

Midbrain encodes sound detection behavior without auditory cortex

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Abstract

Hearing involves analyzing the physical attributes of sounds and integrating the results of this analysis with other sensory, cognitive and motor variables in order to guide adaptive behavior. The auditory cortex is considered crucial for the integration of acoustic and contextual information and is thought to share the resulting representations with subcortical auditory structures via its vast descending projections. By imaging cellular activity in the corticorecipient shell of the inferior colliculus of mice engaged in a sound detection task, we show that the majority of neurons encode information beyond the physical attributes of the stimulus and that the animals' behavior can be decoded from the activity of those neurons with a high degree of accuracy. Surprisingly, this was also the case in mice in which auditory cortical input to the midbrain had been removed by bilateral cortical lesions. This illustrates that subcortical auditory structures have access to a wealth of non-acoustic information and can, independently of the auditory cortex, carry much richer neural representations than previously thought.

eLife assessment

This study demonstrates that neurons receiving inputs from auditory cortex in the inferior colliculus widely encode the outcome of a sound detection task independent of the presence of auditory cortex. This **valuable** study based on imaging of transynaptically labelled neurons provides **convincing** evidence that auditory cortex is necessary neither for sound detection, nor to channel information related to behavioral outcome to the subcortical auditory system. This study will be of wide interest for sensory neuroscientists.

Introduction

Classically, perception is considered to rely on the flow of information from the sensory periphery via a sequence of hierarchically-organized brain structures up to the cortex. The ascending sensory pathways connecting these structures have been studied extensively and much has been learned about how signals are relayed, how features are extracted, and how information is integrated to produce increasingly abstract representations of the sensory environment. These pathways are paralleled by descending pathways that can feed information back to lower-order sensory structures. The fact that descending projections often outnumber their feedforward

counterparts (Sherman, 2007 [↗](#)) attests to their likely importance for brain function. This may include turning an otherwise passive, stimulus-driven device into an active and adaptive brain that is capable of processing sensory input within its behavioral context and, therefore, able to learn and create meaning (Engel et al., 2001 [↗](#); Kraus and White-Schwoch, 2015 [↗](#); Malmierca, Anderson and Antunes, 2015 [↗](#)).

The descending projections of the auditory cortex target all major subcortical stations of the auditory pathway and are among the largest pathways of the brain (Winer, 2006; Bajo and King, 2013 [↗](#); Antunes and Malmierca, 2021 [↗](#)), making them a particularly suitable system for investigating the behavioral and physiological consequences of corticofugal processing. One of their main targets is the inferior colliculus (IC), an obligatory midbrain relay for nearly all ascending auditory input. The corticocollicular projection primarily terminates in the non-lemniscal shell of the IC. The shell encapsulates and is extensively connected with the central nucleus of the IC, which forms part of the tonotopically organized core or lemniscal auditory pathway to the primary auditory cortex. The projection from the auditory cortex to the midbrain was identified almost a century ago (Mettler 1935 [↗](#)) and decades of research have since demonstrated that manipulating the activity of descending projection neurons can alter the collicular representations of multiple sound features, influence adaptive plasticity and perceptual learning, and even trigger an innate flight response (Suga 2008 [↗](#); Nakamoto et al 2008 [↗](#); Bajo et al 2010; Xion et al 2015; Blackwell et al 2020 [↗](#)). However, experimental evidence, especially from behaving animals, that could help explain what information the auditory midbrain and other subcortical sensory structures rely on their cortical input for is still very limited.

Interactions between different sensory pathways occur at multiple processing levels and they are also closely linked with the brain's motor centers and neuromodulatory regions. Indeed, recordings in awake animals have shown that behavior, cognition and brain state can strongly influence activity in the sensory pathways (Schneider and Mooney, 2018; McCormick et al., 2020; Parker et al., 2020 [↗](#)). Consistent with a hierarchical view of sensory processing in which neurons at higher levels carry progressively more complex representations of the world, such contextual influences appear particularly strong in the cortex (Stringer et al., 2019; Musall et al., 2019 [↗](#)) and may to a large extent be the result of intracortical processing (Noudoost et al., 2010 [↗](#); Schneider et al., 2014 [↗](#); Song et al., 2017 [↗](#)). Nevertheless, non-acoustic and contextual variables can also alter sensory processing at subcortical levels, including the IC and particularly its shell (Metzger et al., 2006 [↗](#); Gruters and Groh, 2012; Chen and Song, 2019 [↗](#); Yang et al., 2020 [↗](#); Parras et al., 2017 [↗](#); Sadari et al., 2021 [↗](#); Shaheen et al., 2021 [↗](#)). This raises the possibility that these context-dependent effects may be inherited from the auditory cortex.

To test whether auditory midbrain neurons convey behaviorally-relevant signals that depend on descending cortical inputs, we imaged corticorecipient IC shell neurons in mice engaged in a sound detection task. We found that the activity of most neurons contained information beyond the physical attributes of the sound and that this information could be used to decode the animals' behavior with a high degree of accuracy. Surprisingly, this was the case both in mice with an intact cortex and those in which the auditory cortex had been lesioned. These findings suggest that subcortical auditory structures have access to a wealth of non-auditory information independently of descending inputs from the auditory cortex. Consequently, the contextually-enriched representations that are characteristic of sensory cortices can arise from subcortical processing.

Results

Transient suppression of the auditory cortex impairs sound detection

Our aim was to characterize the activity of neurons in the shell of the IC in animals engaged in sound-guided behavior and assess how this activity is influenced by the input from the auditory cortex. To this end, we trained water-regulated mice on a sound detection task (**Figure 1A**) in which they were rewarded with a drop of water for licking in response to a click sound. Transient pharmacological silencing of auditory cortex using the GABA-A agonist muscimol has been shown to abolish the ability of rodents (Talwar et al., 2001), including head-fixed mice (Li et al., 2017), to perform a sound detection task, making this approach unsuitable for our aim of exploring the role of IC during behavior. We found that optogenetic suppression of cortical activity by photoactivating ChR2-expressing inhibitory neurons in *GAD2-IRES-cre* mice (Lohse et al., 2020) also significantly impaired sound detection performance (**Figure 1B,C**), albeit not to the same degree as pharmacological silencing. Although a control group in which the auditory cortex was injected with an EYFP virus lacking ChR2 would be required to confirm that the altered behavior results from an opsin-dependent perturbation of cortical activity, this result shows that this manipulation is also unsuitable for this study as it would leave us unable to determine whether any changes in the activity of IC neurons arise from removal of their auditory cortical input or are a consequence of alterations in the animals' behavior.

Auditory cortex lesions leave detection ability intact

Several recent studies have shown that in contrast to the disruptive effects of transient silencing, cortical lesions leave performance in some sensory tasks intact (Hong et al., 2018; Ceballo et al., 2019; O'Sullivan et al., 2019). In order to assess how auditory cortex lesions impact sound detection performance, we therefore compared the performance of mice with bilateral lesions of the auditory cortex ($n = 7$) with non-lesioned controls ($n = 9$).

Most corticocollicular neurons project ipsilaterally, with a substantial proportion also sending axons to the contralateral midbrain (Stebbins et al., 2014). The majority of corticocollicular neurons are found in the temporal cortex, and overwhelmingly in the auditory fields, while a small fraction populates adjacent areas, such as the temporal association area (**Figure 2 - figure supplement 1**). After the experiments, we injected a retrogradely-transported viral tracer (rAAV2-retro-tdTomato) into the right IC to determine whether any corticocollicular neurons remained after the auditory cortex lesions (**Figure 2**, **Figure 2 - figure supplement 2**, **Figure 2 - figure supplement 3**). The presence of retrogradely-labeled corticocollicular neurons in non-temporal cortical areas (**Figure 2**) was not the result of viral leakage from the dorsal IC injection sites into the superior colliculus (**Figure 2 - figure supplement 3**).

The ability of the mice to learn and perform the click detection task was evident in increasing hit rates and decreasing false alarm rates across training days (**Figure 3A**, $p < 0.01$, mixed-design ANOVAs). There was no difference between lesioned and non-lesioned mice in their learning speed (**Figure 3A**, $p > 0.05$, mixed-design ANOVAs) or psychometric functions (**Figure 3B**, $p > 0.05$, mixed-design ANOVA). Cortical lesioning thus leaves behavioral sensitivity to clicks intact and therefore provides a means of examining the effects of removing corticocollicular input, albeit non-reversibly, without directly affecting sound detection performance.

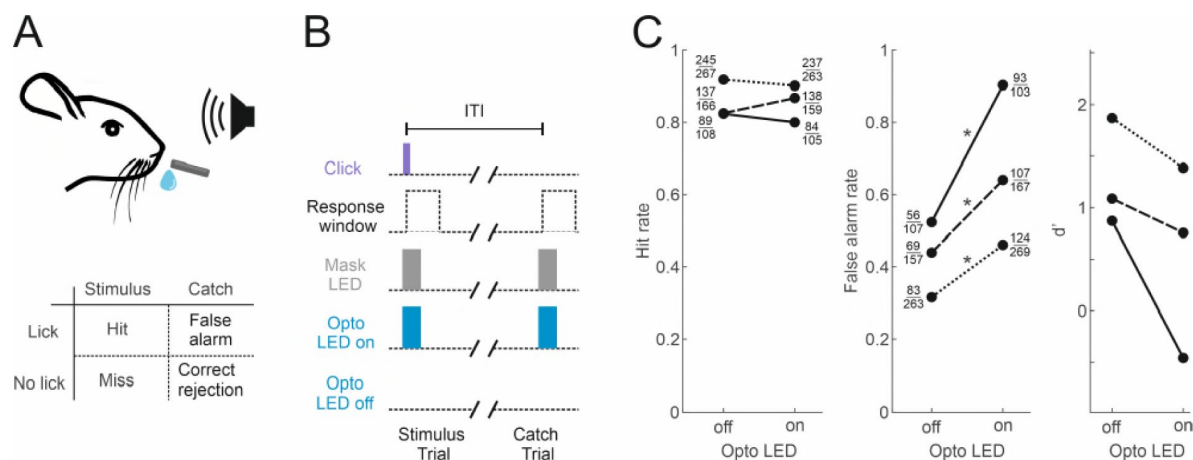


Figure 1.

Optogenetic inactivation of auditory cortex impairs sound detection performance in head-fixed mice. **(A)** Schematic of the click detection task. **(B)** Trial structure for experiments involving optogenetic manipulation. Stimulus trials (click) and catch trials (no click) were randomly interleaved and consecutive trials separated by a randomly varying inter-trial interval (ITI). LEDs placed over each auditory cortex were switched on randomly in half of the stimulus and catch trials to photoactivate the opsin. A separate set of LEDs (Mask LEDs) placed directly in front of the mouse's eyes were switched on in all Opto-on and Opto-off trials to prevent mice from visually registering the light from the photoactivation LEDs. **(C)** Detection performance in trials during which light was shone on the auditory cortex for optogenetic silencing (Opto LED - on) vs control trials (Opto LED - off). Different line styles indicate different mice ($n = 3$). Numbers next to data points indicate numbers of hit and false alarm trials over total number of stimulus and catch trials, respectively. *: $p < 0.001$, two-sided Chi-squared proportion test.

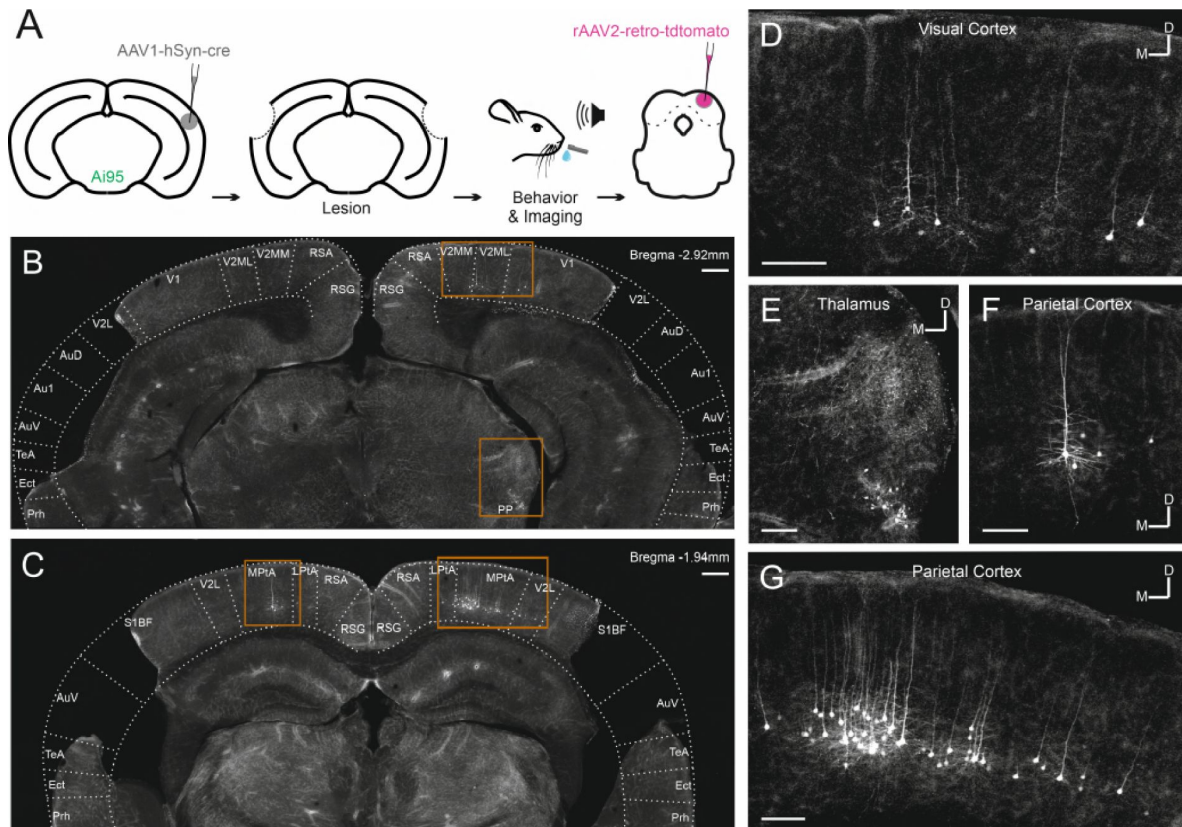


Figure 2.

Retrograde viral tracing of IC-projecting neurons in bilaterally lesioned mice. **(A)** Timeline of experimental procedures. AAV1.hSyn.cre.WPRE was injected into the right auditory cortex of GCaMP6f reporter (Ai95D) mice. This causes transsynaptic delivery of the virus to the IC and expression of GCaMP6f in corticorecipient IC neurons. Several weeks later, the mice underwent bilateral lesioning of auditory cortex either by aspiration or by thermocoagulation (see **Figure 2 - figure supplement 2** for histological sections from a mouse that underwent thermocoagulation) and were implanted with a glass window over the right auditory cortex. Following recovery from this procedure, water access was restricted and, 2-3 days later, behavioral training and imaging commenced. After data collection had been completed, rAAV2-retro-tdTomato was injected in the dorsal IC in order to label corticocollicular neurons that had remained intact. **(B,C)** Coronal sections showing lesion extent at different rostro-caudal positions for one example mouse. Area borders were drawn onto the images according to Paxinos and Franklin (2001). No retrogradely-labeled neurons were found near the lesion borders, suggesting that the auditory cortex had been completely removed. Corticocollicular projections from non-temporal regions as well as thalamocollicular projections remained intact. Scale bars, 200 μ m. **(D)** High magnification image (location shown by the upper rectangle in **B**) showing corticocollicular neurons in visual cortex. Scale bar, 100 μ m. **(E)** High magnification image (location shown by the lower rectangle in **B**) showing thalamocollicular neurons in the peripeduncular nucleus of the thalamus (PP). Scale bar, 100 μ m. **(F,G)** High magnification images (locations shown by the left and right rectangles in **C**, respectively) showing corticocollicular neurons in the parietal cortex. Scale bars, 100 μ m. Cortical area abbreviations: Au1, primary auditory; AuD, secondary auditory, dorsal; AuV, secondary auditory, ventral; Ect, ectorhinal; LPTa, lateral parietal association; MPta, medial parietal association; Prh, perirhinal; RSG, retrosplenial granular; RSA, retrosplenial agranular; S1BF, primary somatosensory, barrel field; TeA, temporal association; V1, primary visual; V2L, secondary visual, lateral; V2ML, secondary visual mediolateral; V2MM, secondary visual mediomedial.

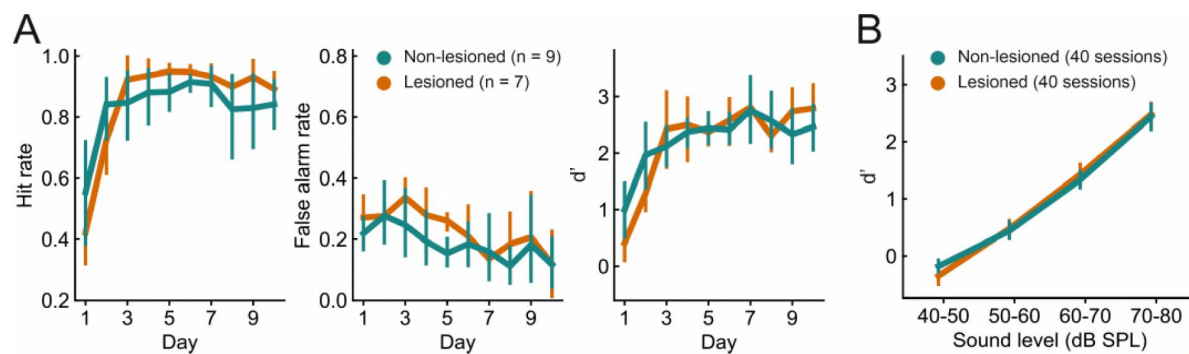


Figure 3.

Lesioned and non-lesioned mice are indistinguishable in their click detection learning rate and sensitivity. **(A)** Hit rate, false alarm rate and d' over time for lesioned and non-lesioned animals. **(B)** d' as a function of sound level. The sound levels used were not identical across all mice and were therefore combined into 10-dB wide bins. Error bars indicate 95% confidence intervals.

Transsynaptic labeling and two-photon calcium imaging of auditory corticorecipient IC neurons

Manipulations of auditory cortical activity can influence the activity of neurons throughout the IC, including the central nucleus (Suga 2008 [↗](#), Nakamoto et al., 2008 [↗](#)), where corticocollicular axons are relatively sparse (Stebbins et al 2014 [↗](#)). The strongest effects, however, tend to be observed in the shell, where cortical input is densest (Nakamoto et al., 2008 [↗](#); Vila et al 2019 [↗](#); Blackwell et al., 2020 [↗](#)). But even here, effects can be subtle (Vila et al., 2019 [↗](#)) or undetectable (Blackwell et al., 2020 [↗](#)), especially for cortical silencing. It is also unclear whether the IC neurons recorded in these studies receive cortical input or not. Therefore, we took a projection-specific approach to record the activity of IC neurons that receive direct input from the auditory cortex. More specifically, we injected AAV1.hSyn.Cre.WPRE, a virus with anterograde transsynaptic spread properties (Zingg et al., 2017 [↗](#)), into the right auditory cortex of, initially, a tdTomato (Ai9) reporter mouse. This resulted in the expression of Cre recombinase and the reporter gene in neurons that receive input from the auditory cortex, including the corticorecipient neurons of the IC (**Figure 4A** [↗](#)). By employing this approach in GCaMP6f (Ai95D) reporter mice, we could target the expression of a calcium indicator to corticorecipient IC neurons. We then proceeded to record the activity of corticorecipient neurons within about 150 μm of the dorsal surface of the IC using two-photon microscopy (**Figure 4B** [↗](#), Video 1).

Corticorecipient IC neurons display heterogeneous response profiles

The activity of individual corticorecipient IC neurons showed distinct response profiles across neurons and trial outcomes (hit vs miss) (**Figure 4C** [↗](#)). While averaging across all neurons cannot capture the diversity of responses, the averaged response profiles suggest that it is mostly trial outcome rather than the acoustic stimulus and neuronal sensitivity to sound level that shapes those responses (**Figure 4 – figure supplement 1** [↗](#)). Indeed, close to half (1272 / 2649) of all neurons showed a statistically significant difference in response magnitude between hit and miss trials, while only a small fraction (97 / 2649) exhibited a significant response to the sound. While the number of sound-responsive neurons is low, it is not necessarily surprising given the moderate intensity and very short duration of the stimuli. For comparison: Using the same transgenics, labeling approach and imaging setup and presenting 200-ms long pure tones at 60 dB SPL with frequencies between 2 kHz and 64 kHz, we typically find that between a quarter and a third of neurons in a given imaging area exhibit a statistically significant response (data not shown).

To capture the heterogeneity of response patterns across all recorded neurons, we used an unsupervised clustering algorithm (Namboodiri et al. 2019 [↗](#)) to group the average responses on hit and miss trials for each neuron. This yielded 10 clusters that displayed different response patterns over the course of the trial (**Figure 5A, B** [↗](#)). Most of the clusters exhibited distinct activity for hit vs miss trials. Some hit trial profiles were characterized by increases or decreases in activity, with a very sharp, short-latency onset, as in clusters 4 and 10 (see **Figure 5 – figure supplement 1** [↗](#) for a scaled version of cluster 10), and others by much more gradual changes in which a peak occurred seconds after the trial onset, as in clusters 5 and 9. Cluster 3, which contained the smallest number of neurons, was an exception in that it showed a transient, short latency response to the stimulus for both trial outcomes. The response profiles of some other clusters, especially clusters 6 and 8, were also qualitatively similar across hit and miss trials and/or only weakly modulated across both trial types.

This suggests that the activity of the majority of neurons in the recorded population contained information beyond the physical properties of the stimulus. Given that licking causes self-generated sounds, IC neurons could, in principle, respond to the sound of licking. However, given

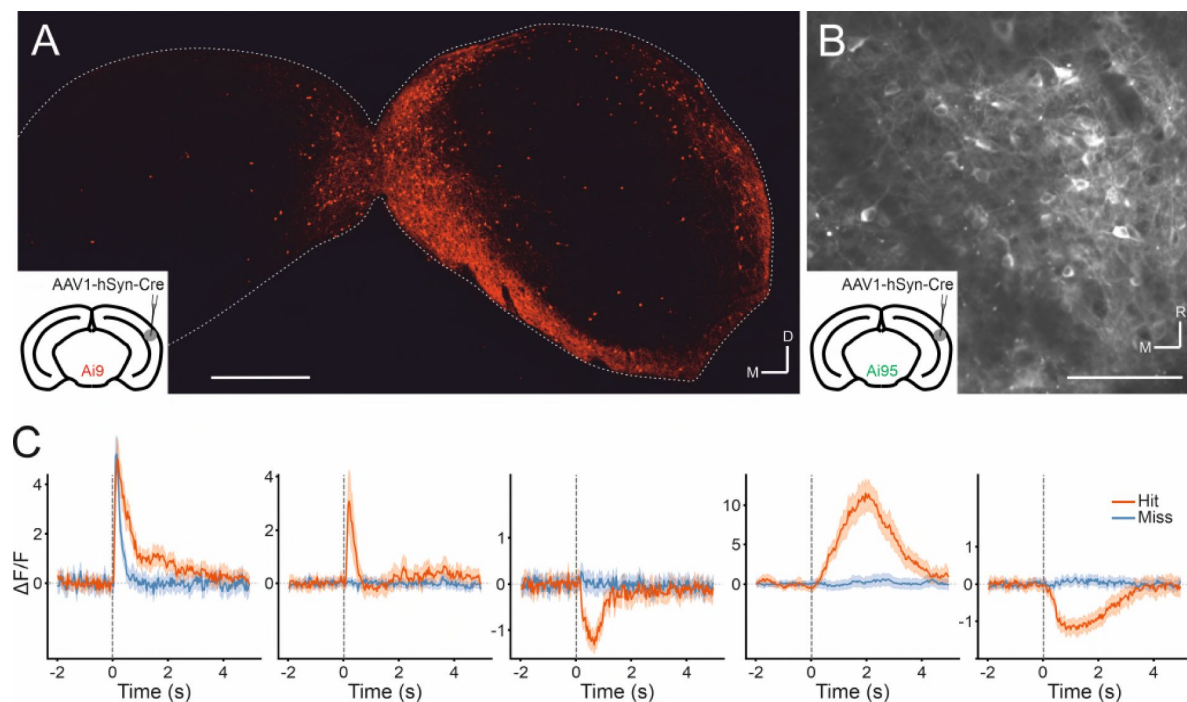


Figure 4.

Transsynaptic targeting and two-photon calcium imaging of corticorecipient IC shell neurons. **(A)** Coronal section of the left and right IC of a tdTomato-reporter (Ai9) mouse in which AAV1.hSyn.Cre.WPRE had been injected into the right auditory cortex three weeks before perfusion. The transsynaptically transported virus drove expression of Cre recombinase and tdTomato in neurons that receive input from the auditory cortex, including the corticorecipient neurons in the IC. tdTomato-labeled neurons were predominantly found in the shell of the ipsilateral (right) IC. Scale bar, 500 μ m. **(B)** In vivo two-photon micrograph taken approximately 100 μ m below the dorsal surface of the right IC of a GCaMP6f-reporter mouse (Ai95D) in which GCaMP6f expression had been driven in corticorecipient IC neurons by injection of AAV1.hSyn.Cre.WPRE into the right auditory cortex. See Video 1 for corresponding video recording. Scale bar, 100 μ m. **(C)** Example average response profiles of five corticorecipient IC neurons for different trial outcomes. Vertical line at time 0 s indicates time of click presentation. Shaded areas represent 95% confidence intervals.

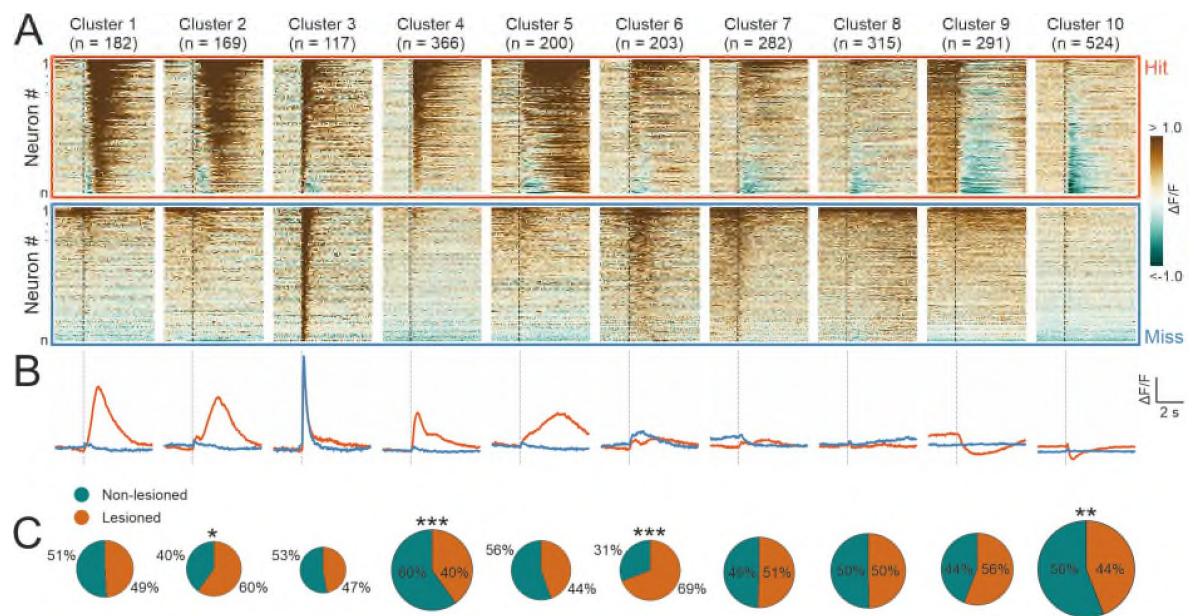


Figure 5.

Corticorecipient IC neurons display heterogeneous response profiles. **(A)** Peri-stimulus time histograms for all neurons in the dataset separated by cluster identity: hit trials (top) vs miss trials (bottom). **(B)** Averaged response profiles obtained by taking the mean across all neurons in a cluster separately for hit (red) and miss (blue) trials. **(C)** Pie charts illustrating the proportion of neurons from lesioned and non-lesioned mice in each cluster. The size of each pie chart is proportional to the total number of neurons in each cluster. Given the unequal number of neurons from lesioned (952 neurons) and non-lesioned (1697 neurons) mice, the pie charts were normalized to the overall sample size such that a 50/50 split indicates a lesioned/non-lesioned distribution that is identical to that of the overall population. Asterisks indicate a significant difference between the lesioned/non-lesioned distribution in the given cluster and that in the overall population. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, two-sided one proportion Z-test.

how quiet these are - estimated to be just 12 dB SPL (Singla et al., 2017 [↗](#)) - and that much of the response to such lick-related sounds is already canceled out at the level of the cochlear nucleus (Singla et al., 2017 [↗](#); but see Shaheen et al., 2021 [↗](#)), it is highly unlikely that lick-related sounds play a major role in driving activity in the IC.

To assess whether certain response profiles depended on auditory cortical input, we compared the ratio of neurons from lesioned vs non-lesioned mice in each cluster to that of the overall recorded population. The number of recorded neurons was unequal for lesioned and non-lesioned mice (952 vs 1697, respectively), reflecting the fact that a greater proportion of imaging sessions in non-lesioned animals were carried out using a larger field of view, which contained larger numbers of neurons (**Figure 5 - figure supplement 2** [↗](#)). To account for this, the percentages shown on the pie charts were normalized to the ratio in the overall population (**Figure 5C** [↗](#)). Neurons from both groups were well represented across all 10 clusters and while a significant difference in the lesioned/non-lesioned ratio was found for four clusters, the difference between the groups was greater than 20% for only one of them. Furthermore, there was a close correspondence between the cluster averages of lesioned and non-lesioned mice (**Figure 5 - figure supplement 3** [↗](#)). This suggests that the IC shell can produce very similar output regardless of whether auditory cortical input is available or not.

Behavior can be accurately decoded from neural activity in lesioned and non-lesioned mice

The average responses of individual neurons in the IC shell exhibited a variety of activity patterns associated with both the stimulus and the trial outcome (**Figure 5A,B** [↗](#)). To gain insight into how these activity patterns can be read out collectively on a trial-by-trial basis, we assessed the relationship between the trial-by-trial network activity and the trial outcome. We trained logistic regression models to classify hit vs miss trials on a trial-by-trial, frame-by-frame basis. As different populations of neurons were recorded in different imaging sessions, the models were trained separately for each session. “Dummy models”, which randomly classified trials while taking into account the probability of hit vs miss trials in a given session, were used as the baseline model performance. If the population activity of the IC shell contained information about the trial outcome, the performance of the models would be significantly above baseline.

In both lesioned and non-lesioned mice, the average model performance was significantly above baseline in classifying hit vs miss trials ($p < 0.05$, one-sided Wilcoxon signed-rank test or paired t-test with Bonferroni correction, **Figure 6A** [↗](#)), showed a temporal profile that is consistent with the dynamics of the activity profiles of some of the clusters, in particular clusters 1, 2, 4, 5, 9, 10 (**Figure 5A,B** [↗](#)), and was not meaningfully affected by differences in sound level distributions between hit and miss trials (**Figure 6 - figure supplement 1** [↗](#)). Additionally, the model performance in non-lesioned mice was significantly better than that in lesioned mice ($p < 0.05$, one-sided Mann-Whitney U test or t-test with Bonferroni correction, **Figure 6A** [↗](#)). The difference in the decoding performance was not the result of the difference in the number of neurons between non-lesioned and lesioned mice (**Figure 6 - figure supplement 2** [↗](#)).

By examining the corticocollicular labeling and referencing the histological sections against a mouse brain atlas (Paxinos and Franklin, 2001), we categorized the mice according to lesion size. Four of the seven lesioned animals had “(near-)complete” lesions, meaning that all (**Figure 2** [↗](#)) or an estimated ~95% (**Figure 2 - figure supplement 2** [↗](#)) of the auditory cortex had been lesioned, while the remaining mice had “partial” lesions, with an estimated 15% - 25% of the auditory cortex left intact. To assess whether the size of the lesions impacted the decoding performance, we compared the model performance between mice that had (near-)complete lesions and mice that had partial lesions. This revealed that the average decoding performance for mice with (near-)complete lesions was significantly better than that measured for mice with partial lesions. While this pattern of results may be unexpected, it is consistent with work showing smaller lesions

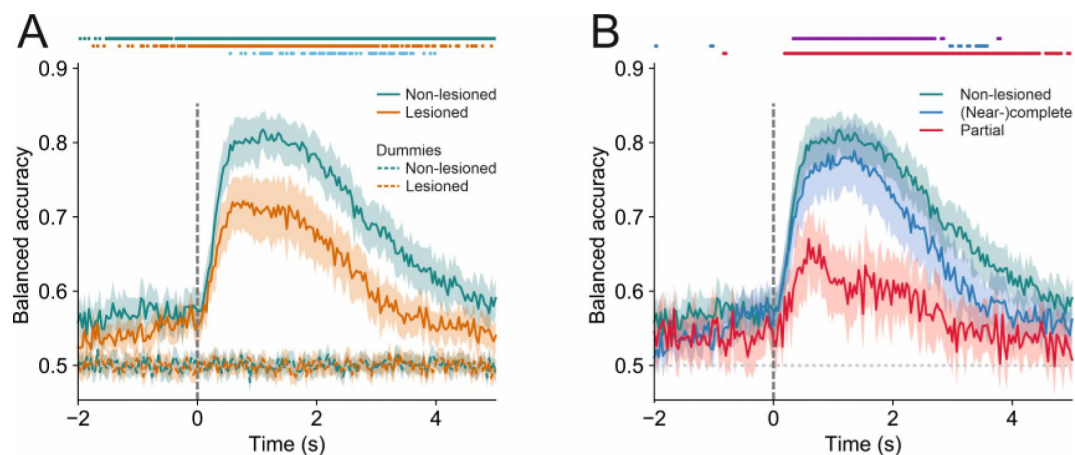


Figure 6.

Trial outcome can be accurately decoded from neural activity in lesioned and non-lesioned mice. **(A)** Average decoding accuracy of logistic regression models as a function of time against dummy models with a score of 0.5 meaning chance performance and a score of 1 being the maximum. Data shown depict the mean model accuracy across 37 (lesioned) and 38 (non-lesioned) sessions, respectively. Dots at the top indicate the timepoints (frames) where the model performance was significantly different between trained and dummy models for non-lesioned mice (teal) or lesioned mice (orange) ($p < 0.05$, one-sided Wilcoxon signed-rank test or paired t-test with Bonferroni correction, depending on whether normality assumption was met), and between the trained models for non-lesioned vs lesioned mice (blue) ($p < 0.05$, one-sided Mann-Whitney U test or t-test with Bonferroni correction, depending on whether normality assumption was met). **(B)** Same as **A** but the average model accuracy is plotted separately for mice with (near-)complete and partial lesions. Dots at the top indicate the timepoints where the model performance was significantly different between partial vs (near-)complete mice (purple), (near-)complete vs non-lesioned mice (blue), and partial vs non-lesioned mice (red) ($p < 0.05$, one-sided Mann-Whitney U test or t-test with Bonferroni correction, depending on whether normality assumption was met). Shaded areas represent 95% confidence intervals.

being associated with greater somatosensory processing deficits (Hong et al., 2018 [↗](#)). Additionally, the decoding performance in mice with (near-)complete lesions was largely indistinguishable from that in mice with an intact auditory cortex. Although the proportion of individual neurons with distinct response magnitudes in hit and miss trials in lesioned mice did not differ from that in non-lesioned mice, it was significantly lower when separating out mice with partial lesions (**Figure 6 – figure supplement 3** [↗](#)). These results imply that the activity of IC shell neurons can contain similar amounts of information about the animal's behavior regardless of whether descending input from the cortex is available or not (**Figure 6B** [↗](#)).

Pre-stimulus activity is predictive of the upcoming trial outcome

Remarkably, decoding accuracy was better than baseline even before stimulus onset. This could reflect changes in the network state that led or contributed to the upcoming trial outcome. For instance, changes in arousal or motivation can alter both the probability that an upcoming stimulus is detected and the activity of neurons in the network (Lee and Dan, 2012 [↗](#), McGinley et al., 2015 [↗](#)). The decoding models might detect such changes in activity, resulting in higher decoding accuracy prior to stimulus onset. Additionally, pre-stimulus differences in hit and miss trial activity could also reflect the anticipation of an upcoming stimulus (Ruth et al., 1974 [↗](#); Nienhuis and Olds, 1978 [↗](#); Metzger et al., 2006 [↗](#)) and the resulting change in attentional state. Inter-trial intervals in our experiments were randomly drawn from a normal distribution with a mean and standard deviation of 8 s and 2 s, respectively, and a lower bound of 3 s. Nevertheless, spontaneous licks did not occur at random times during the peri-catch trial periods following hit trials. Instead, average lick rates approximated the inter-trial interval distribution (**Figure 6 – figure supplement 4A-D** [↗](#)), suggesting that mice learned to adapt their behavior to this distribution and anticipate the timing of upcoming stimuli (**Figure 6 – figure supplement 4E,F** [↗](#)). Assuming that successfully anticipating the timing of an upcoming stimulus confers a greater chance of detecting the stimulus, neurons whose activity reflects that anticipation might be expected to show differences in pre-trial activity between hit and miss trials that could be detected by a decoding model. Note that for the analysis illustrated in **Figures 5** [↗](#) and **6** [↗](#), hit trials were excluded if there were any licks between -500 ms and +120 ms (the latter number representing the lower bound of the animals' lick-latency) relative to stimulus onset, suggesting that changes in pre-stimulus activity cannot be directly related to licking.

Discussion

Imaging auditory corticorecipient neurons in the dorsal shell of the IC in mice trained to perform a sound detection task revealed that the majority of neurons exhibited distinct activity profiles for hit and miss trials, implying that they encode information beyond just the physical attributes of the stimulus. Indeed, using logistic regression models to classify hit vs miss trials, we found that the animals' behavioral choice can be read out from these neurons with a high degree of accuracy. Importantly, the difference in IC activity between hit and miss trials was observed across different sound levels and was not due to a difference in the sound level distribution for these two trial outcomes. Surprisingly, neural activity profiles and the decoding performance were similar in mice in which the auditory cortex had been lesioned bilaterally, suggesting that the midbrain has, independently of the auditory cortex, access to a wealth of non-acoustic information, which may be sufficient to support sound detection behavior.

Auditory corticocollicular axons terminate predominantly in the shell of the IC (Stebbing et al., 2014 [↗](#); Bajo and King, 2013 [↗](#)) and the strongest effects of cortical manipulations have been reported in this region (Nakamoto et al., 2008 [↗](#); Vila et al 2019 [↗](#); Blackwell et al., 2020 [↗](#)). However, these effects can be subtle (Cruces-Solis et al., 2018; Vila et al., 2019 [↗](#)) or undetectable, especially when optogenetic silencing is used (Blackwell et al., 2020 [↗](#)). Because of this and uncertainties over exactly what proportion of neurons in the shell of the IC is innervated by the

auditory cortex and even where the border lies with the underlying central nucleus (Barnstedt et al., 2015), we used an anterograde transsynaptic tagging approach (Zingg et al., 2017) to identify corticorecipient neurons. This therefore maximized the chances of revealing the contribution of descending cortical input to the response properties of these midbrain neurons. We imaged across the optically accessible dorsal surface of the IC down to a depth of about 150 μm below the surface. Consequently, the neurons we recorded were located predominantly in the dorsal cortex. However, identifying the borders between different subdivisions of the IC is not straightforward and we cannot rule out the possibility that some were located in the lateral cortex.

Inferior colliculus neurons exhibit task-related activity

Our recordings from corticorecipient neurons in the IC are consistent with previous studies demonstrating that neural representations of behavioral variables can be found in the auditory midbrain (Ruth et al., 1974; Nienhuis and Olds, 1978; Metzger et al., 2006; Gruters and Groh, 2012; Chen and Song, 2019; Yang et al., 2020; Sadari et al., 2021; Franceschi and Barkat, 2021; Shaheen et al., 2021; Quass et al., 2023). In keeping with responses recorded in the auditory cortex (Francis et al., 2018; Franceschi et al., 2021) and IC (Chen and Song 2019; Yang et al., 2020; Franceschi et al., 2021) of behaving mice, we found that the activity of most neurons was facilitated and about a third were suppressed during the sound detection task. Overall, only a small minority of clusters (mostly cluster 3) in our dataset showed what could be characterized as largely behavior-invariant response profiles to the auditory stimulus. In contrast, a large number of neurons were clearly driven by variables other than the stimulus itself. Their activity may represent the choice (to lick or not to lick) that an animal made, preparatory motor activity, corollary discharge or the reward and the somatosensory or gustatory feedback associated with its consumption, as well as modulation by the animal's cognitive and behavioral state. Due to the task structure used, for the most part, it was not possible to unambiguously assign activity profiles to a particular variable. Nevertheless, we can speculate that neurons with late transients, such as in cluster 5, are more likely to represent corollary discharge and signals associated with the consumption of the reward, while those with very short latency peaks, as in clusters 4 and 10, may represent the animals' choice and/or preparatory motor activity.

When engaged in the detection task, an animal's arousal or motivational state may vary spontaneously or as a result of changes in, for instance, thirst, time of day or time into a session. In addition, cognitive factors, such as expectations about the timing of an upcoming trial (Ruth et al., 1974; Nienhuis and Olds, 1978; Metzger et al., 2006), which mice may have derived by learning the shape of the inter-trial interval distribution, may lead to variations in arousal or attentional state. Pre-trial differences in activity as well as the above-chance decoding performance before trial onset likely reflect the joint impact of those state changes on the activity of IC corticorecipient neurons and detection sensitivity (McCormick et al., 2020).

Contribution of the auditory cortex to task-related activity in the midbrain

Given the massive corticofugal projections that exist within the auditory system (Bajo and King, 2013), we hypothesized that task-related activity in the IC might depend on descending inputs from the auditory cortex. To address this, we imaged corticorecipient IC neurons during the same sound detection task after removing the cortical input. Consistent with previous work in the auditory (O'Sullivan et al., 2019) and somatosensory systems (Hong et al., 2018), we found that transient optogenetic silencing of the auditory cortex impaired sound detection, whereas cortical lesions had no effect on detection behavior, with lesioned mice learning the task as quickly as non-lesioned animals and achieving the same level of performance. In order to determine whether the absence of auditory cortical input alters the activity of IC neurons during sound detection behavior, we therefore focused on mice with bilateral cortical lesions to avoid the potentially confounding effects that reduced detection sensitivity produced by transient cortical silencing might have on the activity of IC neurons. For the same reason, we opted against the more

targeted approach of optogenetic silencing of corticocollicular axons. Furthermore, it would have been difficult to silence the entire corticocollicular projection and the higher light powers required for manipulating axons compared to somata would have risked transmitting light to the cortex or other corticofugal targets, potentially causing behavioral changes and/or sacrificing specificity. Locally silencing corticocollicular axons would also have left indirect transmission via the thalamus between the auditory cortex and IC intact and would have been very challenging to verify. Finally, it has been reported that using optogenetic silencing tools in axons can have unintended consequences (Wiegert et al., 2017 [↗](#)).

In keeping with our findings, numerous studies (reviewed in e.g. Pickles, 1988 [↗](#); Buser and Imbert, 1992 [↗](#)) have shown that simple auditory skills, including the ability of freely moving rats to detect sounds (Kelly, 1970 [↗](#)), are unaffected by the removal of the auditory cortex. However, transient pharmacological silencing of the auditory cortex in freely moving rats (Talwar et al., 2001 [↗](#)), as well as head-fixed mice (Li et al., 2017 [↗](#)), completely abolishes sound detection (but see Gimenez et al., 2015 [↗](#)). The time course of the effects produced by muscimol application (Talwar et al 2001 [↗](#)) suggests that there is a relationship between the size of the behavioral deficit and the degree of cortical inactivation. Consequently, milder impairments may be produced by the optogenetic approaches employed by us and others (Kato et al 2015 [↗](#); O'Sullivan et al., 2019 [↗](#)) because of incomplete suppression of cortical activity. Alternatively, the larger behavioral effects reported following muscimol application may be due to diffusion of the drug to other brain structures, potentially including the IC. Although our results cannot speak directly to the question of whether the preservation of sound detection without auditory cortex reflects a rewiring or repurposing of circuits in the brain, this seems unlikely given that other studies have shown that trained mice achieve pre-lesion performance levels on simple auditory discrimination (Ceballo et al., 2019 [↗](#); O'Sullivan et al., 2019 [↗](#)) or somatosensory detection (Hong et al., 2018 [↗](#)) tasks suddenly and within 48 hours following cortical ablation.

Why then does transient inactivation produce behavioral deficits? One possibility is that disabling the auditory cortex impacts behavior not because it contributes necessary computations or information, but because of the sudden and disruptive removal of tonic excitation (Oberle et al., 2021) to downstream targets (Otchy et al., 2015 [↗](#)) that are indispensable for successful sound detection. In this scenario, normal operation would resume once synaptic scaling (Keck et al., 2013 [↗](#)) had homeostatically restored normal activity in these structures, a process that has been suggested to take up to 48 hours and is consistent with the time course of recovery after lesions (Ceballo et al., 2019 [↗](#); Hong et al., 2018 [↗](#)). Alternatively, several circuits may redundantly support sound detection. Silencing the auditory cortex might then transiently impede sound detection until the relevant downstream decision and motor structures have updated their synaptic weights and/or processing has shifted to the other circuits. Two observations, however, argue against this possibility. First, removing one of several redundant structures should leave some residual function intact and not have the devastating effect that pharmacological cortical silencing achieves (Talwar et al., 2001 [↗](#), Li et al., 2017). Second, other circuits mediating the acousticomotor transformation required for successful sound detection behavior very likely incorporate subcortical auditory structures, including the auditory midbrain. Activity in the IC may trigger actions (Cassedey and Covey, 1996), such as licking, via its direct projections to the superior colliculus, pontine nuclei and the periaqueductal gray (Hufman and Henson, 1990, Wenstrup et al., 1994 [↗](#), Casseday and Covey, 1996 [↗](#); Xiong et al., 2015 [↗](#)) or indirectly via its projections to the auditory thalamus. If cortical lesioning results in a greater weight being placed on the activity in spared subcortical circuits for perceptual judgements, we would expect the accuracy with which trial-by-trial outcomes could be read out from IC neurons to be greater in mice without auditory cortex. However, that was not the case. This could imply that, following cortical lesions, greater weight is placed on structures other than the IC, with the thalamus being an obvious candidate, or that the auditory midbrain, thalamus and cortex are bypassed entirely if

simple acoustic-motor transformations, such as licking a spout in response to a sound, are handled by circuits linking the auditory brainstem and motor thalamus via pedunculo-pontine and midbrain reticular nuclei (Inagaki et al., 2022 [DOI](#)).

Some differences were observed for mice with only partial lesions of the auditory cortex. Those mice had a lower proportion of neurons with distinct response magnitudes in hit and miss trials than mice with (near-)complete lesions. Furthermore, trial outcomes could be read out with lower accuracy from these mice. While this finding is somewhat counterintuitive and is based on only three mice with partial lesions, it has been observed before that smaller lesions can have a more disruptive effect than larger, more complete lesions, in that the time it takes mice to learn a whisker-dependent sensory detection task is anticorrelated with the size of their somatosensory cortex lesion (Hong et al., 2018 [DOI](#)). While the complete destruction of a cortical area severs all its communication with downstream structures, a partial lesion may actually be more disruptive by eradicating normal local processing while at the same time leaving intact some tissue, especially in the deeper output layers, which continues to transmit what are now aberrant activity patterns. The difference in decoding accuracy that we observed in the IC could thus be a consequence of residual and now disruptive cortical input.

Our results show that behavioral variables are encoded by corticorecipient neurons in the dorsal shell of the IC independently of their main source of descending input, the auditory cortex. It therefore seems likely that this region of the auditory midbrain is part of the circuit that supports sound detection behavior in the absence of the auditory cortex. Nevertheless, except for the regions immediately bordering the auditory cortex, corticocollicular neurons located in other areas were left intact. These relatively sparse descending projections to the IC, such as those originating from somatosensory cortical areas (Lohse et al., 2021; Lesicko et al., 2016 [DOI](#)) and parietal cortex may have contributed to the response profiles that we observed. Additional non-acoustic sensory input can reach the IC via brainstem nuclei (Lesicko et al., 2016 [DOI](#); Shore and Zhou, 2006 [DOI](#)) and the superior colliculus (Chen et al., 2020; Coleman and Clerici, 1987 [DOI](#)). The latter, together with input from the substantia nigra (Olazabal and Moore, 1989) and the globus pallidus (Morizumi and Hattori, 1991) may also be a source of motor signals, while state changes may impact on the IC via inputs from neuromodulatory structures, including the locus coeruleus and the subparafascicular, dorsal raphe and tegmental nuclei (Chen et al., 2020, Liu et al., 2023 [DOI](#)).

Conclusion

Behavior is a major determinant of activity in the non-lemniscal auditory midbrain and thus key to understanding how it contributes to hearing. The anatomical feature that defines this structure more than any others is its connection with the auditory cortex. While modulation of IC activity by this descending projection has been implicated in various functions, most notably in the plasticity of auditory processing, we have shown in mice performing a sound detection task that IC neurons show task-related activity in the absence of auditory cortical input. These results therefore emphasize more than ever the need to factor in subcortical processing when considering how the cortex contributes to sound-guided behavior.

Materials and methods

Animals

All experiments were approved by the Committee on Animal Care and Ethical Review at the University of Oxford and were licensed by the UK Home Office (Animal Scientific Procedures Act, 1986, amended in 2012). We used 22 (3 female, 19 male) Ai95 (RCL-GCaMP6f)-D (JAX 024105,

Jackson Laboratories, USA), three (one female, two male) Gad2-IRES-Cre (JAX 010802, Jackson Laboratories, USA), six female Ai9 (RCL-tdT) (JAX 007909, Jackson Laboratories, USA), two female Ai95 (RCL-GCaMP6f)-D X VGAT-cre (JAX 016962, Jackson Laboratories, USA), three female Ai95 (RCL-GCaMP6f)-D X T29-1 (Camk2a-cre, JAX 005359, Jackson Laboratories, USA) and three (one male, two female) C57BL6/NTac.Cdh23 (MRC Harwell, UK) mice. All mice were 9–15 weeks old during data collection. They were maintained on a 12-h light/dark cycle and were housed at 20–24°C with a relative humidity of 45–65%.

Surgeries

For all surgical procedures, mice were premedicated with intraperitoneal injections of dexamethasone (Dexadrenon, 4 mg), atropine (Atrocare, 1 mg) and carprofen (Rimadyl, 0.15 mg) before being anesthetized with isoflurane (1.5–2%) and administered with buprenorphine (Vetergesic, 1 ml/kg) postoperatively. Mice were then placed in a stereotaxic frame (Model 900LS, David Kopf Instruments, CA, USA) and their body temperature was kept constant at 37°C by the use of a heating mat and a DC temperature controller in conjunction with a temperature probe (FHC, ME, USA).

For injections in the auditory cortex of AAV1.hSyn.Cre.WPRE (Penn Vector Core), the skin over this part of the brain was shaved and an incision was made, after which three small holes were drilled (Foredom K.1070, Blackstone Industries, CT, USA) into the skull with a 0.4 mm drill bit and the virus injected using a pulled glass pipette and a custom pressure injection system. In order to express GCaMP6f or tdTomato in IC neurons that receive auditory cortical inputs, a total of 150–200 nl of AAV1.hSyn.Cre.WPRE was injected at three sites in the right auditory cortex of GCaMP6f (Ai95D) or tdTomato (Ai9) reporter mice, respectively, at depths of 450–550 µm below the brain surface. Given the anterograde transsynaptic spread properties of AAV1 (Zingg et al. 2017 [\[17\]](#)), this caused the expression of the desired fluorescent protein in structures that the auditory cortex projects to, including the shell of the IC (**Figure 4A,B** [\[17\]](#)).

In order to prepare GAD2-Ires-Cre mice for the optogenetics experiments, we removed a large flap of skin over the parietal and temporal bones, partially removed the temporal muscles and performed a circular craniotomy of 3 mm diameter over each auditory cortex. We then injected a total of 500 nl of AAV5-EF1a-DIO-hChR2-EYFP, UNC Vector Core) bilaterally across 4 sites and two depths (200 and 600 µm) into the auditory cortex. Each craniotomy was covered with a circular 3 mm glass window that was attached to the edges of the skull with cyanoacrylate glue (Pattex Ultra Gel, Henkel), and the exposed skull was sealed with dental acrylic (C&B Superbond, Sun Medical, Japan) into which a custom steel bar was embedded for head fixation. Experiments commenced approximately three weeks afterwards.

The IC window implantation and cortical lesioning in the Ai95D mice were performed at least three weeks after the injections. The window implantation involved removing a flap of skin over the (inter-)parietal and occipital bone and making a circular 3 mm craniotomy over the midbrain. A 3-mm diameter glass coverslip that had been glued to a ~1 mm tall steel cylinder with 0.5 mm wall thickness was inserted into this craniotomy. The cylinder allowed us to press the glass window gently onto the brain (in order to minimize brain movement during experiments) and was then glued to the edges of the skull. For head fixation, we embedded a custom steel plate in the dental acrylic used to seal the exposed bone.

Lesions were performed as part of the cranial window implantation surgery. In those mice undergoing lesions, we removed a slightly larger flap of skin on both sides in order to expose the temporal bone, detached and deflected and/or partly removed the temporal muscle and then made, on both sides, an elliptical craniotomy over the auditory cortex of ~3 mm (dorsoventral) by 4 mm (rostrocaudal). The exposed tissue was then aspirated (Hong et al., 2018 [\[18\]](#)) with a blunted 19 G needle connected to a suction pump (Eschmann Vp25, UK) or destroyed by thermocoagulation (Ceballo et al., 2019 [\[19\]](#)) with a cauterizer (Small Vessel Cauterizer Kit, FST, Germany) and the piece

of skull that had been removed for the craniotomy was glued (Pattex Ultra Gel) back in place. In some of the lesioned mice, after completion of the imaging, 150 nl of a retrograde viral construct (rAAV2-CAG-tdTomato, UNC Vector Core) was injected into the dorsal IC across two to three sites at depths of 100–400 μm below the brain surface in order to visualize the remaining IC-projecting cortical neurons. The extent of the lesions was estimated from the histological sections and by referencing them against sections from a mouse brain atlas (Paxinos and Franklin, 2001). The experimenters were not blinded to the treatment group, i.e. lesioned or non-lesioned, but they were blind to the lesion size both during the behavior experiments and most of the data processing.

In order to visualize the distribution of IC-projecting neurons in mice without cortical lesions, 150 nl of the retrograde rAAV2-CAG-cre (UNC Vector Core) construct was injected into the dorsal IC of one Ai9 mouse with an intact cortex across three sites at depths of 100–400 μm below the brain surface.

Histology

For histological processing, mice were perfused transcardially, first with phosphate buffered saline (PBS) and then with 4% paraformaldehyde in (PBS), and their brains were sectioned coronally (100 μm thick) with a vibratome (Leica). Images were taken manually using a Leica DMR microscope, a confocal laser scanning microscope (Olympus FV1000) or with an automated slide scanner (Zeiss Axioscan Z1). The brain of one mouse (**Figure 2 - figure supplement 1** [↗](#)) was sectioned and imaged on a custom-built two-photon whole brain tomograph.

Click detection task

Starting 2–3 days before training commenced, the mice were habituated to head fixation in the experimental setup and their access to water was restricted to about ~1 ml per day, bringing their body weight down to about ~85% of the pre-restriction values. During the training phase, the mice were required to report a 0.5 ms broadband click stimulus of 80 dB SPL by licking a waterspout positioned in front of them. Licking within a 1.5-second response window (occasionally this was reduced in duration to discourage excessive licking) triggered an immediate water reward (~2 μl). Stimulus trials and catch (no stimulus) trials were randomly interleaved with an inter-trial interval drawn from a normal distribution with a mean and standard deviation of 8 s and 2 s, respectively, and a lower bound of 3 s. Successful reporting of the sound within the response window was scored a ‘hit’, while failure to respond was scored a ‘miss’. During catch trials, neither licking (‘false alarm’) during the 1.5-second response window nor withholding licking (‘correct rejection’) triggered a reward. To help the mice form an association between sound and reward, they received occasional ‘free’ rewards in stimulus trials during the initial training even when no licking occurred.

Once the mice had achieved a stable level of performance (typically two days with $d' > 1.5$), quieter stimuli (41–71 dB SPL) were introduced. The range of sound levels was adjusted to each animal’s behavioral performance to avoid floor and ceiling effects and could, therefore, differ from mouse to mouse. The behavioral experiments were run using custom MATLAB (MathWorks) scripts interfacing with a National Instruments board (NI USB-6501) for reward delivery and lick registration. The stimuli were presented using Psychtoolbox through a free-field speaker (Vifa, Avisoft Bioacoustics, Germany), positioned about ~15 cm from the snout of the mouse. Stimuli were calibrated using a Pettersson M500 microphone, which was itself referenced to a sound-level calibrator (Iso-Tech SLC-1356). Stimulus levels were calibrated by integrating the recorded RMS of clicks over the mouse hearing range (1–100 kHz) and comparing this to the RMS of stimuli from the reference sound-level calibrator.

In the optogenetics experiments, the behavioral task was identical except that a single sound level (80 dB SPL) was used and on 50% of the trials bilateral photostimulation (20 Hz, 10 ms pulses, 0.2 mW/mm²) was performed via two 470 nm LEDs (CREE-XP-E2, LED-Tech, Germany) positioned above the cranial windows. LED-on and LED-off trials were randomly interleaved and stimulation lasted for 700 ms starting 50 ms before trial onset. Furthermore, masking flashes were presented on all trials from two bright LEDs (60 mW) positioned a few cm in front of the animals' eyes.

Two-photon calcium imaging

Imaging was performed at a depth of 50 μ m – 150 μ m from the IC surface using a commercial two-photon laser-scanning microscope (B-Scope, ThorLabs, VA, USA), a SpectraPhysics Mai-Tai eHP laser (Spectra-Physics, CA, USA) tuned to 930 nm, and a Nikon 16x 0.8 NA objective. Images were acquired with a resolution of 512 by 512 pixels at a rate of ~28 Hz. The size of the field of view was either 500 μ m by 500 μ m or 666 μ m by 666 μ m, which allowed us to, typically, image dozens of corticorecipient IC neurons simultaneously. Each imaging session lasted around 1-2 hours.

Image processing

Rigid and non-rigid image registration, segmentation, neuropil and signal extraction were performed using the Python version of suite2p (Pachitariu et al. 2017 <https://doi.org/10.1016/j.neuroimage.2017.05.031>). Neuropil extraction was performed using default suite2p parameters (<https://suite2p.readthedocs.io/en/latest/settings.html>), neuropil correction was done using a coefficient of 0.7 and calcium $\Delta F/F$ signals were obtained by using the median over the entire fluorescence trace as F0. To remove slow fluctuations in the signal, a baseline of each neuron's entire trace was calculated by Gaussian filtering as well as minimum and maximum filtering using default suite2p parameters. This baseline was then subtracted from the signal. To assess the extent of image displacement in the z-axis, we compared the average of the top and the bottom 500 frames of each spatial principal component (PC) of the registered images for every 8-16 minutes of the recordings. Any region of interest (ROI) with substantial z-axis movement was excluded from further analysis. Sessions in which the majority of ROIs had to be excluded were discarded entirely. Furthermore, in order to specifically assess brain motion caused by the motor component of the task, i.e. the animal's licking, lick-triggered movies of the imaging frames were created for every 8-16 minutes of the recordings. The rationale here is that if licking causes a stereotypical displacement of the imaging plane, this will become apparent when image sequences are averaged across lick events. Specifically, non-registered image sequences surrounding (from 2 s before to 2 s after) lick events were used to produce averaged lick-triggered movies. These lick-triggered movies, as well as non-averaged sequences, were then visually inspected and ROIs were excluded from subsequent analysis if they were affected by substantial z-motion.

Analysis of task-modulated and sound-driven neurons

To identify individual neurons that produced significantly different response magnitudes in hit and miss trials, we calculated the mean activity for each stimulus trial by taking the mean activity over the 5 seconds following stimulus presentation and subtracting the mean activity over the 2 seconds preceding the stimulus during that same trial. A Mann-Whitney U test was then performed to assess whether a neuron showed a statistically significant difference (Benjamini-Hochberg adjusted p-value of 0.05) in response magnitude between hit and miss trials. The analysis was performed using equal numbers of hit and miss trials at each sound level to ensure balanced sound level distributions. If, for a given sound level, there were more hit than miss trials, we randomly selected a sample of hit trials (without substitution) to match the sample size for the miss trials and vice versa. Sound-driven neurons were identified by comparing the mean miss trial activity before and after stimulus presentation. Specifically, we performed a Mann-Whitney U test to assess whether there was a statistically significant difference (Benjamini-Hochberg adjusted p-value of 0.05) between the mean activity over the 2 seconds preceding the stimulus and the

mean activity over the 1 second period following stimulus presentation. This analysis was performed using miss trials with click intensities from 53 dB SPL to 65 dB SPL (many sessions contained very few or no miss trials for higher sound levels).

Clustering analysis

To identify sub-populations of neurons with distinct response profiles, a clustering analysis was performed. While clustering is a useful approach for organizing and visualizing the activity of large and heterogeneous populations of neurons, we need to be mindful that, given continuous distributions of response properties, the locations of cluster boundaries can be somewhat arbitrary and/or reflect idiosyncrasies of the chosen method and thus vary from one algorithm to another. We employed an approach very similar to that described in [Namboodiri et al. \(2019\)](#) because it is thought to produce stable results in high-dimensional neural data ([Hirokawa et al. 2019](#)). For each neuron, the trial-averaged activity was obtained by averaging across all the sound levels presented in a given session separately for hit and miss trials (given the small number of catch trials, approximately one tenth of all trials, this analysis was restricted to stimulus trials only). Differences in the field of view size between sessions resulted in slight differences in frame rate and thus frame duration. Therefore, the activity traces were linearly interpolated to have the same number of data points (193 frames). For each neuron, the trial-averaged activity for miss trials was appended to that for hit trials, producing 386 data points per neuron for a total of 2649 neurons ($n = 1697$ neurons from 40 sessions with 9 non-lesioned mice; $n = 952$ neurons from 40 sessions with 7 lesioned mice). To reduce the dimensionality of this dataset before applying the clustering algorithm, we performed principal components analysis (PCA) along the time axis to capture the temporal response profile for each neuron. Guided by the ‘elbow’ point in a scree plot visualizing the fraction of variance explained by each PC, we decided to project the dataset to the lower dimensional subspace formed by the first 9 PCs.

Spectral clustering was used to cluster the resulting data. The affinity matrix was constructed by computing a graph of nearest neighbors. The hyperparameters of the clustering algorithm, including the number of nearest neighbors and the number of clusters, were optimized by a grid search to maximize the mean Silhouette Score for all samples. The Silhouette Score is a measure of the compactness of individual clusters (intra-cluster distance) and the separation amongst clusters (inter-cluster distance). For a given sample i that belongs to cluster C_I , the Silhouette Score is defined as:

$$S_i = \frac{(b_i - a_i)}{\max(a_i, b_i)}$$

where a_i is the mean distance between sample i and all the other samples in the same cluster, and b_i is the mean distance of sample i to the nearest cluster that sample i is not part of. Let $|C_I|$ and $|C_J|$ be the number of samples belonging to clusters C_I and C_J , and $d(i, j)$ be the distance between samples i and j ; a_i and b_i are defined as:

$$a_i = \frac{1}{|C_I| - 1} \sum_{j \in C_I, i \neq j} d(i, j)$$

$$b_i = \min_{j \neq i} \frac{1}{|C_J|} \sum_{j \in C_J} d(i, j)$$

The resulting clusters from the hyperparameter search were further examined by plotting clusters in pairs against each other with t-distributed Stochastic Neighbor Embedding, a statistical method for visualizing high-dimensional data that involves giving each data point a location in a two or three-dimensional space ([van der Maaten and Hinton 2008](#)).

Population decoding

Logistic regression models were trained on the network activity of each session, i.e., the $\Delta F/F$ values of all ROIs in each session, to classify hit vs miss trials. This was done on a frame-by-frame basis, meaning that each time point (frame) of each session was trained separately. Rather than including all the trials in a given session, only trials of intermediate difficulty were used for the decoding analysis. More specifically, we only included trials across five sound levels, comprising the lowest sound level that exceeded a d' of 1.5 plus the two sound levels below and above that level. That ensured that differences in sound level distributions would be small, while still giving us a sufficient number of trials to perform the decoding analysis. Sessions were only included if there were at least 15 instances for both hit and miss trials. The models were trained with L2 regularization, which gave similar contributions to correlated features (i.e., individual neuronal activity) instead of discarding some of the correlated features that were also related to behaviorally-relevant information. The strength of the regularization for each model was hyperparameter-tuned and the reported results were cross validated. Specifically, neuronal data in each session was split into 5 stratified folds, and each fold preserved the percentage of hit and miss trials in a given session. Four folds were used for cross-validated hyperparameter search (randomized search drawn from the log-uniform distribution between 1×10^{-4} and 1×10^2), and the remaining 1 fold was used for evaluating the model after the best hyperparameters were refitted on the 4 folds of data. To more reliably estimate the model results, the evaluation was done for each of the 5 folds for each session and the average of these 5 results was taken as each session's model performance at each timepoint.

The percentage of hit and miss trials was different in each session, and the number of hit trials often exceeded the number of miss trials. To include as many trials as possible while preventing the models from taking advantage of class imbalances, balancing procedures were performed at both the model-level and the metrics-level. First, logistic regression was trained with the class weights adjusted inversely proportional to the frequency of each trial type in the training data, giving higher weights to the minority class and lower weights to the majority class. Given the total number of trials in the training data N_T , the number of classes N_C , and the number of trials for a given class N_i , the weight for a given class W_i was defined as follows:

$$W_i = W_T / (N_C * N_i)$$

These weights were then applied to the cost function during the training process to increase the penalty for minority class misclassifications and reduce the penalty for majority class misclassifications. Second, to avoid the estimated model performance being inflated due to class imbalance, balanced accuracy (Brodersen et al. 2010 [\[10\]](#)) was used to report the model performance. Balanced accuracy was defined as the arithmetic mean of the true positive rate and the true negative rate. For a model performing equally well on either class, the balanced accuracy is the same as the conventional accuracy (i.e., the number of correct predictions divided by the total number of predictions). However, for a model scoring above chance only because the model takes advantage of the class imbalance (i.e. consistently predicts the majority class), the balanced accuracy is at chance level.

$$\text{Balanced accuracy} = \frac{1}{2} * \left(\frac{N_{\text{true pos.}}}{N_{\text{true pos.}} + N_{\text{true neg.}}} + \frac{N_{\text{true neg.}}}{N_{\text{true neg.}} + N_{\text{true pos.}}} \right)$$

Additionally, dummy models were used as baseline models to compare against the performance of the logistic regression models. Dummy models predicted the class labels (i.e., hit or miss trials) randomly while taking into account the probability of each class.

To assess whether the model performance was correlated with the number of ROIs recorded in a session, Spearman's correlation coefficient was computed between the number of ROIs in a session and the mean model performance over different 1-second time periods relative to stimulus onset (from 2 seconds before to 5 seconds after stimulus onset).

Statistical tests were conducted to compare the model performance between lesioned and non-lesioned mice, as well as between the trained models and dummy models. Since the frame rate varied slightly with the size of the field of view, the numbers of frames (193 - 197 frames) per 7-s trial could be different across sessions. Thus, model performance was linearly interpolated to make all sessions contain the same number of frames before statistical tests were performed at each timepoint. The model performance of each session was cross-validated and averaged across folds, and the statistical tests were performed on the distributions of the sessions' model performance. The Shapiro–Wilk test was used to determine whether a parametric or nonparametric test should be used, using $p < 0.05$ as a criterion. A one-sided Wilcoxon signed-rank test or paired t-test was performed for comparing trained vs dummy models, while a one-sided Mann-Whitney U test or t-test was performed for comparing trained models for different groups of mice. Because of the smaller sample sizes, the statistical tests in **Figure 6B** [↗](#) were carried out after binning the scores for every two timepoints. Statistical significance was defined as $p < 0.05$ after Bonferroni correction.

Data availability

Data will be made available in a public repository.

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Figure supplements

Video 1. Two-photon calcium imaging performed approximately 100 μm below the dorsal surface of the right IC of a GCaMP6f-reporter mouse (Ai95D) engaged in a sound detection task. GCaMP6f expression had been driven in corticorecipient IC neurons by injection of AAV1.hSyn.Cre.WPRE into the right auditory cortex. Video is played at twice the speed of acquisition and corresponds to the micrograph shown in **Figure 4B** [↗](#).

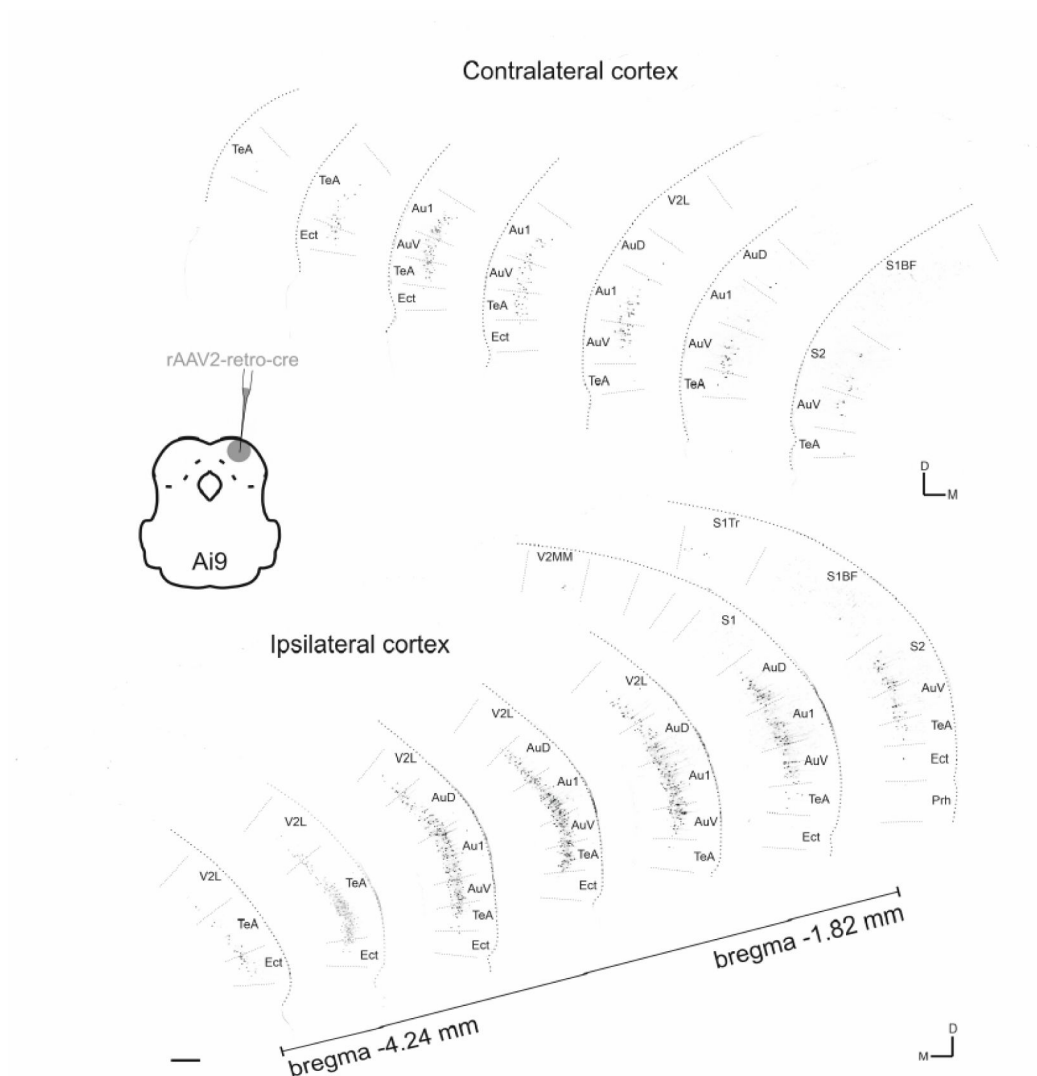


Figure 2 - figure supplement 1.

Contra- and ipsilateral corticocollicular neurons along the rostrocaudal axis. Seven coronal sections are shown from each hemisphere covering approximately 2.5 mm of the rostrocaudal axis. Corticocollicular neurons were labeled by injecting a total of 150 nL of rAAV2-CAG-cre into the dorsal IC (at three sites and several depths from 100 μ m - 400 μ m below the brain surface) of a tdTomato reporter mouse (Ai9). Data were obtained using whole-brain laser scanning two-photon tomography. The resulting images were grayscale inverted and thresholded to remove all background labeling so that they could be more easily arranged into a common figure. Area borders were drawn onto the images according to Paxinos and Franklin (2001). Cortical area abbreviations: Au1, primary auditory; AuD, secondary auditory, dorsal; AuV, secondary auditory, ventral; Ect, ectorhinal; Prh, perirhinal; S1, primary somatosensory; S1BF, primary somatosensory, barrel field; S1Tr, primary somatosensory, trunk region; S2, secondary somatosensory; TeA, temporal association; V2L, secondary visual, lateral. Scale bar, 200 μ m.

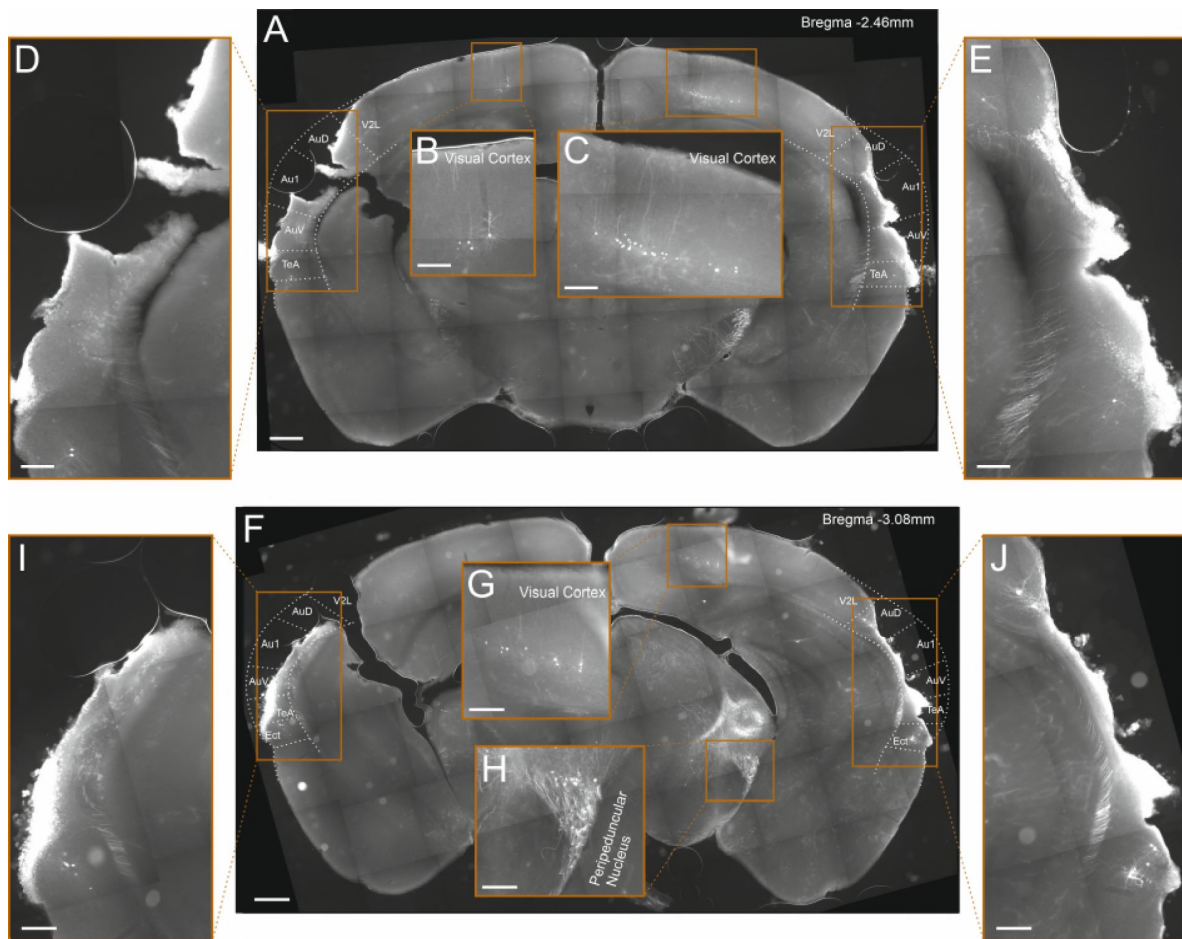


Figure 2 - figure supplement 2.

Lesioning by thermocoagulation. **(A)** Coronal section showing lesion extent in a mouse that had undergone lesioning by thermocoagulation. After data collection had been completed, rAAV2-retro-tdTomato was injected along the dorsal IC in order to label corticocollicular neurons that had remained intact. Area borders were drawn onto the images according to Paxinos and Franklin (2001). Scale bar, 500 μm . **(B,C)** Higher magnification images showing tdTomato-labeled corticocollicular neurons in the left and right visual cortex. Scale bars, 200 μm . **(D,E)** Higher magnification images of the temporal regions surrounding the lesion sites, showing a very small number of residual corticocollicular neurons in the left and right temporal association area and the right dorsal auditory field. Scale bars, 200 μm . **(F)** Same as **A** for a different coronal section of the same mouse. **(G,H)** Higher magnification images showing tdTomato-labeled corticocollicular neurons in the right visual cortex and thalamocollicular neurons in the right peripeduncular nucleus. Scale bars, 200 μm . **(I,J)** Higher magnification images of the temporal regions surrounding the lesion sites showing a very small number of residual corticocollicular neurons in the left and right ectorhinal cortex and the right dorsal auditory field. Scale bars, 200 μm . While the lesion procedure spared some auditory cortex tissue in this animal, its visual appearance and the fact that barely any corticocollicular neurons could be found suggests that this residual tissue was almost completely destroyed. Consequently, we categorized this animal as having a (near)-complete lesion, meaning that 5% or less of the auditory cortex was left intact. Cortical area abbreviations: Au1, primary auditory; AuD, secondary auditory, dorsal; AuV, secondary auditory, ventral; Ect, ectorhinal; TeA, temporal association; V2L, secondary visual, lateral;

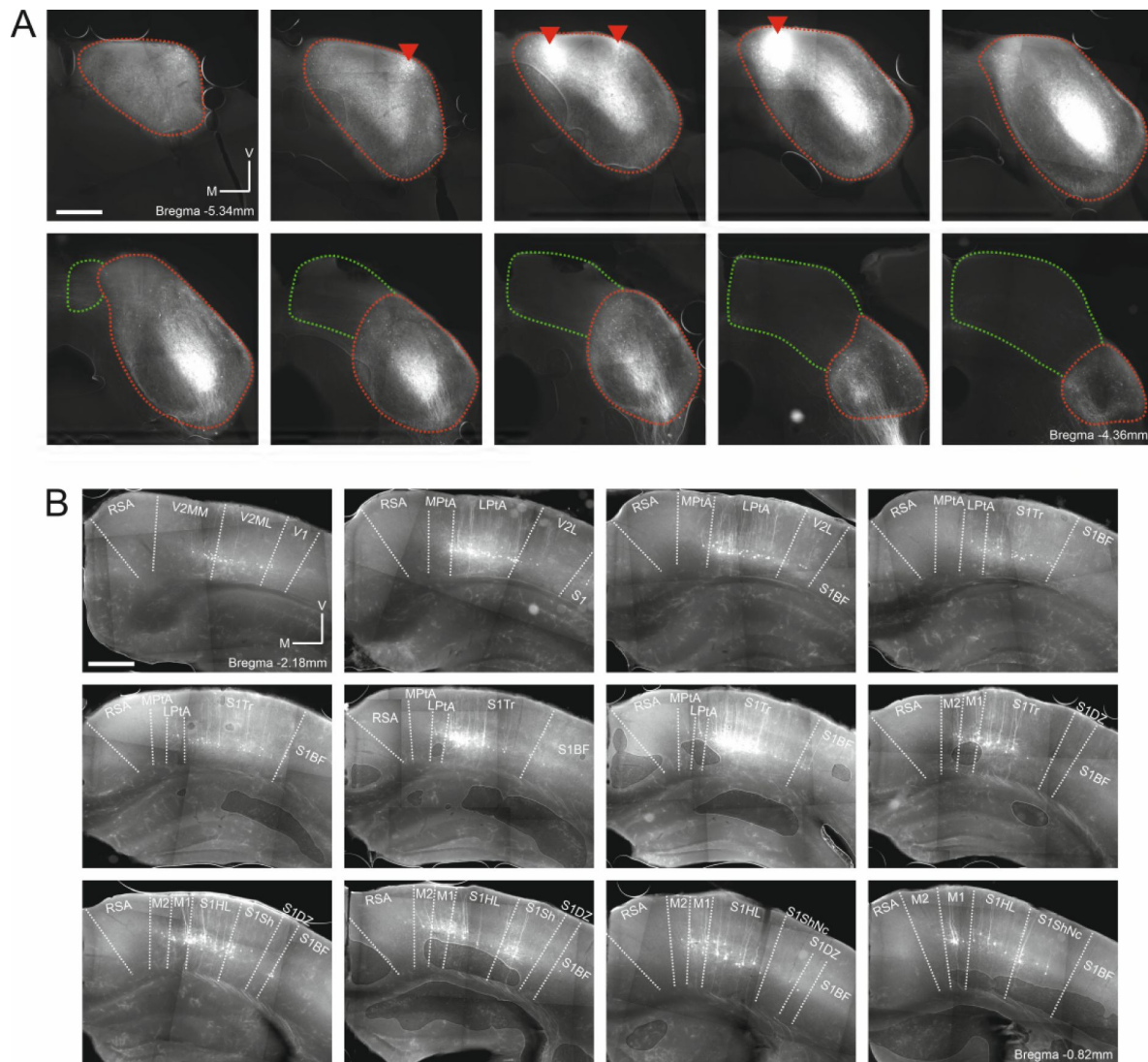


Figure 2 - figure supplement 3.

Retrograde labeling of corticocollicular neurons in non-temporal areas of the cerebral cortex is not the result of viral leakage into the superior colliculus. **(A)** Coronal sections showing the right midbrain of one example mouse (same mouse as in **Figure 2 - figure supplement 2**). Sections are ordered caudo-rostrally from top left to bottom right. Red lines indicate the approximate outline of the inferior colliculus, green lines the approximate outline of the superior colliculus. Red triangles indicate rAAV2-retro-tdTomato injection locations. In addition to the labeling near the injection sites, widespread retrograde labeling is found in the central nucleus of the inferior colliculus. No labeled cell bodies were found in the superior colliculus. Scale bar, 500 μ m. **(B)** Coronal sections showing corticocollicular neurons in non-temporal areas of the right cerebral cortex labeled as a result of the rAAV2-retro-tdTomato injections in the inferior colliculus illustrated in **A**. Sections are ordered caudo-rostrally from top left to bottom right. Area borders were drawn onto the images according to Paxinos and Franklin (2001). Scale bar, 500 μ m. Cortical area abbreviations: LPtA, lateral parietal association; MPtA, medial parietal association; M1: primary motor; M2: secondary motor; RSA, retrosplenial agranular; S1BF, primary somatosensory, barrel field; S1DZ, primary somatosensory, dysgranular region; S1HL, primary somatosensory, hindlimb region; S1Sh, primary somatosensory, shoulder region; S1ShNc, primary somatosensory, shoulder/neck region; S1Tr, primary somatosensory, trunk field; V1, primary visual; V2L, secondary visual, lateral; V2ML, secondary visual mediolateral; V2MM, secondary visual mediomedial.

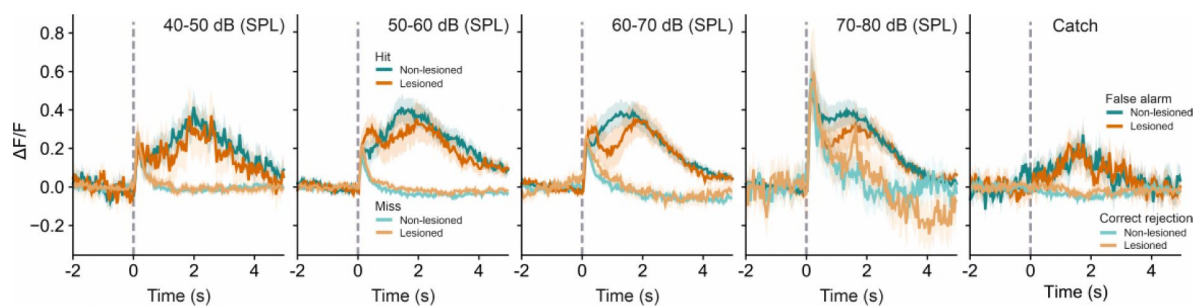


Figure 4 - figure supplement 1.

Averaged response profiles for stimulus and catch trials. Stimulus trials are binned into four different sound level ranges and separated into hit and miss trials. Catch trials are separated into false alarms and correct rejections. Shaded areas represent 95% confidence intervals.

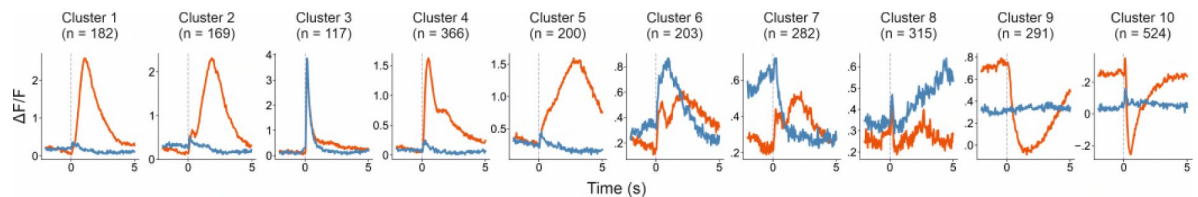


Figure 5 - figure supplement 1.

Rescaled response profiles for each cluster. Averaged response profiles obtained by taking the mean across all neurons in a cluster separately for hit (red) and miss (blue) trials. Same as **Figure 5B** except that here each panel has an individualized y-axis range.

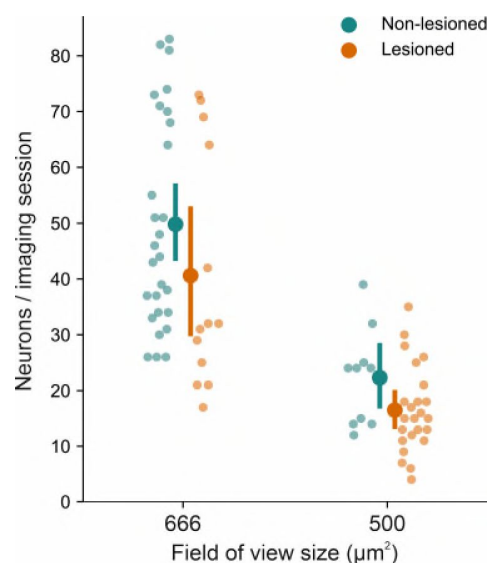


Figure 5 - figure supplement 2.

Number of sessions for each imaging field of view size. A greater number of recordings happened to be made with the larger field of view in non-lesioned (28 of 38) than in lesioned (13 of 37) mice. Consequently, the number of neurons recorded in non-lesioned mice was greater than that recorded in lesioned mice (1697 vs 952). Error bars represent 95% confidence intervals.

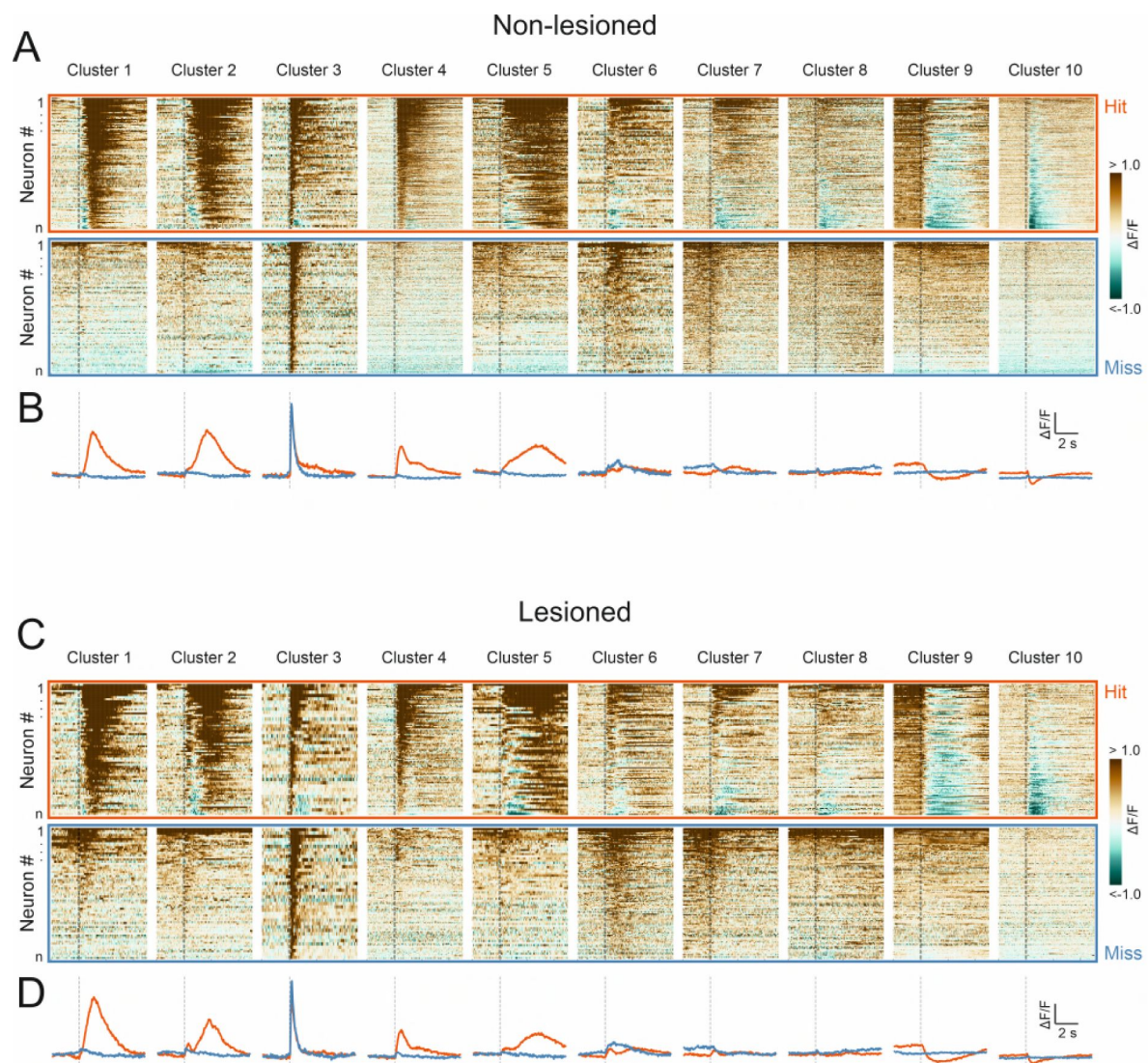


Figure 5 – figure supplement 3.

High correspondence between cluster profiles of lesioned and non-lesioned mice. **(A)** Peri-stimulus time histograms for all neurons recorded in non-lesioned mice separated by cluster identity: hit trials (top) vs miss trials (bottom). **(B)** Averaged response profiles obtained by taking the mean across all neurons in each cluster separately for hit (red) and miss (blue) trials. **(C, D)** Same as **A** and **B** for neurons recorded in lesioned mice.

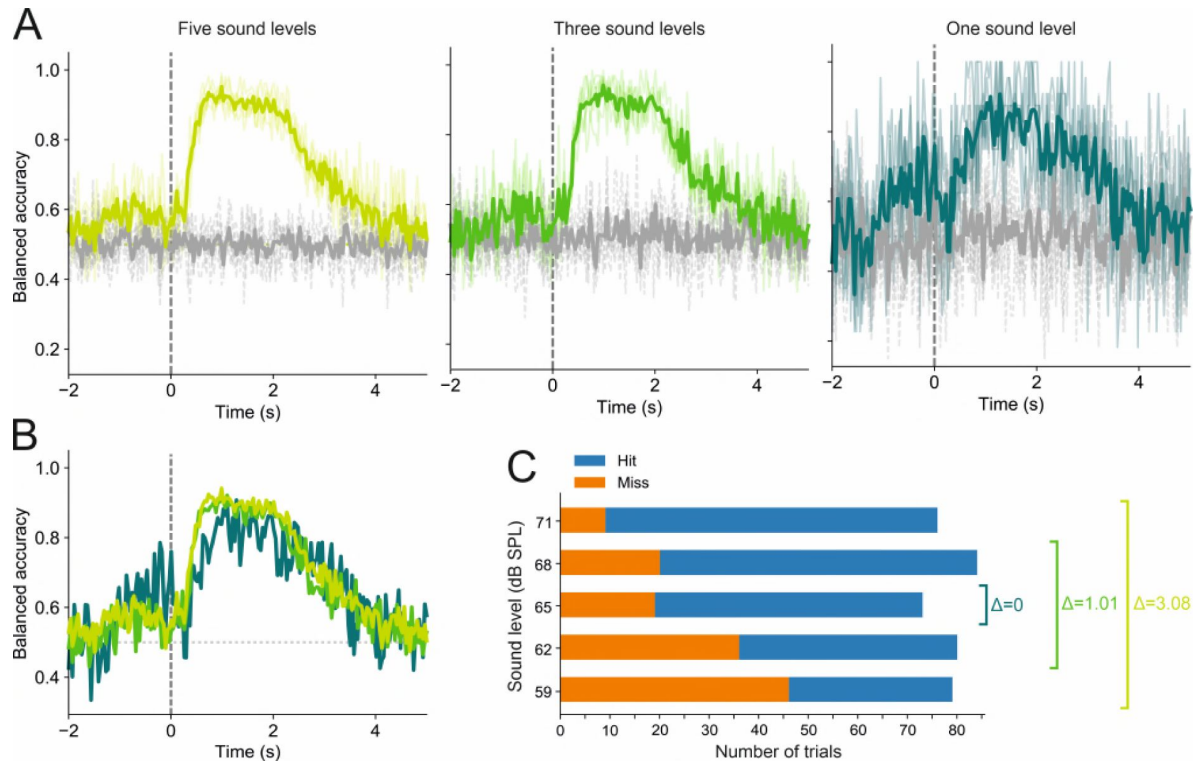


Figure 6 – figure supplement 1.

Trial outcome decoding is not meaningfully affected by differences in sound level distributions between hit and miss trials. **(A)** Decoding results for one imaging session based on trials in which stimuli were presented at five (left), three (middle), or a single sound level (right). Thin colored lines show the results of each of the five cross-validation folds. Thick colored lines indicate averages across all five folds. Gray lines show results for the corresponding dummy models. **(B)** Superimposed averages from **A**. **(C)** Hit and miss trial distributions for each of the five sound levels, as well as the mean sound level difference (Δ) between hit and miss trials for the three decoding conditions shown in **A** and **B**. The mean difference was 3.08 dB, 1.01 dB and 0 dB for the five, three and one sound level condition, respectively.

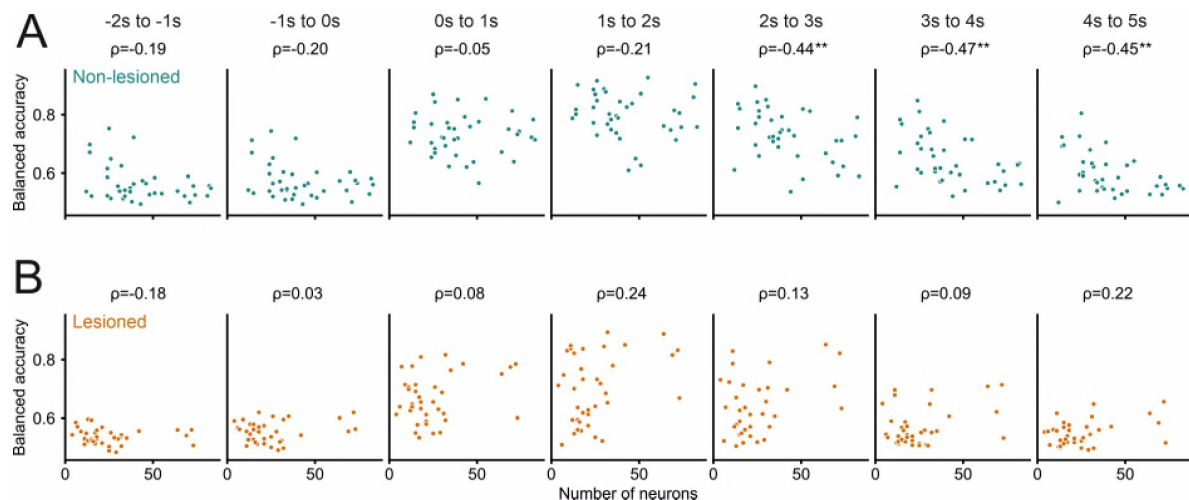


Figure 6 - figure supplement 2.

Greater number of recorded neurons was not associated with better decoding performance. **(A,B)** Decoding performance (balanced accuracy) of the logistic regression models averaged over different 1-s time periods relative to stimulus onset as a function of the number of neurons recorded in a given session. A greater number of neurons obtained in a field of view was not associated with better decoding performance. Values above panels indicate Spearman's rank correlation coefficient ρ . The only statistically significant relationship between the number of recorded neurons and decoding performance was found for late trial periods in non-lesioned mice (A), and indicated that for time periods >2 seconds after stimulus onset a smaller sample size was associated with better decoding performance. **: $p < 0.01$.

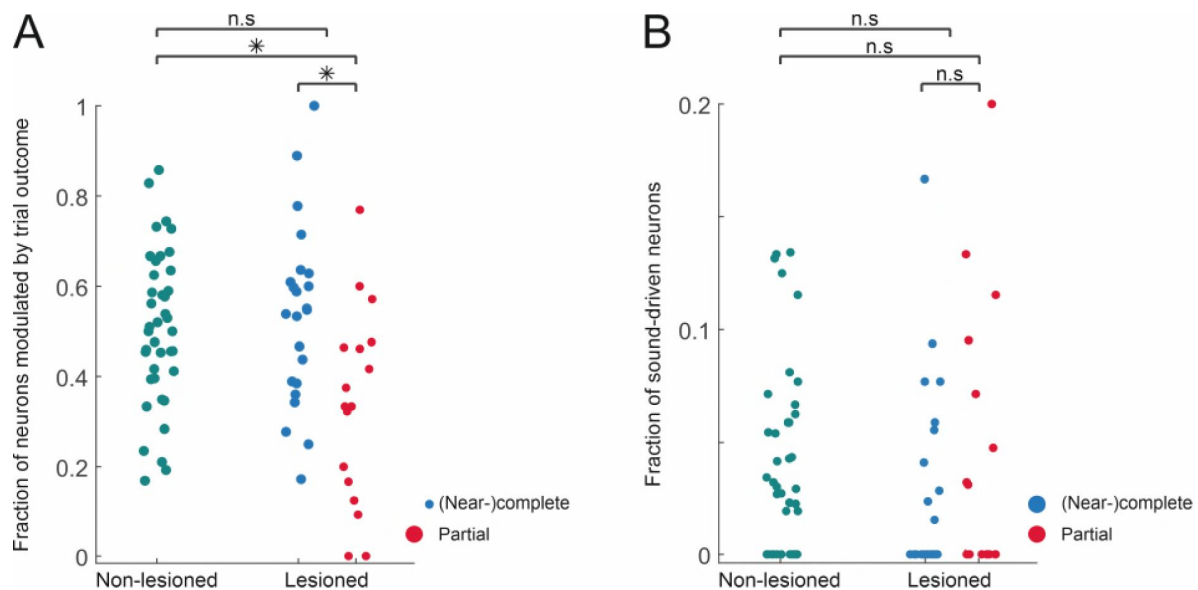


Figure 6 - figure supplement 3.

Similar fractions of task-modulated and sound-driven neurons in lesioned and non-lesioned mice. **(A)** Fraction of neurons per session that exhibit a significant difference in response magnitude between hit and miss trials. **(B)** Fraction of neurons per session that exhibit a significant stimulus response in miss trials. *: $p < 0.01$, Mann Whitney U test.

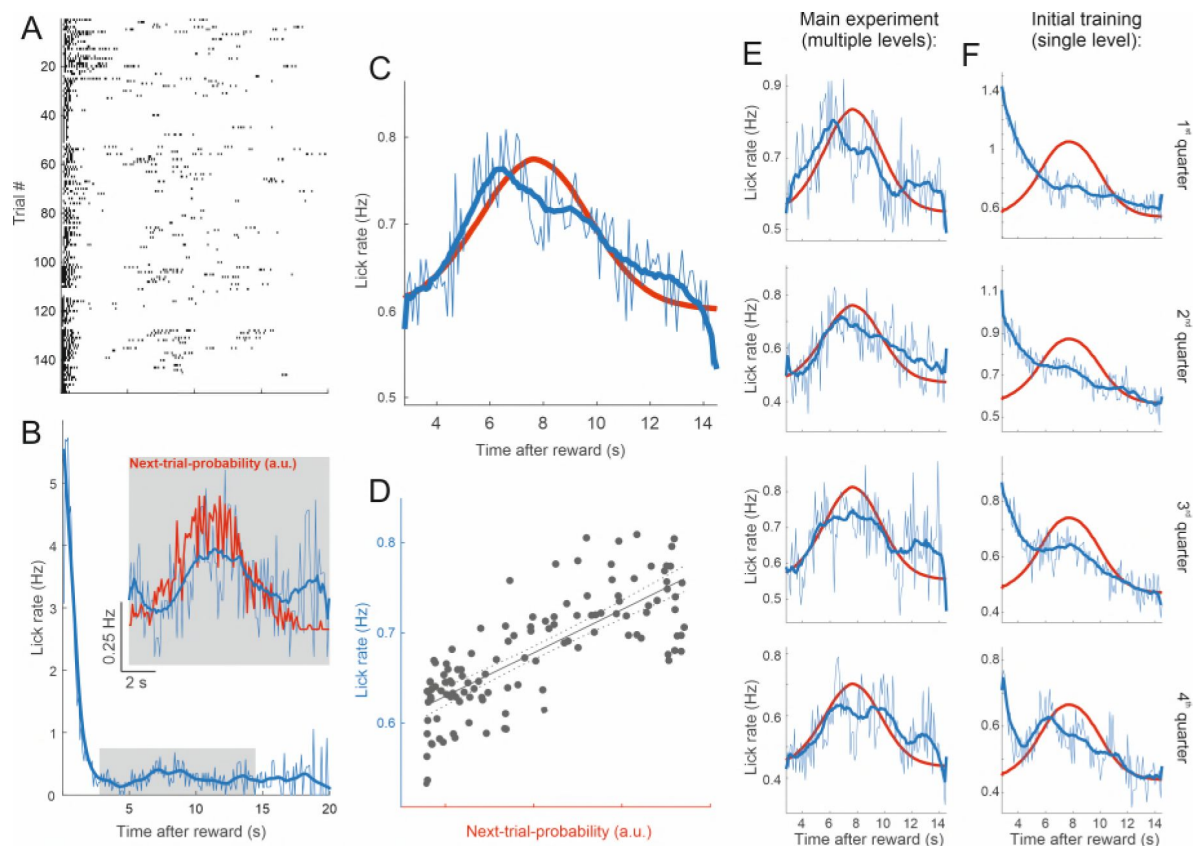


Figure 6 - figure supplement 4.

Lick rates in peri-catch trial periods approximate next-trial-probability. **(A)** Peri-catch trial lick raster for all catch trials that followed a hit trial for one example mouse. The peri-catch trial period was defined as the period from the reward delivery in the hit trial to the onset of the trial following the catch trial. **(B)** Lick rate averaged across the peri-catch trial periods shown in **A** and binned into 100ms wide bins. The thick blue line shows the smoothed (20-point running average) lick rate. The inset gives a magnified view of the average lick rate during the period indicated by the gray rectangle. The red line illustrates the distribution of 'reward-to-next-trial-onset' intervals experienced by the example mouse. Given that licks are plotted time-locked to reward delivery, we plotted the distribution of intervals between reward delivery and onset of the next trial rather than the ITI distribution. In practice the difference between the two is roughly the latency between the stimulus and the first lick and thus barely distinguishable at this scale. As the distribution indicates the probability of the next trial presentation as a function of time since the preceding reward delivery we refer to it as 'next-trial-probability'. **(C)** Same as inset in **B** averaged across all mice. Next-trial-probability was smoothed with a 20-point running average. **(D)** Next-trial-probability as a predictor of lick rate. The dotted lines indicate the 95% confidence bounds around the regression fit. Adjusted $R^2 = 0.59$. Although the next-trial-probability is a good predictor of changes in the average lick rate, the lick rate at the peak of the distribution is merely about a quarter higher than at its tails where next-trial-probability approaches zero. Furthermore, to put the average lick rates into perspective, note that mice tend to lick in bouts, typically consisting of two to six licks in very quick succession (see lick raster in **A**), and that, consequently, the lick rate exceeds the underlying bout rate by a factor of about four. **(E)** Same as **C** but with peri-catch trials binned into four quarters before averaging in order to illustrate changes in lick behavior across different stages of the experiment. **(F)** Same as **E** for all peri-catch trials during the initial training with a single-level stimulus. While the peri-catch trial lick rate profile changed substantially over the course of the initial training (**F**) and started to approximate the stimulus probability distribution towards the end of training, it remained broadly stable throughout the main experiment (**E**). In order to increase the statistical power for this analysis, we included data from several additional mice used in other projects. These additional mice received the same training and performed the same task, but differed from those in the main dataset in that they had a different genetic background and/or had been fitted with a cranial implant for cortical rather than midbrain imaging. N for panels **C-F** = 34 mice.

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Editors

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Reviewer #1 (Public Review):

The inferior colliculus (IC) is the central auditory system's major hub. It integrates ascending brainstem signals to provide acoustic information to the auditory thalamus. The superficial layers of the IC ("shell" IC regions as defined in the current manuscript) also receive a massive descending projection from the auditory cortex. This auditory cortico-collicular pathway has long fascinated the hearing field, as it may provide a route to funnel "high-level" cortical signals and impart behavioral salience upon an otherwise behaviorally agnostic midbrain circuit.

Accordingly, IC neurons can respond differently to the same sound depending on whether animals engage in a behavioral task (Ryan and Miller 1977; Ryan et al., 1984; Slee & David, 2015; Sadari et al., 2021; De Franceschi & Barkat, 2021). Many studies also report a rich variety of non-auditory responses in the IC, far beyond the simple acoustic responses one expects to find in a "low-level" region (Sakurai, 1990; Metzger et al., 2006; Porter et al., 2007). A tacit assumption is that the behaviorally relevant activity of IC neurons is inherited from the auditory cortico-collicular pathway. However, this assumption has never been tested, owing to two main limitations of past studies:

- (1) Prior studies could not confirm if data were obtained from IC neurons that receive monosynaptic input from the auditory cortex.
- (2) Many studies have tested how auditory cortical inactivation impacts IC neuron activity; the consequence of cortical silencing is sometimes quite modest. However, all prior inactivation studies were conducted in anesthetized or passively listening animals. These conditions may not fully engage the auditory cortico-collicular pathway. Moreover, the extent of cortical inactivation in prior studies was sometimes ambiguous, which complicates interpreting modest or negative results.

Here, the authors' goal is to directly test if the auditory cortex is necessary for behaviorally relevant activity in IC neurons. They conclude that surprisingly, task relevant activity in cortico-recipient IC neuron persists in absence of auditory cortico-collicular transmission. To this end, a major strength of the paper is that the authors combine a sound-detection behavior with clever approaches that unambiguously overcome the limitations of past studies.

First the authors inject a transsynaptic virus into the auditory cortex, thereby expressing a genetically encoded calcium indicator in the auditory cortex's postsynaptic targets in the IC. This powerful approach enables 2-photon Ca^{2+} imaging from IC neurons that unambiguously receive monosynaptic input from auditory cortex. Thus, any effect of cortical silencing should be maximally observable in this neuronal population. Second, they abrogate auditory cortico-collicular transmission using lesions of auditory cortex. This "sledgehammer" approach is arguably the most direct test of whether cortico-recipient IC neurons will continue to encode task-relevant information in absence of descending feedback. Indeed, their method circumvents the known limitations of more modern optogenetic or chemogenetic silencing, e.g. variable efficacy.

The authors have revised their manuscript and adequately addressed the major concerns. Although more in depth analyses of these rich datasets are definitely possible, the current results nevertheless stand on their own. Indeed, the work serves as a beacon to move away from the idea that cortico-collicular projections function primarily to impart behavioral relevance upon auditory midbrain neurons. This knowledge inspires a search for alternative explanations as to the role of auditory cortico-collicular synapses in behavior.

<https://doi.org/10.7554/eLife.89950.2.sa2>

Reviewer #2 (Public Review):

Summary:

This study takes a new approach to studying the role of corticofugal projections from auditory cortex to inferior colliculus. The authors performed two-photon imaging of cortico-recipient IC neurons during a click detection task in mice with and without lesions of auditory cortex. In both groups of animals, they observed similar task performance and relatively small differences in the encoding of task-response variables in the IC population.

They conclude that non-cortical inputs to the IC provide can substantial task-related modulation, at least when AC is absent.

Strengths:

This study provides valuable new insight into big and challenging questions around top-down modulation of activity in the IC. The approach here is novel and appears to have been executed thoughtfully. Thus, it should be of interest to the community.

Weaknesses:

There are however, substantial concerns about the interpretation of the findings and limitations to the current analysis. In particular, Analysis of single unit activity is absent, making interpretation of population clusters and decoding less interpretable. These concerns should be addressed to make sure that the results can be interpreted clearly in an active field that already contains a number of confusing and possibly contradictory findings.

<https://doi.org/10.7554/eLife.89950.2.sa1>

Reviewer #3 (Public Review):

Summary:

This study aims to demonstrate that cortical feedback is not necessary to signal behavioral outcome to shell neurons of the inferior colliculus during a sound detection task. The demonstration is achieved in a very clear manner by the observation of the activity of cortico-receptient neurons in animals which have received lesions of the auditory cortex. The experiment shows that neither behavior performance nor neuronal responses are significantly impacted by cortical lesions except for the case of partial lesions which seem to have a disruptive effect on behavioral outcome signaling.

Strengths:

The demonstration of the main conclusions is based on state-of-the-art, carefully controlled methods and is highly convincing. There is an in depth discussion of the different effects of auditory cortical lesions on sound detection behavior.

Weaknesses:

The description of feedback signals could be more detailed although it is difficult to achieve good temporal resolution with the calcium imaging technique necessary for targeting cortico-receptient neurons.

<https://doi.org/10.7554/eLife.89950.2.sa0>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:

We thank the reviewers for the detailed assessment of our work as well as their praise and constructive feedback which helped us to significantly improve our manuscript.

Reviewer #1 (Public Review):

The inferior colliculus (IC) is the central auditory system's major hub. It integrates ascending brainstem signals to provide acoustic information to the auditory thalamus. The superficial layers of the IC ("shell" IC regions as defined in the current manuscript) also receive a massive descending projection from the auditory cortex. This auditory cortico-collicular pathway has long fascinated the hearing field, as it may provide a route to funnel "high-level" cortical signals and impart behavioral salience upon an otherwise behaviorally agnostic midbrain circuit.

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Here, the authors' goal is to directly test if auditory cortex is necessary for behaviorally relevant activity in IC neurons. They conclude that surprisingly, task relevant activity in cortico-recipient IC neuron persists in absence of auditory cortico-collicular transmission. To this end, a major strength of the paper is that the authors combine a sound-detection behavior with clever approaches that unambiguously overcome the limitations of past studies.

First, the authors inject a transsynaptic virus into the auditory cortex, thereby expressing a genetically encoded calcium indicator in the auditory cortex's postsynaptic targets in the IC. This powerful approach enables 2-photon Ca²⁺ imaging from IC neurons that unambiguously receive monosynaptic input from auditory cortex. Thus, any effect of cortical silencing should be maximally observable in this neuronal population. Second, they abrogate auditory cortico-collicular transmission using lesions of auditory cortex. This "sledgehammer" approach is arguably the most direct test of whether cortico-recipient IC neurons will continue to encode task-relevant information in absence of descending feedback. Indeed, their method circumvents the known limitations of more modern optogenetic or chemogenetic silencing, e.g. variable efficacy.

I also see three weaknesses which limit what we can learn from the authors' hard work, at least in the current form. I want to emphasize that these issues do not reflect any fatal flaw of the approach. Rather, I believe that their datasets likely contain the treasure-trove of knowledge required to completely support their claims.

(1) The conclusion of this paper requires the following assumption to be true: That the difference in neural activity between Hit and Miss trials reflects "information beyond the physical attributes of sound." The data presentation complicates asserting this assumption. Specifically, they average fluorescence transients of all Hit and all Miss trials

in their detection task. Yet, Figure 3B shows that mice's d' depends on sound level, and since this is a detection task the smaller d' at low SPLs presumably reflects lower Hit rates (and thus higher Miss rates). As currently written, it is not clear if fluorescence traces for Hits arise from trials where the sound cue was played at a higher sound level than on Miss trials. Thus, the difference in neural activity on Hit and Miss trials could indeed reflect mice's behavior (licking or not licking). But in principle could also be explained by higher sound-evoked spike rates on Hit compared to Miss trials, simply due to louder click sounds. Indeed, the amplitude and decay tau of their indicator GCaMP6f is non-linearly dependent on the number and rate of spikes (Chen et al., 2013), so this isn't an unreasonable concern.

(2) The authors' central claim effectively rests upon two analyses in Figures 5 and 6. The spectral clustering algorithm of Figure 5 identifies 10 separate activity patterns in IC neurons of control and lesioned mice; most of these clusters show distinct activity on averaged Hit and Miss trials. They conclude that although the proportions of neurons from control and lesioned mice in certain clusters deviates from an expected 50/50 split, neurons from lesioned mice are still represented in all clusters. A significant issue here is that in addition to averaging all Hits and Miss trials together, the data from control and lesioned mice are lumped for the clustering. There is no direct comparison of neural activity between the two groups, so the reader must rely on interpreting a row of pie charts to assess the conclusion. It's unclear how similar task relevant activity is between control and lesioned mice; we don't even have a ballpark estimate of how auditory cortex does or does not contribute to task relevant activity. Although ideally the authors would have approached this by repeatedly imaging the same IC neurons before and after lesioning auditory cortex, this within-subjects design may be unfeasible if lesions interfere with task retention. Nevertheless, they have recordings from hundreds to thousands of neurons across two groups, so even a small effect should be observable in a between-groups comparison.

(3) In Figure 6, the authors show that logistic regression models predict whether the trial is a Hit or Miss from their fluorescence data. Classification accuracy peaks rapidly following sound presentation, implying substantial information regarding mice's actions. The authors further show that classification accuracy is reduced, but still above chance in mice with auditory cortical lesions. The authors conclude from this analysis task relevant activity persists in absence of auditory cortex. In principle I do not disagree with their conclusion.

The weakness here is in the details. First, the reduction in classification accuracy of lesioned mice suggests that auditory cortex does nevertheless transmit some task relevant information, however minor it may be. I feel that as written, their narrative does not adequately highlight this finding. Rather one could argue that their results suggest redundant sources of task-relevant activity converging in the IC. Secondly, the authors conclude that decoding accuracy is impaired more in partially compared to fully lesioned mice. They admit that this conclusion is at face value counterintuitive, and provide compelling mechanistic arguments in the Discussion. However, aside from shaded 95% CIs, we have no estimate of variance in decoding accuracy across sessions or subjects for either control or lesioned mice. Thus we don't know if the small sample sizes of partial ($n = 3$) and full lesion ($n = 4$) groups adequately sample from the underlying population. Their result of Figure 6B may reflect spurious sampling from tail ends of the distributions, rather than a true non-monotonic effect of lesion size on task relevant activity in IC.

Our responses to the 'recommendations for the authors' below lay out in detail how we addressed each comment and concern. Besides filling in key information about how our original analysis aimed at minimizing any potential impact of differences in sound level

distributions - namely that trials used for decoding were limited to a subset of sound levels - and which was accidentally omitted in the original manuscript, we have now carried out several additional analyses.

We would like to highlight one of these because it supplements both the clustering and decoding analysis that we conducted to compare hit and miss trial activity, and directly addresses what the reviewer identified as our work's main weakness (a possible confound between animal behavior and sound level distributions) and the request for an analysis that operates at the level of single units rather than the population level. Specifically, we assessed, separately for each recorded neuron, whether there was a statistically significant difference in the magnitude of neural activity between hit and miss trials. This approach allowed us to fully balance the numbers of hit and miss trials at each sound level that were entered into the analysis. The results revealed that a large proportion (close to 50%) of units were task modulated, i.e. had significantly different response magnitudes between hit and miss trials, and that this proportion was not significantly different between lesioned and non-lesioned mice. We hope that this, together with the rest of our responses, convincingly demonstrates that the shell of the IC encodes mouse sound detection behavior even when top-down input from the auditory cortex is absent.

Reviewer #2 (Public Review):

Summary:

This study takes a new approach to studying the role of corticofugal projections from auditory cortex to inferior colliculus. The authors performed two-photon imaging of cortico-recipient IC neurons during a click detection task in mice with and without lesions of auditory cortex. In both groups of animals, they observed similar task performance and relatively small differences in the encoding of task-response variables in the IC population. They conclude that non-cortical inputs to the IC provide can substantial task-related modulation, at least when AC is absent. Strengths:

This study provides valuable new insight into big and challenging questions around top-down modulation of activity in the IC. The approach here is novel and appears to have been executed thoughtfully. Thus, it should be of interest to the community.

Weaknesses: There are, however, substantial concerns about the interpretation of the findings and limitations to the current analysis. In particular, Analysis of single unit activity is absent, making interpretation of population clusters and decoding less interpretable. These concerns should be addressed to make sure that the results can be interpreted clearly in an active field that already contains a number of confusing and possibly contradictory findings.

Our responses to the 'recommendations for the authors' below lay out in detail how we addressed each comment and concern. Several additional analyses have now been carried out including ones that operate at the level of single units rather than the population level, as requested by the reviewer. We would like to briefly highlight one here because it supplements both the clustering and decoding analysis that we conducted to compare hit and miss trial activity and directly addresses what the other reviewers identified as our work's main weakness (a possible confound between animal behavior and sound level distributions). Specifically, we assessed, separately for each recorded neuron, whether there was a statistically significant difference in the magnitude of neural activity between hit and miss trials. This approach allowed us to fully balance the numbers of hit and miss trials at each sound level that were entered into the analysis. The results revealed that a large proportion (close to 50%) of units were task modulated, i.e. had significantly different response magnitudes between hit and miss trials, and that this proportion was not significantly different between lesioned and non-lesioned mice. We hope that this, together

with the rest of our responses, convincingly demonstrates that the shell of the IC encodes mouse sound detection behavior even when top-down input from the auditory cortex is absent.

Reviewer #3 (Public Review):

Summary:

This study aims to demonstrate that cortical feedback is not necessary to signal behavioral outcome to shell neurons of the inferior colliculus during a sound detection task. The demonstration is achieved by the observation of the activity of cortico-recipient neurons in animals which have received lesions of the auditory cortex. The experiment shows that neither behavior performance nor neuronal responses are significantly impacted by cortical lesions except for the case of partial lesions which seem to have a disruptive effect on behavioral outcome signaling. Strengths:

The experimental procedure is based on state of the art methods. There is an in depth discussion of the different effects of auditory cortical lesions on sound detection behavior. Weaknesses:

The analysis is not documented enough to be correctly evaluated. Have the authors pooled together trials with different sound levels for the key hit vs miss decoding/clustering analysis? If so, the conclusions are not well supported, as there are more misses for low sound levels, which would completely bias the outcome of the analysis. It would possible that the classification of hit versus misses actually only reflects a decoding of sound level based on sensory responses in the colliculus, and it would not be surprising then that in the presence or absence of cortical feedback, some neurons responds more to higher sound levels (hits) and less to lower sound levels (misses). It is important that the authors clarify and in any case perform an analysis in which the classification of hits vs misses is done only for the same sound levels. The description of feedback signals could be more detailed although it is difficult to achieve good temporal resolution with the calcium imaging technique necessary for targeting cortico-recipient neurons.

Our responses to the ‘recommendations for the authors’ below lay out in detail how we addressed each comment and concern. Besides filling in key information about how our original analysis aimed at minimizing any potential impact of differences in sound level distributions - namely that trials used for decoding were limited to a subset of sound levels - and which was accidentally omitted in the original manuscript, we have now carried out several additional analyses to directly address what the reviewer identified as our work’s main weakness (a possible confound between animal behavior and sound level distributions). This includes an analysis in which we were able to demonstrate for one imaging session with a sufficiently large number of trials that limiting the trials entered into the decoding analysis to those from a single sound level did not meaningfully impact decoding accuracy. We would like to highlight another new analysis here because it supplements both the clustering and decoding analyses that we conducted to compare hit and miss trial activity and addresses the other reviewers’ request for an analysis that operates at the level of single units rather than the population level. Specifically, we assessed, separately for each recorded neuron, whether there was a statistically significant difference in the magnitude of neural activity between hit and miss trials. This approach allowed us to fully balance the numbers of hit and miss trials at each sound level that were entered into the analysis. The results revealed that a large proportion (close to 50%) of units were task modulated, i.e. had significantly different response magnitudes between hit and miss trials, and that this proportion was not significantly different between lesioned and non-lesioned mice. We hope that this, together with the rest of our responses, convincingly demonstrates

that the shell of the IC encodes mouse sound detection behavior even when top-down input from the auditory cortex is absent.

Reviewer #1 (Recommendations For The Authors):

Thank you for the opportunity to read your paper. I think the conclusion is exciting. Indeed, you indicate that perhaps contrary to many of our (untested) assumptions, task-relevant activity in the IC may persist in absence of auditory cortex.

As mentioned in my public review: Despite my interest in the work, I also think that there are several opportunities to significantly strengthen your conclusions. I feel this point is important because your work will likely guide the efforts of future students and post-docs working on this topic. The data can serve as a beacon to move the field away from the (somewhat naïve) idea that the evolved forebrain imparts behavioral relevance upon an otherwise uncivilized midbrain. This knowledge will inspire a search for alternative explanations. Indeed, although you don't highlight it in your narrative, your results dovetail nicely with several studies showing task-relevant activity in more ventral midbrain areas that project to the IC (e.g., pedunculo pontine nuclei; see work from Hikosaka in monkeys, and more recently in mice from Karel Svoboda's lab).

Thanks for the kind words.

These studies, in particular the work by Inagaki et al. (2022) outlining how the transformation of an auditory go signal into movement could be mediated via a circuit involving the PPN/MRN (which might rely on the NLL for auditory input) and the motor thalamus, are indeed highly relevant.

We made the following changes to the manuscript text.

Line 472: "...or that the auditory midbrain, thalamus and cortex are bypassed entirely if simple acoustic motor transformations, such as licking a spout in response to a sound, are handled by circuits linking the auditory brainstem and motor thalamus via pedunculo pontine and midbrain reticular nuclei (Inagaki et al., 2022)."

The beauty of the eLife experiment is that you are free to incorporate or ignore these suggestions. After all, it's your paper, not mine. Nevertheless, I hope you find my comments useful.

First, a few suggestions to address my three comments in the public review.

Suggestion for public comment #1: An easy way to address this issue is to average the neural activity separately for each trial outcome at each sound level. That way you can measure if fluorescence amplitude (or integral) varies as a function of mice's action rather than sound level. This approach to data organization would also open the door to the additional analyses for addressing comment #2, such as directly comparing auditory and putatively non-auditory activity in neurons recorded from control and lesioned mice.

We have carried out additional analyses for distinguishing between the two alternative explanations of the data put forward by the reviewer: That the difference in neural activity between hit and miss trials reflects a) behavior or b) sound level (more precisely: differences in response magnitude arising from a higher proportion of high-sound-level trials in the hit trial group than in the miss trial group). If the data favored b), we would expect no difference in activity between hit and miss trials when plotted separately for each sound level. The new Figure 4 - figure supplement 1 indicates that this is not the case. Hit and miss trial activity are clearly distinct even when plotted separately for different sound levels, confirming that this difference in activity reflects the animals' behavior rather than sensory information.

Changes to manuscript.

Line 214: “While averaging across all neurons cannot capture the diversity of responses, the averaged response profiles suggest that it is mostly trial outcome rather than the acoustic stimulus and neuronal sensitivity to sound level that shapes those responses (Figure 4 – figure supplement 1).”

Additionally, we assessed for each neuron separately whether there was a significant difference between hit and miss trial activity and therefore whether the activity of the neuron could be considered “task-modulated”. To achieve this, we used equal numbers of hit and miss trials at each sound level to ensure balanced sound level distributions and thus rule out any potential confound between sound level distributions and trial outcome. This analysis revealed that the proportion of task-modulated neurons was very high (close to 50%) and not significantly different between lesioned and non-lesioned mice (Figure 6 - figure supplement 3).

Changes to the manuscript.

Line 217: “Indeed, close to half (1272 / 2649) of all neurons showed a statistically significant difference in response magnitude between hit and miss trials...”

Line 307: “Although the proportion of individual neurons with distinct response magnitudes in hit and miss trials in lesioned mice did not differ from that in non-lesioned mice, it was significantly lower when separating out mice with partial lesions (Figure 6 – figure supplement 3).”

Differences in the distributions of sound levels in the different trial types could also potentially confound the decoding into hit and miss trials. Our original analysis was actually designed to take this into account but, unfortunately, we failed to include sufficient details in the methods section.

Changes to the manuscript.

Line 710: “Rather than including all the trials in a given session, only trials of intermediate difficulty were used for the decoding analysis. More specifically, we only included trials across five sound levels, comprising the lowest sound level that exceeded a d' of 1.5 plus the two sound levels below and above that level. That ensured that differences in sound level distributions would be small, while still giving us a sufficient number of trials to perform the decoding analysis.”

In this context, it is worth bearing in mind that a) the decoding analysis was done on a frame-by-frame basis, meaning that the decoding score achieved early in the trial has no impact on the decoding score at later time points in the trial, b) sound-driven activity predominantly occurs immediately after stimulus onset and is largely over about 1 s into the trial (see cluster 3, for instance, or average miss trial activity in Figure 4 – figure supplement 1), c) decoding performance of the behavioral outcome starts to plateau 500-1000 ms into the trial and remains high until it very gradually begins to decline after about 2 s into the trial. In other words, decoding performance remains high far longer than the stimulus would be expected to have an impact on the neurons' activity. Therefore, we would expect any residual bias due to differences in the sound level distribution that our approach did not control for to be restricted to the very beginning of the trial and not to meaningfully impact the conclusions derived from the decoding analysis.

Finally, we carried out an additional decoding analysis for one imaging session in which we had a sufficient number of trials to perform the analysis not only over the five (59, 62, 65, 68, 71 dB SPL) original sound levels, but also over a reduced range of three (62, 65, 68 dB SPL)

sound levels, as well as a single (65 dB SPL) sound level (Figure 6 - figure supplement 1). The mean sound level differences between the hit trial distributions and miss trial distributions for these three conditions were 3.08, 1.01 and 0 dB, respectively. This analysis suggests that decoding performance is not meaningfully impacted by changing the range of sound levels (and sound level distributions), other than that including fewer sound levels means fewer trials and thus noisier decoding.

Changes to manuscript.

Line 287: "...and was not meaningfully affected by differences in sound level distributions between hit and miss trials (Figure 6 – figure supplement 1)."

Suggestion for public comment #2: Perhaps a solution would be to display example neuron activity in each cluster, recorded in control and lesioned mice. The reader could then visually compare example data from the two groups, and immediately grasp the conclusion that task relevant activity remains in absence of auditory cortex. Additionally, one possibility might be to calculate the difference in neural activity between Hit and Miss trials for each task-modulated neuron. Then, you could compare these values for neurons recorded in control and lesion mice. I feel like this information would greatly add to our understanding of cortico-collicular processing.

I would also argue that it's perhaps more informative to show one (or a few) example recordings rather than averaging across all cells in a cluster. Example cells would give the reader a better handle on the quality of the imaging, and this approach is more standard in the field. Finally, it would be useful to show the y axis calibration for each example trace (e.g. Figure 5 supp 1). That is also pretty standard so we can immediately grasp the magnitude of the recorded signal.

We agree that while the information we provided shows that neurons from lesioned and nonlesioned groups are roughly equally represented across the clusters, it does not allow the reader to appreciate how similar the activity profiles of neurons are from each of the two groups. However, picking examples can be highly subjective and thus potentially open to bias. We therefore opted instead to display, separately for lesioned and non-lesioned mice, the peristimulus time histograms of all neurons in each cluster, as well as the cluster averages of the response profiles (Figure 5 - figure supplement 3). This, we believe, convincingly illustrates the close correspondence between neural activity in lesioned and non-lesioned mice across different clusters. All our existing and new figures indicate the response magnitude either on the figures' y-axis or via scale/color bars.

Changes to manuscript.

Line 254: "Furthermore, there was a close correspondence between the cluster averages of lesioned and non-lesioned mice (Figure 5 – figure supplement 3)."

Furthermore, we've now included a video of the imaging data which, we believe, gives the reader a much better handle on the data quality than further example response profiles would.

Changes to manuscript.

Line 197: "...using two-photon microscopy (Figure 4B, Video 1)."

Suggestion for public comment #3: In absence of laborious and costly follow-up experiments to boost the sample size of partial and complete lesion groups, it may be more prudent to simply tone down the claims that lesion size differentially impacts decoding accuracy. The results of this analysis are not necessary for your main claims.

Our new results on the proportions of ‘task-modulated’ neurons (Figure 6 - figure supplement 3) across different experimental groups show that there is no difference between non-lesioned and lesioned mice as a whole, but mice with partial lesions have a smaller proportion of taskmodulated neurons than the other two groups. While this corroborates the results of the decoding analysis, we certainly agree that the small sample size is a caveat that needs to be acknowledged.

Changes to manuscript.

Line 477: “Some differences were observed for mice with only partial lesions of the auditory cortex.

Those mice had a lower proportion of neurons with distinct response magnitudes in hit and miss trials than mice with (near-)complete lesions. Furthermore, trial outcomes could be read out with lower accuracy from these mice. While this finding is somewhat counterintuitive and is based on only three mice with partial lesions, it has been observed before that smaller lesions...”

A few more suggestions unrelated to public review:

Figure 1: This is somewhat of an oddball in this manuscript, and its inclusion is not necessary for the main point. Indeed, the major conclusion of Fig 1 is that acute silencing of auditory cortex impairs task performance, and thus optogenetic methods are not suitable to test your hypothesis. However, this conclusion is also easily supported from decades of prior work, and thus citations might suffice.

We do not agree that these data can easily be substituted with citations of prior published work. While previous studies (Talwar et al., 2001, Li et al., 2017) have demonstrated the impact of acute pharmacological silencing on sound detection in rodents, pharmacological and optogenetic silencing are not equivalent. Furthermore, we are aware of only one published study (Kato et al., 2015) that investigated the impact of optogenetically perturbing auditory cortex on sound detection (others have investigated its impact on discrimination tasks). Kato et al. (2015) examined the effect of acute optogenetic silencing of auditory cortex on the ability of mice to detect the offsets of very long (5-9 seconds) sounds, which is not easily comparable to the click detection task employed by us. Furthermore, when presenting our work at a recent meeting and leaving out the optogenetics results due to time constraints, audience members immediately enquired whether we had tried an optogenetic manipulation instead of lesions. Therefore, we believe that these data represent a valuable piece of information that will be appreciated by many readers and have decided not to remove them from the manuscript.

A worst case scenario is that Figure 1 will detract from the reader's assessment of experimental rigor. The data of 1C are pooled from multiple sessions in three mice. It is not clear if the signed-rank test compares performance across $n = 3$ mice or $n = 13$ sessions. If the latter, a stats nitpicker could argue that the significance might not hold up with a nested analysis considering that some datapoints are not independent of one another. Finally, the experiment does not include a control group, gad2-cre mice injected with a EYFP virus. So as presented, the data are equally compatible with the pessimistic conclusion that shining light into the brain impairs mice's licking. My suggestion is to simply remove Figure 1 from the paper. Starting off with Figure 3 would be stronger, as the rest of the study hinges upon the knowledge that control and lesion mice's behavior is similar.

Instead of reporting the results session-wise and doing stats on the d' values, we now report results per mouse and perform stats on the proportions of hits and false alarms separately for

each mouse. The results are statistically significant for each mouse and suggest that the differences in d' are primarily caused by higher false alarm rates during the optogenetic perturbation than in the control condition.

Changes to manuscript.

New Figure 1.

We agree that including control mice not expressing ChR2 would be important for fully characterizing the optogenetic manipulation and that the lack of this control group should be acknowledged. However, in the context of this study, the outcome of performing this additional experiment would be inconsequential. We originally considered using an optogenetic approach to explore the contribution of cortical activity to IC responses, but found that this altered the animals' sound detection behavior. Whether that change in behavior is due to activation of the opsin or simply due to light being shone on the brain has no bearing on the conclusion that this type of manipulation is unsuitable for determining whether auditory cortex is required for the choice-related activity that we recorded in the IC.

Changes to manuscript.

Line 106: "Although a control group in which the auditory cortex was injected with an EYFP virus lacking ChR2 would be required to confirm that the altered behavior results from an opsin-independent perturbation of cortical activity, this result shows that this manipulation is also unsuitable..."

Figure 2, comment #1: The micrograph of panel B shows the densest fluorescence in the central IC. You interpret this as evidence of retrograde labeling of central IC neurons that project to the shell IC. This is a nice finding, but perhaps a more relevant micrograph would be to show the actual injection site in the shell layers. The rest of Figure 2 documents the non-auditory cortical sources of forebrain feedback. Since non-auditory cortical neurons may or may not target distinct shell IC sub-circuits, it's important to know where the retrograde virus was injected. Stylistic comment: The flow of the panels is somewhat unorthodox. Panel A and B follow horizontally, then C and D follow vertically, followed by E-H in a separate column. Consider sequencing either horizontally or vertically to maximize the reader's experience.

Figure 2, comment #2: It would also be useful to show more rostral sections from these mice, perhaps as a figure supplement, if you have the data. I think there is a lot of value here given a recent paper (Olthof et al., 2019 Jneuro) arguing that the IC receives corticofugal input from areas more rostral to the auditory cortex. So it would be beneficial for the field to know if these other cortical sources do or do not represent likely candidates for behavioral modulation in absence of auditory cortex.

Figure 2, comment #3: You have a striking cluster of retrogradely labeled PPC neurons, and I'm not sure PPC has been consistently reported as targeting the IC. It would be good to confirm that this is a "true" IC projection as opposed to viral leakage into the SC. Indeed, Figure 2, supplement 2 also shows some visual cortex neurons that are retrogradely labeled. This has bearing on the interpretations, because choice-related activity is rampant in PPC, and thus could be a potential source of the task relevant activity that persists in your recordings. This could be addressed as the point above, by showing the SC sections from these same mice.

All IC injections were made under visual guidance with the surface of the IC and adjacent brain areas fully exposed after removal of the imaging window. Targeting the IC and steering clear of surrounding structures, including the SC, was therefore relatively straightforward.

We typically observed strong retrograde labeling in the central nucleus after viral injections into the dorsal IC and, given the moderate injection volume (~50 nL at each of up to three sites), it was also typical to see spatially fairly confined labeling at the injection sites. For the mouse shown in Figure 2, we do not have further images of the IC. This was one of the earliest mice to be included in the study and we did not have access to an automatic slide scanner at the time. We had to acquire confocal images in a ‘manual’ and very time-consuming manner and therefore did not take further IC images for this mouse. We have now included, however, a set of images spanning the whole IC and the adjacent SC sections for the mouse for which we already show sections in Figure 2 - figure supplement 2. These were added as Figure 2 - figure supplement 3A to the manuscript. These images show that the injections were located in the caudal half of the IC and that there was no spillover into the SC - close inspection of those sections did not reveal any labeled cell bodies in the SC. Furthermore, we include as Figure 2 - figure supplement 3B a dozen additional rostral cortical sections of the same mouse illustrating corticocollicular neurons in regions spanning visual, parietal, somatosensory and motor cortex. Given the inclusion of the IC micrographs in the new supplementary figure, we removed panel B from Figure 2. This should also make it easier for the reader to follow the sequencing of the remaining panels.

Changes to manuscript.

New Figure 2 - figure supplement 3.

Line 159: “After the experiments, we injected a retrogradely-transported viral tracer (rAAV2-retrotdTomato) into the right IC to determine whether any corticocollicular neurons remained after the auditory cortex lesions (Figure 2, Figure 2 – figure supplement 2, Figure 2 – figure supplement 3). The presence of retrogradely-labeled corticocollicular neurons in non-temporal cortical areas (Figure 2) was not the result of viral leakage from the dorsal IC injection sites into the superior colliculus (Figure 2 – figure supplement 3).”

Line 495: “...projections to the IC, such as those originating from somatosensory cortical areas (Lohse et al., 2021; Lesicko et al., 2016) and parietal cortex may have contributed to the response profiles that we observed.

Figure 5 (see also public review point #2): I am not convinced that this unsupervised method yields particularly meaningful clusters; a grain of salt should be provided to the reader. For example, Clusters 2, 5, 6, and 7 contain neurons that pretty clearly respond with either short latency excitation or inhibition following the click sound on Hits. I would argue that neurons with such diametrically opposite responses should not be "classified" together. You can see the same issue in some of Namboodiri/Stuber's clustering (their Figure 1). It might be useful to make it clear to the reader that these clusters can reflect idiosyncrasies of the algorithm, the behavior task structure, or both.

We agree.

Changes to manuscript.

Line 666: “While clustering is a useful approach for organizing and visualizing the activity of large and heterogeneous populations of neurons, we need to be mindful that, given continuous distributions of response properties, the locations of cluster boundaries can be somewhat arbitrary and/or reflect idiosyncrasies of the chosen method and thus vary from one algorithm to another. We employed an approach very similar to that described in Namboodiri et al. (2019) because it is thought to produce stable results in high-dimensional neural data (Hirokawa et al. 2019).”

Methods:

How was a "false alarm" defined? Is it any lick happening during the entire catch trial, or only during the time period corresponding to the response window on stimulus trials?

The response window was identical for catch and stimulus trials and a false alarm was defined as licking during the response window of a catch trial.

Changes to manuscript.

Line 598: "During catch trials, neither licking ('false alarm') during the 1.5-second response window ..."

L597 and so forth: What's the denominator in the conversion from the raw fluorescence traces into $\Delta F/F$? Did you take the median or mode fluorescence across a chunk of time? Baseline subtract average fluorescence prior to click onset? Similarly, please provide some more clarification as to how neuropil subtraction was achieved. This information will help us understand how the classifier can decode trial outcome from data prior to sound onset.

Signal processing did not involve the subtraction of a pre-stimulus period.

Changes to manuscript.

Line 629: "Neuropil extraction was performed using default suite2p parameters (<https://suite2p.readthedocs.io/en/latest/settings.html>), neuropil correction was done using a coefficient of 0.7, and calcium $\Delta F/F$ signals were obtained by using the median over the entire fluorescence trace as F0. To remove slow fluctuations in the signal, a baseline of each neuron's entire trace was calculated by Gaussian filtering in addition to minimum and maximum filtering using default suite2p parameters. This baseline was then subtracted from the signal."

Was the experimenter blinded to the treatment group during the behavior experiments? If not, were there issues that precluded blinding (limited staffing owing to lab capacity restrictions during the pandemic)? This is important to clarify for the sake of rigor and reproducibility.

Changes to manuscript.

Line 574: "The experimenters were not blinded to the treatment group, i.e. lesioned or non-lesioned, but they were blind to the lesion size both during the behavior experiments and most of the data processing."

Minor:

L127-128: "In order to test...lesioned the auditory cortex bilaterally in 7 out of 16 animals". I would clarify this by changing the word animals to "mice" and 7 out of 16 by stating $n = 9$ and $n = 7$ are control and lesion groups, respectively.

Agreed.

Changes to manuscript.

Line 129: "...compared the performance of mice with bilateral lesions of the auditory cortex ($n = 7$) with non-lesioned controls ($n = 9$)"

L225-226: You rule out self-generated sounds as a likely source of behavioral modulation by citing Nate Sawtell's paper in the DCN. However, Stephen David's lab suggested that in

marmosets, post sound activity in central IC may in fact reflect self-generated sounds during licking. I suggest addressing this with a nod to SVD's work (Singla et al., 2017; but see Shaheen et al., 2021).

Agreed.

Changes to manuscript.

Line 243: "(Singla et al., 2017; but see Shaheen et al., 2021)"

Line 238 - 239: You state that proportions only deviate greater than 10% for one of the four statistically significant clusters. Something must be unclear here because I don't understand: The delta between the groups in the significant clusters of Fig 5C is (from left to right) 20%, 20%, 38%, and 12%. Please clarify.

Our wording was meant to convey that a deviation "from a 50/50 split" of 10% means that each side deviates from 50 by 10% resulting in a 40/60 (or 60/40) split. We agree that that has the potential to confuse readers and is not as clear as it could be and have therefore dropped the ambiguous wording.

Changes to manuscript.

Line 253: "...the difference between the groups was greater than 20% for only one of them."

L445: I looked at the cited Allen experiment; I'd be cautious with the interpretation here. A monosynaptic IC->striatum projection is news to me. I think Allen Institute used an AAV1-EGFP virus for these experiments, no? As you know, AAV1 is quite transsynaptic. The labeled fibers in striatum of that experiment may reflect disynaptic labeling of MGB neurons (which do project to striatum).

Agreed. We deleted the reference to this Allen experiment.

L650: Please define "network activity". Is this the fluorescence value for each ROI on each frame of each trial? Averaged fluorescence of each ROI per frame? Total frame fluorescence including neuropil? Depending on who you ask, each of these measures provides some meaningful readout of network activity, so clarification would be useful.

Changes to manuscript.

Line 707: "Logistic regression models were trained on the network activity of each session, i.e., the $\Delta F/F$ values of all ROIs in each session, to classify hit vs miss trials. This was done on a frame-by-frame basis, meaning that each time point (frame) of each session was trained separately.

Figure 3 narrative or legend: Listing the F values for the anova would be useful. There is pretty clearly a main effect of training session for hits, but what about for the false alarms? That information is important to solidify the result, and would help more specialized readers interpret the d-prime plot in this figure.

Agreed. There were significant main effects of training day for both hit rates and false alarm rates (as well as d').

Changes to manuscript.

Line 165: "The ability of the mice to learn and perform the click detection task was evident in increasing hit rates and decreasing false alarm rates across training days (Figure 3A, $p < 0.01$,

mixed-design ANOVAs).”

In summary, thank you for undertaking this work. Your conclusions are provocative, and thus will likely influence the field's direction for years to come.

Thank you for those kind words and valuable and constructive feedback, which has certainly improved the manuscript.

Reviewer #2 (Recommendations For The Authors):

MAJOR CONCERNS

(1) (Fig. 5) What fraction of individual neurons actually encode task-related information in each animal group? How many neurons respond to sound? The clustering and decoding analyses are interesting, but they obscure these simple questions, which get more directly at the main questions of the study. Suggested approach: For a direct comparison of AC-lesioned and -non-lesioned animals, why not simply compare the mean difference between PSTH response for each neuron individually? To test for trial outcome effects, compare Hit and Miss trials (same stimulus, different behavior) and for sound response effects, compare Hit and False alarm trials (same behavior, different response). How do you align for time in the latter case when there's no stimulus? Align to the first lick event. The authors should include this analysis or explain why their approach of jumping right to analysis of clusters is justified.

We have now calculated the fraction of neurons that encode trial outcome by comparing hit and miss trial activity. That fraction does not differ between non-lesioned animals and lesioned animals as a whole, but is significantly smaller in mice with partial lesions. The author's suggestion of comparing hit and false alarm trial activity to assess sound responsiveness is problematic because hit trials involve reward delivery and consumption. Consequently, they are behaviorally very different from false alarm trials (not least because hit trials tend to contain much more licking). Therefore, we calculated the fraction of neurons that respond to the acoustic stimulus by comparing activity before and after stimulus onset in miss trials. We found no significant difference between the non-lesioned and lesioned mice or between subgroups.

We have addressed these points with the following changes to the manuscript:

Line 217: “Indeed, close to half (1272 / 2649) of all neurons showed a statistically significant difference in response magnitude between hit and miss trials, while only a small fraction (97 / 2649) exhibited a significant response to the sound.”

Line 307: “Although the proportion of individual neurons with distinct response magnitudes in hit and miss trials in lesioned mice did not differ from that in non-lesioned mice, it was significantly lower when separating out mice with partial lesions (Figure 6 – figure supplement 3).”

Line 648: “Analysis of task-modulated and sound-driven neurons. To identify individual neurons that produced significantly different response magnitudes in hit and miss trials, we calculated the mean activity for each stimulus trial by taking the mean activity over the 5 seconds following stimulus presentation and subtracting the mean activity over the 2 seconds preceding the stimulus during that same trial. A Mann-Whitney U test was then performed to assess whether a neuron showed a statistically significant difference (Benjamini-Hochberg adjusted p-value of 0.05) in response magnitude between hit and miss trials. The analysis was performed using equal numbers of hit and miss trials at each sound level to ensure balanced sound level distributions. If, for a given sound level, there were more hit than miss trials, we randomly selected a sample of hit trials (without substitution) to match the sample size for

the miss trials and vice versa. Sounddriven neurons were identified by comparing the mean miss trial activity before and after stimulus presentation. Specifically, we performed a Mann-Whitney U test to assess whether there was a statistically significant difference (Benjamini-Hochberg adjusted p-value of 0.05) between the mean activity over the 2 seconds preceding the stimulus and the mean activity over the 1 second period following stimulus presentation.”

Some more specific concerns about focusing only on cluster-level and population decoding analysis are included below.

(2) (L 234) "larger field of view". Do task-related or lesion-dependent effects depend on the subregion of IC imaged? Some anatomists would argue that the IC shell is not a uniform structure, and concomitantly, task-related effects may differ between fields. Did coverage of IC subregions differ between experimental groups? Is there any difference in task related effects between subregions of IC? Or maybe all this work was carried out only in the dorsal area? The differences between lesioned and non-lesioned animals are relatively small, so this may not have a huge impact, but a more nuanced discussion that accounts for observed or potential (if not tested) differences between regions of the IC.

The specific subregion coverage could also impact the decoding analysis (Fig 6), and if possible it might be worth considering an interaction between field of view and lesion size on decoding.

Each day we chose a new imaging location to avoid recording the same neurons more than once and aimed to sample widely across the optically accessible surface of the IC. We typically stopped the experiment only when there were no more new areas to record from. In terms of the depth of the imaged neurons, we were limited by the fact that corticorecipient neurons become sparser with depth and that the signal available from the GCaMP6f labeling of the Ai95 mice becomes rapidly weaker with increasing distance from the surface. This meant that we recorded no deeper than 150 μm from the surface of the IC. Consequently, while there may have been some variability in the average rostrocaudal and mediolateral positioning of imaging locations from animal to animal due to differences between mice in how much of the IC surface was visible, cranial window positioning, and in neuronal labeling etc, our dataset is anatomically uniform in that all recorded neurons receive input from the auditory cortex and are located within 150 μm of the surface of the IC. Therefore, we think it highly unlikely that small sampling differences across animals could have a meaningful impact on the results.

Given that there is no consensus as to where the border between the dorsal and external/lateral cortices of the IC is located and that it is typically difficult to find reliable anatomical reference points (the location of the borders between the IC and surrounding structures is not always obvious during imaging, i.e. a transition from a labeled area to a dark area near the edge of the cranial window could indicate a border with another structure, but also the IC surface sloping away from the window or simply an unlabeled area within the IC), we made no attempt to assign our recordings from corticorecipient neurons to specific subdivisions of the IC.

Changes to manuscript.

Line 195: “We then proceeded to record the activity of corticorecipient neurons within about 150 μm of the dorsal surface of the IC using two-photon microscopy (Figure 4B, Video 1).”

Line 375: “We imaged across the optically accessible dorsal surface of the IC down to a depth of about 150 μm below the surface. Consequently, the neurons we recorded were located predominantly in the dorsal cortex. However, identifying the borders between different subdivisions of the IC is not straightforward and we cannot rule out the possibility that some were located in the lateral cortex.”

(3) (L 482-483) "auditory cortex is not required for the task-related activity recording in IC neurons of mice performing a sound detection task". Most places in the text are clearer, but this statement is confusing. Yes, animals with lesions can have a "normal"-looking IC, but does that mean that AC does not strongly modulate IC during this behavior in normal animals? The authors have shown convincingly that subcortical areas can both shape behavior and modulate IC normally, but AC may still be required for IC modulation in non-lesioned animals. Given the complexity of this system, the authors should make sure they summarize their results consistently and clearly throughout the manuscript.

The reviewer raises an important point. What we have shown is that corticorecipient dorsal IC neurons in mice without auditory cortex show neural activity during a sound detection task that is largely indistinguishable from the activity of mice with an intact auditory cortex. In lesioned mice, the auditory cortex is thus not required. Whether the IC activity of the non-lesioned group can be shaped by input from the auditory cortex in a meaningful way in other contexts, such as during learning, is a question that our data cannot answer.

Changes to manuscript.

Line 508: "While modulation of IC activity by this descending projection has been implicated in various functions, most notably in the plasticity of auditory processing, we have shown in mice performing a sound detection task that IC neurons show task-related activity in the absence of auditory cortical input."

LESSER CONCERNS

(L. 106-107) "Optogenetic suppression of cortical activity is thus also unsuitable..." It appears that behavior is not completely abolished by the suppression. One could also imagine using a lower dose of muscimol for partial inactivation of AC feedback. When some behavior persists, it does seem possible to measure task-related changes in the IC. This may not be necessary for the current study, but the authors should consider how these transient methods could be applied usefully in the Discussion. What about inactivation of cortical terminals in the IC? Is that feasible?

Our argument is not that acute manipulations are unsuitable because they completely abolish the behavior, but because they significantly alter the behavior. Although it would not be trivial to precisely measure the extent of pharmacological cortical silencing in behaving mice that have been fitted with a midbrain window, it should be possible to titrate the size of a muscimol injection to achieve partial silencing of the auditory cortex that does not fully abolish the ability to detect sounds. However, such an outcome would likely render the data uninterpretable. If no effect on IC activity was observed, it would not be possible to conclude whether this was due to the fact that the auditory cortex was only partially silenced or that projections from the auditory cortex have no influence on the recorded IC activity. Similarly, if IC activity was altered, it would not be possible to say whether this was due to altered descending modulation resulting from the (partially) silenced auditory cortex or to the change in behavior, which would likely be reflected in the choice-related activity measured in the IC.

Silencing of corticocollicular axons in the IC is potentially a more promising approach and we did devote a considerable amount of time and effort to establishing a method that would allow us to simultaneously image IC neurons while silencing corticocollicular axons, trying both eNpHR3.0 and Jaws with different viral labeling approaches and mouse lines. However, we ultimately abandoned those attempts because we were not convinced that we had achieved sufficient silencing or that we would be able to convincingly verify this.

Furthermore, axonal silencing comes with its own pitfalls and the interpretation of its consequences is not straightforward. Given that our discussion already contains a section (line 421) on axonal silencing, we do not feel there would be any benefit in adding to that.

(Figure 1). Can the authors break down the performance for FA and HR, as they do in Fig. 3? It would be helpful to know what aspect of behavior is impaired by the transient inactivation.

Good point. Figure 1 has been updated to show the results separately for hit rates, false alarms and d' . The new figure indicates that the change in d' is primarily a consequence of altered false alarm rates. Please also see our response to a related comment by reviewer #1.

Changes to manuscript.

New figure 1.

(Figure 4 legend). Minor: Please clarify, what is time 0 in panel C? Time of click presentation?

Yes, that is correct.

Changes to manuscript.

Line 209: "Vertical line at time 0 s indicates time of click presentation."

(L. 228-229). There has been a report of lick and other motor related activity in the IC - e.g., see Shaheen, Slee et al. (J Neurosci 2021), the timing of which suggests that some of it may be acoustically driven.

Thanks for pointing this out. Shaheen et al., 2021 should certainly have been cited by us in this context as well as in other parts of the manuscript.

Changes to manuscript.

Line 243: "(Singla et al., 2017; but see Shaheen et al., 2021)"

Also, have the authors considered measuring a peri-lick response? The difference between hit and miss trials could be perceptual or it could reflect differences in motor activity. This may be hard to tease apart, but, for example, one can test whether activity is stronger on trials with many licks vs. few licks?

(L. 261) "Behavior can be decoded..." similar or alternative to the previous question of evoked activity, can you decode lick events from the population activity?

The difference between hit and miss trial activity almost certainly partially reflects motor activity associated with licking. This was stated in the Discussion, but to make that point more explicitly, we now include a plot of average false alarm trial activity, i.e. trials without sound (catch trials) in which animals licked (but did not receive a reward).

Given a sufficient number of catch trials, it should be possible to decode false alarm and correct rejection trials. However, our experiment was not designed with that in mind and contains a much smaller number of catch trials than stimulus trials (approximately one tenth the number of stimulus trials), so we have not attempted this.

Changes to manuscript.

New Figure 4 - figure supplement 1.

(L. 315) *"Pre-stimulus activity..." Given reports of changes in activity related to pupil-indexed arousal in the auditory system, do the authors by any chance have information about pupil size in these datasets?*

Given that all recordings were performed in the dark, fluctuations in pupil diameter were relatively small. Therefore, we have not made any attempt to relate pupil diameter to any of the variables assessed in this manuscript.

(L. 412) *"abolishes sound detection". While not exactly the same task, the authors might comment on Gimenez et al (J Neurophys 2015) which argued that temporary or permanent lesioning of AC did not impair tone discrimination. More generally, there seems to be some disagreement about what effects AC lesions have on auditory behavior.*

Thank you for this suggestion. Gimenez et al. (2015) investigated the ability of freely moving rats to discriminate sounds (and, in addition, how they adapt to changes in the discrimination boundary). Broadly consistent with later reports by Ceballo et al. (2019) (mild impairment) and O'Sullivan et al. (2019) (no impairment), Gimenez et al. (2015) reported that discrimination performance is mildly impaired after lesioning auditory cortex. Where the results of Gimenez et al. (2015) stand out is in the comparatively mild impairments that were seen in their task when they used muscimol injections, which contrast with the (much) larger impairments reported by others (e.g. Talwar et al., 2001; Li et al., 2017; Jaramillo and Zador, 2014).

Changes to manuscript.

Line 433: "However, transient pharmacological silencing of the auditory cortex in freely moving rats (Talwar et al., 2001), as well as head-fixed mice (Li et al., 2017), completely abolishes sound detection (but see Gimenez et al., 2015)."

(L. 649) *"... were generally separable" Is the claim here that the clusters are really distinct from each other? This is unexpected, and it might be helpful if the authors could show this result in a figure.*

The half-sentence that this comment refers to has been removed from the methods section. Please also see a related comment by reviewer #1 which prompted us to add the following to the methods section.

Changes to manuscript.

Line 666: "While clustering is a useful approach for organizing and visualizing the activity of large and heterogeneous populations of neurons we need to be mindful that, given continuous distributions of response properties, the locations of cluster boundaries can be somewhat arbitrary and/or reflect idiosyncrasies of the chosen method and thus vary from one algorithm to another. We employed an approach very similar to that described in Namboodiri et al. (2019) because it is thought to produce stable results in high-dimensional neural data (Hirokawa et al. 2019)."

Reviewer #3 (Recommendations For The Authors):

(1) *The authors must absolutely clarify if the hit versus misses decoding and clustering analysis is done for a single sound level or for multiple sound levels (what is the fraction of trials for each sound level?). If the authors did it for multiple sound levels they should redo all analyses sound-level by sound-level, or for a single sound level if there is one that dominates. No doubt that there is information about the trial outcome in IC, but it should not be over-estimated by a confound with stimulus information.*

This is an important point. The original clustering analysis was carried out across different sound levels. We have now carried out additional analysis for distinguishing between two alternative explanations of the data, which were also raised by reviewer #1. – that the difference in neural activity between hit and miss trials could reflect a) the animals' behavior or b) relatively more hit trials at higher sound levels, which would be expected to produce stronger responses. If the data favored b), we would expect no difference in activity between hit and miss trials when plotted separately for different sound levels. The new figure 4 - figure supplement 1 indicates that that is not the case. Hit and miss trial activity are clearly distinct even when plotted separately for different sound levels, confirming that this difference in activity reflects the animals' behavior rather than sensory information.

We made the following changes to manuscript.

Line 214: “While averaging across all neurons cannot capture the diversity of responses, the averaged response profiles suggest that it is mostly trial outcome rather than the acoustic stimulus and neuronal sensitivity to sound level that shapes those responses (Figure 4 – figure supplement 1).”

Differences in the distributions of sound levels in the different trial types could also potentially confound the decoding into hit and miss trials. Our analysis actually aimed to take this into account but, unfortunately, we failed to include sufficient details in the methods section.

Changes to manuscript.

Line 710: “Rather than including all the trials in a given session, only trials of intermediate difficulty were used for the decoding analysis. More specifically, we only included trials across five sound levels, comprising the lowest sound level that exceeded a d' of 1.5 plus the two sound levels below and above that level. That ensured that differences in sound level distributions would be small, while still giving us a sufficient number of trials to perform the decoding analysis.”

In this context, it is worth bearing in mind that a) the decoding analysis was done on a frame-by-frame basis, meaning that the decoding score achieved early in the trial has no impact on the decoding score at later time points in the trial, b) sound-driven activity predominantly occurs immediately after stimulus onset and is largely over about 1 s into the trial (see cluster 3, for instance, or average miss trial activity in figure 4 - figure supplement 1), c) decoding performance of the behavioral outcome starts to plateau 500-1000 ms into the trial and remains high until it very gradually begins to decline after about 2 s into the trial. In other words, decoding performance remains high far longer than the stimulus would be expected to have an impact on the neurons' activity. Therefore, we would expect any residual bias due to differences in the sound level distribution that our approach did not control for to be restricted to the very beginning of the trial and not to meaningfully impact the conclusions derived from the decoding analysis.

Furthermore, we carried out an additional decoding analysis for one imaging session in which we had a sufficient number of trials to perform the analysis not only over the five (59, 62, 65, 68, 71 dB SPL) original sound levels, but also over a reduced range of three (62, 65, 68 dB SPL) sound levels, as well as a single (65 dB SPL) sound level (Figure 6 - figure supplement 1). The mean sound level difference between the hit trial distributions and miss trial distributions for these three conditions were 3.08, 1.01 and 0 dB, respectively. This analysis suggests that decoding performance is not meaningfully impacted by changing the range of sound levels (and sound level distributions) other than that including fewer sound levels means fewer trials and thus noisier decoding.

Changes to manuscript.

Line 287: "...and was not meaningfully affected by differences in sound level distributions between hit and miss trials (Figure 6 – figure supplement 1)."

Finally, in order to supplement the decoding analysis, we determined for each individual neuron whether there was a significant difference between the average hit and average miss trial activity. Note that this was done using equal numbers of hit and miss trials at each sound level to ensure balanced sound level distributions and to rule out any potential confound of sound level. This revealed that the proportion of neurons containing "information about trial outcome" was generally very high, close to 50% on average, and not significantly different between lesioned and non-lesioned mice.

Changes to manuscript.

Line 307: "Although the proportion of individual neurons with distinct response magnitudes in hit and miss trials in lesioned mice did not differ from that in non-lesioned mice, it was significantly lower when separating out mice with partial lesions (Figure 6 – figure supplement 3)."

Line 648: "Analysis of task-modulated and sound-driven neurons. To identify individual neurons that produced significantly different response magnitudes in hit and miss trials, we calculated the mean activity for each stimulus trial by taking the mean activity over the 5 seconds following stimulus presentation and subtracting the mean activity over the 2 seconds preceding the stimulus during that same trial. A Mann-Whitney U test was then performed to assess whether a neuron showed a statistically significant difference (Benjamini-Hochberg adjusted p-value of 0.05) in response magnitude between hit and miss trials. The analysis was performed using equal numbers of hit and miss trials at each sound level to ensure balanced sound level distributions. If, for a given sound level, there were more hit than miss trials we randomly selected a sample of hit trials (without substitution) to match the sample size for the miss trials and vice versa."

(2) I have the feeling that the authors do not exploit fully the functional data recorded with two-imaging. They identify several cluster but do not describe their functional differences. For example, cluster 3 is obviously mainly sensory driven as it is not modulated by outcome. This could be mentioned. This could also be used to rule out that trial outcome is the results of insufficient sensory inputs. Could this cluster be used to predict trial outcome at the onset response? Could it be used to predict the presence of the sound, and with which accuracy. The authors discuss a bit the different cluster type, but in a very elusive manner. I recognize that one should be careful with the use of signal analysis methods in calcium imaging but a simple linear deconvolution of the calcium dynamic who help to illustrate the conclusions that the authors propose based on peak responses. It would also be very interesting to align the clusters responses (deconvolved) to the timing of licking and rewards event to check if some clusters do not fire when mice perform licks before the sound comes. It would help clarify if the behavioral signals described here require both the presence of the sound and the behavioral action or are just the reflection of the motor command. As noted by the authors, some clusters have late peak responses (2 and 5). However, 2 and 5 are not equivalent and a deconvolution would evidence that much better. 2 has late onset firing. 5 has early onset but prolonged firing.

We agree with the reviewer's statement that "cluster 3 is obviously mainly sensory driven". In the Discussion we refer to cluster 3 as having a "largely behaviorally invariant response profile to the auditory stimulus" (line X), which is consistent with the statement of the reviewer. With regard to the reviewer's suggestion to describe the "functional differences" between the clusters, we would like to refer to the subsequent three sentences of the same paragraph in which we speculate on the cognitive and behavioral variables that may underlie

the response profiles of different clusters. Given the limitations imposed by the task structure, we do not think it is justified to expand on this.

We have added an additional analysis in order to explicitly address the question of which neurons are sound responsive (please also see response to point 3 below and to point 1 of reviewer #2). That trial outcome could be predicted on the basis of only the sound-responsive neurons' activity during the initial period of the trial ("predict trial outcome at the onset response") is unlikely given their small number (only 97 of 2649 neurons show a statistically significant sound-evoked response) and given that only a minority (42/98) of those sound-driven neurons are also modulated by trial outcome within that initial trial period (i.e. 0-1s after stimulus onset; data not shown).

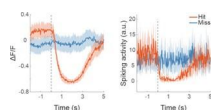
Changes to manuscript.

Line 219: "..., while only a small fraction (97 / 2649) exhibited a significant response to the sound."

Line 658: "Sound-driven neurons were identified by comparing the mean miss trial activity before and after stimulus presentation. Specifically, we performed a Mann-Whitney U test to assess whether there was a statistically significant difference (Benjamini-Hochberg adjusted p-value of 0.05) between the mean activity over the 2 seconds preceding the stimulus and the mean activity over the 1 second period following stimulus presentation. This analysis was performed using miss trials with click intensities from 53 dB SPL to 65 dB SPL (many sessions contained very few or no miss trials at higher sound levels)."

While calcium traces represent an indirect measure of neural activity, deconvolution does not necessarily provide an accurate picture of the spiking underlying those traces and has the potential to introduce additional problems. For instance, deconvolution algorithms tend to perform poorly at inferring the spiking of inhibited neurons (Vanvalleghem et al., 2021). Given that suppression is such a prominent feature of IC activity and is evident both in our calcium data as well as in the electrophysiology data of others (Franceschi and Barkat, 2021), we decided against using deconvolved spikes in our analyses. See also the side-by-side comparison below of the hit and miss trial activity of one example neuron based on either the calcium trace (left) or deconvolved spikes (right) (extracted using the OASIS algorithm (Friedrich et al., 2017) incorporated into suite2p (Pachitariu et al., 2016)).

Author response image 1.



(3) Along the same line, the very small proportion of really sensory driven neurons (cluster 3) is not discussed. Is it what one would expect in typical shell or core IC neurons?

As requested by reviewer #2 and mentioned in response to the previous point, we have now quantified the number of neurons in the dataset that produced significant responses to sound (97 / 2649). For a given imaging area, the fraction of neurons that show a statistically significant change in neural activity following presentation of a click of between 53 dB SPL and 65 dB SPL rarely exceeded ten percent. While that number is low, it is not necessarily surprising given the moderate intensity and very short duration of the stimuli. For comparison: Using the same transgenics, labeling approach and imaging setup and presenting 200-ms long pure tones at 60 dB SPL with frequencies between 2 kHz and 64 kHz,

we typically find that between a quarter and a third of neurons in a given imaging area exhibit a statistically significant response (data not shown).

Changes to manuscript.

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Line 220: “While the number of sound-responsive neurons is low, it is not necessarily surprising given the moderate intensity and very short duration of the stimuli. For comparison: Using the same transgenics, labeling approach and imaging setup and presenting 200-ms long pure tones at 60 dB SPL with frequencies between 2 kHz and 64 kHz, we typically find that between a quarter and a third of neurons in a given imaging area exhibit a statistically significant response (data not shown).”

(4) In the discussion, the interpretation of different transient and permanent cortical inactivation experiment is very interesting and well balanced given the complexity of the issue. There is nevertheless a comment that is difficult to follow. The authors state:

If cortical lesioning results in a greater weight being placed on the activity in spared subcortical circuits for perceptual judgements, we would expect the accuracy with which trial-by-trial outcomes could be read out from IC neurons to be greater in mice without auditory cortex. However, that was not the case.

However, there is no indication that the activity they observe in shell IC is causal to the behavioral decision and likely it is not. There is also no indication that the behavioral signals seen by the authors reflect the weight put on the subcortical pathway for behavior. I find this argument handwavy and would remove it.

While we are happy to amend this section, we would not wish to remove it because a) we believe that the point we are trying to make here is an important and reasonable one and b) because it is consistent with the reviewer’s comment. Hopefully, the following will make this clearer: In order for the mouse to make a perceptual judgment and act upon it - in the context of our task, hearing a sound and then licking a spout - auditory information needs to be read out and converted into a motor command. If the auditory cortex normally plays a key role in such perceptual judgments, cortical lesions would require the animal to base its decisions on the information available from the remaining auditory structures, potentially including the auditory midbrain. This might result in a greater correspondence between the mouse’s behavior and the neural activity in those structures. That we did not observe this outcome for the IC could mean that the auditory cortex did not contribute to the relevant perceptual judgments (sound detection) in the first place. Therefore, no reweighting of signals from the other structures is necessary. Alternatively, greater weight might be placed exclusively on structures other than the auditory midbrain, e.g. the thalamus. The latter would imply that the contribution of the IC remains the same. This includes the possibility that the IC shell does not play a causal role in the behavioral decision – in either control mice or mice with cortical lesions – as suggested by the reviewer.

Changes to manuscript.

Line 471: “This could imply that, following cortical lesions, greater weight is placed on structures other than the IC, with the thalamus being the most likely candidate, ..”

| (5) *In Fig. 5 the two colors used in B and C are the same although they describe different categories.*

The dark green and ‘deep orange’ we used to distinguish between non-lesioned and lesioned in Figure 5C are slightly lighter than the colors used to distinguish between these two categories in other figures and therefore might be more easily confused with the blue and red in Figure 5B. This has been changed.