NOISE-INDUCED MILD HEARING LOSS AND COGNITION IN YOUNG MALE WISTAR RATS

Fanny Sophia Bunn: S3826236

Faculty of Medical Sciences, University of Groningen

WMBC902-29: Minor Thesis

Supervisor: Joelle Jagersma

Primary Examiner: prof. S. Pyott

Second Examiner: prof. J.D.A. Olivier

February, 2023

Abstract

The pervasive use of listening devices has raised growing concerns about developmental and educational difficulties following noise-induced hearing loss in children and adolescents. Recent human and rodent studies have suggested a link between noise-induced mild hearing loss and cognitive impediments in young adults that reach well beyond auditory and verbal working memory processes. To date, the exact cognitive consequences following mild hearing loss caused by noise exposure in young adults remain elusive. Therefore, the current study investigated the effects of mild hearing loss induced by noise exposure on spatial, recognition, and social recognition memory in young male Wistar rats. Results showed no significant differences in memory performances between rats with noise-induced mild hearing loss and controls. The study's findings, however, are limited by further analysis showing that behavioral observations partially failed to exploit rodents' tendency for novelty over-familiarity. These results imply that rats did either not remember the task or the task failed to measure memory performances due to methodological drawbacks. Overall, findings suggest no significant impact of noise-induced mild hearing loss on spatial, recognition and social recognition memory. However, follow-up studies should refine the methodological limitations of the current study and assess the replicability of its findings.

Introduction

A growing body of literature has highlighted educational and developmental difficulties in children and adolescents with hearing loss (Davidson et al., 2019; Marshall et al., 2015; Bess et al., 1998). In fact, auditory deprivation in form of deafness has demonstrated lasting changes in verbal and auditory working memory processes in young children, associated with white matter alterations in the auditory cortex (Davidson et al., 2019, Simon et al., 2020). Specifically, children with hearing loss have shown difficulties in working memory related to storing and processing verbal information (Davidson et al., 2019). Moreover, deaf children's non-verbal working memory has been demonstrated to be impacted by their loss of auditory input, implying that auditory impairment may reach well beyond verbal and auditory working memory processes (Marshall et al., 2015).

Working memory appears to be in the foreground of language processing affected by hearing deprivation, however, processes such as recognition memory and spatial memory seem to be impacted as well (Meister et al., 2016; Park et al., 2016; Liu et al., 2016: Chang et al., 2019). For instance, impaired listeners exhibited difficulties in speech recognition related to word formation and word recall (Meister et al., 2016). Similarly, a murine model has evidenced diminished recognition memory performances in the novel object recognition task (NOR) in mice with moderate hearing loss (Park et al., 2016). Furthermore, research has evidenced reduced spatial memory and hampered hippocampal neurogenesis in young

rodents following noise-induced hearing loss (Park et al., 2016; Liu et al., 2016; Manohar et al., 2020; Tao et al., 2015). These findings corroborate that hearing loss affects cognitive processes in considerably greater complexity involving spatial and recognition memory processes.

Interestingly, hearing loss seems to affect episodic and semantic long-term memory differently from short-term memory processes (Rönnberg et al., 2011; Rönneberg et al., 2014). Specifically, Rönneberg et al. (2011) investigated the relationship between auditory acuity and various memory functions using a language understanding model and found that hearing loss is negatively related to episodic and semantic long-term memory but not to short-term memory. This was followed by a later study investigating non-auditory encoding performances (Rönneberg et al., 2014). Their findings revealed that the effects of functional hearing loss are more pronounced in episodic long-term memory than working memory or short-term memory, also in non-auditory memory processes. The results of Rönneberg et al. (2014) strongly implicate that long-term memory in form of episodic memory is affected to a greater extent than short-term memory in both auditory and non-auditory encoding. However, previous studies highlighting the role of verbal and non-verbal working memory difficulties in patients with hearing loss suggest otherwise (Davidson et al., 2019; Marshall et al., 2015). In light of the inconclusive findings, further research is needed to shed light on differences in short-term and long-term memory mechanisms affected by hearing loss.

Communication difficulties associated with hearing loss and impaired cognitive processing have been shown to place a burden on sociability. Specifically, hearing loss has been shown to result in increased social isolation due to difficulties in emotional speech recognition (Meister et al., 2016; Lauer et al., 2018; Arlinger S., 2003). Specifically, Meister et al. (2016) evidenced an interplay between hearing loss and reduced speech recognition with regard to word object formation and word recall. A rodent study further demonstrated that hearing loss resulted in reduced social interaction such as sniffing, following, and grooming in 2-10 months young rats, possibly caused by impaired hearing of vocalizations in higher frequencies (Lauer et al., 2018). Despite several studies underscoring the relevance of hearing loss in deprived emotional speech processing and reduced social interaction, the relationship and cognitive underpinnings between the two remain poorly understood. Therefore, further studies are warranted to examine the social consequences of hearing loss.

Whilst a causal relationship between hearing loss and impaired cognition remains elusive, the association of dementia with age-related hearing loss in older adults provides additional evidence supporting a link between impaired cognitive function and hearing loss. Specifically, research has shown an increased risk

of dementia in patients with hearing loss compared to healthy control (Lin et al., 2013). Moreover, hearing loss has been shown to exaggerate symptoms of mild cognitive impairments and accelerate the onset of dementia pathology (Bucholc et al., 2021). In turn, patients using hearing aids were at significantly lower risk of developing symptoms of mild cognitive impairment and dementia compared to those without a hearing aid (Bucholc et al., 2021). The findings suggest a potential causal relationship between hearing loss and cognitive decline in the elderly. However, less is known about the younger population. Therefore, it would be worthwhile to further investigate the question of correlation and causality in the younger population.

Especially, the pervasive use of listening devices and their potential risk of noise-induced hearing loss in young people raises concerns (Harrison et al., 2012; Daniel et al., 2007; Bess et al., 1998; Calcus et al., 2019). In fact, an estimated 12.5% of children and adolescents and 17% of adults have reported hearing damage that was caused by excessive noise exposure through the use of listening devices (Harrison et al., 2012). A comparative study has reported children's difficulty with a series of educational and functional test measures following mild-sensorineural hearing loss (Bess et al., 1998). Moreover, mild-to-moderate hearing loss during childhood has been shown to yield lasting changes in neural processing, as shown by the delayed auditory evoked response to sounds (Calcus et al., 2019). These findings instigate the developmental and educational complications of noise-induced auditory deprivation, especially in the younger generation. A question remains, however, to what extent mild-hearing loss caused by perpetuating noise exposure has an impact on cognition in the younger generation. Therefore, further empirical evidence is needed to determine the severity of cognitive consequences in individuals with mild sensorineural hearing loss caused by excessive noise exposure.

To fill the gaps in the current literature, the present study aimed at elucidating to what extent noise-induced mild hearing loss affects memory processes at a young age. The study, therefore, employed a rodent model using young male Wistar rats 3–11 weeks of age. Female rats were excluded from the cohort to avoid hormonal/menstrual interferences during observational task performances. Based on literature highlighting the relevance of noise exposure on sensorineural hearing loss and cognition, the study induced mild hearing loss through noise exposure three weeks after birth, which was subsequently examined by the auditory brainstem response at several time points (ABR) (Reijntjes et al., 2018). Cognitive abilities of spatial memory (Liu et al., 2016; Chang et al., 2019) recognition memory (Meister et al., 2016; Park et al., 2016), and social recognition memory (Lauer et al., 2018; Meister et al., 2016) were examined by means of behavioral assessments. Findings on the relationship between noise-induced

hearing loss and cognitive consequences in animal models will be essential to understand the trajectory of cognitive deficits in children and young adults reporting mild hearing loss.

Objectives and Hypotheses

To investigate the effects of noise-induced mild-hearing loss on memory processes in young male Wistar rats, three distinct cognitive measures were performed. Firstly, the object location task served to determine whether young male Wistar rats with mild auditory loss performed more poorly in spatial memory compared to their healthy controls. Previous research by Liu et al. (2016), Manohar et al. (2020), and Tao et al. (2015) has implicated the role of hearing loss in reduced spatial memory capacity. In line with their findings, it is expected that rats with mild hearing loss perform less well in the spatial memory task compared to controls.

Literature by Meister et al. (2016) and Park et al. (2016) has delineated the role of hearing loss in impaired recognition memory. Correspondingly, the current study utilized the novel object recognition task to examine object recognition memory in rats with mild hearing loss. In light of previous findings, noised rats are expected to perform more poorly in recognition memory compared to controls.

According to studies highlighting the relevance of hearing loss in social isolation due to impaired speech recognition and emotional processing (Meister et al., 2016; Lauer et al., 2018), the last behavioral measure served to investigate social recognition through the social recognition task. Overall, rats with mild hearing loss were expected to exhibit reduced social recognition compared to the healthy controls.

Findings by Rönneberg et al. (2014) highlighted differences in long-term and short-term memory processes in individuals with hearing loss. Therefore, the current study aimed at elucidating whether long-term or short-term memory processes are affected differently in rats with mild hearing loss compared to healthy controls. In light of the controversies emerging from the literature, the current study's expectation remains explorative. Overall, it is expected that controls perform better in the short-term (1-hour time interval) than in the long-term task (12-hour time interval), as memory might be more easily retrievable following the short-term interval compared to the long-term interval.

Materials and Methods

Animals and Dam Housing

A total of 7 pregnant female Wistar rats were ordered from ENVIGO (HsdCpb:WU). The offspring were housed together with their mothers until weaning on postnatal day 21. Male pups were separated from

female pups on day 1 or 2 after parturition. Overall, male pups were housed in groups of four. To discriminate within the litter, the animals were ear-clipped prior to the first ABR procedure with the following combinations: left, right, left right, left left, right right. The pups were housed in standard cages on a human light/dark cycle, starting at 7am-7pm. Standard food pellets, water, and nesting material were available ad libitum. The cage was cleaned once a week. Rats were weighed once a week and after each experimental procedure. Housing and care were in accordance with Annex III of Directive 2010/63/EU. All experiments were approved by the animal ethics committee of the University of Groningen and University Medical Center Groningen and complied with the guidelines for animal experiments from the UG/UMCG and Netherlands animal welfare law.

Design

Initially, each cage consisted of four male rats, constituting two controls and two rats with mild hearing loss. However, due to the early death of a male pub suffering from an anesthetic overdose, the total cohort consisted of 23 rats. In addition, one cage consisted of two male rats used as stimulus animals in later behavioral observation of the social recognition task.

Procedure

Prior to noising, ABR was performed 3 weeks after birth serving as the baseline condition (Figure 1). This was followed by noising of 12 of the 23 rats 4 weeks after birth. The first behavioral examination (object location task) was performed in week 5, and the ABR measure was followed in week 6. The novel object recognition task took place in week 8, followed by the social recognition task in week 10. The experimental procedure was finalized in week 11 with the last ABR examination and the subsequent decapitation and preservation of the brains and inner ears.

Week	1	2	3	4	5	6	7	8	9	10	11
Pregnant females arriving			ABR & ear clipping	Noising	Object Location Task	ABR		Novel Object Recognition Task		Social Recognition Task	ABR + Brain collection

Fig. 1. Timeline of the experimental procedure encompassing a total of 11 weeks.

Auditory Brainstem Response

Calibration

The speaker was calibrated at an intensity of 114 dB at click and pure tones (2, 4, 8, 16, 32 kHz) at an alternating phase. Calibration equipment was used in line with (Reijntjes et al., 2018). The high-pass filter was set to 30 Hz whereas the low-pass filter was set to 3000 Hz. Electrode recordings were amplified 100×. Stimuli duration was set to 21.10/s rate on 100 u duration (click) and 5000 µs (tone bursts). During the ABR, the stimuli were presented starting at an intensity of 20 dB, increasing up to 90 dB with 5 dB increments.

Preparation

Rats were first weighed, then anesthetized intraperitoneally with Ketamidor (375 µl/ml), Dexdomitor (250 µl/ml), and saline (375 µl/ml). 2µl of the Ketamidor/Dexdomitor solution was injected per gram per rat. If the procedure endured longer than two hours, animals received 1/4th of the original dose to remain asleep. As an anesthesia antagonist, Antisedan (200 µl/ml) diluted in 800 µl/ml saline was administered following completion of the ABR (approx. 20-45 minutes). Saline was further injected in most of the rats to promote recovery. Rats were placed in a heating unit during recuperation.

Rats were checked by the toe pinch following anesthesia injection ~2-10 min. The animal's eyes were covered with a protective ophthalmic ointment to keep the eyes moist during anesthesia. The rat was placed in a soundproof chamber in front of an open open-field speaker (Visaton DHT 8 S). Each rat was placed at an equal distance from the speaker. For hearing threshold evaluation, three electrodes were positioned below the pinna of the left ear (reference electrode), subdermal at the forehead (active electrode), and below the right ear (ground electrode) (Reijntjes et al., 2018). Channels were set to either 1,1 or 3,3. Impedance was checked through the channel device and ABR acquisition phase of the hardware and software Intelligent Hearing Systems (IHS). Overall, the rejection rate of the sweeps was supposed to be not higher than 10%. If the rejection rate of the sweeps was too high, the electrodes were re-adjusted, and calibration was repeated.

Threshold Determination

The threshold in decibels was determined across the frequency range of 2, 4, 8, 16, 32 kHz and click (Reijntjes et al., 2018). The absolute threshold was visually inspected and determined by the first detectable waveform 1 starting at the lowest intensity, where the first peak was distinguishable from the noise and persisted in higher intensities thereafter. The analysis was executed subjectively by two observers. Threshold shift was defined by the difference between individual thresholds 2- and 7-weeks

post-noising (PN) compared to baseline (Reijntjes et al., 2018).

Noising

At 4 weeks of age, hearing loss was induced via continuous white broadband noise exposure at 100 dB SPL for two hours under anesthesia in a soundproofed chamber (Reijntjes et al., 2018). Controls were anesthetized and placed in the sound-attenuated room for two hours without noise exposure.

Tissue Collection

Following the last ABR procedure at week 11, rats remained anesthetized for decapitation and subsequent dissection of the brain and inner ear. Prior to decapitation, rats were placed in a chamber with isoflurane to ensure insensitivity during the dissection procedure. The left and right hemispheres were dissected apart from each other. The tissue was frozen in ethanol and dry ice, while the inner ear was fixed in 4% paraformaldehyde (PFA) in PBS (pH 7.4) for two hours at degree room temperature. The tissue was stored in an 80-degree freezer.

Behavioral Observation

Setup and Procedure

The arena was 100x100 cm large with objects placed at a 50 cm distance from each other (Figure 3). Orientation marks were fixed on the inside of the wall at each side of the square (Figure 3). Object location (OL), novel object recognition (NOR), and social recognition test (SR) were following a similar procedure as described elsewhere (Sik et al., 2003; Olivier et al., 2008). First, the animals were habituated for 5 minutes to an open-field environment as shown in Figure 3. The following day, rats were tested twice during two separate trials. In the first trial (familiarization trial), rats were introduced to two identical objects in symmetrical positions in the OL and NOR (Figure 4,5), while in the SR, rats were introduced to a single rat (Figure 6). Following completion of the first trial, animals were reintroduced to the arena (trial 2) after one hour (short-term inter-trial interval (ST ITI)) or 12 hours (long-term inter-trial interval (ST ITI)). Thereby, the environment has changed through the replacement of one of the two objects with either its location (OL), characteristic (NOR), or an addition of another stimulus animal (SR). Every trial of the OL and NOR consisted of a duration of 3 minutes, whereas the duration of each trial in the SR was 10 minutes. Rats were exposed to the object combinations in balanced positions to avoid biased tendencies for a particular location or object.

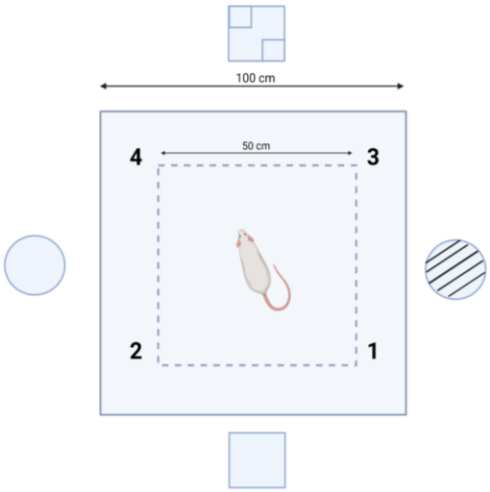

Fig. 3 Schematic representation of behavioral observation environment of the object location task and novel object recognition task. Created with Biorender.com.

Object Location Task

The experimentation of the object location (OL) task was performed 1 week PN (week 5 after birth). Following habituation, animals were placed in the arena with two identical objects (trial 1). During the second trial of either short-term or long-term ITI, rats were reintroduced to the identical objects with one object placed on a different location (diagonally from the familiar object). Overall, object combinations consisted of 2x4 variations: two different objects on four varying positions (1,2,3,4) (Figure 4).

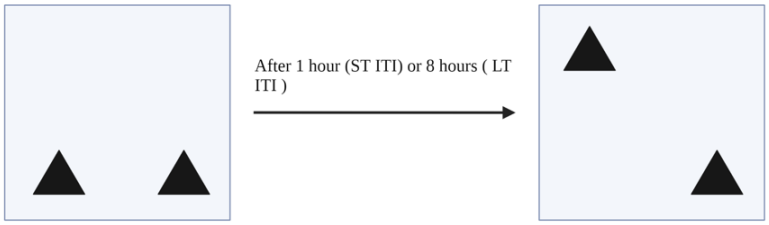

Fig. 4 Schematic representation of the object location test with an inter-trial interval of either 1 hour (short-term) or 8 hours (long-term). Created with Biorender.com.

Novel Object Recognition Test

The novel object recognition task was executed in week 5 PN (week 8 after birth) and followed a similar procedure as described in the object location task, despite the replacement of a different object instead of a different location in trial 2 (Figure 5). Overall, rats were exposed to a variation of four different object characteristics in balanced positions (1,2 or 3,4).

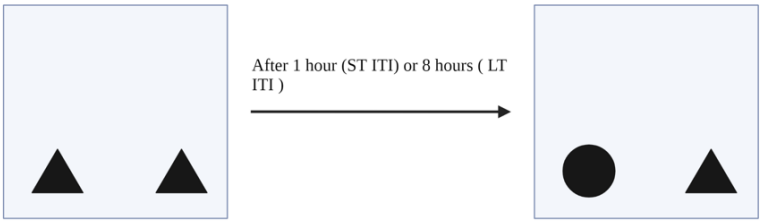

Fig. 5 Schematic representation of the object location test with an inter-trial interval of either 1 hour (short-term) or 8 hours (long-term) in between. Created with Biorender.com.

Social Recognition Test

The SR was performed 5 weeks PN (week 10 after birth). Alike the OL and NOR, the familiarization trial consisted of a single stimulus rat, whereas trial 2 consisted of the familiar rat of trial 1 and a novel stimulus rat (Figure 6). For the experimental procedure, two stimulus rats were used that did not encounter the animal before.

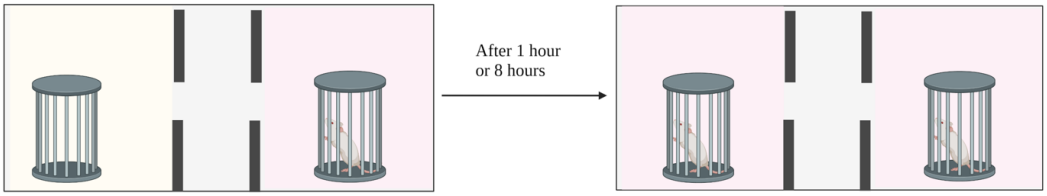

Fig. 6 Schematic representation of the social recognition test with an inter-trial interval of either 1 hour (short-term) or 8 hours (long-term). Created with Biorender.com.

Recording

The behavioral observations were video recorded from above the arena. The behavioral measures occurred under constant bright light illumination. Exploring behavior was defined as sniffing, touching the object with the nose, and leaning onto the object/subject with no more than 2 cm distance, while turning around or sitting next to the object was not considered explorative (Antunes et al., 2012; Sik et al., 2003). To avoid any olfactory disturbance, objects were cleaned with a 10% ethanol solution between each of the subject's trials.

Scoring

Behavioral observations were scored manually using the Behavioral Observation Interactive Software version 8.7. Scoring of the video started at the time when the door of the observation room was closed. The scoring was completed after 3 minutes for the OL and NOR tasks and after 10 min for the SR task. The corresponding key was pressed when the animal was actively exploring the object (i.e. sniffing the

object, leaning on the object) and stopped immediately after exploration. Start and stop events were defined as state events. The cumulative value of the state events determined the total exploration time for each object/location/stimulus animal.

Measures of Interest

Measures of interest were the exploration time of the novel object/subject (*en)* and familiar object/subject (*ef*), as well as the total exploration time (*E*) of both novel and familiar objects/subjects (Table 1). If rats explored for less than 5 seconds both objects, or only explored one of the two objects, these trials were removed from the analysis to avoid confounded data (Şık et al. 2003; Olivier et al., 2008). The discrimination index (*d2*) was calculated by subtracting the exploration time of the familiar object (*ef*) from the exploration time of the novel object (*en*) during trial 2, divided by the total exploration time (*E*) during trial 2 (Table 1) (Rutten et al. 2007; Şık et al. 2003; Olivier et al., 2008). The discrimination index (*d2*) is a measure to determine rodents' intrinsic preference for novelty over-familiarity while correcting for the total exploration time. A positive value implies more time spent exploring the novel object, whereas a discrimination index of zero implies equal time spent with both objects. Conversely, a negative value implies more time spent exploring the familiar object over the novel one. All behavioral observations exploit the rodents' preference for novelty over-familiarity if they remember the initial exposure of trial 1.

Table 1. Measures involved in the Behavioral Observations

---

e = total observation time of object
en = novel object
ef = familiar object
E = total observation time of *en* + *ef*
d1 = *en* – *ef*
d2 = *d1/E*

Data Preparation and Statistical Analysis

The homogeneity assumption of the variance was tested with Lavene's test ($p>0.5$). Data with $p < .05$ was analyzed non-parametrically with the Mann-Whitney U test for simple group comparison. The normality assumption was examined using the Shapiro-Wilk test and q-q plot.

ABR

The design of the ABR analysis consisted of a two-way ANOVA. The variable of interest was hearing threshold (dB) subjected to three factors, namely, treatment (noised vs controls) time (baseline, 2 weeks PN, 7 weeks PN), and frequency (kHz). The aim was to confirm that noised rats exhibited hearing loss. Therefore, noised rats were expected to exhibit higher thresholds (dB) in higher frequencies compared to the control group (Reijntjes et al., 2019).

Behavioral Observations

To test whether noise mild-induced hearing loss affects memory performance in young male Wistar rats, simple group comparison was performed using a student sample t-test for each, ST and LT ITI. The variables of interest were total exploration time (*E*), and the discrimination index (*d2*) for each of the treatment groups (noised vs controls). Total exploration time (*E*) served as a control variable to test whether noised and control rats differed in total exploration time. The discrimination index served as a preference index for novelty over-familiarity (Sik et al., 2003).

Controlling for Rodents' Novelty Over-Familiarity Preference

To interpret the data in correct manner, further analysis was necessary to examine whether the behavioral tasks did exploit the rodents' tendency for exploring novel objects/subjects over familiar ones. If the tasks did not exploit the inherent preference for novelty over-familiarity, the behavioral measure might not have been sufficiently robust to measure memory function. Thus, further analysis was necessary to test whether rats, particularly, the control group, exhibited indeed a bias for novelty. The variable of interest was the total observation time for each, novel (*en*), and familiar object/subject (*ef*). Two-way ANOVA served to determine whether there was a significant difference in exploration time between the novel vs familiar object (preference) within each treatment group (noised vs controls), while controlling for short-term (ST) vs long-term (LT) ITI.

Results

Rats exposed to Noise have elevated High-Frequency ABR Thresholds

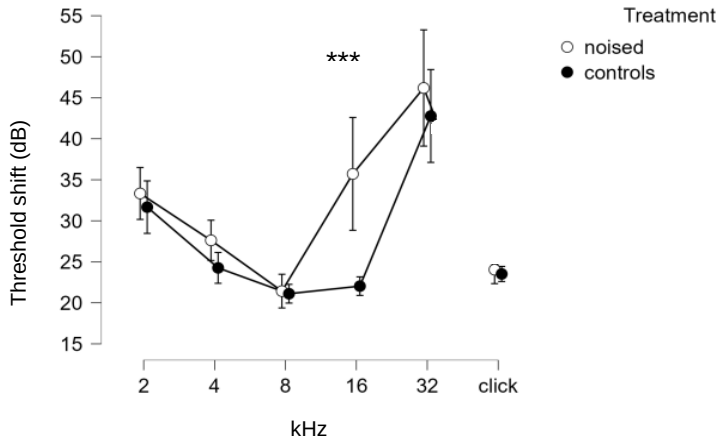

Fig.7. Graph summarizing the overall threshold shift (y-axis) across tested frequencies (x-axis) in both, noised and control rats.

Comparing Baseline and 2 Weeks PN

Timewise comparison between the baseline and 2 weeks PN was performed across factors treatment (noised vs controls) and frequency (kHz). Analysis of variances revealed significant differences between baseline and 2 weeks PN in hearing threshold (dB) $F(1, 156) = 28.708$, $p < 0.001$, $\eta p2 = .0061$. Moreover, analysis of variance revealed a main effect of treatment $F(1, 156) = 4.789$, $p = 0.030$, $\eta p2 = 0.010$, implying that there are differences in hearing threshold (dB) between noised and control rats. Moreover, there were differences in hearing threshold (dB) between frequencies presented (2, 4, 8, 16, 32 kHz and click) $F(5, 156) = 41.746$, $p < 0.001$, $\eta p2 = 0.442$.

Further analysis of simple main effects of factor time revealed differences in 16 kHz ($p < 0.001$) and 32 kHz ($p < 0.001$) between baseline and 2 weeks PN in both, noised and control rats. When looking at factor treatment as simple main effect, noised and control rats differed in hearing threshold of 16 kHz ($p < 0.001$), but not 32 kHz ($p = 0.580$) (Figure 8). In other words, noised and control rats showed a positive threshold (dB) shift from baseline to 2 weeks PN in 16 kHz and 32 kHz frequency. However, between-subject comparisons of noised vs control rats showed only a difference in the hearing threshold at 16 kHz but not in 32 kHz frequency 2 weeks PN compared to baseline.

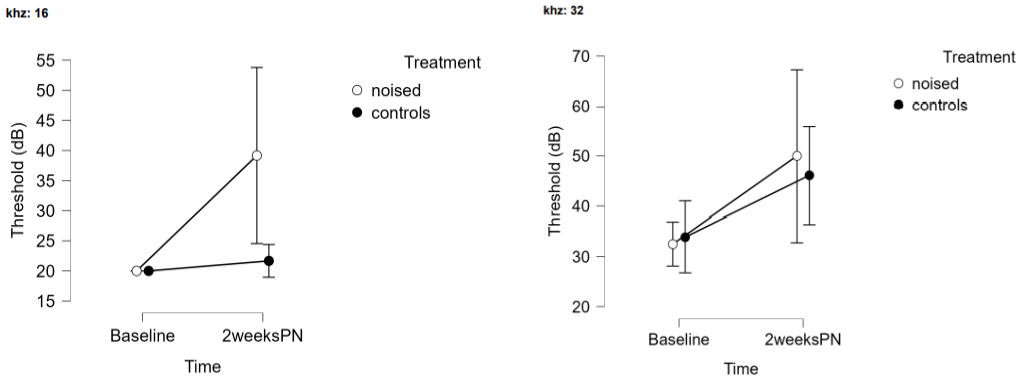

Fig. 8. Graphs depicting a shift in absolute threshold (dB) from baseline to 2 weeks PN in both noised and control treatment conditions in 16 kHz and 32 kHz.

Comparing Baseline and 7 Weeks PN

Seven weeks PN to baseline comparison revealed significant differences with respect to hearing threshold (dB) $F(1, 192) = 78.986$, $p < 0.001$, $\eta p2 = .145$. Results further indicated main effects of the treatment condition (noised vs controls) $F(1, 192) = 7.680$, $p = 0.006$, $\eta p2 = 0.014$, and frequency (kHz) $F(5, 192) = 40.458$, $p < 0.001$, $\eta p2 = 0.372$. Moreover, there were significant interaction effects between frequency (kHz) and treatment $F(5, 192) = 2.535$, $p = 0.030$, $\eta p2 = 0.023$ as well as frequency (kHz) and time $F(5, 192) = 6.433$, $p < 0.001$, $\eta p2 = 0.059$.

When looking at the simple main effects with respect to factor time, noised and control rats showed an increase in absolute threshold (dB) from baseline to 7 weeks PN in frequencies of 16 kHz ($p < 0.001$), 32 kHz ($p < 0.001$), and 2 kHz ($p < 0.001$). However, differences in threshold (dB) between noised and control rats were only seen at 16 kHz ($p < 0.001$), not at 32 kHz ($p = 0.199$), or 2 kHz ($p = 1.00$) (Figure 9).

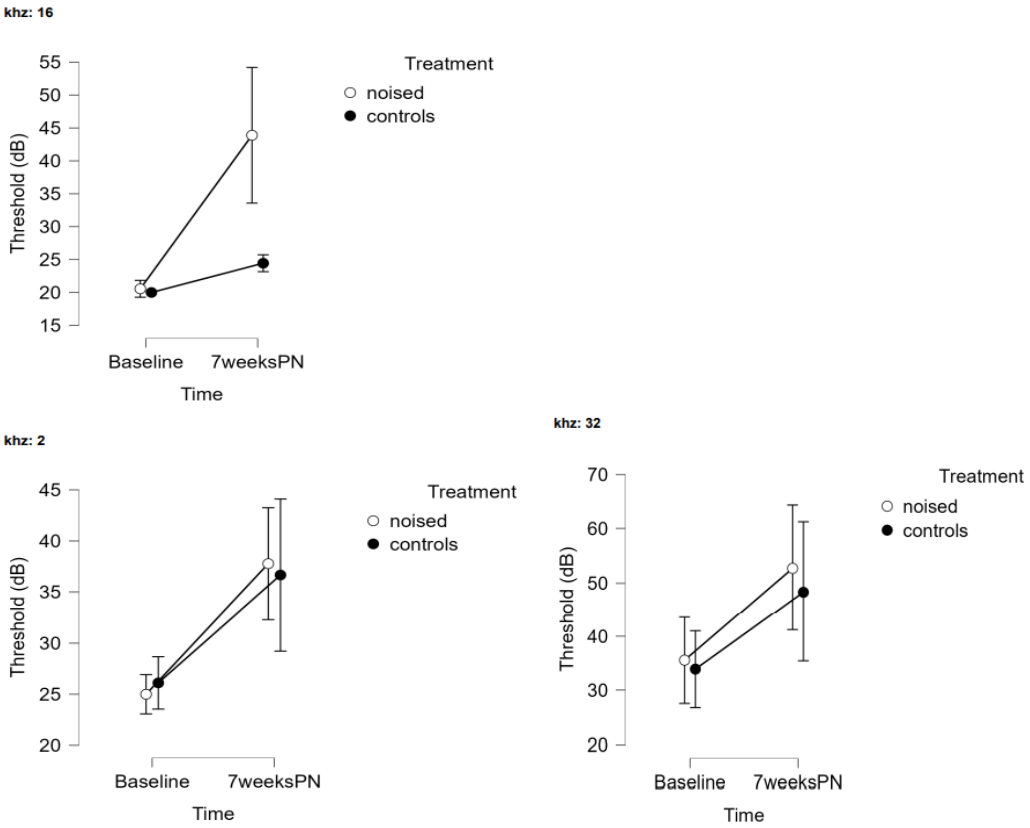

Fig. 9. Graphs depicting a shift in absolute threshold (dB) from baseline to 7 weeks PN in both noised and control rats in 2 kHz, 16 kHz, and 32 kHz.

Hearing Loss does not affect Memory Performance in the OL, NOR, and SR task

Object Location

Noised and control rats did not differ in total exploration time spent on the objects (t = -0.218, p = 0.830, d = -0.096) or in preference for novelty over-familiarity (*d2*), (U = 58.00, p = .808, r = 0.074) in the ST ITI (Fig. 10 A). Similarly, noised and control rats did not differ in the LT ITI in total exploration time (t = -0.336, p = 0.741, d = - 0.151) or discrimination index (*d2*) (t = -0.053, p = 0.958, d = -0.024), (Figure 10 B).

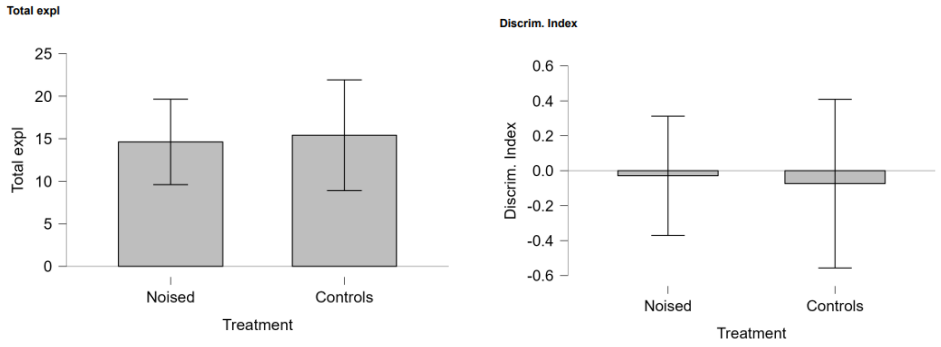

Fig. 10 A. Total exploration time (sec) and discrimination index in noised and control rats in the ST ITI.

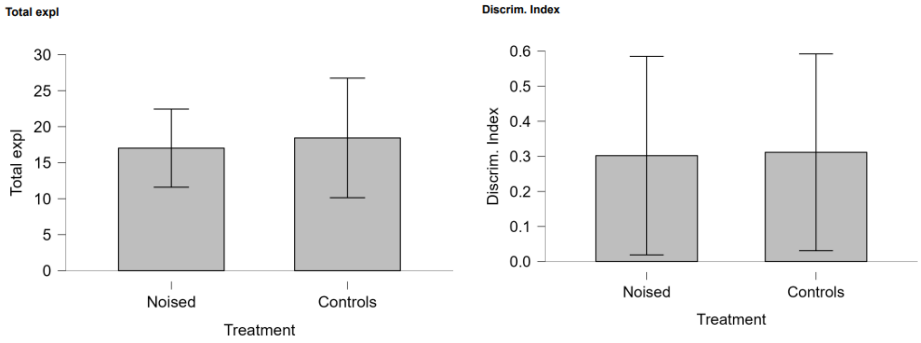

Fig. 10 B. Total exploration time (sec) and discrimination index in noised and control rats in LT ITI.

Novel Object Recognition

Data analysis of the novel object recognition task revealed no differences between the noised and control rats with respect to total exploration time (t = 0.206, p = 0.6829, d = 0.086) or discrimination index (t = 0.864, p = 0.397, d = 0.364) in the ST ITI. Similarly, noised and control rats did not differ in total exploration time (U = 66.00, p = .722, r = 0.10) or in preference for novelty over-familiarity (t = 0.634, p = 0.533, d = 0.271) in the LT ITI (Figure 11).

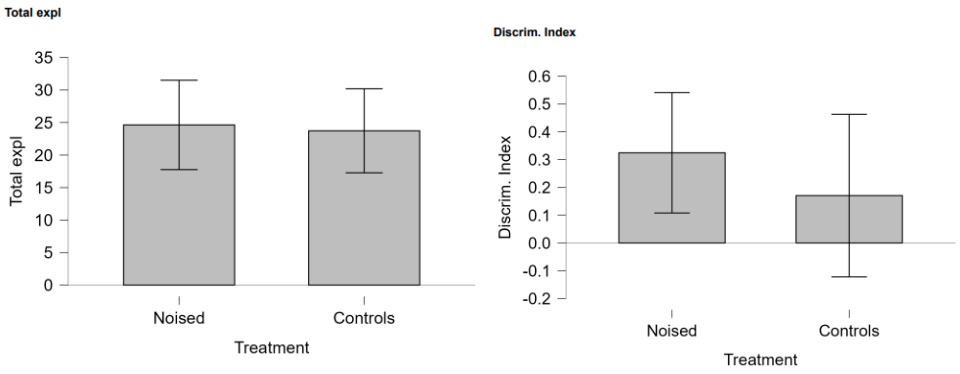

Fig. 11 A. Total exploration time (sec) and discrimination index in noised and control rats in the ST ITI.

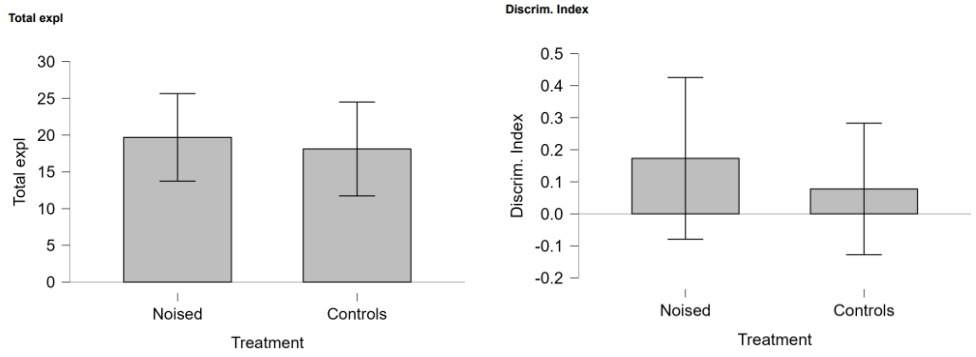

Fig. 11 B. Total exploration time (sec) and discrimination index in noised and control rats in LT ITI.

Social Recognition

Simple group comparison between the noised and control rats in the social recognition task revealed no differences in total exploration (t = -0.847, p = 0.407, d = -0.354) or discrimination index in the ST ITI (t = 0.887, p = 0.385, d = 0.370) (Figure 12 A). Similarly, there were no differences between the noised and control rats in the LT ITI in exploration time (t = -0.885, p = 0.386, d = -0.369) or discrimination index (t = -0.907, p = 0.374, d = -0.374), respectively (Figure 12 B).

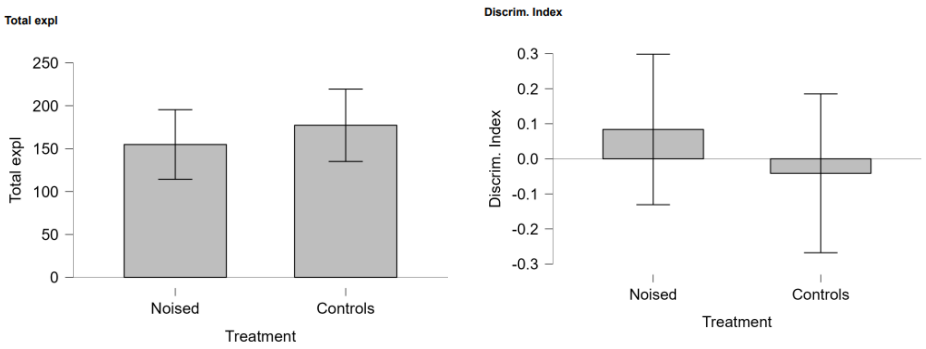

Fig. 12 A. Total exploration time (sec) and discrimination index in noised and control rats in the ST ITI.

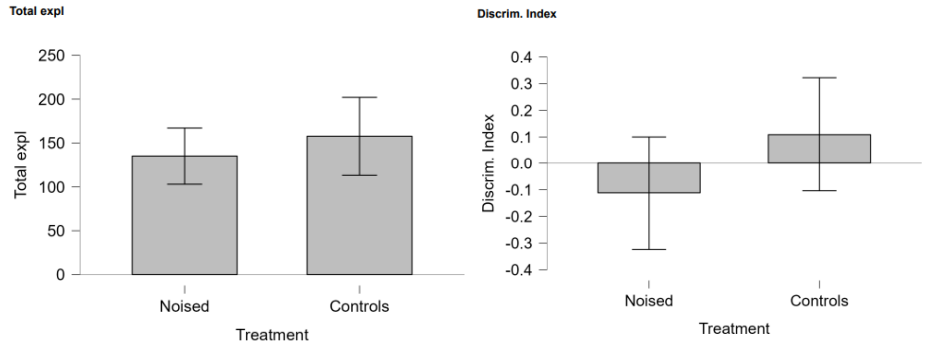

Fig. 12 B. Total exploration time (sec) and discrimination index in noised and control rats in LT ITI.

Behavioral Measures only partially exploit Rodents' Novelty Over-Familiarity Preference

Object Location Test

Analysis of Variance including the factors preference, treatment, and ITI indicated no main effects. In other words, there was no difference in exploration time between familiar and novel objects $F(1, 74) = 3.246$, $p = 0.076$, $\eta p2 = 0.038$, nor was there a difference in exploration time with regard to treatment group $F(1, 74) = 0.126$, $p = 0.724$, $\eta p2 = 0.001$ or between ST and LT ITI $F(1, 74) = 0.923$, $p = 0.340$, $\eta p2 = 0.011$. Results have shown an interaction effect between Preference and ITI $F(1, 74) = 5.847$, $p = 0.018$, $\eta p2 = 0.069$, suggesting that time spent on a familiar vs novel object depended on whether the ITI was short-term (1 hour) or long-term (12 hours) (Figure 13).

Simple main effects revealed that the preference for a novel object depended on LT ITI ($p= 0.004$), but not on ST ITI ($p= 0.661$). In other words, both, noised ($p = 0.038$) and control animals ($p = 0.041$)

preferred the novel over the familiar object in the LT ITI session (12 hours), but not in the ST ITI session (1 hour) (p = 0.974), (p = 0.581), respectively.

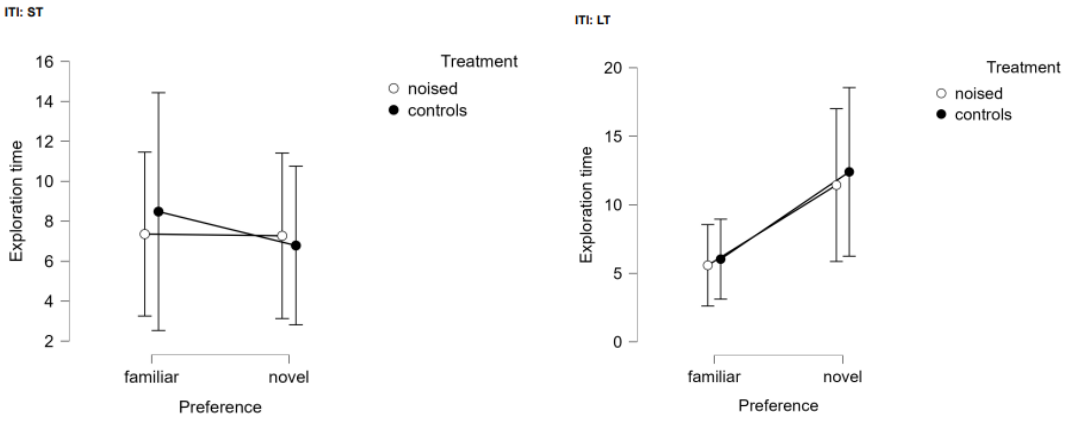

Fig. 13. Group comparison of the OL task between total exploration time (sec) for familiar vs novel object while controlling for treatment group in ST ITI and LT ITI.

Novel Object Recognition Test

Analysis of variance revealed significant main effects in both, preference F(1, 84) = 8.886, p = 0.004, ηp2 = 0.086, and ITI F(1, 84) = 4.295, p = 0.041, ηp2 = 0.041, despite no significant difference between the treatment conditions (noised vs control) F(1, 84) = 0.096, p = 0.758, ηp2 = 9.222×10^{-4}.

Further analysis of simple main effects showed that noised rats exhibited a preference for novelty in ST ITI (p< 0.001), but not in the LT ITI (p= 0.219). Contrary, control rats exhibited no difference in exploration time between the novel (p= 2.212) vs familiar objects (p= 0.214), in either ST or LT ITI (Figure 14).

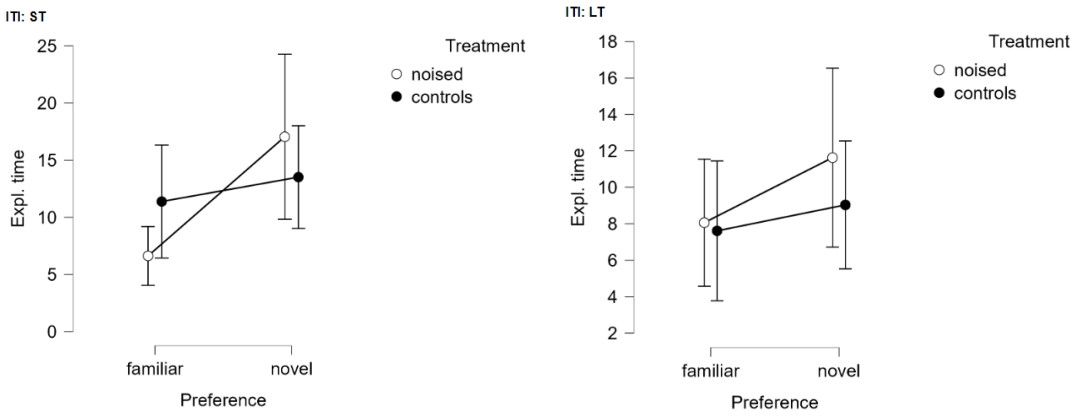

Fig. 14. Group comparison of the NOR between total exploration time (sec) for familiar vs novel object while controlling for treatment group in ST ITI and LT ITI.

Social Recognition test

Analysis of variance did not reveal any significant main or interaction effects of factor preference F(1, 84) = 0.025, $p = 0.875$, $\eta p2 = 2.838 \times 10^{-4}$, treatment F(1, 84) = 0.965, $p = 0.329$, $\eta p2 = 0.011$ or ITI F(1, 84) = 1.430, $p = 0.235$, $\eta p2 = 0.016$, suggesting that the prior assumption of rodents' tendency for novelty could not be met (Figure 15).

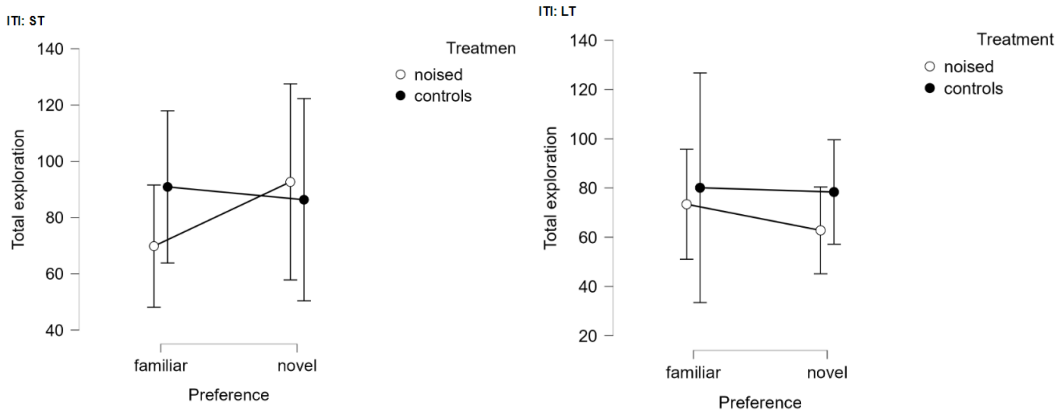

Fig. 15. Group comparison of the NOR between total exploration time (sec) for familiar vs novel object while controlling for factor treatment in ST ITI and LT ITI.

Discussion

The current study investigated whether mild noise-induced hearing loss leads to reduced memory performance in young male Wistar rats. Findings revealed that mild induced hearing loss does not result in reduced memory performance in spatial memory, object recognition, and social recognition memory in young male Wistar rats, thus, contradicting previous findings by Meister et al. (2016), Park et al. (2016) Liu et al. (2016) Chang et al. (2019). A question remains, however, whether these outcomes are valid considering that the behavioral experiments might not have sufficiently exploited the rodents' intrinsic preference for novelty over-familiarity (Sik et al., 2003).

The ABR analysis confirmed that noise-exposed rats indeed exhibited mild hearing loss, specifically at 16 kHz frequency. Although these findings are in line with previous literature by Reijntjes et al. (2018), further analysis has revealed no differences between noised and control rats in the higher frequency of 32 kHz after 2 or 7 weeks PN. However, it is worth mentioning that there has been a general threshold shift in both, noised and control rats in 32 kHz and 2 kHz 7 weeks PN compared to baseline. These findings suggest that the highest (32 kHz) and lowest (2 kHz) frequencies lay in the outer range of hearing acuity in healthy rats at an age of 11 weeks. Specifically, the threshold shift from baseline to 7 weeks PN suggests that the hearing range narrows with time, and that age might be a relevant factor playing a role in hearing acuity in young male Wistar rats (Bielefeld et al., 2010).

With regard to the OL, it is worth noting that results of the ST ITI have shown a discrimination index below a positive value (around zero) in noised and control rats, despite indicating a positive value in the LT ITI in both treatment groups. These findings suggest that rats did not remember the initial trial 1 after one hour (ST ITI), despite being able to memorize the initial trial 1 after 8 hours (LT ITI) which is in parallel with findings by Rönneberg et al. (2014). However, the results beg the question of whether the time interval of 1 hour (ST ITI) might have been too short for sufficient memory consolidation and retrieval. In light of this notion, it is worth noting that rats performed the memory tasks during the human circadian rhythm. Presumably, sleep deprivation might have, therefore, interfered with memory consolidation to a greater extent during the ST ITI (performed in the morning after 1 hour) than the LT ITI (performed in the afternoon 8 hours later). Furthermore, the ST ITI of the OL has been the first memory task which the rats performed. Therefore, despite prior habituation to the arena, the task environment may have been too novel to yield adequate attention to the actual task. Lastly, rats were only five weeks old during the first observational task of the OL, possibly further influencing the attention capacity during the memory tasks. To this end, the OL might have been less suitable for testing spatial memory cognition in Wistar rats at an early age of 5 weeks and against their natural sleep cycle.

Despite positive discrimination indexes in both noised and control rats during the ST and LT ITI of the NOR, results of further analysis of rodents' novelty preference have shown that the differences in exploration time between the novel vs familiar objects were neither significant in control rats in the ST and LT ITI, nor in the noised rats in the LT ITI. These findings suggest that the preference for novelty over-familiarity and the resulting positive discrimination index might be due to chance. Particularly, noised rats have only shown a preference for novel objects in the ST ITI but not LT ITI, whereas the control rats exhibited no novelty preference in either ST or LT ITI. Similarly, in the SR, noised and control rats have shown no differences in exploration time between the novel and familiar stimulus animals. These findings raise the question of whether the behavioral measures, specifically, the NOR and SR were sufficiently robust to measuring object and social recognition memory, or whether the rats simply did not remember the initial trial.

Implications and Future Suggestion

Considering the outcomes of the behavioral observations, alternative memory tasks such as the Morris water maze, or the delayed radial arm memory task might be more applicable for testing memory cognition in young male Wistar rats (Liu et al., 2016). Furthermore, performing the tasks during the rodent nocturnal rhythm might reduce the risk of sleep deprivation and, therefore, possible interference with memory performance (Colavito et al., 2013). Moreover, the repeated administration of anesthesia involving ketamine might have influenced the animal's cognitive performance, as exemplified in a study by Pitsikas et al. (2020). Lastly, mild-induced hearing loss might result in only subtle changes in the auditory-sensual processing, and impact memory processes to a lesser extent than moderate hearing loss or deafness (Liu et al., 2016). Accordingly, there might be greater differences in recognition memory in the event of moderate-to-absolute hearing loss. Overall, the experimental setup in this model needs to be carefully considered when interpreting the results of the behavioral observations. Moreover, amendments are necessary to avoid the limitations of the current design in follow-up studies and future behavioral observations.

Conclusion

In light of the study's findings, rats with noise-induced mild hearing loss did not seem to differ in their memory performance compared to the controls. Despite epidemiological studies highlighting children's difficulties in education following mild-hearing loss, the overall link between noise-induced mild hearing loss and memory cognition remains elusive and may imply to be rather correlational than causational. However, a conclusion stating no link between the two cannot be drawn as the behavioral measures appear to be partially invalid. Instead, future studies should employ alternative behavioral tests that might

prove greater robustness in measuring memory cognition.

References

Antunes, M., & Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cognitive processing*, *13*(2), 93–110. https://doi.org/10.1007/s10339-011-0430-z

Arlinger S. (2003). Negative consequences of uncorrected hearing loss--a review. International journal of audiology, 42 Suppl 2, 2S17–2S20.

Bess, F. H., Dodd-Murphy, J., & Parker, R. A. (1998). Children with minimal sensorineural hearing loss: prevalence, educational performance, and functional status. Ear and hearing, 19(5), 339–354. https://doi.org/10.1097/00003446-199810000-00001

Bielefeld, E. C., Tanaka, C., Chen, G. D., & Henderson, D. (2010). Age-related hearing loss: is it a preventable condition?. *Hearing research*, *264*(1-2), 98–107. https://doi.org/10.1016/j.heares.2009.09.001

Bucholc, M., McClean, P. L., Bauermeister, S., Todd, S., Ding, X., Ye, Q., Wang, D., Huang, W., & Maguire, L. P. (2021). Association of the use of hearing aids with the conversion from mild cognitive impairment to dementia and progression of dementia: A longitudinal retrospective study. Alzheimer's & dementia (New York, N. Y.), 7(1), e12122. https://doi.org/10.1002/trc2.12122

Calcus, A., Tuomainen, O., Campos, A., Rosen, S., & Halliday, L. F. (2019). Functional brain alterations following mild-to-moderate sensorineural hearing loss in children. eLife, 8, e46965. https://doi.org/10.7554/eLife.46965

Chang, M., Kim, H. J., Mook-Jung, I., & Oh, S. H. (2019). Hearing loss as a risk factor for cognitive impairment and loss of synapses in the hippocampus. Behavioural brain research, 372, 112069. https://doi.org/10.1016/j.bbr.2019.112069

Colavito, V., Fabene, P. F., Grassi-Zucconi, G., Pifferi, F., Lamberty, Y., Bentivoglio, M., & Bertini, G. (2013). Experimental sleep deprivation as a tool to test memory deficits in rodents. *Frontiers in systems neuroscience*, *7*, 106. https://doi.org/10.3389/fnsys.2013.00106

Daniel E. (2007). Noise and hearing loss: a review. *The Journal of school health*, *77*(5), 225–231. https://doi.org/10.1111/j.1746-1561.2007.00197.x

Davidson, L. S., Geers, A. E., Hale, S., Sommers, M. M., Brenner, C., & Spehar, B. (2019). Effects of Early Auditory Deprivation on Working Memory and Reasoning Abilities in Verbal and Visuospatial Domains for Pediatric Cochlear Implant Recipients. Ear and hearing, 40(3), 517–528. https://doi.org/10.1097/AUD.0000000000000629

Harrison R. V. (2012). The prevention of noise induced hearing loss in children. International journal of pediatrics, 2012, 473541. https://doi.org/10.1155/2012/473541

Lauer, A. M., Larkin, G., Jones, A., & May, B. J. (2018). Behavioral Animal Model of the Emotional Response to Tinnitus and Hearing Loss. Journal of the Association for Research in Otolaryngology : JARO, 19(1), 67–81. https://doi.org/10.1007/s10162-017-0642-8

Liu, L., Shen, P., He, T. et al. Noise induced hearing loss impairs spatial learning/memory and hippocampal neurogenesis in mice. Sci Rep 6, 20374 (2016). https://doi.org/10.1038/srep20374

Lin, F. R., Yaffe, K., Xia, J., Xue, Q. L., Harris, T. B., Purchase-Helzner, E., Satterfield, S., Ayonayon, H. N., Ferrucci, L., Simonsick, E. M., & Health ABC Study Group (2013). Hearing loss and cognitive decline in older adults. JAMA internal medicine, 173(4), 293–299. https://doi.org/10.1001/jamainternmed.2013.1868

Manohar, S., Adler, H. J., Chen, G. D., & Salvi, R. (2020). Blast-induced hearing loss suppresses hippocampal neurogenesis and disrupts long term spatial memory. *Hearing research*, *395*, 108022. https://doi.org/10.1016/j.heares.2020.108022

Marshall, C., Jones, A., Denmark, T., Mason, K., Atkinson, J., Botting, N., & Morgan, G. (2015). Deaf children's non-verbal working memory is impacted by their language experience. Frontiers in psychology, 6, 527. https://doi.org/10.3389/fpsyg.2015.00527

Meister, H., Schreitmüller, S., Ortmann, M., Rählmann, S., & Walger, M. (2016). Effects of Hearing Loss and Cognitive Load on Speech Recognition with Competing Talkers. Frontiers in psychology, 7, 301. https://doi.org/10.3389/fpsyg.2016.00301

Niskar, A. S., Kieszak, S. M., Holmes, A. E., Esteban, E., Rubin, C., & Brody, D. J. (2001). Estimated prevalence of noise-induced hearing threshold shifts among children 6 to 19 years of age: the

Third National Health and Nutrition Examination Survey, 1988-1994, United States. Pediatrics, 108(1), 40–43. https://doi.org/10.1542/peds.108.1.40

Olivier, J. D., Jans, L. A., Korte-Bouws, G. A., Korte, S. M., Deen, P. M., Cools, A. R., Ellenbroek, B. A., & Blokland, A. (2008). Acute tryptophan depletion dose dependently impairs object memory in serotonin transporter knockout rats. *Psychopharmacology*, *200*(2), 243–254. https://doi.org/10.1007/s00213-008-1201-0

Park, S. Y., Kim, M. J., Sikandaner, H., Kim, D. K., Yeo, S. W., & Park, S. N. (2016). A causal relationship between hearing loss and cognitive impairment. Acta oto-laryngologica, 136(5), 480–483. https://doi.org/10.3109/00016489.2015.1130857

Pitsikas, N., & Carli, M. (2020). Ketamine disrupted storage but not retrieval of information in male rats and apomorphine counteracted its impairing effect. *Neuroscience letters*, *737*, 135321. https://doi.org/10.1016/j.neulet.2020.135321

Ramsteijn, A. S., Van de Wijer, L., Rando, J., van Luijk, J., Homberg, J. R., & Olivier, J. D. A. (2020). Perinatal selective serotonin reuptake inhibitor exposure and behavioral outcomes: A systematic review and meta-analyses of animal studies. *Neuroscience and biobehavioral reviews*, *114*, 53–69. https://doi.org/10.1016/j.neubiorev.2020.04.010

Reijntjes, D. O. J., Schubert, N. M. A., Pietrus-Rajman, A., van Dijk, P., & Pyott, S. J. (2018). Changes in spontaneous movement in response to silent gaps are not robust enough to indicate the perception of tinnitus in mice. *PloS one*, *13*(8), e0202882. https://doi.org/10.1371/journal.pone.0202882

Rönnberg, J., Danielsson, H., Rudner, M., Arlinger, S., Sternäng, O., Wahlin, A., & Nilsson, L. G. (2011). Hearing loss is negatively related to episodic and semantic long-term memory but not to short-term memory. *Journal of speech, language, and hearing research : JSLHR*, *54*(2), 705–726. https://doi.org/10.1044/1092-4388(2010/09-0088)

Rönnberg, J., Hygge, S., Keidser, G., & Rudner, M. (2014). The effect of functional hearing loss and age on long- and short-term visuospatial memory: evidence from the UK biobank resource. *Frontiers in aging neuroscience*, *6*, 326. https://doi.org/10.3389/fnagi.2014.00326

Shukla, A., Harper, M., Pedersen, E., Goman, A., Suen, J. J., Price, C., Applebaum, J., Hoyer, M., Lin, F. R., & Reed, N. S. (2020). Hearing Loss, Loneliness, and Social Isolation: A Systematic Review.

Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery, 162(5), 622–633. https://doi.org/10.1177/0194599820910377

Sik, A., van Nieuwehuyzen, P., Prickaerts, J., & Blokland, A. (2003). Performance of different mouse strains in an object recognition task. *Behavioural brain research*, *147*(1-2), 49–54. https://doi.org/10.1016/s0166-4328(03)00117-7

Simon, M., Campbell, E., Genest, F., MacLean, M. W., Champoux, F., & Lepore, F. (2020). The Impact of Early Deafness on Brain Plasticity: A Systematic Review of the White and Gray Matter Changes. *Frontiers in neuroscience*, *14*, 206. https://doi.org/10.3389/fnins.2020.00206

Tao, S., Liu, L., Shi, L., Li, X., Shen, P., Xun, Q., Guo, X., Yu, Z., & Wang, J. (2015). Spatial learning and memory deficits in young adult mice exposed to a brief intense noise at postnatal age. *Journal of otology*, *10*(1), 21–28. https://doi.org/10.1016/j.joto.2015.07.001