Orbital Cortex, S1; Primary Somatosensory Cortex, LV; Lateral Ventricle, AuD; Secondary Auditory Cortex Dorsal, A1; Primary Auditory Cortex, AC; Auditory Cortex, PFC; Prefrontal Cortex, nBM; Nucleus Basalis of Meynert, B; Basal Nucleus of Meynert, 3V; Third Ventricle; R; Rostral, C; Caudal, M; Medial, L; Lateral).

Fig. 2. Auditory stimuli evoked strong MUA in PFC and AC during passive sound presentation in mice.

A: Example MU from PFC that shows an increase (left) or a decrease (right) in firing rate during the sound stimulus. **B:** Example MU from AC that shows an increase (left) or a decrease (right) in firing rate during the sound stimulus. **C, D:** Mean population MUA in PFC (**C**) and AC (**D**) during the sound presentation (left) and the proportion of responsive MUs for each region (right). **E:** Mean normalized PFC MUA from all animals across all depth locations from the pre-infusion condition. Note that all regions show an increase in

firing rate with prelimbic (PrL) exhibiting the largest increase in firing rate (* = $p < 0.05$). **F:** Mean population MUA from granular and extragranular layers in AC and mean population MUA from the PFC from the pre-infusion period. (Cg; Cingulate Cortex, PrL; Prelimbic cortex, IL; Infralimbic Cortex, MO; Medial Orbital Cortex).

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Fig. 3. Auditory input produced robust ERPs in PFC and AC during passive sound presentation in mice.

A: Representative ERP response to auditory stimuli across all channels in the PFC (i) and AC (ii). **B:** Population ERP from PFC (i) and AC (ii), in response to auditory stimuli. **C:** ERP onset latency along the dorsal-ventral axis of PFC (i) and along the rostro-caudal axis of AC (ii). (solid lines are mean, and shaded areas are mean $\pm$ s.e.m. **D:** Histogram of ERP onset latencies from PFC (i) and the granular layers of AC (ii).

Fig. 4. Effects of cholinergic antagonism on LFP power and optogenetically induced cortical desynchrony in AC and PFC.

A: Diagram illustrating recording/infusion configuration in AC in a horizontal section. Compass denotes rostral-caudal and medial-lateral axis. Inner shanks are highlighted yellow and outer shanks are highlighted green. Pipette location is noted in blue. **B:** Population spectrum of LFP power on inner shanks in AC before (black) and after (red) infusion of high dose scopolamine during the inter-trial interval in the absence of stimuli or optogenetic activation of cholinergic NB (left). Bar height represents population mean $\pm$ s.e.m

normalized to pre-infusion baseline across all mice recorded (right) and compares ACSF (blue) to scopolamine (red). Outer sites are darkly shaded relative to inner sites (* = $p < 0.05$). **C:** Cortical desynchrony produced by optogenetic activation of cholinergic NB. Trial averaged, baseline normalized spectrograms from AC (left) or PFC (right) during optical stimulation. The ratio of low frequency power (1–10 Hz) to high frequency power (10–100 Hz) for AC and PFC is shown below (bottom). **D:** Population spectrogram of AC LFP during optogenetic activation of cholinergic NB prior to high dose scopolamine infusion (left), and following infusion (right). **E:** Change in AC LFP power by frequency range during optogenetic stimulation in the presence of antagonists or vehicle. Values are expressed as percentages of pre infusion power (mean $\pm$ s.e.m). Outer sites are darkly shaded relative to inner sites (* = $p < 0.05$).

Fig. 5. Effects of muscarinic blockade on CSD profiles in AC.

A: ERPs in AC overlaid on CSD calculated from laminar ERP in a representative animal. **B:** Population sound-evoked CSD response from the channel exhibiting the largest thalamocortical response during pre-infusion period (mean $\pm$ s.e.m.). Red dotted line represents 0 mV/mm. Zero crossings were used to define an early window (0–72 ms exhibiting granular layer current sinks) and a late window (72–186 ms exhibiting granular layer source). **C,D:** CSD responses across the four probes before (**C**) and after high-dose scopolamine infusion (**D**) separated by shank location. Bar graphs represent the calculated RMS across all channels of the CSD in the early and late windows as defined in (**B**). Diagram illustrates recording site being shown and placement relative to infusion location in AC. Inner shanks (2&3) are closest to infusion pipette while 1&4 are furthest from the infusion site. Inner shanks are also highlighted yellow and outer shanks are highlighted green. Shank 1 is the most rostral placement while shank 4 is the most caudal. **E:** CSD response from the channel with the largest thalamocortical response from both inner and outer shanks before (black) scopolamine infusion and after (red) high dose scopolamine infusion (mean $\pm$ s.e.m). **F:** Change in net current of the CSD following cholinergic

antagonist or ACSF infusions from the early (left) and late (right) windows. Bar height represents population mean, error bars are $\pm$ s.e.m. (* = $p < 0.05$).

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Fig. 6. Endogenous muscarinic signaling modulates the strength of MUA in AC during sound presentation.

A: Diagram illustrating recording/infusion configuration and location of electrodes shown on the right. Inner shanks are highlighted yellow and outer shanks are highlighted green. Granular channels are highlighted. Granular layer MU population responses from inner and outer shanks before (black) and after (red) high dose scopolamine infusion (right) differences relative to the pre-sound firing rate. **B:** Integrated MUA from the granular layers from the early and late windows identified from CSD analysis (early: 0–72 ms, late 72–180 ms). Bar graphs are differences relative to the pre infusion baseline and represent changes in firing rate following infusion of ACSF or scopolamine (* = $p < 0.05$). **C:** Diagram illustrating recording/infusion configuration and location of signals shown on the right. Extragranular channels are highlighted. MUA from extragranular channels on the inner (left) and outer (right) shanks before (black) or after (red) high dose scopolamine infusion. **D:** Same as B, for extragranular layers. Figures are shown as population mean $\pm$ s.e.m. (* = $p < 0.05$).

Fig. 7. Scopolamine infusion in AC reduced ERP amplitude and spiking activity in PFC.

A: Example sound evoked PFC ERP responses before (black) and after (blue) local infusion of ACSF (i) or high dose scopolamine (red) in AC (ii). Solid lines indicate mean, and shaded areas are mean $\pm$ s.e.m. **B:** ERP amplitude after infusion of different drugs normalized to pre-infusion amplitude. Error bars are $\pm$ s.e.m. **C:** Normalized MUA responses before (black) and after (red) infusion of ACSF (i) or high dose scopolamine (Cii). Lines are mean, and shaded areas are mean $\pm$ s.e.m. **D:** Comparison of the change in normalized MUA for all drug conditions. Bar graphs show mean $\pm$ s.e.m. (* = $p < 0.05$; ** = $p < 0.01$). **E:** Mean

population PFC MU response across channels from the pre-infusion (left), post-Infusion (middle) and pre-post difference (right). F: Reduction in normalized firing rate by PFC subregion. All regions showed significant reductions from baseline that did not differ from one another. (Cg; Cingulate Cortex, Prl; Prelimbic cortex, IL; Infralimbic Cortex, MO; Medial Orbital Cortex).

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