Reversible inactivation of ferret auditory cortex impairs spatial and non-spatial hearing

Stephen M. Town[1*], Katherine C. Wood[2], Katarina C. Poole[1] & Jennifer K. Bizley[1*]

Author Affiliations:

[1]Ear Institute, University College London, London, UK.

[2] Department of Otorhinolaryngology: HNS, Department of Neuroscience, University of Pennsylvania, Philadelphia, PA.

Corresponding Authors:

Stephen Town (s.town@ucl.ac.uk), UCL Ear Institute, 332 Gray's Inn Road, London, UK

Jennifer Bizley (j.bizley@ucl.ac.uk), UCL Ear Institute, 332 Gray's Inn Road, London, UK

Abstract

A central question in auditory neuroscience is how far brain regions are functionally specialized for processing specific sound features such as sound location and identity. In auditory cortex, correlations between neural activity and sounds support both the specialization of distinct cortical subfields, and encoding of multiple sound features within individual cortical areas. However, few studies have tested the causal contribution of auditory cortex to hearing in multiple contexts. Here we tested the role of auditory cortex in both spatial and non-spatial hearing. We reversibly inactivated the border between middle and posterior ectosylvian gyrus using cooling (n = 2) or optogenetics (n=1) as ferrets discriminated vowel sounds in clean and noisy conditions. Animals with cooling loops were then retrained to localize noise-bursts from multiple locations and retested with cooling. In both ferrets, cooling impaired sound localization and vowel discrimination in noise, but not discrimination in clean conditions. We also tested the effects of cooling on vowel discrimination in noise when vowel and noise were colocated or spatially separated. Here, cooling exaggerated deficits discriminating vowels with colocalized noise, resulting in increased performance benefits from spatial separation of sounds and thus stronger spatial release from masking during cortical inactivation. Together our results show that auditory cortex contributes to both spatial and non-spatial hearing, consistent with single unit recordings in the same brain region. The deficits we observed did not reflect general impairments in hearing, but rather account for performance in more realistic behaviors that require use of information about both sound location and identity.

Introduction

A central question in neuroscience is to what extent, and where, is the brain functionally organized into specialised units, or distributed networks of interacting regions (Bowers, 2017; Földiák, 2009; Quian Quiroga, 2016). In hearing research, this debate has played an important role in our understanding of auditory cortex, where it has been proposed that two functionally specialized streams branch from primary auditory cortex into specialized 'what' and 'where' streams carrying information about the identity and location of sounds (Rauschecker and Scott, 2009; Rauschecker and Tian, 2000; van der Heijden et al., 2018). The 'what' and 'where' pathways extend over separate ventral and dorsal regions of non-primary auditory cortex, before moving to separate areas of prefrontal cortex, either directly in the case of the ventral pathway, or indirectly via parietal cortex in the dorsal pathway (Kaas and Hackett, 2000; Romanski et al., 1999).

In humans, causal evidence for the distinction between spatial and non-spatial pathways comes from selective deficits in sound localization or recognition following brain lesions (Clarke et al., 2002, 2000). Transcranial magnetic stimulation in anterior or posterior non-primary auditory cortex also delays reaction times of healthy listeners discriminating amplitude modulation or sound location respectively (Ahveninen et al., 2013). In animal models, inactivation of the anterior or posterior auditory fields of cat auditory cortex produce selective deficits in pattern discrimination and sound localization respectively (Lomber and Malhotra, 2008).

The division of spatial and non-spatial processing in auditory cortex is not always clear however. For example, auditory cortical lesions in humans do not always produce deficits in spatial or non-spatial hearing (Adriani et al., 2003). Furthermore, when one records at the activity of individual neurons, spatial information is present in the responses of cells from a wide variety of auditory cortical fields (Middlebrooks et al., 1998; Middlebrooks and Pettigrew, 1981; Stecker et al., 2005). In the ferret, this spatial information overlaps with information about other perceptual attributes of sound, including pitch, timbre, level and voicing (Bizley et al., 2009; Town et al., 2018; Walker et al., 2011). This interdependent, or mixed, selectivity is consistent with models that predict the joint encoding of both spatial and spectrotemporal sound features (Młynarski, 2015) and may offer computational benefits for reconstructing sounds (Johnston et al., 2020; Macellaio et al., 2020).

Both primary and non-primary areas of ferret auditory cortex are necessary for sound localization (Nodal et al., 2012; Smith et al., 2004; Wood et al., 2017), but it is unknown if the same brain regions also play a causal role in the identification of sounds. Here, we tested whether auditory cortex is necessary for both spatial and non-spatial hearing by training ferrets to perform multiple listening tasks involving speech sound (vowel) discrimination in various contexts (clean conditions, with co-located or spatially separated noise) and sound localization. During behaviour, we used cooling to reversibly inactivate a candidate area of auditory cortex in which neurons show tuning to low sound frequencies relevant for both spatial and non-spatial hearing.

Results

Cortical inactivation in ferrets

We examined the role of auditory cortex in behavior using cortical inactivation via cooling in two ferrets (F1311 and F1509) or stimulation of inhibitory interneurons using optogenetics in one ferret (F1706).

Cooling reduces the temperature of neurons, reversibly suppressing spiking responses (Lomber et al., 1999; Wood et al., 2017). To reduce the temperature of auditory cortex during behavioral testing, we implanted cooling loops over the boundary between middle and posterior Ectosylvian gyrus (MEG and PEG respectively) that contain primary (A1, AAF) and non-primary fields (PSF, PPF)(**Fig. 1**). Loops were cooled to a target temperature of between 8 and 12°C bilaterally in all tasks, and also unilaterally in sound localization (**Supplementary Fig. 1**). Temperature mapping in earlier work from our lab showed that this level of cooling should affect cortical tissue within the immediate vicinity of the loop without spreading beyond the ectosylvian gyrus (Wood et al., 2017). Given the size and location of the loops, and previous data documenting the effects of cooling on spiking activity (Coomber et al., 2011; Lomber et al., 1999; Wood et al., 2017), inactivation should cover a substantial area of MEG and the low frequency border with PEG, as well as a smaller portion of the low frequency Anterior Ectosylvian (AEG).

Optogenetic suppression of cortical activity was tested only during vowel discrimination in noise. One ferret (F1706) received injection of an adeno-associated virus (AAV2-mDlx-ChR2-mCherry) (Dimidschstein et al., 2016) to induce expression of Channelrhodopsin 2 (ChR2) by GABAergic interneurons in left and right auditory cortex. This animal was also implanted with an optic fibre over the same MEG/PEG boundary so that blue light (463 nm) could be delivered to auditory cortex during behavioral testing. Cooling was applied on a session-by session basis, whereas the temporal resolution of optogenetics allowed us to interleave individual trials with and without light.

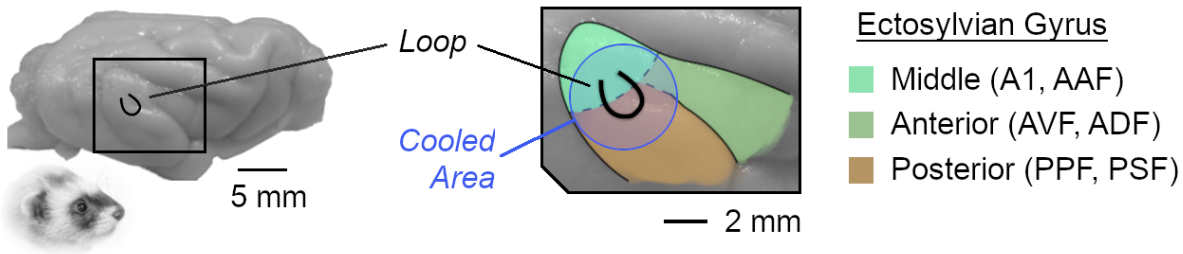
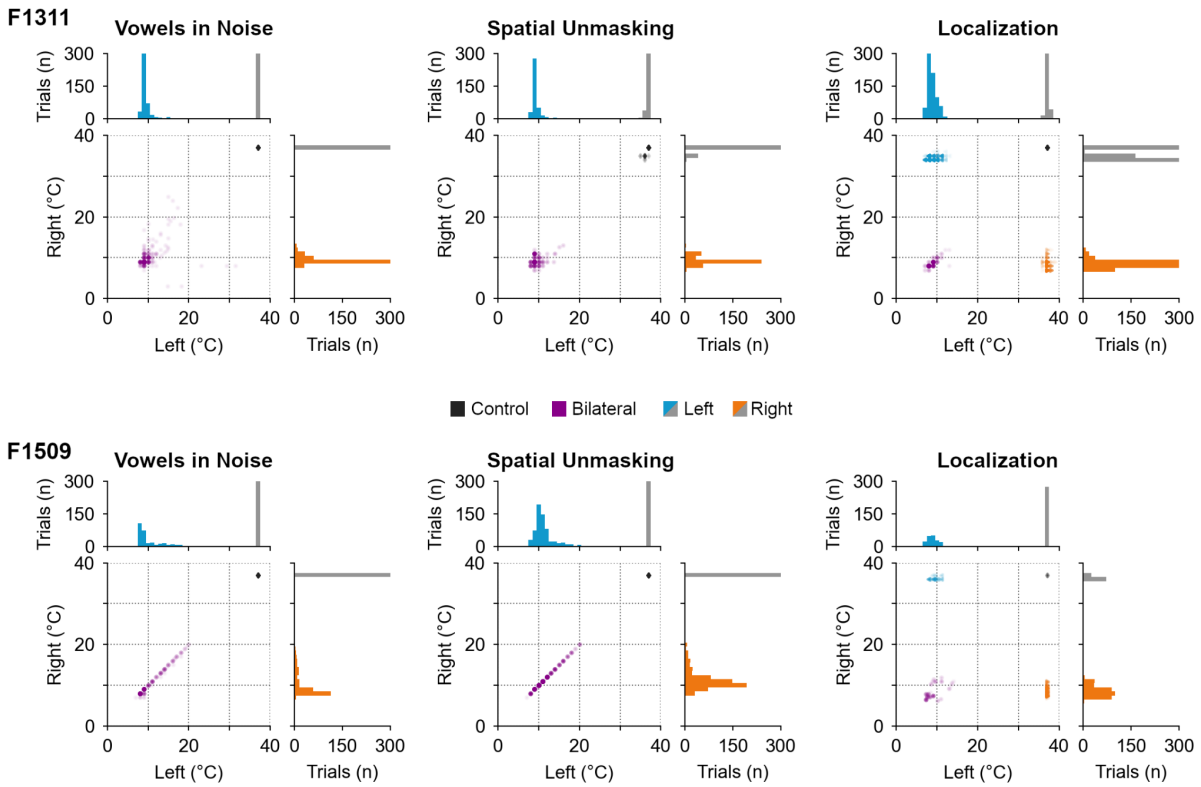


Figure 1. Inactivation of auditory cortex via cooling. Anatomical location of ferret auditory cortex at which cooling loops were implanted (F1311 & F1509) over the border between middle and posterior Ectosylvian Gyrus. Blue shading indicates estimated coverage of inactivation.



Supplementary Figure 1. Distribution of cortical temperatures during bilateral (all tasks) and unilateral cooling (localization). Scatterplots show temperatures on individual trials.

Auditory cortex is required for vowel discrimination in noise but not clean conditions

We trained ferrets to discriminate between two vowel sounds (/u/ and /e/) varying in sound level, either presented in broadband noise or clean conditions without noise. Two animals were tested with vowels in noise and clean conditions during cooling, and in control sessions without cooling (F1311 and F1509; **Fig. 2A-B**). For vowel discrimination in noise, we also tested a third animal in which control trials were interspersed with trials where 463 nm light was delivered to drive activity of GABAergic interneurons via ChR2 (F1509; **Fig. 2C**).

In both cooling and optogenetic modulation of auditory cortex, we observed an impairment of vowel discrimination in noise with experimental treatment (cooling or light delivery). Across SNRs, performance discriminating sounds in noise was worse during cooling than control sessions (change in performance [cooled-control]: F1311 = -11.0%, F1509 = -20.2%) and worse on trials when light was delivered ([Light: On - Off]: F1706 = -12.5%). For each animal, the decline in performance with cortical inactivation was significantly greater than expected by chance (Permutation test with Holm-Bonferroni correction for multiple comparisons, F1311: $p < 0.001$, F1509: $p = 0.002$, F1706: $p = 0.007$). In contrast, vowel discrimination in clean conditions was not affected by cooling in either animal tested (F1311 = +5.49%, F1509 = -1.43%; F1509 not tested in clean conditions) and these differences were not significantly greater than expected by chance (permutation test, $p > 0.2$).

To assess changes in vowel discrimination with cortical inactivation across subjects, we compared single trial performance using a mixed-model logistic regression with ferret as a random effect, and

in which background noise (clean vs. noise), target sound level and experimental treatment (test [cooled or light-on] vs. control [warm or light-off]) were contrasted as fixed effects. We observed a significant interaction between noise condition and experimental treatment ($\beta = -0.732$, $p = 0.004$) reflecting the selective impairment in task performance in noise with cortical inactivation. There was also a significant main effect of noise ($\beta = -0.352$, $p = 0.011$) that captured both the general impairment of performance caused by adding noise, and a significant interaction between noise condition and sound level / SNR ($\beta = 0.04$, $p = 0.010$) that reflected the dependence of performance on SNR in noise that was otherwise absent when animals discriminated vowels equally well across sound levels in clean conditions. There was no main effect of treatment ($\beta = 0.083$, $p = 0.601$) reflecting the absence of a broader hearing impairment from cooling/light delivery. Thus both at the individual and group levels, our results indicate that auditory cortex makes a selective, causal contribution to vowel discrimination in noise.

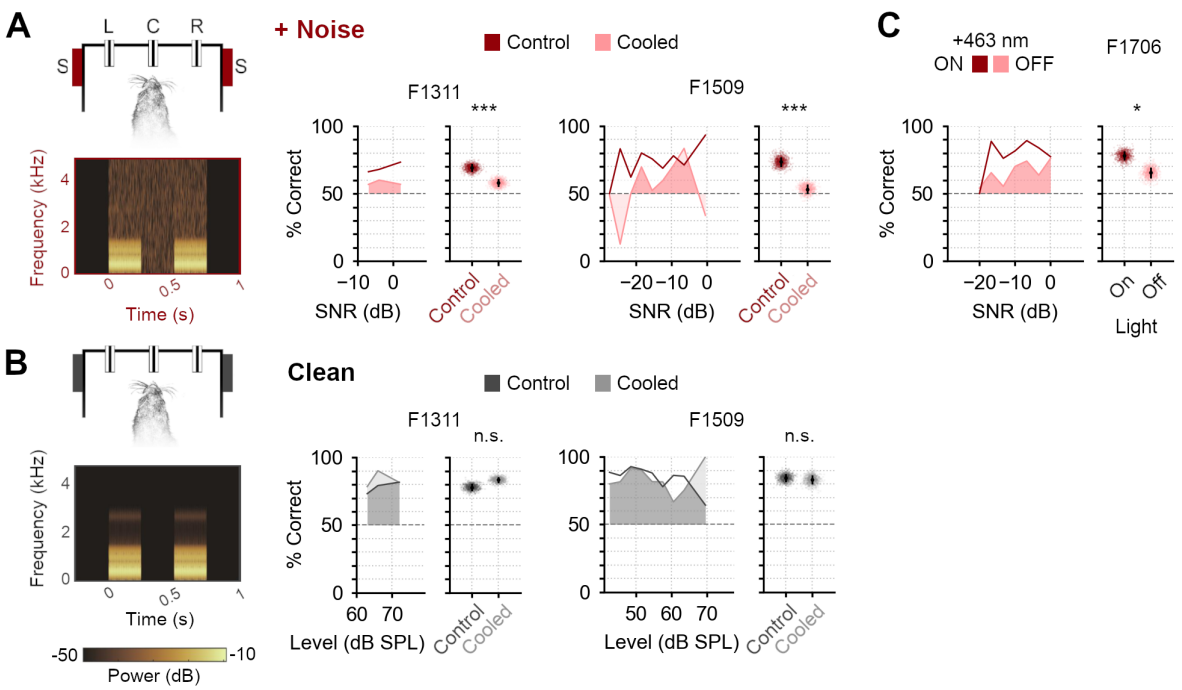


Figure 2. Effects of cooling on vowel discrimination in noise and clean conditions. (A-B) Task design and performance of ferrets tested with cortical cooling during discrimination of vowels in noise (**A**) and in clean conditions (**B**). Schematics show two-choice design in which ferrets discriminate vowels (/u/ or /ɛ/). Labels show response port locations (L, C and R), with sounds presented from both speakers (S) on the left and right of the head. Spectrograms show vowels (e.g. two 250 ms tokens of /u/, separated by 250 ms interval) in clean conditions and with additive broadband noise. Line plots show mean bootstrapped performance as a function of cooling and SNR / sound level. Scatter plots show performance across all SNRs / sound levels during cooled and control conditions for each bootstrap (red/grey; n = 1000 iterations), with mean and standard deviation of performance across bootstrap resampling shown in black. (**C**) Performance of additional ferret tested with light delivery during vowel discrimination in noise. Across plots, asterisks indicate significant differences between cooled and control performance (permutation tests, Holm-Bonferroni corrected for multiple comparisons: * $p < 0.05$, *** $p < 0.001$).

Spatial release from masking during auditory cortical inactivation

Many everyday listening conditions require the use of both spatial and non-spatial information; spatial release from masking is a widely demonstrated example in which presentation of a target signal at a distance from sources of masking improves discrimination (Bronkhorst, 2015; Litovsky, 2012). We therefore examined whether spatial separation of vowel and broadband noise improved ferrets ability to discriminate vowel identity, both in control conditions and during cortical cooling (n=2 ferrets). In tests of spatial release from masking, vowel and noise were either co-located at the left or right speaker, spatially separated by 180° by presenting vowels and noise from different speakers, or vowels were presented alone from a single speaker in clean conditions (**Fig. 3A-D**)

We first asked if we could replicate the selective impairment in vowel discrimination in noise but not clean conditions identified earlier in Figure 2 in tests with a single speaker. As before, cooling impaired performance on trials when vowels were presented with co-located noise but not when vowels were presented in quiet from a single speaker on the left or right of the head (**Fig. 3B, D**). Modelling behavior with logistic regression (on the odds of responding correctly to vowels in clean conditions or colocalized noise, in cooled and control conditions, with ferret as a random effect) confirmed a significant main effect of cooling ($\beta = -0.656$, $p < 0.001$) and interaction between cooling and presence of noise ($\beta = 0.683$, $p = 0.015$).

We then asked if spatial separation of vowel and noise improved performance discriminating vowels in noise, and if any benefit was affected by cooling. In both cooled and control conditions, spatial separation improved performance of both animals implanted with cooling loops (**Fig. 3E**). However, the performance benefit of spatial separation was small, and not statistically significant (Logistic regression on the odds of responding correctly to vowels in colocalized or spatially separated noise, in cooled and control conditions, $p > 0.05$). There was no interaction between cooling and stimulus conditions; however, we did identify a significant main effect of cooling ($\beta = -0.621$, $p = 0.001$). To further explore these differences further, we therefore analysed performance in control and cooled sessions separately.

In control conditions, task performance improved when vowel and noise were spatially separated for both animals (**Fig. 3B**)(mean change in performance across bootstrapping, separated-colocalized; F1311: +1.05%, F1509: +3.08%). The benefits of spatial separation were small, but representative of a broader cohort of ferrets tested in similar conditions (**Supplementary Figure 2**, n = 4, change in performance; F1201 = +2.78%, F1203 = +2.61%, F1216 = +0.94%, F1217 = +6.28%). However, these changes in performance with spatial separation were not statistically significant - either when considering the two subjects implanted with loops, or all animals (Logistic regression on responses to vowels in colocalized or spatially separated noise, in control conditions only: $p > 0.05$).

In cooled conditions, we observed the strongest spatial release from masking (mean change in performance across bootstrapping, separated-colocalized; F1311: +12.5%, F1509: +6.25%). These large benefits were reflected by a statistically significant effect of spatial separation on performance across animals (Logistic regression on responses to vowels in colocalized or spatially separated noise, in cooled conditions only, $\beta = 0.557$, $p = 0.014$). Our results indicate that spatial release from masking can occur during auditory cortical inactivation, and were primarily driven by the lower performance of animals on trials with colocalized noise during cooling, while performance discriminating spatially separated vowels and noise remained relatively stable.

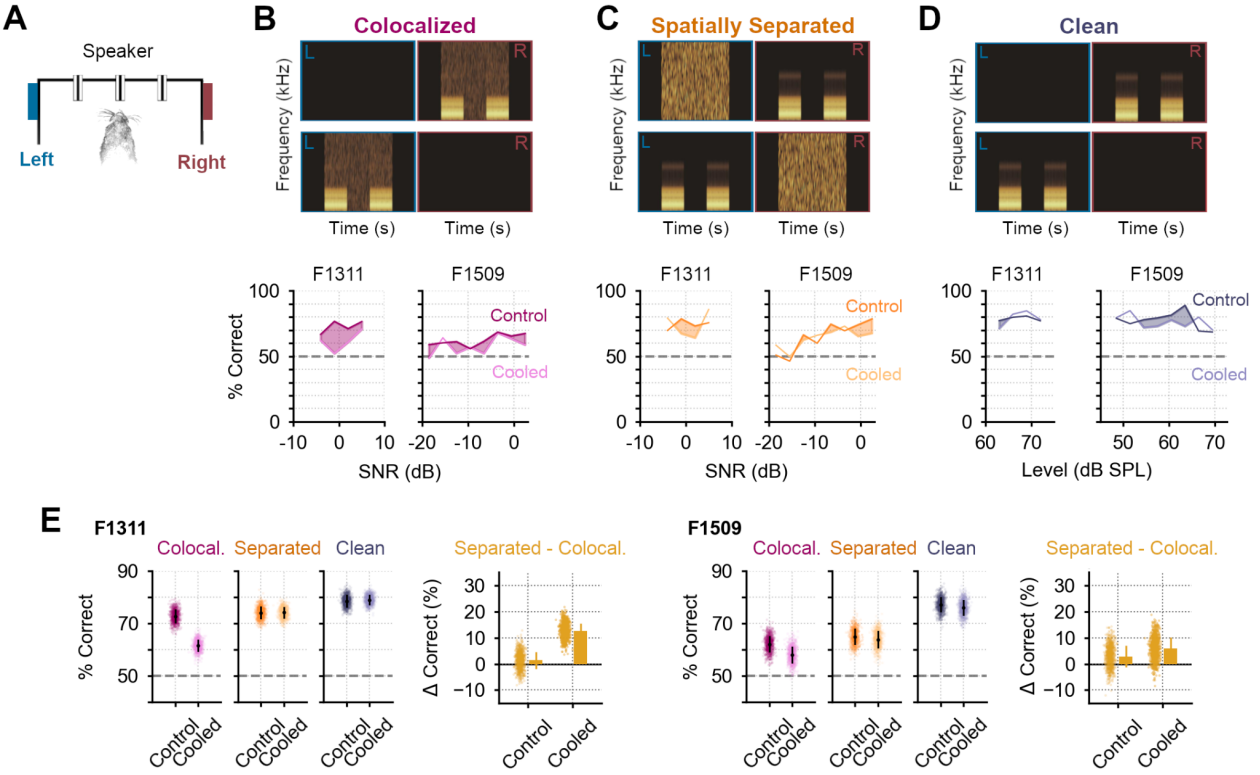
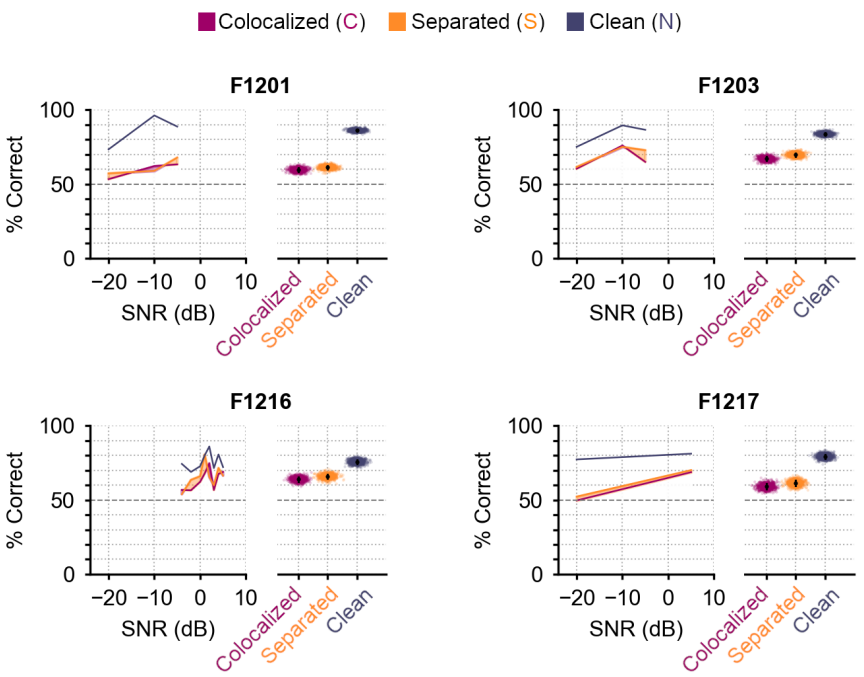


Figure 3. Effects of cooling on spatial release from masking. (A) Schematic illustrating the position of the two speakers from which vowels and/or noise were presented. **(B-D)** Spectrograms illustrating each stimulus condition, with performance of each ferret as a function of SNR in control and cooled testing shown below. Data shown as mean performance across bootstrapping (n=10^3 iterations). **(E)** Performance of each ferret across SNRs / sound level during cooled conditions, with spatial release from masking calculated as performance in separated - colocalized noise. Scatterplots show individual bootstrap results, with bars showing mean and standard deviation across bootstrap resampling.



Supplementary Figure 2: Further examples of spatial release from masking. Data from four further ferrets (F1201, F1203, F1216 and F1217) discriminating vowels in colocalized or spatially separated noise. Line plots show performance by vowel level; scatter plots show bootstrapped performance across all sound levels tested, with mean and standard deviation across bootstrap resampling shown in black.

A shared role for auditory cortex in sound localization and vowel discrimination in noise

Our results so far point towards a specific role for auditory cortex in the separation of co-localized sound sources (here the target vowel and masking noise). To determine whether the region of auditory cortex that we cooled was also involved in spatial hearing, we retrained the two ferrets implanted with cooling loops in a sound localization task.

Sound localization was tested using an approach to target task that has been widely used, in which subjects were required to hold their head at a response port in the center of a speaker ring in order to initiate presentation of a target sound from one of several speakers around the test chamber (**Fig. 4A**). Animals then reported the sound's location by visiting the response ports placed closest to the target speaker. We tested sound localization during control conditions, when auditory cortex was cooled bilaterally, and also during unilateral cooling of either the left or right auditory cortex only.

Bilateral cooling impaired sound localization in both ferrets, with performance (percent correct) being lower (**Fig. 4B**)(change in performance [cooled-control]: F1311 = -14.8%, F1509 = -12.9%) and errors being larger during cooling (**Fig. 4C**)(change in absolute error [cooled-control]: F1311 = +7.68°, F1509 = +16.7°). We modelled the effects of bilateral cooling on single trial performance using logistic regression (with ferret as random effect, and treatment [cooled/control] as fixed effect) and confirmed that cooling was associated with a significant decrease in probability of making a correct response ($\beta = 0.574$, $p < 0.001$). We also compared the effects of bilateral cooling on the magnitude of (unsigned) errors using a generalized linear mixed model (GLMM; using Poisson distributions and log link function, with ferret as random effect, and treatment [cooled/control] as fixed effect) and found that cooling was associated with significantly larger differences between target and response location ($\beta = -0.417$, $p < 0.001$).

Unilateral cooling impaired localization of sounds in the contralateral hemifield of space (**Fig. 4D-E**). Specifically, cooling the left auditory cortex resulted in poorer performance reporting sound location on the right side of space; whereas cooling the right auditory cortex resulted in worse performance localizing sounds in the left side of space. The opposing effects of cooling left and right auditory cortex was reflected by a significant interaction between cooled hemisphere and speaker hemifield when modelling the probability of responding correctly (Logistic regression on odds of responding correctly, with cooled hemisphere and speaker hemifield as fixed effects, and ferret as random effect: $\beta = 0.717$, $p < 0.001$) or the magnitude of errors (Poisson GLMM cooled hemisphere and speaker hemifield as fixed effects, and ferret as random effect: $\beta = -0.595$, $p < 0.001$). In both analyses, there was no main effect of cooled hemisphere ($p > 0.05$), reflecting the similar overall performance of animals when either left or right auditory cortex was cooled.

Our results demonstrate a role for auditory cortex in ferrets' ability to report the specific position of sounds in continuous space; continuous sound localization can be contrasted with less complex tasks in which listeners report whether a sound is to the left or right of the head (sound lateralization). Previous studies have reported that sound localization deficits following auditory cortical inactivation do not extend to sound lateralization, and that during inactivation, listeners remain able to discriminate left from right. We assessed this in our data by examining the pattern of errors within or between hemifields; asking whether ferrets reported the location of speakers as being on the correct left or right side of space, even if the precise location of response did not match the exact speaker location. Both ferrets showed accurate lateralization during both control conditions (**Supplementary**

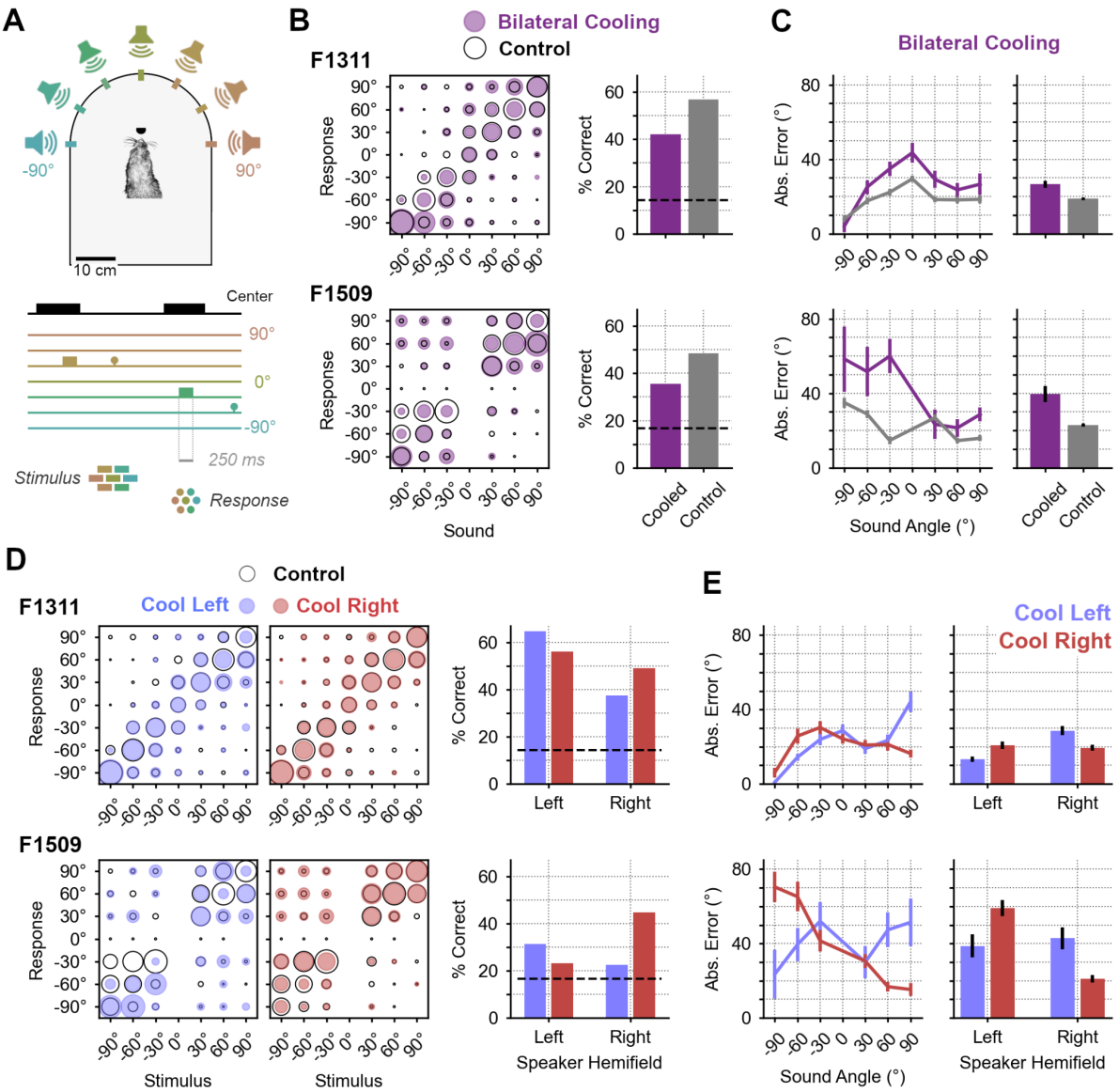
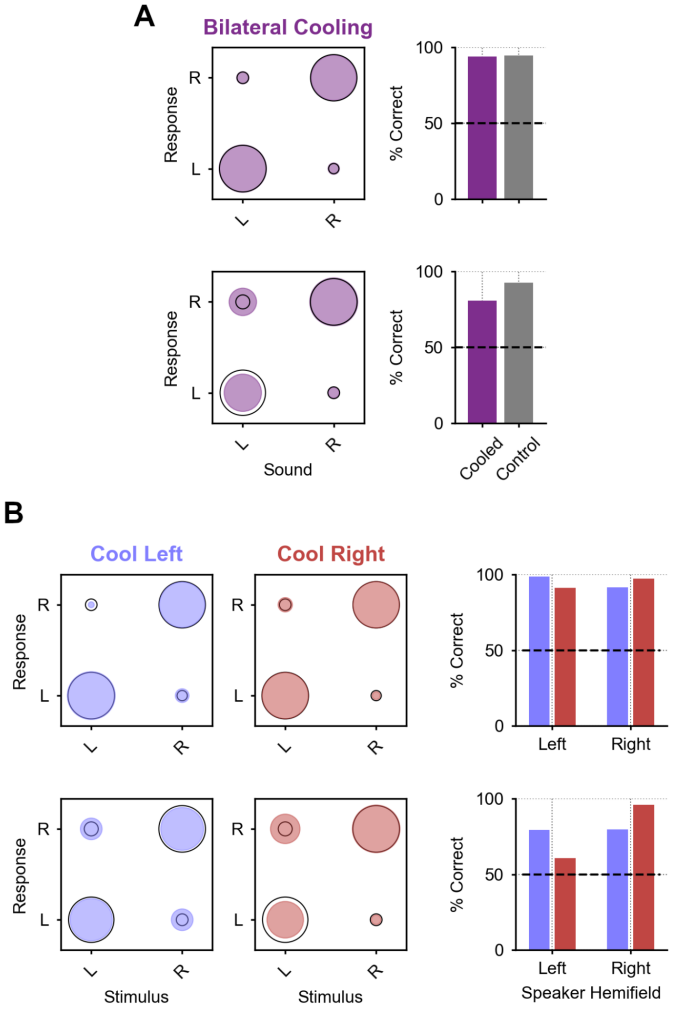


Figure 4. Effects of cooling on sound localization. (A) Sound localization task in which ferrets reported the location of sounds from one of several speakers in frontal space. **(B)** Localization performance during control and bilateral cooling for each ferret. *Left:* Bubble plots showing the joint distribution of behavioral responses for each speaker and response location. Data points show performance in control conditions (Unfilled black: F1311 = 1690 trials, F1509 = 1220 trials) and bilateral cooling (Filled purple: F1311 = 294 trials, F1509 = 115 trials). (**C**) Magnitude of response errors as a function of sound location (line plots) and across locations (bars). Data shown as mean ± s.e.m. across trials performed in control conditions (purple) and bilateral cooling (grey). **(D)** Localization performance during unilateral cooling for each ferret. Data points show performance when cooling left (Filled blue: F1311 = 476 trials, F1509 = 97 trials) or right auditory cortex (Filled red: F1311 = 536 trials, F1509 = 294 trials), with performance in control conditions shown for reference (Unfilled black). (**E**) Magnitude of response errors as a function of sound location (line plots) and across locations (bars) during unilateral cooling. Data shown as mean ± s.e.m. across trials performed when cooling left (blue) or right (red) auditory cortex only.

Fig. 3: control performance, F1311: 94.8%, F1509: 92.7%) and bilateral cooling (F1311: 93.9%, F1509: 80.9%), with cooling having no significant effect on performance (Logistic regression with cooling as fixed effect and ferret as random effect; Effect of cooling: β = 0.232, p = 0.082).



Supplementary Figure 3. Effects of cooling on sound lateralization (A) Lateralization performance during control and bilateral cooling for each ferret. *Left:* Bubble plots showing the joint distribution of behavioral responses for speakers on the left and right sides of the central platform. Data points show performance in control conditions (Unfilled black: F1311 = 1397 trials, F1509 = 1215 trials) and bilateral cooling (Filled purple: F1311 = 245 trials, F1509 = 115 trials). Trials on which sounds were presented from the midline (0°) were not included in this analysis. **(B)** Lateralization performance during unilateral cooling for each ferret. Data points show performance when cooling left (Filled blue: F1311 = 388 trials, F1509 = 97 trials) or right auditory cortex (Filled red: F1311 = 442 trials, F1509 = 294 trials), with performance in control conditions shown for reference (Unfilled black).

Discussion

Our results, summarized in **Table 1**, demonstrate that both vowel discrimination in noise and sound localization depend on a common region of ferret auditory cortex, and that cortical inactivation via cooling leads to selective behavioral deficits in both tasks, while leaving intact other forms of hearing such as vowel discrimination in clean conditions and spatial release from masking.

Table 1: Summary of Results.

Ferret	Vowel Discrimination	Vowel Discrimin. in Noise	Spatial Unmasking	Sound Localization
F1311	Unaffected	Impaired	Present	Impaired
F1509	Unaffected	Impaired	Present	Impaired
F1706	Not tested	Impaired	Not tested	Not tested

Selection of cortical region for inactivation

We implanted cooling loops (or optic fibres) over the MEG/PEG border region of ferret auditory cortex; a region in which neurons are predominantly tuned to low sound frequencies (0.15 to 4 kHz)(Bizley et al., 2005) and known to contain a high proportion of vowel responsive and spatially tuned neurons (Bizley et al., 2009; Town et al., 2018, 2017). We reasoned that this area would therefore be most likely to contribute to vowel discrimination, even though the effects of cooling would cover both primary and non-primary fields of auditory cortex. We also believed that this low-frequency area may play an important role in encoding interaural timing cues for sound localization, given low sound frequencies are also particularly relevant for inter-aural timing cues that ferrets exploit in spatial hearing (Wood et al., 2019). These motivations thus shaped the strategy by which we identified multiple roles for auditory cortex in specific behaviors that future studies can refine by inactivating specific auditory cortical subfields.

In any perturbation study, the extent of inactivation is a critical consideration; the size of cooling loops used here reflected a compromise between the need to inactivate sufficient numbers of neurons to observe behavioral deficits, and avoid unintended spread of cooling to subcortical structures (Coomber et al., 2011). Previous data from our lab has shown that the cooling loops we used induce spatially-restricted heat loss that limits the reduction in spiking activity to the cortical layers surrounding the loop (Wood et al., 2017). In the current study, ferrets' ability to discriminate vowels in clean conditions during bilateral cooling, to benefit from spatial release from masking and to localize sounds in the contralateral hemisphere during unilateral cooling, all indicate that the cooling protocol we used was unlikely to affect structures outside the cortex and did not affect ferrets' general hearing, motor ability or capacity to engage in behavioral tasks.

Earlier cooling studies in cats have used multiple loops to identify distinct contributions of specific non-primary areas to spatial and non-spatial hearing (Lomber and Malhotra, 2008). It is possible that such distinctions also exist in ferrets, and that smaller loops targeting specific subfields may reveal

distinct causal contributions to spatial and non-spatial hearing. However, neurons with mixed selectivity to multiple sound features are found in most regions of ferret auditory cortex (Bizley et al., 2009; Walker et al., 2011) and impairments in sound localization are observed during inactivation of either primary and non-primary ferret auditory cortex (Nodal et al., 2012). Together with our present results, these data suggest that function is broadly distributed across ferret auditory cortex and thus that more localized inactivation would result in smaller behavioral deficits in both sound localization and vowel discrimination in noise.

Hearing with and without auditory cortex

Our results confirm the widely observed role of auditory cortex in sound localization in carnivores (Kavanagh and Kelly, 1987; Malhotra et al., 2008; Smith et al., 2004; Wood et al., 2017), while the ability of ferrets to discriminate vowels in clean conditions is consistent with similar behavior in cats with lesions of primary and secondary auditory cortex (Dewson, 1964). Thus, although auditory cortical neurons are modulated by vowel timbre (Bizley et al., 2009), such sensitivity may be redundant and thus not always causally relevant.

A role for auditory cortex in vowel discrimination became evident when we added noise to vowels; such findings are consistent with a broader trends from research with both cats and non-human primates, in which auditory cortical lesions impair fine spectrotemporal resolution but have limited effects on coarser disriminations (Dewson et al., 1969; Diamond et al., 1962; Evarts, 1952; Goldberg and Neff, 1961; Heffner and Heffner, 1986; Massopust et al., 1965). Interpreting lesion studies requires caution, due to the potential for recovery of function; however our results were obtained using reversible methods for which there was minimal opportunity for recovery during cooling (see also **Supplementary Fig. 4**). Our data also go beyond lesion studies of individual behaviors, to show that the same cortical areas in the same subjects are required for both spatial and non-spatial hearing, while also demonstrating that spatial release from masking does not require intact auditory cortical function. The preservation of release from masking during cooling, coupled with impaired sound localization is also consistent with the idea of separate mechanisms for spatial stream segregation and discrimination of sound location (Middlebrooks and Onsan, 2012).

The finding that spatial separation of target vowels and noise maskers protects performance from cooling suggests that subcortical structures can parse the noise and vowel into separate streams. In stark contrast, substantial performance deficits were observed for co-located vowel and noise, emphasizing the importance of auditory cortex in segregating competing sound sources (Bizley and Cohen, 2013; Mesgarani and Chang, 2012). In our results, the spatial separation of target and masker into opposing hemifields may result in the representation of the vowel being comparable to that in clean conditions in the hemisphere contralateral to the vowel, and help animals to compensate for the lack of cortical scene analysis that is critical for resolving co-located sound sources. Future work must use finer grained spatial separation of target and masker to determine whether the role of auditory cortex in resolving co-located sound sources extends to nearby (but not identical) locations.

The effects of release from masking that we observed were small, relative to the benefit of removing masking entirely. The limited effectiveness of spatial release from masking in our experiments may result from the use of broadband noise that primarily introduces energetic rather than informational masking. Spatial release from masking is strongest when informational masking arises (Brungart,

2001; Jones and Litovsky, 2011) and so combining cortical inactivation with more closely related sounds (e.g. overlapping speech) may reveal stronger release from masking in future experiments.

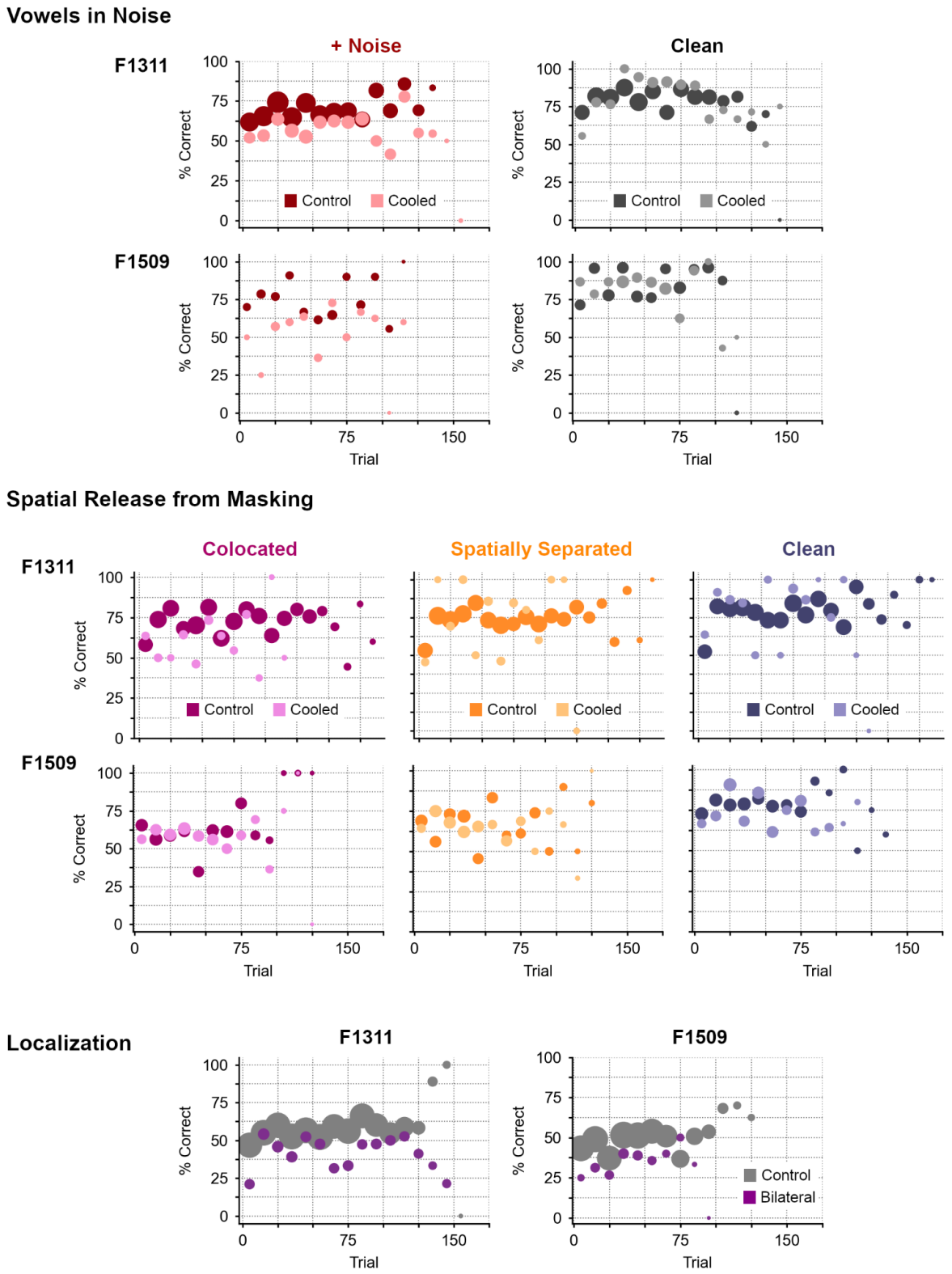
A notable feature of our results, along with the general pattern in the literature on hearing impairments following auditory cortical inactivation, is the preserved ability of animals to perform some sound-based tasks (e.g. vowel discrimination in clean conditions). These findings suggest that there is either substantial redundancy in the auditory system that allows alternative pathways to take over task performance, or that information at earlier stages of the auditory system, in this case about vowel identity (Blackburn and Sachs, 1990; Moore and Cashin, 1974; Schebesch et al., 2010), can access brain areas that coordinate behavior and is sufficient for discriminations that have already been learnt (Ponvert and Jaramillo, 2019). Our use of reversible inactivation via cooling, which operates on the timescale of minutes / individual test sessions, suggests that any redundant pathways must come into use rapidly, integrate seamlessly with normal decision-making processes and occur with minimal need for learning. Analysis of ferret behavior during cooling did not reveal any indications of learning during cortical inactivation (**Supplementary Figure 4**).

Understanding how signals in auditory cortex are integrated into behavior is also critical for determining how the deficits in spatial and non-spatial hearing arise, as there is no guarantee that impairments observed in vowel discrimination in noise and sound localization arose through the same mechanisms. Cooling suppresses the activity of neurons, and so we might infer that the absence of spiking degrades cell assemblies that downstream neurons rely on for informed auditory decision making. Such downstream centers may be located elsewhere in areas such as the prefrontal cortex (Kaas and Hackett, 2000; Romanski et al., 1999) or the striatum (Znamenskiy and Zador, 2013). To ascertain the underlying causes of the deficits we have observed, it will be necessary to combine auditory cortical inactivation with neural recording in such downstream areas, or to perform targeted manipulations of specific neural pathways.

A causal role for areas showing mixed selectivity in perception?

We targeted inactivation to the area of auditory cortex in which neurons have previously shown mixed selectivity for sound location and vowel identity (Bizley et al., 2009; Town et al., 2018; Walker et al., 2011). Such mixed selectivity has been observed widely, including across the auditory system (Chambers et al., 2014; Cohen et al., 2004; Downer et al., 2017; O'Connor et al., 2010; Yi et al., 2019) and may reflect a general process through which neural systems meet the demands of complex and flexible behaviors (Jazayeri and Afraz, 2017; Rigotti et al., 2013). Our results show that an area of the brain tuned to multiple sound features makes a causal contribution to multiple forms of hearing, and are consistent with broader predictions about the involvement of mixed selectivity in behavior (Fusi et al., 2016).

Mixed selectivity expands the range of dimensions through which neural populations can potentially represent sounds, and so it may be possible to recover detailed information about diverse stimulus sets from population activity in auditory cortex. However, our ability to observe the use of such information in animal behavior is still limited, as most behavioral tasks are low-dimensional (i.e. they have only one or two independent variables along which subjects act)(Gao and Ganguli, 2015). By testing effects of cortical inactivation on both spatial and non-spatial hearing in the same subjects, we have taken some of the first steps towards expanding the study of auditory behavior to higher dimensions that may be necessary to understand the role of mixed selectivity in everyday hearing.



Supplementary Figure 4. Effects of cooling as a function of trial within session. Data shown for each task and ferret (F1311/F1509) as performance measured within bins of 10 trials across sessions, with marker size indicating sample size. Correction trials not included.

Methods

Subjects

We trained and tested seven pigmented ferrets (0.5-5 years old, all female) in behavioral tasks. Ferrets were water-restricted prior to testing and received a minimum of 60ml/kg of water per day, either during task performance or supplemented as a wet mash made from water and ground high-protein pellets. Subjects were tested in morning and afternoon sessions on each day for up to five days in a week. The weight and water consumption of all animals was measured throughout the experiment. Regular otoscopic examinations were made to ensure the cleanliness and health of ferrets' ears.

Animals were maintained in groups of two or more ferrets in enriched housing conditions. Following behavioral testing, two ferrets were chronically implanted with cooling loops for cortical inactivation (F1311, and F1506) and one ferret was implanted with optic fibres for light delivery (F1706). All experimental procedures were approved by local ethical review committees (Animal Welfare and Ethical Review Board) at University College London and The Royal Veterinary College, University of London and performed under license from the UK Home Office (Project License 70/7267) and in accordance with the Animals (Scientific Procedures) Act 1986.

Stimuli

Vowel discrimination

Vowels were synthesized in MATLAB (MathWorks, USA) using an algorithm adapted from Malcolm Slaney's Auditory Toolbox (https://engineering.purdue.edu/~malcolm/interval/1998-010/) that simulates vowels by passing a click train through a biquad filter with appropriate numerators such that formants are introduced in parallel. In the current study, four formants (F1-4) were modelled: /u/ (F1-4: 460, 1105, 2857, 4205 Hz), /ɛ/ (730, 2058, 2857, 4205 Hz), /a/ (936, 1551, 2975, 4263 Hz) and /i/ (437, 2761, 2975, 4263 Hz). Ferrets were only trained to discriminate between a pair of vowels: either /e/ and /u/ (F1201, F1203, F1217, F1509 and F1706), or /a/ and /i/ (F1216 and F1311). All vowels were generated with a 200 Hz fundamental frequency.

Vowels were presented in clean conditions as two tokens (250 ms duration) of the same identity, separated with an interval of 250 ms (**Fig. 2A**). Here, two vowel tokens were used for consistency with previous work (Bizley et al., 2013a; Town et al., 2015). Sounds (two tokens, 250 ms each with a 250 ms interval) were presented through loudspeakers (Visaton FRS 8) positioned on the left and right sides of the head at equal distance and approximate head height. These speakers produced a smooth response (± 2 dB) from 200 to 20000 Hz, with a 20 dB drop-off from 200 to 20 Hz when measured in an anechoic environment using a microphone positioned at a height and distance equivalent to that of the ferrets in the testing chamber. All vowel sounds were passed through an inverse filter generated from calibration of speakers to golay codes (Zhou et al., 1992). Clean conditions were defined as the background sound level measured within the sound-attenuating chamber in which the task was performed in the absence of stimulus presentation (22 dB SPL) .

Vowels were also presented with additive broadband noise fixed at 70 dB SPL and restricted in time to the period of stimulus presentation (0 to 750 ms after onset of the first vowel token). Noise was generated afresh on each trial. Onsets of both vowel sounds and noise were ramped using a 5 ms

cosine function. Here, the temporal profile of noise was chosen so that trials with clean and noisy sounds could be interleaved in the same test session. During initial experiments on vowel discrimination in noise, vowels and noise were played from both left and right speakers; however during later investigation of spatial unmaksing, vowels were presented from either left or right speaker but not both (with noise occurring either on the same speaker, opposite speaker or not at all).

Sound localization

Auditory stimuli were broadband noise bursts of differing durations (F1509: 700 ms; F1311: 250 ms or 100 ms) cosine ramped with 5-ms duration at the onset and offset and low-pass filtered below 22 kHz (finite impulse response filter <22 kHz, 70 dB attenuation at 22.2 kHz). Noise bursts were generated afresh on each trial in MATLAB at a sampling frequency of 48848.125 Hz and presented from one of seven speakers (Visaton FRS SC 5.9) positioned at 30° intervals. Note that one ferret (F1509) was not tested with sounds from the central speaker (0°). Across trials, stimuli were presented at one of three pseudorandomly selected intensities (57, 61.5 and 66 dB SPL).

Speakers were calibrated to produce a flat response from 200 Hz to 25 kHz using Golay codes, presented in an anechoic environment, to construct inverse filters (Zhou et al., 1992). All the speakers were matched for level using a microphone positioned upright at the level of the ferret head in the centre of the semi-circle. Calibrations were performed with a condenser microphone (Model 4191, Brüel and Kjær), a TDT System 3 RX8 signal processor and/or a Brüel and Kjær 3110–003 measuring amplifier.

Task design

Behavioural tasks, data acquisition, and stimulus generation were all automated using custom software running on personal computers, which communicated with TDT real-time signal processors (Vowel discirmination: RZ6, Sound localization: RX8).

Vowel discrimination

Ferrets were trained to discriminate the synthetic vowel sounds within a custom-built double-walled sound attenuating chamber (IAC Acoustics Ltd.) lined with acoustic foam. The chamber contained a wire-frame pet-cage with three response ports housing infra-red sensors that detected the ferret's presence. On each trial, the ferret was required to approach the center spout and hold head position for a variable period (0 – 500 ms) before stimulus presentation. Animals were required to maintain contact with the center spout until 250 ms after the presentation of the first token, at which point they could respond at left or right response ports. Correct responses were rewarded with water while incorrect responses led to a brief time out (3 - 8 s), followed by correction trials in which the previous trial was repeated until a correct response was given. To encourage animals to maintain a steady head position at the center spout during sound presentation, a water reward was also given at trial onset on a small proportion (10%) of randomly chosen trials.

Sound localization

Ferrets were trained and tested in a second behavioral chamber that consisted of a custom-built D-shaped box surrounded by an array of seven speakers at 30˚ intervals (**Fig. 4A**). Each speaker had a response spout located in front (8.5 cm in front of the speaker; 15.5 cm from the centre of the box) at which animals could report sound location and obtain water rewards. A further spout was also

placed at the centre of the arena to initiate stimulus presentation. This spout was offset from the centre by 3 cm to ensure the animal's head was aligned at the centre of the speaker ring, with the interaural axis in line with the -90˚ and 90˚ speakers. Outside the training box, an LED (15 cm from the floor) was used to indicate trial availability. The test arena was housed in a custom built sound attenuating chamber (90 cm high x 90 cm wide x 75 cm deep, Zephyr Products Ltd, UK) lined with 45 mm acoustic foam.

Behavioral training

Vowel discrimination

Subjects were trained to discriminate a pair of vowels through a series of stages of increasing difficulty. When first introduced to the training apparatus, animals were rewarded with water if they visited any spout. Once subjects had associated the spouts with water, a contingency was introduced in which the subject was required to hold the head at the central spout for a short time (501–1001 ms) before receiving a reward. The central spout activation initiated a trial period in which a nose-poke at either peripheral spout was rewarded.

Following acquisition of the basic task structure (typically two to three sessions), sounds were introduced. On each trial, two repeats of a single vowel sound (each 250 ms in duration with a 250 ms interval) were played after the animal first made contact with the spout with a variable delay (0–500 ms). A trial was initiated if the subject's head remained at the spout for the required hold time, plus an additional 500 ms in which the first token of the sound and subsequent interval were played. Following trial initiation, vowel sounds were looped (i.e. played repeatedly) until the ferret completed the trial by visiting the "correct" peripheral spout to receive a reward. Nose-pokes at the "incorrect" peripheral spout were not rewarded or punished at this stage and incorrect responses did not terminate trials. If the animal failed to visit the correct spout within a specified period after initiating a trial (25–60 s), that trial was aborted and the animal could begin the next trial.

Once animals were completing trials frequently, the consequences of incorrect responses were altered so that incorrect responses terminated the current trial. Subjects were then required to return to the center spout to initiate a correction trial in which the same stimulus was presented. Correction trials were included to prevent animals from biasing their responses to only one spout and were repeated until the animal made a correct response.After a minimum of two sessions in which errors terminated trials, a timeout (5–15 s) punishment was added to incorrect responses. Timeouts were signaled by a burst of broadband noise (100 ms), and the center spout was disabled for the duration of the timeout, preventing initiation of further trials.

Once subjects could discriminate repeated sounds on consecutive sessions with a performance of 80%, looping of sounds was removed so that subjects were presented with only two vowel sounds during the initiation of the trial at the center spout. When ferrets correctly identified 80% of vowel pairs in two consecutive sessions, the animal was considered to be ready for testing in noise. For the animals here, presentation of sounds in noise was a part of a wider suite of tests involving discrimination of vowels in a range of conditions (e.g. varying in location, voicing etc.)(Town et al., 2018, 2015). Note that beyond experience through testing, ferrets did not receive specific training to discriminate vowels in noise.

Sound localization

In contrast to vowel discrimination, training in sound localization took place after animals were implanted with cooling loops, and following completion of all testing in vowel discrimination. Ferrets (F1311 and F1509) were first trained to hold at the spout in the center of the localization arena to initiate presentation of a series of repeating 1000 ms noise bursts (500 ms interval) from one speaker. The animal was allowed to leave the central spout after the first burst, after which stimulus repeated until a correct response was made at the peripheral spout nearest the presenting speaker. Responses at other spouts had no effect at this stage, but premature departures from the center triggered a short (1 sec) timeout.

Once ferrets were accustomed to the task (identified by regular returning to the start spout after receiving water from target locations), errors were introduced so that trials were terminated when animals reported at the wrong peripheral spout. The ferret was then required to initiate a new trial, on which the same stimulus was presented (correction trial) until a correct response was made. Timeouts were then introduced for incorrect responses and were increased from 1 to between 5 and 7 seconds. During this training phase, we also increased the hold time required at the central spout before stimulus presentation, initially upto 500 ms during training and then 1000 ms during testing.

Once ferrets reached ≥ 60% correct, the stimulus was reduced to a single noise burst and subsequently the stimulus duration was reduced. Ferrets were ready for testing at these durations once their performance stabilised (~3–4 weeks); for one ferret (F1311) we could reduce sound duration to between 250 and 100 ms with stable performance, however time constraints on the lifetime of the cooling implant required that we use a longer duration (700 ms) for the second animal (F1509).

Cortical inactivation using cooling

Loop implantation

Cortical inactivation experiments were performed using an approach developed by Wood et al. (2017): Two ferrets (F1311 & F1509) were implanted with cooling loops made from 23 gauge stainless steel tubing bent to form a loop shape approximately the size of primary auditory cortex. At the base of the loop, a micro-thermocouple made from twisting together PFA insulated copper (30 AWG; 0.254 mm) and constantan wire (Omega Engineering Limited, Manchester, UK), was soldered and secured with araldite. Thermocouple wires were soldered to a miniature thermocouple connector (RS components Ltd, UK) and secured with epoxy resin.

Loops were surgically implanted over the border between middle and posterior ectosylvian gyrus, corresponding to the boundary between primary and non-primary auditory cortex. Consistent with previous studies (Wood et al., 2017), we did not map the boundaries of auditory cortical subfields prior to loop placement. Cortical mapping may damage brain tissue, potentially triggering compensatory mechanisms that might mask causal roles in task performance. Placement of cooling loops was therefore based on our extensive experience targeting this area for electrode placements (Bizley et al., 2013b, 2009; Town et al., 2018). Surgery was performed in sterile conditions under general anesthesia, induced by a single intramuscular injection of diazepam (0.4 ml/kg, 5 mg/ml; Hameln) and ketamine (Ketaset; 0.25 ml/kg, 100 mg/ml; Fort Dodge Animal Health, Kent, UK). Animals were intubated and ventilated, and anesthesia was then maintained with 1-3% isoflurane in

oxygen throughout the surgery. Animals were provided with subcutaneous injections of atropine (0.09 ml/kg, 600 µl/ml) and dexamethasone (0.25 ml/kg), as well as surgical saline intravenously, while vital signs (body temperature, end-tidal CO2, Oxygen saturation and electrocardiogram) were monitored throughout surgery.

General anesthesia was supplemented with local analgesics (Marcaine, 2 mg/kg, AstraZeneca) injected at the point of midline incision. Under anaesthesia, the temporal muscle overlying the skull was retracted and largely removed, and a craniotomy was made over the ectosylvian gyrus. The dura over the gyrus was then opened to allow placement of the cooling loop on the surface of the brain. The loop was shaped during surgery to best fit the curvature of the cortical surface prior to placement, and was then embedded within silicone elastomer (Kwik-Sil, World Precision Instruments) around the craniotomy, and dental cement (Palacos R+G, Heraeus) on the head. Bone screws (stainless steel, 19010-100, Interfocus) were also placed along the midline and rear of the skull (two per hemisphere) to anchor the implant. Implant anchorage was also facilitated by cleaning the skull with citric acid (0.1 g in 10 ml distilled water) and application of dental adhesive (Supra-Bond C&B, Sun Medical). Some skin was then removed in order to close the remaining muscle and skin smoothly around the edges of the implant. Pre-operative, peri-operative and post-operative analgesia and anti-inflammatory drugs were provided to animals under veterinary advice. Animals were allowed to recover for at least one month before resuming behavioral testing and beginning cortical inactivation experiments.

Cooling during behavior

To reduce the temperature of the cortical tissue surrounding the loop, cooled ethanol (100%) was passed through the tube using an FMI QV drive pump (Fluid Metering, Inc., NY, USA) controlled by a variable speed controller (V300, Fluid Metering, Inc., NY, USA). Ethanol was carried to and from the loop on the animal's head via FEP and PTFE tubing (Adtech Polymer Engineering Ltd, UK) insulated with silicon tubing and, where necessary, bridged using two-way connectors (Diba Fluid Intelligence, Cambridge, UK). Ethanol was cooled by passage through a 1 meter coil of PTFE tubing held within a Dewar flask (Nalgene, NY, USA) containing dry ice and ethanol. After passing through the loop to cool the brain, ethanol was returned to a reservoir that was open to the air.

For a cooling session, the apparatus was first `pre-cooled' before connecting an animal by pumping ethanol through spare cooling loops (i.e. loops that were not implanted in an animal) until loop temperatures fell below 0°C. The animal was then connected to the system, using the implanted thermocouples to monitor loop temperature at the cortical surface. The temperature was monitored online using a wireless transfer system (UWTC-1, Omega Engineering Ltd., Manchester, UK) or wired thermometer, and pump flow rates adjusted to control loop temperature. Loops over both left and right auditory cortex were connected during bilateral cooling (all tasks), whereas only left or right loop was connected during unilateral cooling (sound localization only).

For F1311, the animal was connected to the system and cooling began before the behavioral session, with the subject held by the experimenter and rewarded with animal treats (Nutriplus gel, Virbac, UK) while cooling pumps were turned on and loop temperatures reduced over five to ten minutes. When loop temperatures reached ≤12°C, the animal was placed in the behavioral arena and testing began. In contrast, F1509 would not perform tasks after being rewarded by the experimenter and so behavioral sessions were started and cortical temperature slowly reduced during task performance.

Trials performed by the animal before the loops reached ≤ 20°C were excluded from analysis. (**Supplementary Figure 1** shows the full distribution of cortical temperatures for each animal in each task).

For both animals, cooling took place while the animals were free to move without interaction with the experimenter and within the same apparatus as used for previous behavioral testing. The behavioral tasks during cooling were unchanged from those already described; i.e. the same ranges of sound levels were used, correction trials were included and the same reward contingencies were used. For each trial in the task, the time of stimulus onset was recorded and cross-referenced with temperature records so that any trials in which cortical temperature was above threshold during a cooling session could be removed offline. During testing, the experimenter was blind to stimuli presented on each trial.

Optogenetic manipulation of auditory cortex

A further subject (F1706) was injected bilaterally in auditory cortex with an adenovirus to induce expression of ChannelRhodopsin 2 in GABAergic interneurons using the mDlx promotor (AAV2.mDlx.ChR2-mCherry-Fishell3.WPRE.SV40, Addgene83898, UPenn Vector Core)(Dimidschstein et al., 2016). For each auditory cortex (i.e. left and right), injections were placed at two sites in the boundary area between middle and posterior Ectosylvian gyrus, and within each site, injections were made at two depths (500 and 800 µm below the cortical surface). Four injections were thus made in total, with 1 µl injected each time.

Following viral delivery, we implanted an optrode (Neuronexus, Ann Arbor, MI, USA) in each auditory cortex to deliver light. During testing, light was delivered from a 463 nm DPSS laser (Shanghai Laser & Optics Century Ltd. China) with a steady-state power of 40 mW, measured at fibre termination before the optrode using an S140C integrating sphere photodiode sensor (ThorLabs, Germany).

In behavioral testing, light was delivered on 50% of test trials (with the exception of the first test session in which the laser was presented on all test trials); however, all correction trials took place without light delivery. On each trial that light was presented, we used short pulses (10 ms duration, presented at 10 Hz) that began 100 ms before sound onset, and continued until 100 ms after sound offset. For this animal, we were only able to test the effect of light delivery on vowel discrimination in noise, as a failure in the implant precluded testing of vowel discrimination in clean conditions, or with stimuli used to study spatial release from masking or sound localization.

Data analysis

All analyses excluded responses on correction trials, or trials where ferrets failed to respond within the required time (60 s). For all tests of vowel discrimination, we also required a minimum number of trials (n=10) in both cooled and control conditions to include a sound level / SNR value in the analysis.

Temperature measurements were obtained on each trial for loops over left and right auditory cortex, and the animal was considered to be cooled if the average loop temperature was ≤ 20°C (bilateral cooling). In unilateral cooling, cooling was considered to be achieved if the relevant loop was ≤ 20°C. The threshold for cooling was based on previous work demonstrating the suppression of neural activity below this temperature (Jasper et al., 1970; Lomber et al., 1999).

Statistical analysis of effects of stimulus manipulation (e.g. presence of noise) and cooling used generalized linear mixed models fitted using the *lme4* library in R (Bates et al., 2015). The details of each model are outlined below; however, in general, analysis of behavioral performance (correct vs. incorrect responses) was based on logistic regression in which the GLMM used binomial distribution and logit link function settings. For each model, we used ferret as a random factor and reported the magnitude of coefficients (β) associated with fixed effects of interest (e.g. effect of cooling) and probability (p) that the coefficient was drawn from a distribution centered about zero.

Vowel Discrimination in Noise

Data shown in Figure 2 indicate bootstrapped performance of animals, where bootstrapping was achieved by randomly sampling data with equal numbers of each vowel and sound level / SNR (when showing data across level / SNR). Bootstrapping was performed 10^3 times, with samples drawn with replacement on each iteration.

To analyse the effects of cooling / light delivery, we compared behavioral performance of each animal across multiple sessions: The effects of cooling were measured on paired testing sessions performed on the same day (F1509) or unpaired sessions collected over the same time period (F1311). Optogenetics provided more refined temporal control than cooling, allowing us to compare performance on trials within the same test session, with and without light delivery (F1706).

For the analysis of F1509, we added an additional constraint to the analysis to address floor effects in control performance. Floor effects arose when this animal occasionally performed poorly during specific control sessions, and thus lowered the baseline from which potential effects of cooling could be observed. For each day (the level at which control and cooled sessions were paired), we identified sound levels / SNRs for which control performance was less than 60%, and excluded both cooled and control trials with these levels. This procedure was also used when analysing spatial release from masking (see below).

Logistic regression was used to compare the effect of treatment (cooling / light delivery) on vowel discrimination in noise and clean conditions across subjects. Treatment and noise condition were included in the model as main effects, with ferret as a random effect. For each animal, we also used permutation tests to assess whether the effect of cooling/light delivery on vowel discrimination in noise, and in clean conditions was larger than would be expected by chance. Permutation tests were conducted by randomly shuffling treatment labels across trials, and recalculating the difference in performance between cooled/light on and control/light off trials. Shuffling was repeated 10^3 times, so that we could report the probability of achieving an equal or larger suppression in performance by random chance ($p < 0.05$). Results were corrected for multiple comparisons using the Holm-Bonferroni correction (Holm, 1979).

Spatial Release from Masking

Data shown in Figure 3. indicate bootstrapped performance (10^3 iterations) discriminating vowels in each stimulus condition (colocalized, spatially separated or clean), where samples were taken with replacement with equal numbers of vowel locations, vowel identities, and where relevant sound level / SNR. In addition to performance in each stimulus condition, we also measured spatial release from masking on each iteration as the difference in performance when vowel and noise were spatially separated (separated - colocated; Fig. 3E).

Logistic regression was used to assess the influence of cooling and stimulus type on odds of making a correct response. To assess the effect of cooling on vowel discrimination in colocated or clean conditions, we modelled performance using stimulus (clean|colocated), cooling (cooled | control) and vowel location (left | right) as fixed effects and ferret as a random effect, with the full model (i.e. interactions between all fixed effects) being fitted.

To model spatial release from masking, we modelled performance using stimulus (separated|colocated), cooling (cooled | control) and vowel location (left | right) as fixed effects and ferret as a random effect, with the full model (i.e. interactions between all fixed effects) being fitted. To test for spatial release from masking in control and cooled conditions separately, the data were filtered for the relevant condition and the effect of cooling was removed from the model specification before refitting.

Sound Localization

Performance localizing sounds was analysed using the percentage of trials on which animals correctly reported the target stimulus position (**Fig. 4B** and **4D**), as well as the response error, measured as the absolute difference between target and response location (**Fig. 4C** and **3E**). For F1311, we included responses to sounds of 100 ms and 250 ms duration, and sampled a random subset of data to ensure equal numbers of trials with each sound duration were included for each cooling condition.

To measure the effect of bilateral cooling on sound localization across animals, we modelled the proportion of correct trials using logistic regression with cooling as a main effect (cooled | control) and ferret as a random effect. We also used the same design (cooling as main effect, ferret as random effect) to model the absolute magnitude of errors with a GLMM that used poisson distribution and log link functions to accommodate non-negative data.

To assess the effect of unilateral cooling on localization of sounds from the left and right sides of the speaker ring, we used similar GLMM designs for modelling the proportion of correct trials, and absolute error. However, the cooling term specified the cooled hemisphere (left | right) and models were extended to include stimulus hemifield (left | right) as a main effect, with the full model fitted to include interactions.

Data Availability

All code and data associated with the project is available at:

https://github.com/stephentown42/cooling_auditory_cortex

Competing Interests

No competing interests declared.

Acknowledgements

We would like to thank Dr. Joseph Sollini for assistance in developing the optogenetic approach and Dr Tara Etherington for assistance with data collection during cortical cooling.

Data associated with sound localization of one subject (F1311) has been previously reported in (Wood et al., 2017)

This research was funded in whole, or in part, by the Wellcome Trust [a Wellcome Trust / Royal Society Sir Henry Dale Fellowship to JKB, Grant number 098418/Z/12/A], a Royal Society Dorothy Hodgkin Fellowship to JKB and the BBSRC (BB/H016813/1). For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

References

Adriani M, Maeder P, Meuli R, Thiran AB, Frischknecht R, Villemure J-G, Mayer J, Annoni J-M, Bogousslavsky J, Fornari E, Thiran J-P, Clarke S. 2003. Sound recognition and localization in man: specialized cortical networks and effects of acute circumscribed lesions. *Exp Brain Res* **153**:591–604. doi:10.1007/s00221-003-1616-0

Ahveninen J, Huang S, Nummenmaa A, Belliveau JW, Hung A-Y, Jääskeläinen IP, Rauschecker JP, Rossi S, Tiitinen H, Raij T. 2013. Evidence for distinct human auditory cortex regions for sound location versus identity processing. *Nat Commun* **4**:1–8. doi:10.1038/ncomms3585

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using **lme4**. *J Stat Softw* **67**. doi:10.18637/jss.v067.i01

Bizley JK, Cohen YE. 2013. The what, where and how of auditory-object perception. *Nat Rev Neurosci* **14**:693–707. doi:10.1038/nrn3565

Bizley JK, Nodal FR, Nelken I, King AJ. 2005. Functional organization of ferret auditory cortex. *Cereb Cortex N Y N 1991* **15**:1637–1653. doi:10.1093/cercor/bhi042

Bizley JK, Walker KMM, King AJ, Schnupp JWH. 2013a. Spectral timbre perception in ferrets: discrimination of artificial vowels under different listening conditions. *J Acoust Soc Am* **133**:365–376. doi:10.1121/1.4768798

Bizley JK, Walker KMM, Nodal FR, King AJ, Schnupp JWH. 2013b. Auditory cortex represents both pitch judgments and the corresponding acoustic cues. *Curr Biol CB* **23**:620–625. doi:10.1016/j.cub.2013.03.003

Bizley JK, Walker KMM, Silverman BW, King AJ, Schnupp JWH. 2009. Interdependent encoding of pitch, timbre, and spatial location in auditory cortex. *J Neurosci Off J Soc Neurosci* **29**:2064–2075. doi:10.1523/JNEUROSCI.4755-08.2009

Blackburn CC, Sachs MB. 1990. The representations of the steady-state vowel sound /e/ in the discharge patterns of cat anteroventral cochlear nucleus neurons. *J Neurophysiol* **63**:1191–1212. doi:10.1152/jn.1990.63.5.1191

Bowers JS. 2017. Grandmother cells and localist representations: a review of current thinking. *Lang Cogn Neurosci* **32**:257–273. doi:10.1080/23273798.2016.1267782

Bronkhorst AW. 2015. The cocktail-party problem revisited: early processing and selection of multi-talker speech. *Atten Percept Psychophys* **77**:1465–1487. doi:10.3758/s13414-015-0882-9

Brungart DS. 2001. Informational and energetic masking effects in the perception of two simultaneous talkers. *J Acoust Soc Am* **109**:1101–1109. doi:10.1121/1.1345696
Chambers AR, Hancock KE, Sen K, Polley DB. 2014. Online Stimulus Optimization Rapidly Reveals Multidimensional Selectivity in Auditory Cortical Neurons. *J Neurosci* **34**:8963–8975. doi:10.1523/JNEUROSCI.0260-14.2014
Clarke S, Bellmann A, Meuli RA, Assal G, Steck AJ. 2000. Auditory agnosia and auditory spatial deficits following left hemispheric lesions: evidence for distinct processing pathways. *Neuropsychologia* **38**:797–807. doi:10.1016/S0028-3932(99)00141-4
Clarke S, Bellmann Thiran A, Maeder P, Adriani M, Vernet O, Regli L, Cuisenaire O, Thiran J-P. 2002. What and Where in human audition: selective deficits following focal hemispheric lesions. *Exp Brain Res* **147**:8–15. doi:10.1007/s00221-002-1203-9
Cohen YE, Russ BE, Gifford GW, Kiringoda R, MacLean KA. 2004. Selectivity for the Spatial and Nonspatial Attributes of Auditory Stimuli in the Ventrolateral Prefrontal Cortex. *J Neurosci* **24**:11307–11316. doi:10.1523/JNEUROSCI.3935-04.2004
Coomber B, Edwards D, Jones SJ, Shackleton TM, Goldschmidt J, Wallace MN, Palmer AR. 2011. Cortical inactivation by cooling in small animals. *Front Syst Neurosci* **5**:53. doi:10.3389/fnsys.2011.00053
Dewson JH. 1964. Speech Sound Discrimination by Cats. *Science* **144**:555–556. doi:10.1126/science.144.3618.555
Dewson JH, Pribram KH, Lynch JC. 1969. Effects of ablations of temporal cortex upon speech sound discrimination in the monkey. *Exp Neurol* **24**:579–591. doi:10.1016/0014-4886(69)90159-9
Diamond IT, Goldberg JM, Neff WD. 1962. Tonal discrimination after ablation of auditory cortex. *J Neurophysiol* **25**:223–235. doi:10.1152/jn.1962.25.2.223
Dimidschstein J, Chen Q, Tremblay R, Rogers SL, Saldi G-A, Guo L, Xu Q, Liu R, Lu C, Chu J, Grimley JS, Krostag A-R, Kaykas A, Avery MC, Rashid MS, Baek M, Jacob AL, Smith GB, Wilson DE, Kosche G, Kruglikov I, Rusielewicz T, Kotak VC, Mowery TM, Anderson SA, Callaway EM, Dasen JS, Fitzpatrick D, Fossati V, Long MA, Noggle S, Reynolds JH, Sanes DH, Rudy B, Feng G, Fishell G. 2016. A viral strategy for targeting and manipulating interneurons across vertebrate species. *Nat Neurosci* **19**:1743–1749. doi:10.1038/nn.4430
Downer JD, Rapone B, Verhein J, O'Connor KN, Sutter ML. 2017. Feature-Selective Attention Adaptively Shifts Noise Correlations in Primary Auditory Cortex. *J Neurosci Off J Soc Neurosci* **37**:5378–5392. doi:10.1523/JNEUROSCI.3169-16.2017
Evarts EV. 1952. Effect of auditory cortex ablation on frequency discrimination in monkey. *J Neurophysiol* **15**:443–448. doi:10.1152/jn.1952.15.6.443
Földiák P. 2009. Neural Coding: Non-Local but Explicit and Conceptual. *Curr Biol* **19**:R904–R906. doi:10.1016/j.cub.2009.08.020
Fusi S, Miller EK, Rigotti M. 2016. Why neurons mix: high dimensionality for higher cognition. *Curr Opin Neurobiol*, Neurobiology of cognitive behavior **37**:66–74. doi:10.1016/j.conb.2016.01.010
Gao P, Ganguli S. 2015. On simplicity and complexity in the brave new world of large-scale neuroscience. *Curr Opin Neurobiol*, Large-Scale Recording Technology (32) **32**:148–155. doi:10.1016/j.conb.2015.04.003
Goldberg JM, Neff WD. 1961. Frequency discrimination after bilateral ablation of cortical auditory areas. *J Neurophysiol* **24**:119–128. doi:10.1152/jn.1961.24.2.119
Heffner HE, Heffner RS. 1986. Effect of unilateral and bilateral auditory cortex lesions on the discrimination of vocalizations by Japanese macaques. *J Neurophysiol* **56**:683–701. doi:10.1152/jn.1986.56.3.683
Holm S. 1979. A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Stat* **6**:65–70.
Jasper HH, Shacter DG, Montplaisir J. 1970. The effect of local cooling upon spontaneous and evoked electrical activity of cerebral cortex. *Can J Physiol Pharmacol* **48**:640–652. doi:10.1139/y70-094

Jazayeri M, Afraz A. 2017. Navigating the Neural Space in Search of the Neural Code. *Neuron* **93**:1003–1014. doi:10.1016/j.neuron.2017.02.019
Johnston WJ, Palmer SE, Freedman DJ. 2020. Nonlinear mixed selectivity supports reliable neural computation. *PLOS Comput Biol* **16**:e1007544. doi:10.1371/journal.pcbi.1007544
Jones GL, Litovsky RY. 2011. A cocktail party model of spatial release from masking by both noise and speech interferers. *J Acoust Soc Am* **130**:1463–1474. doi:10.1121/1.3613928
Kaas JH, Hackett TA. 2000. Subdivisions of auditory cortex and processing streams in primates. *Proc Natl Acad Sci* **97**:11793–11799. doi:10.1073/pnas.97.22.11793
Kavanagh GL, Kelly JB. 1987. Contribution of auditory cortex to sound localization by the ferret (Mustela putorius). *J Neurophysiol* **57**:1746–1766. doi:10.1152/jn.1987.57.6.1746
Litovsky R. 2012. Spatial release from masking. *Acoust Today* **8**:18–25.
Lomber SG, Malhotra S. 2008. Double dissociation of “what” and “where” processing in auditory cortex. *Nat Neurosci* **11**:609–616. doi:10.1038/nn.2108
Lomber SG, Payne BR, Horel JA. 1999. The cryoloop: an adaptable reversible cooling deactivation method for behavioral or electrophysiological assessment of neural function. *J Neurosci Methods* **86**:179–194. doi:10.1016/S0165-0270(98)00165-4
Macellaio MV, Liu B, Beck JM, Osborne LC. 2020. Why sensory neurons are tuned to multiple stimulus features. doi:10.1101/2020.12.29.424235
Malhotra S, Stecker GC, Middlebrooks JC, Lomber SG. 2008. Sound Localization Deficits During Reversible Deactivation of Primary Auditory Cortex and/or the Dorsal Zone. *J Neurophysiol* **99**:1628–1642. doi:10.1152/jn.01228.2007
Massopust LC, Barnes HW, Verdura J. 1965. Auditory frequency discrimination in cortically ablated monkeys. *J Aud Res* **5**:85–93.
Mesgarani N, Chang EF. 2012. Selective cortical representation of attended speaker in multi-talker speech perception. *Nature* **485**:10.1038/nature11020. doi:10.1038/nature11020
Middlebrooks JC, Onsan ZA. 2012. Stream segregation with high spatial acuity. *J Acoust Soc Am* **132**:3896–3911. doi:10.1121/1.4764879
Middlebrooks JC, Pettigrew JD. 1981. Functional classes of neurons in primary auditory cortex of the cat distinguished by sensitivity to sound location. *J Neurosci Off J Soc Neurosci* **1**:107–120.
Middlebrooks JC, Xu L, Eddins AC, Green DM. 1998. Codes for sound-source location in nontonotopic auditory cortex. *J Neurophysiol* **80**:863–881. doi:10.1152/jn.1998.80.2.863
Młynarski W. 2015. The Opponent Channel Population Code of Sound Location Is an Efficient Representation of Natural Binaural Sounds. *PLOS Comput Biol* **11**:e1004294. doi:10.1371/journal.pcbi.1004294
Moore TJ, Cashin JL. 1974. Response patterns of cochlear nucleus neurons to excerpts from sustained vowels. *J Acoust Soc Am* **56**:1565–1576. doi:10.1121/1.1903480
Nodal FR, Bajo VM, King AJ. 2012. Plasticity of spatial hearing: behavioural effects of cortical inactivation. *J Physiol* **590**:3965–3986. doi:10.1113/jphysiol.2011.222828
O’Connor K, Yin P, Petkov C, Sutter M. 2010. Complex Spectral Interactions Encoded by Auditory Cortical Neurons: Relationship Between Bandwidth and Pattern. *Front Syst Neurosci* **4**:145. doi:10.3389/fnsys.2010.00145
Ponvert ND, Jaramillo S. 2019. Auditory Thalamostriatal and Corticostriatal Pathways Convey Complementary Information about Sound Features. *J Neurosci* **39**:271–280. doi:10.1523/JNEUROSCI.1188-18.2018
Quian Quiroga R. 2016. Neuronal codes for visual perception and memory. *Neuropsychologia*, Special Issue: Functional Selectivity in Perceptual and Cognitive Systems - A Tribute to Shlomo Bentin (1946-2012) **83**:227–241. doi:10.1016/j.neuropsychologia.2015.12.016
Rauschecker JP, Scott SK. 2009. Maps and streams in the auditory cortex: nonhuman primates illuminate human speech processing. *Nat Neurosci* **12**:718–724. doi:10.1038/nn.2331
Rauschecker JP, Tian B. 2000. Mechanisms and streams for processing of “what” and “where” in auditory cortex. *Proc Natl Acad Sci* **97**:11800–11806. doi:10.1073/pnas.97.22.11800

Rigotti M, Barak O, Warden MR, Wang X-J, Daw ND, Miller EK, Fusi S. 2013. The importance of mixed selectivity in complex cognitive tasks. *Nature* **497**:585–590. doi:10.1038/nature12160

Romanski LM, Tian B, Fritz J, Mishkin M, Goldman-Rakic PS, Rauschecker JP. 1999. Dual streams of auditory afferents target multiple domains in the primate prefrontal cortex. *Nat Neurosci* **2**:1131–1136. doi:10.1038/16056

Schebesch G, Lingner A, Firzlaff U, Wiegrebe L, Grothe B. 2010. Perception and neural representation of size-variant human vowels in the Mongolian gerbil (Meriones unguiculatus). *Hear Res* **261**:1–8. doi:10.1016/j.heares.2009.12.016

Smith AL, Parsons CH, Lanyon RG, Bizley JK, Akerman CJ, Baker GE, Dempster AC, Thompson ID, King AJ. 2004. An investigation of the role of auditory cortex in sound localization using muscimol-releasing Elvax. *Eur J Neurosci* **19**:3059–3072. doi:10.1111/j.0953-816X.2004.03379.x

Stecker GC, Harrington IA, Macpherson EA, Middlebrooks JC. 2005. Spatial Sensitivity in the Dorsal Zone (Area DZ) of Cat Auditory Cortex. *J Neurophysiol* **94**:1267–1280. doi:10.1152/jn.00104.2005

Town SM, Atilgan H, Wood KC, Bizley JK. 2015. The role of spectral cues in timbre discrimination by ferrets and humans. *J Acoust Soc Am* **137**:2870–2883. doi:10.1121/1.4916690

Town SM, Brimijoin WO, Bizley JK. 2017. Egocentric and allocentric representations in auditory cortex. *PLOS Biol* **15**:e2001878. doi:10.1371/journal.pbio.2001878

Town SM, Wood KC, Bizley JK. 2018. Sound identity is represented robustly in auditory cortex during perceptual constancy. *Nat Commun* **9**:1–15. doi:10.1038/s41467-018-07237-3

van der Heijden K, Rauschecker JP, Formisano E, Valente G, de Gelder B. 2018. Active Sound Localization Sharpens Spatial Tuning in Human Primary Auditory Cortex. *J Neurosci Off J Soc Neurosci* **38**:8574–8587. doi:10.1523/JNEUROSCI.0587-18.2018

Walker KMM, Bizley JK, King AJ, Schnupp JWH. 2011. Multiplexed and robust representations of sound features in auditory cortex. *J Neurosci Off J Soc Neurosci* **31**:14565–14576. doi:10.1523/JNEUROSCI.2074-11.2011

Wood KC, Town SM, Atilgan H, Jones GP, Bizley JK. 2017. Acute Inactivation of Primary Auditory Cortex Causes a Sound Localisation Deficit in Ferrets. *PLOS ONE* **12**:e0170264. doi:10.1371/journal.pone.0170264

Wood KC, Town SM, Bizley JK. 2019. Neurons in primary auditory cortex represent sound source location in a cue-invariant manner. *Nat Commun* **10**:1–15. doi:10.1038/s41467-019-10868-9

Yi HG, Leonard MK, Chang EF. 2019. The Encoding of Speech Sounds in the Superior Temporal Gyrus. *Neuron* **102**:1096–1110. doi:10.1016/j.neuron.2019.04.023

Zhou B, Green DM, Middlebrooks JC. 1992. Characterization of external ear impulse responses using Golay codes. *J Acoust Soc Am* **92**:1169–1171. doi:10.1121/1.404045

Znamenskiy P, Zador AM. 2013. Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination. *Nature* **497**:482–485. doi:10.1038/nature12077