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Osama Hussein April 9th, 2023

Identifying Brain Regions that Enable Auditory Localization in Freely Moving Mice.

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2023

Abstract

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People and animals can localize sounds while moving around their environment, but the neural circuits for this are unknown. One candidate structure is the auditory cortex, which is involved in higher-order processing of sound. This project tries to identify the brain structures that allow mice to perform, learn, and adapt to localizing sound while freely moving. We trained freely moving mice in a new behavioral task that challenges them to find a reward port that is playing sound. We used surgical aspiration lesions to test the role of the auditory cortex, and computational anatomy to locate the lesions in the brain. We found that the complete bilateral removal of auditory cortex and nearby structures strongly impaired the ability to perform this task, but that partial removal had a smaller effect. Therefore, we conclude that the ability to perform this task is distributed over auditory cortex and nearby structures.

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Introduction and Background

Auditory localization is the ability to localize the origin of sound from any direction or distance, which helps us make decisions related to where we are in time and space. For instance, we can use localization to identify the direction of traffic when crossing the street, allowing us to effectively navigate our environment. Importantly, localization allows for the separation of complex acoustic stimuli, such as vocalizations, which is vital for survival and communication (Wood et al., 2019). For example, in a social setting, we require auditory localization to discriminate or identify what someone is saying when other people are speaking at the same time, which is called the cocktail party effect (Bronkhorst, 2015).

Moreover, in nature, animals, and humans both move freely. Importantly, their heads and bodies aren't bound or constricted to any particular direction. However, in a laboratory setting animals and humans are often studied in fixed-head or restrained positions, which is neither natural position for animals nor humans. While these positions allow us to investigate complex neural circuits that cannot be observed otherwise; these positions create undue stress for animals and research subjects which can result in behavior that is only observed in unnatural conditions (Schwarz et al., 2010). In nature, humans can rotate their heads upwards of 90 degrees to the right and the left, and other animals such as owls can rotate their heads at a maximum of 270 degrees. The ability of organisms to move the body to better process sensory information is called active sensing. Active sensing involves two main processes: perception and action (Yang et al., 2016). Perception is the cognitive process by which we interpret information about the world, and action is the mechanism by which we obtain information about the world (Yang et al., 2016). However, recent studies of the auditory system ignore active sensing by modeling sound localization and hearing in head-fixed mice (Parker et al., 2020). Given that neither mice nor humans naturally behave with their heads restrained in place, there is a knowledge gap about active localization in the auditory system. We sought to address this knowledge gap by developing a behavioral model of active hearing in mice.

The auditory cortex (AC) is a part of the brain that processes complex auditory information. In humans, it is located in the temporal lobe of the cerebral cortex (Mangold & Das, 2022). In mice, the AC is divided into multiple regions: the primary auditory field (AUDp), the ventral auditory field (AUDv), the dorsal auditory field (AUDd), and the posterior auditory field (AUDpo) (Tsukano et al., 2016). However, AUDp has been identified to play a key role in sound

perception as it is one of the first cortical structures to process sound in the brain (Bowen et al., 2020). Importantly, AUDp has also been shown to encode the location of sound sources (Wood et al., 2019).

On a neural level, individual neurons in the AC have been shown to encode the spatial location of sounds, meaning that they respond differently based on the location of a sound (Wood et al., 2019). However, other structures like the inferior colliculus also encode sound location, so it is not clear whether the AC is necessary for any particular auditory task (Slee & Young, 2011). In fact, during the identification of simple sounds subcortical structures can substitute for the AC; however, AC is essential for complex sound identification (Ceballo et al., 2019). These studies in head-fixed mice suggest that AC is required for complex but not simple tasks, but it is unknown whether freely moving sound localization is simple enough to not engage AC or sufficiently complex enough to engage AC.

A parallel study has examined whether the motor cortex is necessary for skill learning. In mice, the motor cortex is required for learning a timed lever-pressing task, but not for performing that task after learning, due to consolidation in subcortical pathways (Kawai et al., 2015). This suggests that the primary role of the motor cortex is for learning, not performing, skilled behaviors. Despite this, it is unknown whether cortex is involved in performing tasks that combine skilled body movement with sensory processing. Another experiment in ferrets indicated that connections between AUDp and the inferior colliculus are necessary for recovery from single-sided hearing loss (Bajo et al., 2010), indicating that auditory learning is consolidated in other structures, not only the AUDp. Finally, another experiment examined whether lesions in the somatosensory cortex can affect the acquisition and performance of mice in facial whisker object detection task. The experiment showed that the acquisition and performance of the mice on the learned whisker task were not affected as a result of the primary somatosensory cortex lesion (Hong et al., 2018). Thus, for object detection, other surrounding structures can pick up the function of the primary somatosensory cortex, but this is unknown for the auditory cortex. In summary, prior work demonstrates that cortex is dispensable for simple discrimination but essential for motor learning, recovery from sensory loss, and complex discrimination.

To address these knowledge gaps, we developed an auditory localization task that challenges mice to localize sound while freely moving. Due to the novelty of this task, it is

unknown if it requires the auditory cortex. We tested this by lesioning the auditory cortex after mice learned the task. We also lesioned the visual cortex as a control. We hypothesized that the auditory cortex, but not the visual cortex, is required for good performance in our auditory task. We found that large lesions, but not small lesions, significantly impaired performance. We conclude that the ability to perform this task is distributed over the auditory cortex and nearby structures.

Methods

Animals. We tested two cohorts of mice in this study. The first cohort comprised five CBA/CaJ mice, two female and three male, purchased from Jackson Laboratories. The second cohort comprised eight CBA/CaJ x C57BL/6 hybrid mice, three females and five males, all bred in our animal facility. Mice selected for the study are usually 70 days of age or older.

Motivation paradigm. The mice are placed on a water restriction and receive a fixed ration of water every day. The water ration is calculated on the first day of restriction using the baseline weight for every mouse on the day that they are moved from *ad libitum* water to a restriction cage. They receive water rewards for correct trials during the behavioral task, and if they do not complete enough trials correctly to receive their daily ration during the task, we provide the remainder to them after training. Throughout their training, the mice are weighed daily to assess weight stability with respect to their baseline weight. Mice falling below the 80% threshold of their baseline weight require immediate intervention by permanently increasing their daily water ration by 0.1 mL to maintain their weight over time. Mice below 75% of their baseline weight would be immediately removed from the study, although this never happened during our study.

Behavioral Training. The mice go through four phases of behavioral shaping before they are moved on to the full auditory task. These stages are habituation, manually baited poketrain, group poketrain, and individualized poketrain, each lasting from 3 to 4 days. During the habituation process, the mice are habituated to handling. The mice are then water deprived, starting the next stage, manually baited poketrain. In this stage, mice are placed for the first time in an octagonal arena with a nose poke and a speaker on each wall (Fig. 1A-B). We manually bait the nose pokes using water to allow the mice to establish nose pokes as a water source (Fig. 1C). Next, the mice move to group poketrain, in which they may use any of the nose pokes to get water as a group. This allows some group learning between the mice. The next stage is

individualized poketrain where the mice are individually trained in the nose poke mechanism. This allows them to further establish the nose pokes as a reliable water source. After completing this process, the mice move on to the full auditory localization task. During the localization task, a sound (bandpassed white noise) is played out of one of the eight speakers on the walls of the octagonal arena (Fig. 1A). The mice have to actively localize this sound because they are only rewarded for poking the corresponding nose poke.

Lesion Surgeries. Brain lesion surgeries involve removing or damaging a brain region of interest in order to test how it affects behavior. For our purposes, we lesioned the auditory cortex bilaterally in some animals and the visual cortex bilaterally in other animals as a control (Fig. 2). The visual cortex was chosen as a control region because vision is not necessary for the task. Therefore, this control tests whether the effect of the lesion on performance is due to a side effect of invasive brain surgery, or to the specific brain region under test. During the lesion surgery, we used a stereotax to determine the locations of the auditory cortex and the visual cortex using predetermined coordinates with respect to the bregma reference point. The coordinates for the auditory cortex are -2.7mm posterior and 5.0mm lateral with respect to bregma. The coordinates for the visual cortex are -3.5mm posterior and 2.2 lateral also with respect to bregma. In the first AC lesion cohort, the lesions were made to 2mm in length and width and 1mm in depth, spanning most but not all of the auditory cortex (comprising AUDp, AUDd, AUDv, and AUDpo). However, one mouse in the first AC cohort lesion was lesioned at 1mm to test for behavioral differences. In the second cohort, all lesions were made to be 1mm in length and width and 1 mm in depth, whether in the auditory or visual cortex. The lesions were conducted via vacuum aspiration where we withdraw tissue using a sterile needle attached to a vacuum tube. Once the lesion was made the craniotomy was covered with biocompatible silicone sealant (Kwik Cast) to seal off the skull and the skin was sutured. The mice recover for 4-5 days with *ad libitum* water to eliminate any complications due to water deprivation. Once recovered, the mice are tested on the localization task for a minimum of 5 days.

Histology. After the behavioral testing period in the post-lesion mice, the mice are perfused to harvest the brain. The brain needs to be harvested in a timely fashion to prevent the accumulation of scar tissue at the site of the lesion. Once harvested the brain is cryoprotected in sucrose and frozen to prepare for sectioning. The brain is sectioned using a cryostat set at 50 μm, and the slides are cover slipped the following day. The slides are then imaged using the Keyence

microscope. These images are then cropped and rotated in FIJI (Schindelin et a, 2012) and then aligned with the Allen Mouse Brain Atlas using the ABBA software (https://biop.github.io/ijp-imagetoatlas/), to determine the location of the lesion. After aligning each slice with the atlas, we identified which brain regions were damaged in each slice to generate tables and plots that showcase the location of the damage.

Results:

Scoring the Behavior. To score our behavior we have three important metrics: Fraction Correct (FC), Rank of Correct Port (RCP), and Number of trials (n). FC is the number of correct trials divided by the total number of trials. A correct trial is one in which the mouse went to a port playing sound before any of the other six ports, not including the port that was rewarded on the previous trials (Fig. 3). Because the mouse chooses between seven ports on each trial, chance performance is calculated as one over seven or 0.143, which is the performance that the mice can achieve by random guessing without knowing the full rules of the task (Table 1). Mice tend to perform at the chance for the first few training sessions but over time performance increases above chance as they learn the task (Fig. 4 & 5). Similarly, RCP indicates the number of incorrect ports poked before poking the correct port. Chance level performance for RCP is calculated as a mean RCP value of 3. Finally, n indicates the number of trials each mouse performs over the training session (Table 1). Over time as the mice become accustomed to the motivation paradigm the mice tend to perform more trials per session, but after recovery from surgery and during post-lesion testing n trials tend to drop slightly (Fig. 6).

In any given trial, a mouse can only receive an FC value of 1 or 0 for that particular trial. An FC value of 1 indicates a correct trial while an FC value of 0 indicates an incorrect trial. In a given trial, if a mouse correctly identifies the sound source upon its first poke into the correct port, it only receives an FC value of 1, consequently, the mouse also receives an RCP value of 0 (Fig. 3A). However, if a mouse does not correctly localize the sound upon the first poke, it only receives a fraction correct of 0 and an RCP value ranging from 1 to 6 (Fig 3B-C). In this instance, a new trial does not begin until the mouse is able to find the correct sound source. This can be after the mouse pokes a second poke or after poking multiple pokes in a row. Generally, the mice tend to get better at finding the correct port from the first poke as training sessions

increase over time, which drives higher FC, lower RCP, and higher n trials values across training sessions (Fig. 4 & 5).

Mice can learn active sound localization behavior. We have demonstrated through multiple cohorts that the mice can learn this auditory behavior over time. Figures 4 and 5 show that the mice can go from chance performance on the task to medium and high performance after a few weeks of training. Other than helping us keep track of mouse training sessions, these learning curves allow us to select mice for lesioning. Generally, we chose mice to lesion after their FC performance had plateaued. Following the general trend in these learning curves, we observed that the average FC performance value for both of the cohorts tends to be between 0.4 and 0.6 across all training sessions (Fig. 4 & 5). The average RCP performance varies slightly across both of the cohorts but tends to range from 2 to 1 or below (Fig. 4 & 5). While we observed that some mice tend to perform outstandingly on all of the learning curves, there are some mice that fall behind, and those mice were not lesioned until they have an FC performance value of 0.4. All of the mice in all of the cohorts were lesioned.

We additionally trained mice to perform more complex versions of the task. In a spatial variant, mice had to localize sound that was distributed over multiple nearby speakers. In an attention-demanding variant, mice had to localize sounds of one frequency and ignore another frequency. Mice also learned these more complex tasks (data not shown), but all lesion experiments were performed in the standard version of the task.

Effects of lesioning AC and VC. We observed mixed results when comparing the two cohorts of AC lesions. The first cohort was trained during the summer while the second trained during the fall. In the first cohort, we observed that lesioning AC resulted in a profound effect where the mice performed poorly and never recovered (Fig. 6). We observed that after the lesion FC performance in the first cohort fluctuated around chance performance (1/7) and did not show any signs of recovery (Fig. 6A). RCP performance also was indicative of the same effect where the mice did not recover, and their performance hovered around the chance performance of 3 (Fig. 6B). Interestingly, the number of trials was also impacted by the lesion. The mice did not seem to perform as many trials before the lesion after recovery and testing (Fig. 6C). This is an interesting result given that the deprivation protocol was the same after the testing.

Going into lesioning the second cohort we predicted that the effect of lesioning AC in the second cohort was going to be the same as lesioning the first cohort. However, we observed that

the performance of the recovered mice during surgery was profoundly different in the second cohort. The lesion had only a medium effect on performance in this cohort when compared to the first cohort (Fig. 6). Moreover, the number of trials in the second cohort tended to follow the same trend as the first cohort where the number of trials decreased tremendously during the first few post-lesion testing sessions (Fig. 6C). However, unlike the first cohort, the number of trials increased as the post lesioning testing sessions were conducted, even though the same restriction protocol was used during the post-lesion testing period.

We observed that lesioning the visual cortex (VC) did not impact the behavior of the mice after recovery (Fig. 6). In fact, some of the mice performed better after lesioning their visual cortex. In other words, lesioning the VC showed that the FC, RCP, and number of trials in all the mice did not change. This confirmed that the effects of AC lesions above could not be due to a side effect of the surgery but must be due to a specific role of AC itself.

Statistically, we also observed that the mice with the largest lesion size showed the most significant impact on performance. Using Fisher's Exact Test we statistically compared the number of correct trials on the last training session versus every testing after the lesion recovery (Table 2 & 3). AC cohort 1 mouse 1 and mouse 3 lesions were both made to be 2 mm these two lesions showed the largest impact on performance. For these two animals, the analysis showed that every testing session was statistically significant compared to all the other mice in all the other cohorts. An interesting point of comparison is that within the first cohort, the one mouse with the smaller 1 mm size lesion showed testing sessions that were not statistically significant as opposed to the other mice within the same cohort; all with the larger lesions showed statistical significance for all testing sessions. The second AC cohort mice show similar statistical significance to the smaller size AC cohort lesion. The VC lesions do not show statistical significance for any of the testing sessions.

Computational Anatomy. We performed computational anatomy to address the location of these lesions. Some tissue was lost in histological processing and other tissue was lost by an oversight, but we provide anatomical results for four mice out of seven tested mice. This includes one AC lesion from the first cohort and two AC lesions and one VC lesion from the second cohort (Fig. 7).

The AC lesions from the first cohort were intended to be 2 mm, compared to 1 mm in the second cohort. However, one lesion from the first cohort was made to be 1mm, and even with

this smaller lesion, the same profound performance effect was observed. Furthermore, only one mouse from the first AC cohort was analyzed with computational anatomy. The AC lesion from this one mouse was 2mm and it spanned a greater area to include the following brain regions on both the right and left side: SSp, SSs, AUDd, AUDp, AUDpo, AUDv, TEa, ECT, PERI, ENTI, VISp, VISrl, VISal, VISI, VISli, and VISpor (Fig. 7A, Table. 4). This is a total of 16 regions that were damaged (Fig. 8). No subcortical regions were damaged. This mouse showed a large behavioral impairment.

The next two analyzed lesions from the second cohort were intended to be 1 mm. The first mouse spanned regions AUDp, AUDd, AUDpo, AUDv, TEa on the right side and SSp, SSs, AUDp, AUDd, AUDpo, AUDv, Tea, ECT, PERI, ENTI on the left side (Fig. 7C, Table 4). The second mouse spanned regions AUDp, AUDd, AUDpo, AUDv, and TEa both on the left and right side (Fig 7D, Table 4). The total number of damaged areas for both of these mice is 11 and 5. We note that both of these mice also sustained damage to the hippocampus, although we did not further quantify this. These mice showed mild or no behavioral impairment.

The analyzed VC lesion revealed that regions RSPagl, SSp-tr, VISam, VISa, VISrl, VISpm, VISp, and VIS were damaged on the right side, while regions VISal, VISrl, VISp, VISpm, VISam, RSPag, RSPd were damaged on the left side (Fig. 7B, Table 4). This mouse showed no behavioral impairment at all.

Discussion:

We observed variable effects of the auditory cortex lesion between animals, and we considered the possibility that this might be explained by variations in the shape and size of the lesion between animals. To accurately understand the results of our lesions we conducted computational anatomy using the ABBA software.

The primary result that we found was that mice with smaller AC lesions showed moderate or mild behavioral effects, whereas the mouse with the largest lesion showed one of the largest behavioral effects. The majority of first cohort auditory cortex lesions were intended to be 2 mm, with only one mouse having a lesion of 1mm and showing the same profound effect of the lesion as the other two 2 mm lesioned mice. In comparison, the second cohort lesions were all intended to be 1 mm only showing a medium effect. Given this contradiction, between the two cohorts, we are not able to address the reason behind the profound effect of the 1 mm mouse lesion in the first AC cohort given that we were unable to analyze its anatomy for technical

reasons. However, the medium effect of the second cohort lesion can be explained by the fact that these lesions are much smaller, with minimal regional damage to the auditory cortex.

When compared to the first cohort, the second cohort lesions appear to span fewer structures than the first cohort lesions. This indicates that a larger broader lesion allows for more damage across a larger area of the cortex, thus minimizing the chance that other structures can serve as backups to the learned skill behavior. This is in accordance with the mass action principle that states that impairments in learned tasks are due to the amount of tissue damage and not a particular location in the cortex (Kolb and Whishaw 1988). This principle means that the entirety of the cortex participates in behavior, and the larger lesions profoundly impacted performance since they damaged the entirety of AC as well as surrounding structures.

Moreover, the varying size of our lesions led to some unexpected conclusions. Some of the mice in the second cohort had substantial hippocampal damage but showed no behavioral impairment. The mouse that showed a large behavioral impairment had no hippocampal damage. Thus, we find that the hippocampus is likely uninvolved in this task, despite the hippocampus' known role in spatial navigation.

There are other possibilities that might explain the results we found. First, the mice in the second cohort have been trained for a longer period of time than those from the first cohort. In the second cohort, the mice were introduced to the task but did not learn it within the desired time, so we decided to make the task a little easier by making the noise in the task a little louder and slower. Once we implemented this change the mice in the second cohort were able to learn the task quickly, which might explain why the mice in the second cohort were performing very high before the lesion when compared to the mice in the first cohort before the lesion. Furthermore, this change might have resulted in the task being extremely easy for the mice to perform in the second cohort that the mice might not have needed to rely on AC as much as other structures. This means that with the easier version of the task the mice might have consolidated the learned task in other brain regions that can handle the task without too much help from AC.

Another possibility that might have impacted our results from the first cohort to the second cohort is that the mice strains were different. In the first cohort we purchased our mice were CBA/CaJ mice purchased from Jackson Laboratories; however, in the second cohort, we bred our mice in the lab, which were the F1 generation of a CBA/CaJ and a C57 cross. This might have resulted in the different results that we saw when comparing the two cohorts.

Lastly, the medium effect in the second cohort might have just been that with the louder and faster sound, the identification of the sound during the task or the performance might just not be where AC is involved. However, AC can be directly involved in learning this easier version of the task but is not necessary for performing well on an easier version of the auditory localization task.

In conclusion, we found that the mices' performance on our auditory task is very related to the amount of AC that is lost. If the AC and the surrounding regions are completely lost like in the first cohort lesions, then it is very plausible to assume that complete loss of AC can result in complete loss of performance on the simple auditory task. However, partial loss of the AC such as in the second cohort lesions can result in a medium loss of performance. This suggests that the AC might be dispensable, in that the remaining parts of AC could account for the loss of cortex, thus minimally affecting performance on the task if the AC sustained minimal damage.

Tables and Figures

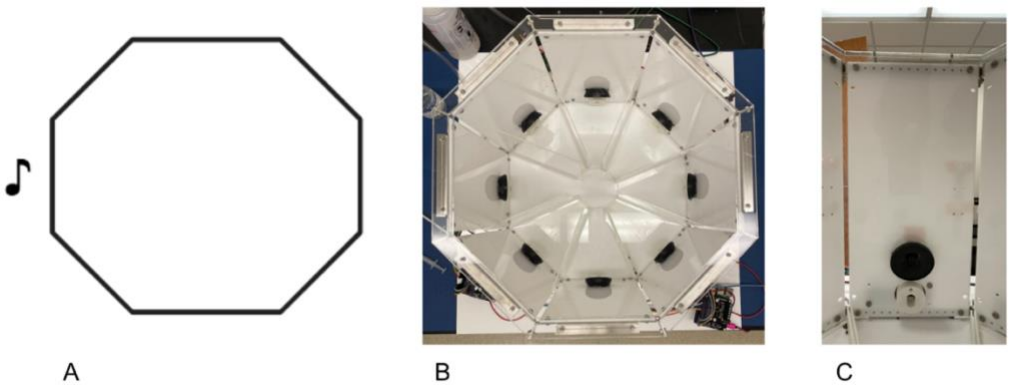


Figure 1. Auditory Localization Task

A. Auditory Localization Task.
B. Top-down view of the Octagon (training arena).
C. Point of view of the mouse inside the octagon. The speaker is shown above the nose poke.

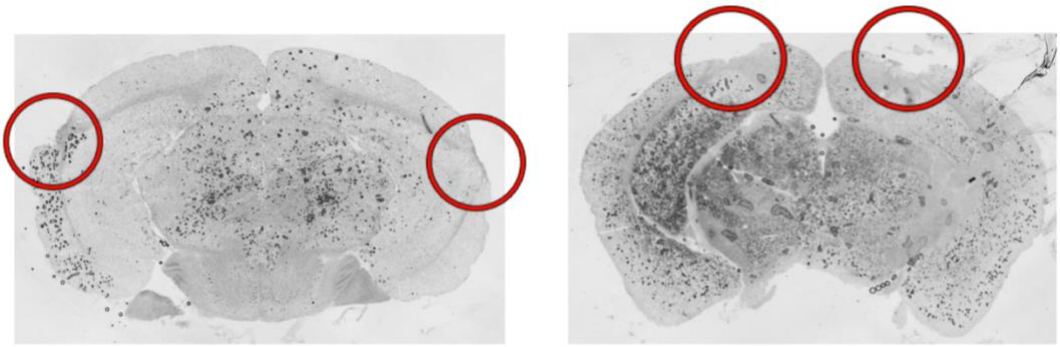


Figure 2. Histology sections showing Auditory Cortex Lesion and Visual Cortex Lesion Location.

A. Auditory Cortex (AC) Lesion is Highlighted in Red.
B. Visual Cortex (VC) Lesion is Highlighted in Red.

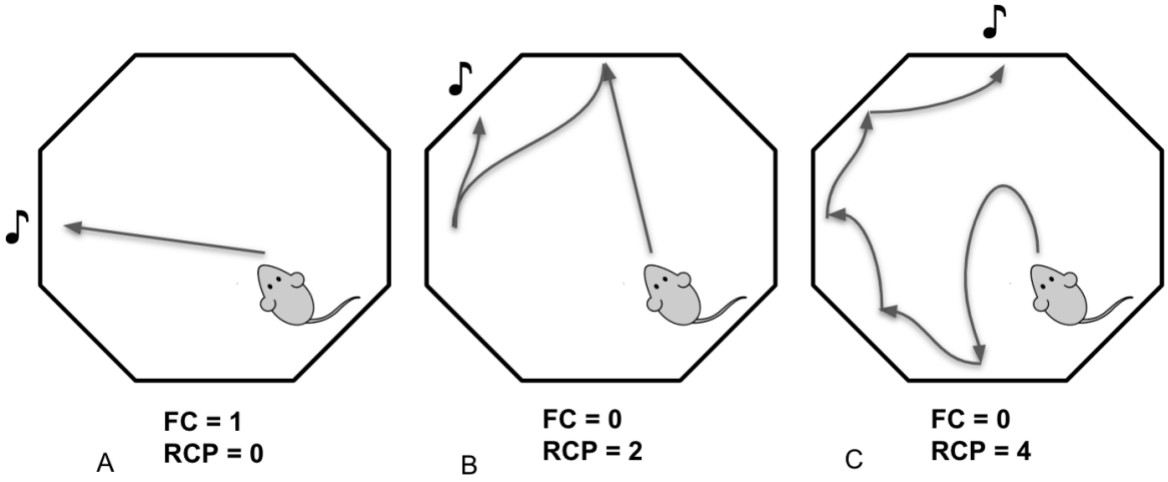
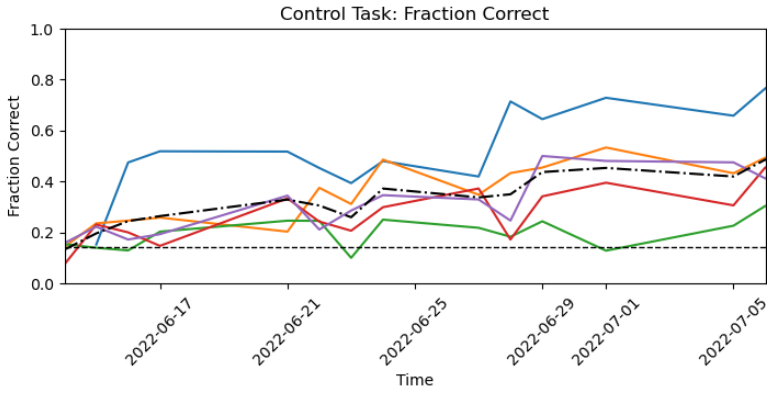


Fig 3. Schematic Illustrating Scoring of the Auditory Behavior Task.

A. Schematic illustrating a correct trial where FC is 1 and RCP is 0
B. Schematic illustrating an incorrect trial where FC is 0 and RCP is 3
C. Schematic illustrating an incorrect trial where FC is 0 and RCP is 4

A



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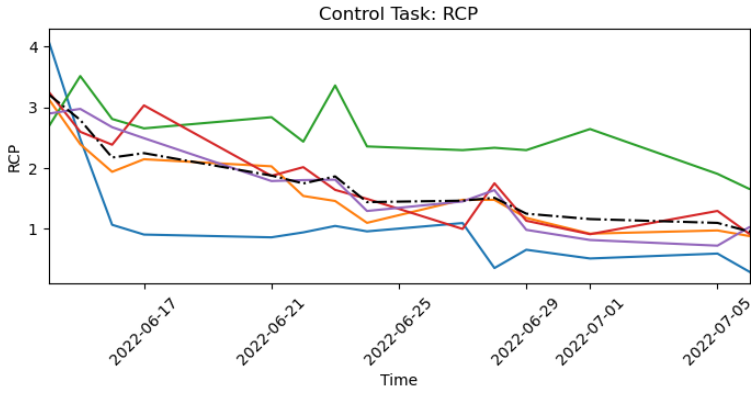

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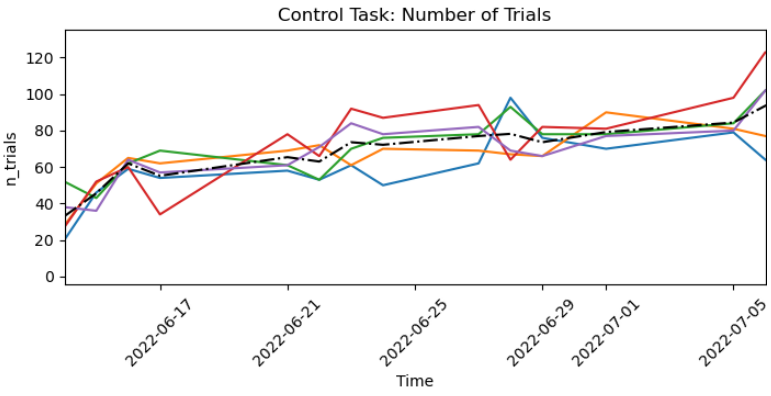
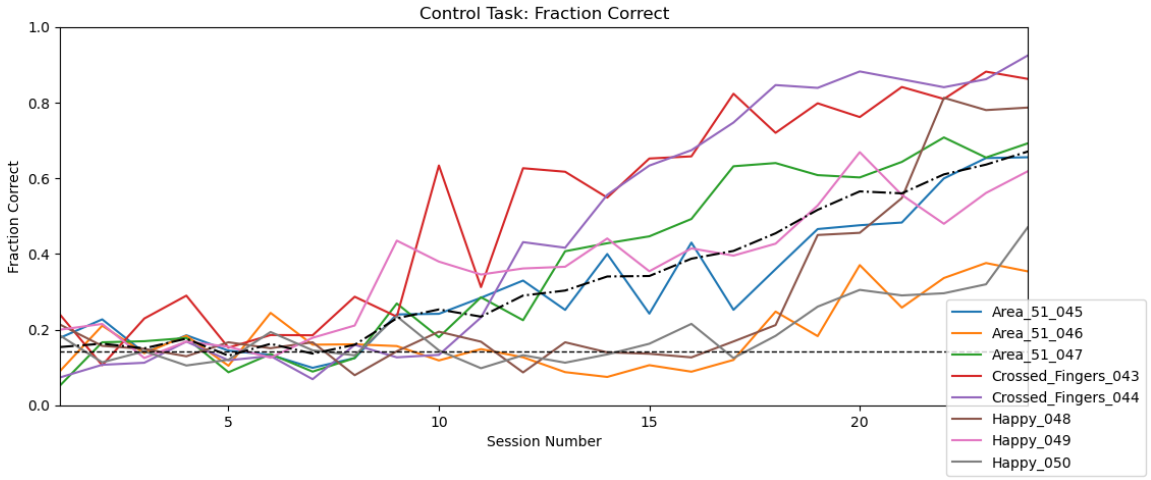


Figure 4. Learning curves for the First AC Lesion Cohort Before the Lesion.

A. Fraction Correct (FC) learning curve for the first AC lesion cohort.
B. Rank of Correct Port (RCP) learning curve for the first AC lesion cohort.
C. Number of Trials (n) learning curve for the first AC lesion cohort.

A



B

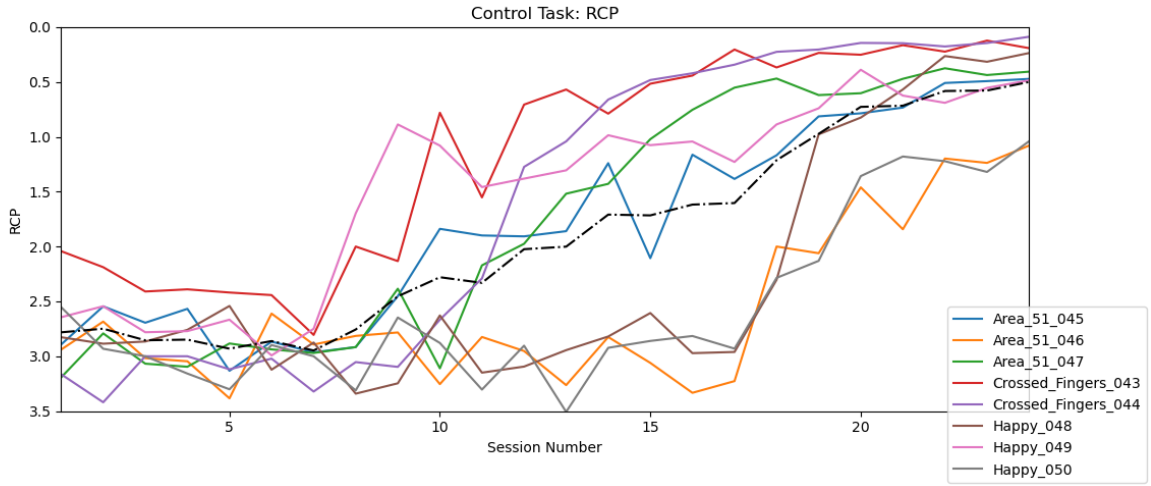
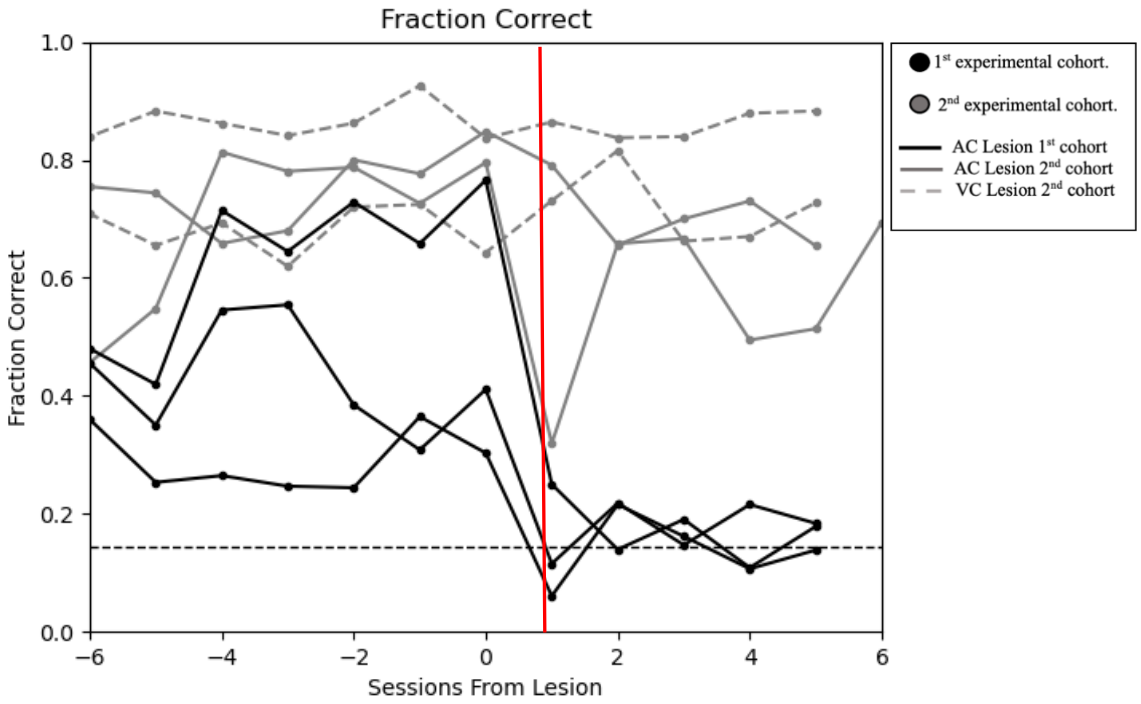


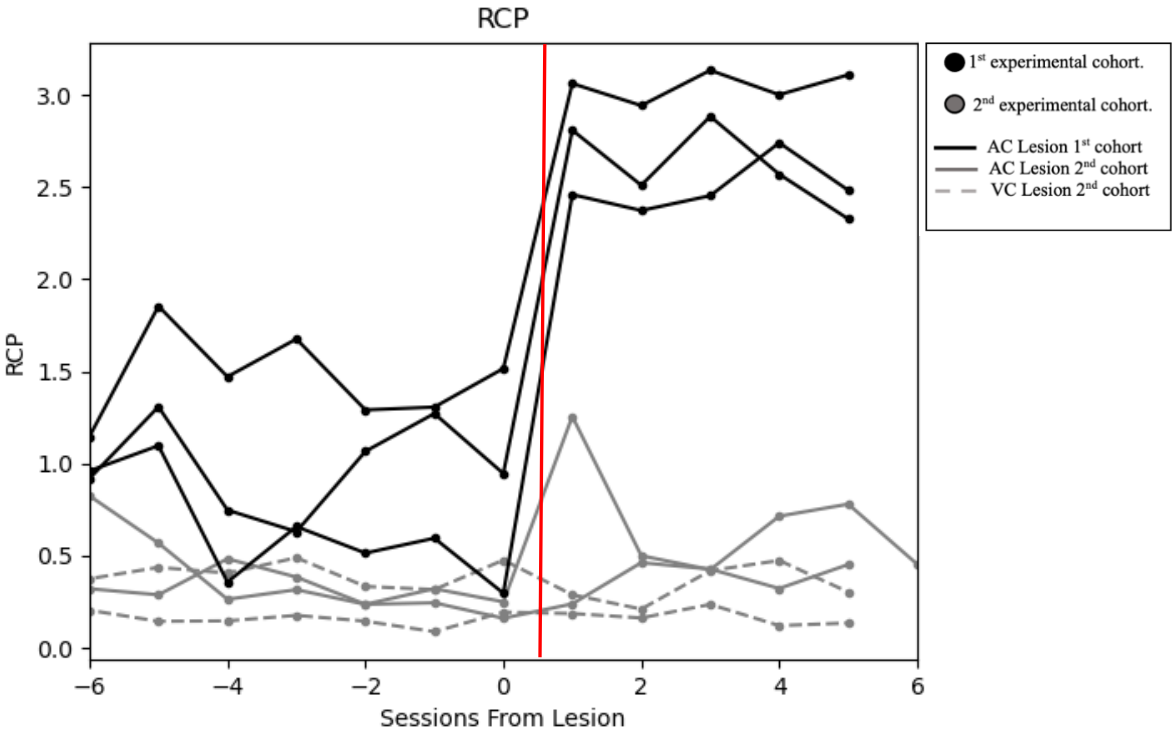
Figure 5. Learning curves For the Second AC and VC Lesion Cohort Before the Lesion.

A. Fraction Correct (FC) learning curve for the second AC and VC lesion cohort.
B. Rank of Correct Port (RCP) learning curve for the second AC and VC lesion cohort.

A


Fraction Correct
Fraction Correct
Sessions From Lesion
1st experimental cohort.
2nd experimental cohort.
AC Lesion 1st cohort
AC Lesion 2nd cohort
VC Lesion 2nd cohort

B


RCP
RCP
Sessions From Lesion
1st experimental cohort.
2nd experimental cohort.
AC Lesion 1st cohort
AC Lesion 2nd cohort
VC Lesion 2nd cohort

C

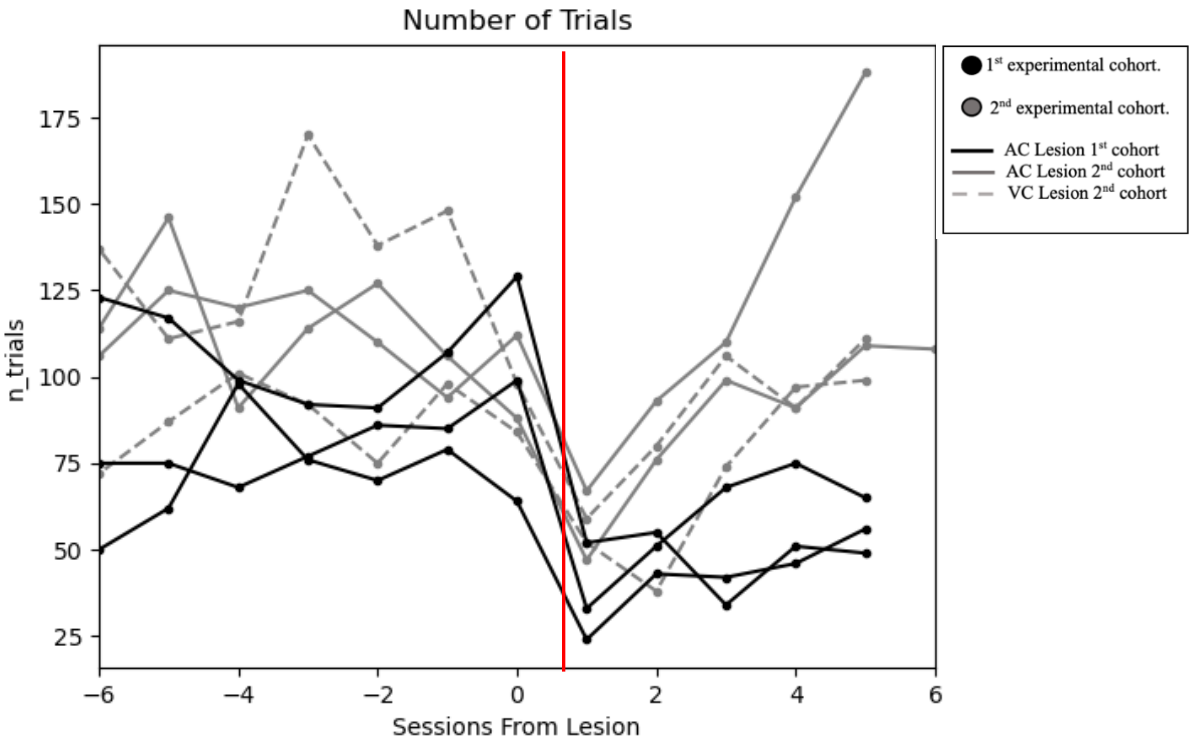
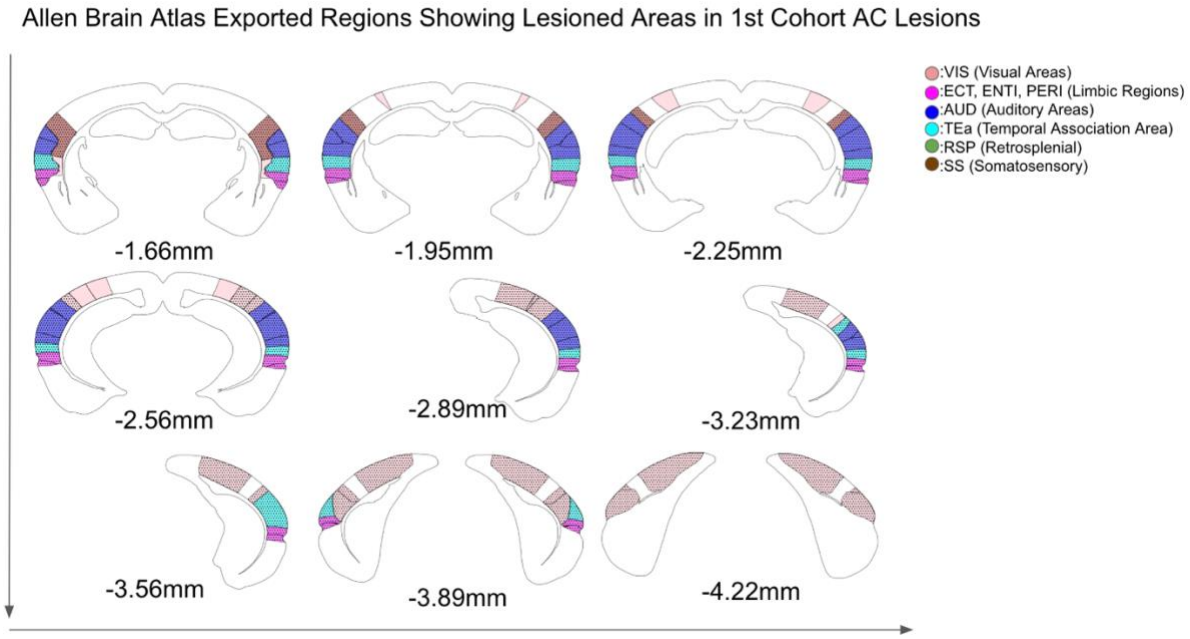


Figure 6. Lesion Analysis Figures. These figures are showing five training sessions before the lesion and five testing sessions after recovery from the lesion. The red line indicates testing sessions after the lesion.

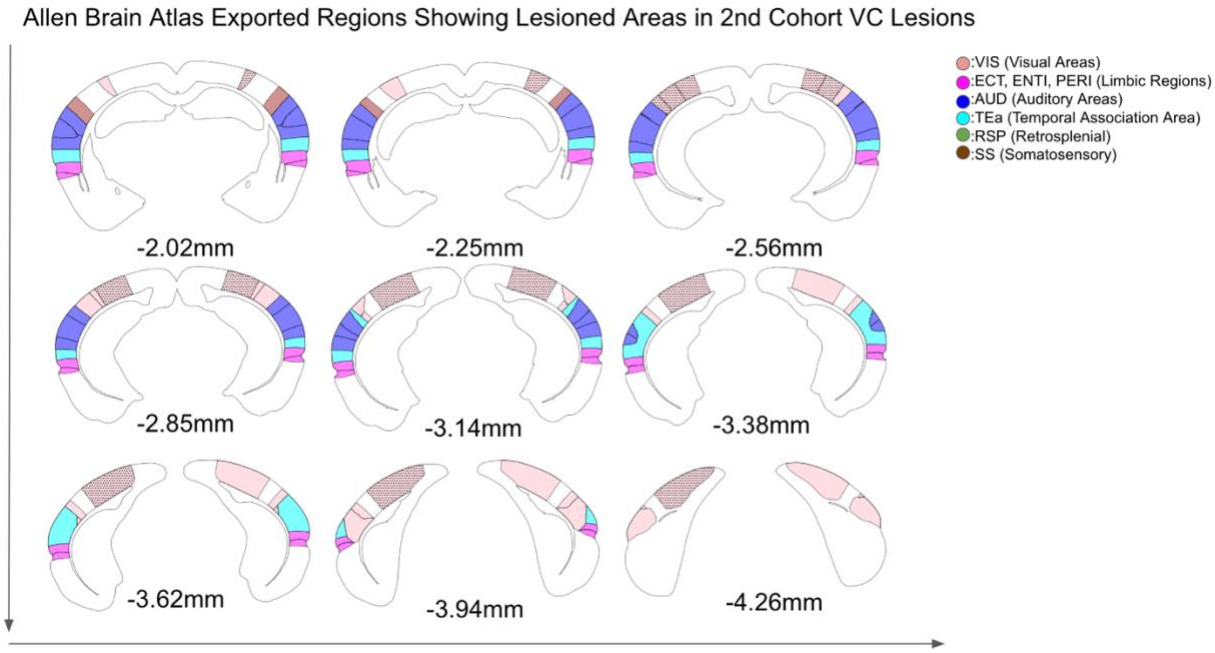
A. FC analysis showing post-lesion testing performance for first and second cohorts.
B. RCP analysis showing post-lesion testing performance for first and second cohorts.
C. N trials analysis showing post-lesion testing performance for first and second cohorts.

*AC = Auditory Cortex

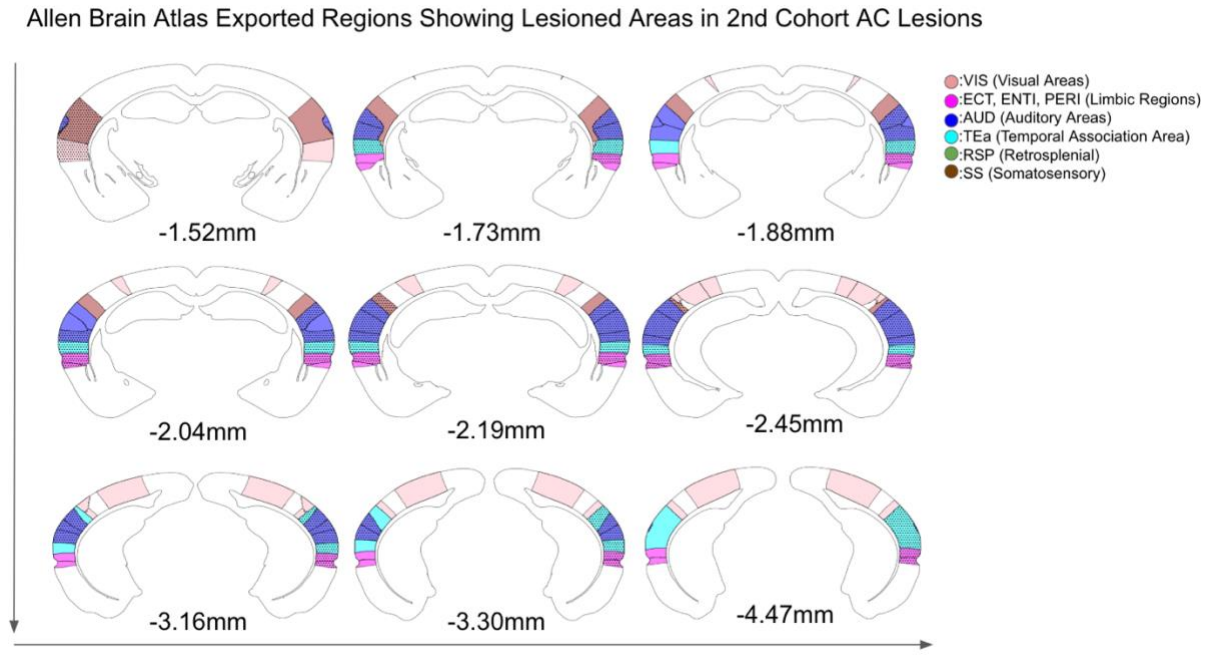
A


Allen Brain Atlas Exported Regions Showing Lesioned Areas in 1st Cohort AC Lesions
:VIS (Visual Areas)
:ECT, ENTI, PERI (Limbic Regions)
:AUD (Auditory Areas)
:TEa (Temporal Association Area)
:RSP (Retrosplenial)
:SS (Somatosensory)
-1.66mm
-1.95mm
-2.25mm
-2.56mm
-2.89mm
-3.23mm
-3.56mm
-3.89mm
-4.22mm

B


Allen Brain Atlas Exported Regions Showing Lesioned Areas in 2nd Cohort VC Lesions
:VIS (Visual Areas)
:ECT, ENTI, PERI (Limbic Regions)
:AUD (Auditory Areas)
:TEa (Temporal Association Area)
:RSP (Retrosplenial)
:SS (Somatosensory)
-2.02mm
-2.25mm
-2.56mm
-2.85mm
-3.14mm
-3.38mm
-3.62mm
-3.94mm
-4.26mm

C



D

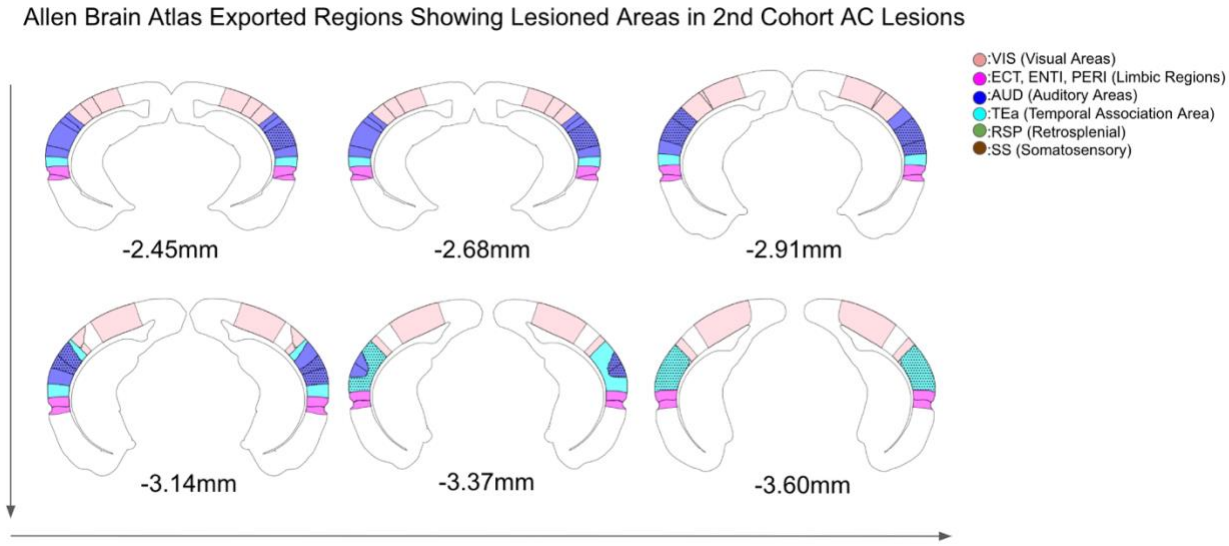


Figure 7. Allen Mouse Brain Atlas Exported Regions Showing Lesioned Areas for Each Mouse.

A. Mouse lesioned regions for AC Cohort 1 Mouse 1
   Cortex is missing on the left side for some slices
B. Mouse lesioned regions for VC Cohort 2 Mouse 1
C. Mouse lesioned regions for AC Cohort 2 Mouse 1
D. Mouse lesioned regions for AC Cohort 2 Mouse 2

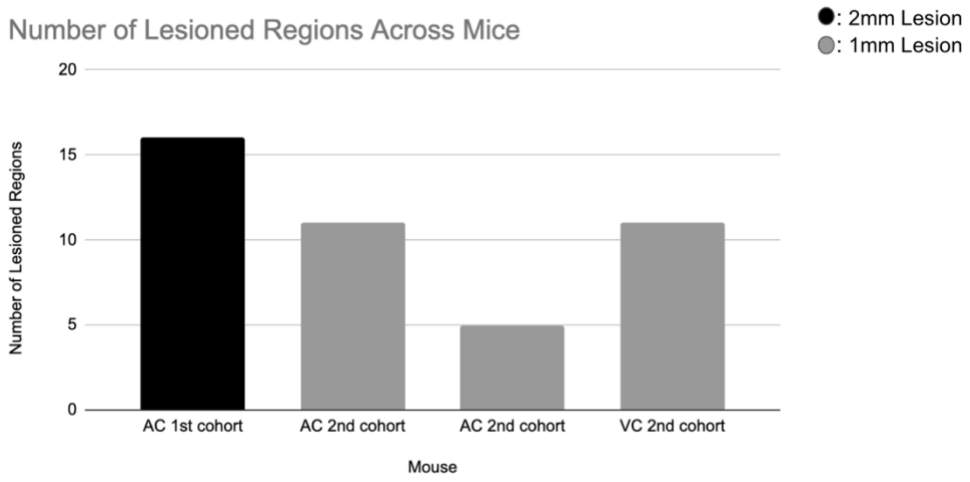


Figure 8: Bar Plot Showing the Number of Lesioned Areas Across All Mice. The figure also indicates which mice had a 1 mm or 2 mm lesion.

Fraction Correct (FC)	**Rank of Correct Port (RCP)**	**Number of Trials (n)**
1, 0, 0, 0, 0, 0, 0	0, 1, 2, 3, 4, 5, 6	(n)
Chance level is 1/7	Chance level is a mean of 3	No chance level
Indicates if a trial is correct	Indicates the number of incorrect ports poked before poking the correct port	Indicates the number of trials in any training session

Table 1. Breakdown of the Metrics Used to Score the Behavioral Task.

*	p < .05
**	p < .01
***	p < .001

Summary of Fisher's Analysis Statistical Significance Table

Mouse	Session 1	Session 2	Session 3	Session 4	Session 5
AC Cohort 1 Mouse 1 (2mm)	***	***	***	***	***
AC Cohort 1 Mouse 2 (1mm)	**		*	**	*
AC Cohort 1 Mouse 3 (2mm)	***	*	**	*	**
AC Cohort 2 Mouse 1 (1mm)	***			**	**
AC Cohort 2 Mouse 2 (1mm)		**	**	*	***
VC Cohort 2 Mouse 1 (1mm)					
VC Cohort 2 Mouse 2 (1mm)					

Table 2. Table Summary of Fisher's Analysis Statistical Significance Results. Yellow indicates significance. 1 mm or 2 mm indicates the lesion size for each mouse.

A

Data of Fisher's Analysis Statistical Significance Table	p-value
AC Cohort 1 Mouse 1	1.51E-05
AC Cohort 1 Mouse 2	4.35E-03
AC Cohort 1 Mouse 3	9.15E-05
AC Cohort 2 Mouse 1	7.70E-08
AC Cohort 2 Mouse 2	4.15E-01
VC Cohort 2 Mouse 1	3.50E-01
VC Cohort 2 Mouse 2	8.19E-01

B

Data of Fisher's Analysis Statistical Significance Table	p-value
AC Cohort 1 Mouse 1	9.04E-11
AC Cohort 1 Mouse 2	3.34E-01
AC Cohort 1 Mouse 3	1.78E-02
AC Cohort 2 Mouse 1	5.35E-02
AC Cohort 2 Mouse 2	1.69E-03
VC Cohort 2 Mouse 1	8.65E-02
VC Cohort 2 Mouse 2	1.00E+00

C

Data of Fisher's Analysis Statistical Significance Table	p-value
AC Cohort 1 Mouse 1	8.15E-09
AC Cohort 1 Mouse 2	4.45E-02
AC Cohort 1 Mouse 3	4.47E-03
AC Cohort 2 Mouse 1	5.08E-02
AC Cohort 2 Mouse 2	1.00E-02
VC Cohort 2 Mouse 1	1.00E+00
VC Cohort 2 Mouse 2	1.00E+00

D

Data of Fisher's Analysis Statistical Significance Table	p-value

AC Cohort 1 Mouse 1	2.53E-12
AC Cohort 1 Mouse 2	2.71E-03
AC Cohort 1 Mouse 3	1.56E-02
AC Cohort 2 Mouse 1	4.19E-05
AC Cohort 2 Mouse 2	2.44E-02
VC Cohort 2 Mouse 1	8.75E-01
VC Cohort 2 Mouse 2	5.33E-01

E

Data of Fisher's Analysis Statistical Significance Table	p-value
AC Cohort 1 Mouse 1	6.90E-11
AC Cohort 1 Mouse 2	1.58E-02
AC Cohort 1 Mouse 3	4.74E-03
AC Cohort 2 Mouse 1	4.93E-05
AC Cohort 2 Mouse 2	2.71E-04
VC Cohort 2 Mouse 1	3.33E-01
VC Cohort 2 Mouse 2	4.23E-01

Table 3: Data for Fisher's Analysis

A. Data for Fisher's analysis for performance on the last training session before the lesion surgery compared to performance on the first session after the lesions.
B. Same as A comparing the last training session to the second training session.
C. Same as A comparing the last training session to the third training session.
D. Same as A comparing the last training session to the fourth training session.
E. Same as A comparing the last training session to the training session.

*AC = Auditory Cortex
*VC = Visual Cortex

Mouse	Number of lesioned regions	Damage Regions (Right)	Damage Regions (Left)	Hippocampal damage
AC Cohort 1 Mouse 1	16	SSp, SSs, AUDd, AUDp, AUDpo AUDv, TEa, ECT, PERI, ENTI, VISp, VISrl, VISal, VISI, VISli, VISpor	SSp, SSs, AUDd, AUDp, AUDpo AUDv, TEa, ECT, PERI, ENTI, VISp, VISrl, VISal, VISI, VISli, VISpor	(No on left or right)
AC Cohort 2 Mouse 1	11	AUDd, AUDp, AUDpo, AUDv, TEa, ECT, PERI, ENTI, VISpor	SSp, SSs, AUDp, AUDd, AUDpo, AUDv, TEa, ECT, PERI, ENTI	(Both left and right)
AC Cohort 2 Mouse 2	5	AUDp, AUDd, AUDpo, AUDv, TEa	AUDp, AUDd, AUDpo, AUDv, TEa	(Both left and right)
VC Cohort 2 Mouse 1	11	RSPagl, SSp-tr, VISam, VISa, VISrl, VISpm, VISp, VIS	VISal, VISrl, VISp, VISpm, VISam, RSPag, RSPd	(Both left and right)

Table 4: Aggregate of all Damaged Brain Areas. The table specifies the lesion type, the experimental cohort, the intended lesion size, and the presence of hippocampal damage.
*AC = Auditory Cortex
*VC = Visual Cortex

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