The blinking eye: A window into tinnitus

Lars Rogenmoser ^{1,2*}, Paweł Kuśmierek ¹, Denis Archakov ¹, Josef P. Rauschecker ¹

¹ Department of Neuroscience, Georgetown University Medical Center, 3970 Reservoir Road, Washington DC 20057, USA

² Department of Medicine, University of Fribourg, Chemin du Musée 8, 1700, Fribourg, Switzerland

*Corresponding author. E-mail address: lars.rogenmoser@unifr.ch (L. Rogenmoser)

Abstract

Tinnitus is a prevalent untreatable audiological disorder, characterized by the perception of phantom sound. Despite longstanding research with animals, its underlying pathophysiology remains poorly understood. The obstacle in progressing in the field may lie in both the current species of choice and the available measurements of tinnitus for animals. To provide fresh impetus, we developed a novel tinnitus-verification technique applicable to rhesus monkeys. Tinnitus was induced via salicylate administration in two rhesus monkeys, and was confirmed by applying a specific eye-blinking procedure: Blinks, as monitored using EMG, were triggered via puffs of air towards the cheek, and their modulation was studied as a function of preceding tones with various frequency and intensity conditions. The advantage in using a tactile reflex-inducing stimulus lies in its non-auditory modality, bypassing potential confounding factors of hearing loss and hyperacusis. Interference effects on the blink modulation pattern was interpreted as tinnitus, and the interfering frequency of the preceding interfering tone as tinnitus frequency. A cross-validation in a sample of tinnitus patients revealed interfering effects of the preceding tone at the specific frequency range corresponding to their own tinnitus frequency, as independently determined by audiologists. This interference effect increased as a function of their individual tinnitus loudness. In conclusion, the present work demonstrates a considerable transferability of our newly established tinnitus-verification technique from non-human primates to human tinnitus patients.

1. Introduction

Tinnitus is a widespread medical condition with the core symptom of hearing phantom noise (Lockwood et al., 2002). It affects about 15% of the general and up to 45% of the older population (Chang et al., 2019; Henry et al., 2005; Jafari et al., 2019), debilitating the patient sometimes with accompanying cognitive-affective and somatic symptoms (Neff et al., 2021; Salazar et al., 2019; Shargorodsky et al., 2010). In worst cases, this condition may even lead to suicidal tendencies (Szibor et al., 2019). While the phantom noise is usually perceived in the ears, it is rather generated by the brain (Berliner et al., 1992; Fahy et al., 2002; Magliulo et al., 2000). Generally, tinnitus is initiated by hearing loss caused by damage of cochlear hair cells together with ribbon synapses and/or spiral ganglion cells (Bauer et al., 2007; Kaltenbach et al., 2004; Singer et al., 2013). This aberration may result from genetic abnormalities, injury, exposure to loud noise or ototoxic substances and/or from degenerative processes associated with aging (Cunningham and Tucci, 2017). The partial loss of peripheral auditory input leads to an imbalance that triggers plastic compensatory mechanisms within the auditory system. As a result, phantom noise is introduced into auditory perception, i.e., tinnitus emerges. As such, tinnitus can be described as a maladaptive plasticity effect (Rauschecker, 1999; Shore et al., 2016), which is documented as tonotopic remapping (Dietrich et al., 2001; Mühlnickel et al., 1998; Wienbruch et al., 2006) or more broadly as change, mainly of gain, in the cellular firing rate or in neural synchrony throughout the auditory pathways (Eggermont, 2015; Noreña, 2011). However, an impaired auditory system alone is not solely responsible for the pathophysiology of tinnitus, and it hardly determines its course or the impact of the suffering on the patient (De Ridder et al., 2011; Leaver et al., 2012). A large body of evidence suggests that in addition to the auditory system particular perception-unrelated brain structures and networks are involved in the pathophysiology as well. Human and animal studies identified cerebellar structures contributing to the generation of tinnitus (Bauer et al., 2013; Brozoski et al., 2017; Chen et al., 2015; Mennink et al., 2018). Tinnitus-related changes have further been identified in limbic (i.e., amygdala, hippocampus, parahippocampus, nucleus accumbens, striatum) and paralimbic (i.e., insula, cingulate) regions that likely account for the affective, attentive and

mnemonic processes implicated in the pathophysiology (Burton et al., 2012; De Ridder et al., 2006; Landgrebe et al., 2009; Leaver et al., 2011; Mühlau et al., 2006; Schecklmann et al., 2013; Schmidt et al., 2018; Vanneste et al., 2010). These changes at the paralimbic and limbic level likely contribute to the mechanisms underlying the conscious awareness and appraisal of the phantom noise. Finally, human imaging studies linked a wide range of cortical non-auditory regions (i.e., temporo-parietal and prefrontal) to impairment of top-down control implicated in tinnitus (Giraud et al., 1999; Leaver et al., 2012, 2011; Paraskevopoulos et al., 2019; Schmidt et al., 2018; Seydell-Greenwald et al., 2012; Vanneste et al., 2010). Multiple studies have revealed that the ventromedial prefrontal cortex (vmPFC) undergoes significant volume loss in tinnitus patients (Leaver et al., 2012, 2011; Mühlau et al., 2006). This morphological change likely disables top-down gating and inhibition normally exerted by vmPFC on auditory centers, thus preventing normally available suppression of phantom noise (Rauschecker et al., 2015, 2010).

Despite the usage of various animal models over the last three decades, the exact mechanisms causing and driving tinnitus are still far from understood, as to this day we are still unable to accurately predict from the outcome of hearing damage the profile, let alone the course or even the emergence rate of tinnitus. Small mammals, such as the cat and various rodent species (i.e., mouse, rat, gerbil, hamster, guinea pig, and chinchilla) are currently used as subjects in tinnitus studies (Noreña and Eggermont, 2003; von der Behrens, 2014). However, developing the appropriate tinnitus-verification technique turned out to be challenging (Brozoski and Bauer, 2016). Currently, the most commonly used one is a technique referred to as Gap-Prepulse Inhibition of the Acoustic Startle (GPIAS) (Turner et al., 2006). In this procedure, the natural Acoustic Startle Reflex (ASR) is experimentally modulated by preceding the startle burst with short gaps embedded in an auditory noise stimulus. Analogous to the mechanism of Prepulse Inhibition (Koch, 1999; Swerdlow et al., 1999), short gaps embedded in a background of ongoing noise are known to inhibit the ASR as well (Ison, 1982). The principle of the GPIAS argues that it is possible to objectively measure the presence of tinnitus by configuring the ongoing background noise to mimic the phantom (tinnitus) noise: If the background noise mimics tinnitus, the phantom sound should “fill in” the gaps and make them less

perceptible, consequently reducing the inhibition of the ASR and thus providing an objective marker of tinnitus. However, the validity of the GPIAS technique has been increasingly criticized given that not all findings could be explained with the “filling-in-the-gap” argument and, most importantly, their results failed to translate to actual tinnitus patients (An et al., 2014; Boyen et al., 2015; Campolo et al., 2013; Fournier and Hébert, 2013; Galazyuk and Hébert, 2015; Ku et al., 2017; Mahmoudian et al., 2013).

In hope of providing fresh impetus, we pilot tested a novel tinnitus-verification technique on a species more closely related to us: the rhesus monkey (*Macaca mulatta*), a nonhuman primate (NHP). We used a specific Eye-Blinking (EB) paradigm to assess salicylate-induced tinnitus in two NHPs and actual tinnitus in a sample of human patients. In this paradigm, we deliver puffs of air towards the cheek that either are preceded by tones or not. Given that inputs of polysensory sources are known to modulate the excitability triggering the blink reflex (Miwa et al., 1998; Valls-Sole, 2012), our model hypothesizes analogous to the GPIAS that tinnitus interferes with the effect of the tone on the puff-evoked blink response if its properties match the phantom sound.

2. Methods

All animal and human procedures were approved by the Institutional Animal Care and Use Committee and the Institutional Review Board of Georgetown University, respectively. All human participants gave written informed consent before participating in this study.

2.1 Animals

Two male NHPs (monkey C and monkey S; 14 and 7 years of age, respectively) were used in this study, each implanted with a headpost (Applied Prototype) secured to the skull with ceramic screws (Thomas Recording) or titanium cortical bone screws (Veterinary Orthopedic Implants), plastic strips, and bone cement (Osteobond, Zimmer). All surgical procedures were conducted under aseptic conditions using isoflurane anesthesia (1–2%) following pre-anesthetic medication with ketamine (10–15 mg/kg) and midazolam (0.1–0.5 mg/kg), with postoperative analgesic and prophylactic antibiotic treatment. Prior data

acquisition, the NHPs were trained to lie in sphinx position restrained by a primate chair (Applied Prototype) while being head-fixed. Data acquisition took place with this position in a sound-attenuated chamber (IAC, Bronx, NY).

2.1.1 Animal protocol

Data acquisition involved Eye-Blinking (EB) and Auditory Brainstem Response (ABR) recordings, which were performed in multiple daily sessions. Per session, ABRs were recorded in order to determine the hearing thresholds, which were used to calibrate the subsequent EB setup. Three blocks each of 6 sessions were carried out. The first block constituted baseline recordings. In the second block, salicylate was administered in order to induce tinnitus. Salicylate (200 mg/kg) in form of Aspirin powder (Vedco, Inc) mixed with food (e.g., yoghurt, honey, peanut butter) was administered to the animal every evening (7 pm) preceding the next day of ABR and EB recording. In total, the drug administration was done 7 days in a row, whereby data acquisition took place on the second treatment day and the five subsequent ones. The third block constituted follow-up recordings, and it was carried out 2-3 weeks after the final salicylate dose was administered.

2.1.2 Auditory Brainstem Response (ABR) recordings

ABR recordings took place early in the morning (5-8 a.m.), when the NHP was still sleepy. The chamber was kept dimmed in order to maintain the NHP's somnolent state, which was monitored via CCTV camera. Three silver-chloride (Ag/AgCl) cup electrodes (ELT-4830, EEG Sales LLC) were attached at the center of the supraorbital ridge (non-inverting input) and at both mastoids (inverting input and ground, respectively) using electrode gel (101, Biopac systems) together with adhesive tape. Auditory stimuli consisted of 1/3-octave band-passed noise bursts centered at 1, 2, 4, 8 and 16 kHz with respective lengths based on the 2-1-2 cycle (i.e., rise-plateau-fall) principle (Stapells and Oates, 1997). They were delivered in alternating polarities (i.e., condensation and rarefaction) at a rate of 25/s via a loudspeaker (MSP3; Yamaha) which was placed about 20 cm in front of the animal. Stimuli-evoked responses were amplified

(gain 100,000x) and band-pass filtered (100–1,500 Hz, CED 1902). The sound pressure level (SPL) was set at 75 dB, and was then systematically decreased in 15-dB steps (CED 3505) until the evoked responses were no longer visually observable in averaged signal. The SPL was then increased in 3-dB steps until the responses were visually observable again, determining the hearing threshold at a given frequency. Each stimulus was repeated a minimum of 2000 times to obtain reliable average responses.

2.1.3 Eye-Blinking (EB) recordings

Blinks were monitored by recording the electromyography (EMG) under the right eye in response to airpuffs delivered towards the left cheek. Three Ag/AgCl cup electrodes (ELT-4830, EEG Sales LLC) were attached according to a standard human protocol (Blumenthal et al., 2005), namely two below the orbicularis oculi muscle under the right eye (inverting and non-inverting input) and one on the forehead (ground). Electrode gel (101, Biopac systems) and adhesive tape were used to ensure attachment. The airpuffs were delivered with a pressure of 30 psi and duration of 10 ms. They were either delivered alone (probability rate of 0.25) or with a tone (probability rate of 0.75), preceding the puffs with 50 ms. The tones consisted of 1/3-octave band noises centered at 1, 2, 4, 8 and 16 kHz with a length of 20 ms (Blackman fade-in/outs of 5 ms). Per frequency condition, the tones were presented at 3 different sensation levels (SL; each with a probability rate of 0.25), which were threshold-adjusted based on the ABR outcomes. The threshold-adjusted intensities were 30, 6 and -6 dB SL. The 30 dB SL provided a condition of clear audibility, the 6 dB SL one overlapping with the range of expected tinnitus loudness (Fowler, 1938; Graham and Newby, 1962; Henry, 2016; Reed, 1960; Roeser and Price, 1980) and the -6 dB SL a condition of inaudibility. The three sensation levels together with the “no-tone” condition were randomized within one frequency block. Each condition was presented 12 times, and the inter-trial interval was jittered between 5 and 25 s. The order of the blocks was randomized. The tones were delivered via loudspeaker (MSP3; Yamaha), which was placed about 20 cm on front of the animal. The airpuffs were controlled via transistor–transistor logic signals using a solenoid valve (#003-0111-900, Parker Hannifin model), and were delivered from an air tank through a nozzle pointed towards the animal’s left cheek. Puff-evoked EMG responses

were amplified (gain 1,000x) band-pass filtered (10–500 Hz, CED 1902).

2.2 Humans

In order to determine transferability of the tinnitus-verification technique, the same ABR and EB recordings were performed in samples of tinnitus patients and matched control subjects (each $N = 11$). The stimulus material, filter settings and presentation protocols were identical to the ones used for the NHPs. The two human samples were comparable regarding age (patients: $M = 49$, $SD = 16.91$, range 20-75; controls: $M = 46$, $SD = 14.66$, range 26-66; $t_{19,6} = 0.44$, $P = 0.66$) and the distribution of the sexes (9 male patients and 5 male controls; Fisher's Exact test, $P = 0.18$). The patients perceived their tinnitus at an average frequency of 8.73 kHz ($SD = 2$, range 6-14 kHz) with an average loudness of 10.18 dB SL ($SD = 6.23$, range 2-20 dB SL), as independently revealed by standardized frequency and loudness matching by certified audiologists at MedStar Georgetown University Hospital. The impact of the tinnitus on their daily living, as measured by the Tinnitus Handicap Inventory (THI) (Newman et al., 1996), varied among patients, ranging from slight to catastrophic ($M = 21.45$, $SD = 89$, range 4-86 THI scores). The patients' individual characteristics on tinnitus are detailed in Table 1.

The participants received two appointments: the first one for determining the hearing thresholds and the second one for EB recordings. All testing took place in a sound-attenuated chamber (IAC, Bronx, NY). Before performing the ABR recording, the participants underwent a behavioral audiogram with the specific ABR stimuli. The participants gave feedback by raising their hand when they were able to hear the presented stimuli, which were manipulated in the same stair-wise manner, determining the behavioral hearing thresholds at a given frequency. During ABR recordings, the chamber was kept dimmed. The participants lied on a stretcher with their eyes closed, and were encouraged to take a nap. The stimuli were delivered binaurally via Sennheiser HD200 headphones. The EB recordings took place in a sitting relaxed position. Airpuffs were delivered via a nozzle pointing towards the left cheek that was attached to the Sennheiser HD200 headphones. The headphones delivered the preceding tones. For both the ABR and EB recordings, three Ag/AgCl cup electrodes (ELT-4830, EEG Sales LLC) were applied using the same

configuration as for the NHPs.

2.3 Stimuli presentation and data recording

In both humans and NHPs, stimuli presentation and data recording were controlled by a Power1401 laboratory interface (CED) together with the Spike2 software. All SPLs were calibrated using a sound level meter (Bruel & Kjaer 2235). Electrode impedances were kept below 5 k Ω , as monitored via EL-Check (Biopac systems). In the NHPs, the surface of the recording spots was shaved and cleaned using alcohol wipes. In both, non-conductive skin cells were removed by gently rubbing the spots with abrasive pads (ELPAD, Biopac systems).

2.4 Data analyses

Data processing and analyzes were conducted using Spike2 (v. 9.10) and Matlab (v. R2018b). The raw EMG data for each subject were rectified. Per airpuff response, epochs were created, ranging from -10 to 170 ms after puff exposure. A baseline correction relative to the “-10 to 0 ms” segment was applied. Condition-wise, these epochs were averaged. The maximal values were extracted from the averaged puff-evoked responses. Within each frequency block (i.e., 1, 2, 4, 8, 16 kHz), the blink modulation was calculated by subtracting the maximal value pertaining to the “no-tone” condition respectively from the maximal values pertaining to the condition with the tone of each intensity level (i.e., 30, 6, -6 dB SL). These difference scores were converted into absolute values, and then logarithmically transformed. In sum, the formula for computing the blink modulation as a function of the preceding tone was:

$$\text{Blink modulation} = \log(|\text{Puff}_{\text{tone}} - \text{Puff}_{\text{no-tone}}|)$$

These scores were imported into the SPSS software (v. 28) for statistical analyses. To explore for interference effects of tinnitus, the scores were frequency-wise subjected to analyses of variance (ANOVA) with the 3-level within-factor(s) “intensity” (i.e., 30, 6, -6 dB SL) and either “recording block” (i.e., baseline, salicylate, follow-up) for NHPs or the 2-level between-factor “group” (i.e., patients, controls) for humans. For each NHP, the statistical analyzes were conducted separately, whereby the session days served

as the unit of observation.

3. Results

3.1 NHP findings

The individual ABR audiograms revealed by both NHPs across the recording days are depicted in Fig 1. Apparent in both NHPs are the elevated hearing thresholds mostly at the upper frequency range in response to the salicylate administration and its reversal in the follow-up days.

The grand-averaged EMG blink responses for each NHP to all conditions of frequency, intensity and recording block can be found in Fig. S1 and Fig S2. Their individual blink modulation scores are depicted in Fig. 2, and the ANOVA results are detailed in Table 2. Both NHPs exhibited highly significant main effects of the factor “intensity” across all frequency conditions ($P \leq 0.001$). An interference on the NHPs’ eye-blinking behavior was apparent at the 16 kHz condition, as revealed by the significant interaction effects between the factors “intensity” and “recording block” (C: $P < 0.005$; S: $P < 0.05$). These interaction effects were due to the specific recording block with salicylate administration, as in comparison to the baseline and follow-up scores, the blink modulation under the influence of salicylate varied differently as a function of the intensity level. Particularly, the puff-evoked blinks preceded by the 16 kHz tones within the loudness range of tinnitus (i.e., 6 dB SL) did not modulate more strongly than the ones preceded by the inaudible (i.e., -6 dB SL) tones of the same frequency (C: $t_5 = 0.41$, $P = 0.70$; S: $t_5 = 2.15$, $P = 0.08$), whereas the blinks recorded in the baseline and follow-up blocks at the same frequency did (C and S: $t_5 > 2.5$, $P < 0.05$). In addition to these interaction effects, monkey C demonstrated significant main effects of the factor “recording block”, mainly at the upper frequency range (i.e., 4-16 kHz).

3.2 Human findings

The ABR and behavioral audiograms of both samples are depicted in Fig. 3. Both samples were comparable regarding their hearing profile, as revealed by a mixed ANOVA with “group” (i.e., patients, controls) as between-factor and “frequency” (i.e., 1, 2, 4, 8, 16 kHz) and “audiogram” (i.e., ABR, behavioral

testing) as within factors. Indeed, the ANOVA revealed a highly significant effect of frequency ($F_{4,80} = 19.27$, $P < 3.9 \times 10^{-11}$), none of audiogram ($F_{1,20} = 0.04$, $P = 0.85$) and no significant group effect ($F_{1,20} = 0.12$, $P = 0.73$) or any significant group-interaction effects (group x frequency x audiogram: $F_{4,20} = 0.61$, $P = 0.22$; group x frequency: $F_{4,20} = 1.74$, $P = 0.17$; group x audiogram: $F_{1,20} = 0.47$, $P = 0.88$). The thresholds across all frequencies correlated between the two audiometric methods (patients: $r_{11} > 0.6$, $P < 0.03$; controls: $r_{11} > 0.7$, $P < 0.02$).

The grand-averaged EMG blink responses for each group to all conditions of frequency and intensity can be found in Fig. S3. Their individual blink modulation scores are depicted in Fig. 4, and the ANOVA results are detailed in Table 3. The ANOVAs revealed highly significant main effects of the factor “intensity” across all frequency conditions ($P < 3.9 \times 10^{-5}$). An interference on the eye-blinking behavior was spotted only at the 8 kHz condition, as revealed by the highly significant group and group-interaction effects ($P < 0.01$). The frequency of the preceding tones within this specific condition largely overlapped with the frequency range at which the patients perceived their tinnitus ($M = 8.73$, $SD = 2$ kHz; see Table 1). In comparison to the controls, the patients exhibited overall reduced blink modulations (i.e., group effect) and additionally an altered blink modulation pattern (i.e., group-interaction effect). This pattern was reflected by the suspension of stronger puff-evoked blink modulations when preceded by the 8 kHz-tones set within the loudness range of tinnitus (i.e., 6 dB) than when preceded by the inaudible (i.e., -6 dB) tones of the same frequency ($t_{10} = 0.03$, $P = 0.98$). In contrast, the puff-evoked blink modulations of the controls preceding the 8 kHz-tones set at 6 dB SL overshoot the ones preceding the tones set at -6 dB of the same frequency ($t_{10} = 2.90$, $P = 0.02$). Finally, the patients demonstrated a negative correlation between tinnitus loudness and the puff-evoked blink modulation preceded by the 8 kHz-tone set at 6 dB SL ($r_{11} = -0.59$, $P = 0.03$; see Fig. 5). No other significant correlations were found between tinnitus loudness and the blink modulation of any condition constellation ($r_{11} < \pm 0.43$, $P > 0.07$).

4. Discussion

In the present work, we pilot tested a novel tinnitus-verification technique applicable to the NHP

as an animal model of tinnitus. In both our NHPs, the procedure was able to identify an interference effect of tinnitus on the eye-blinking behavior in response to the salicylate induction. This interference effect was characterized by altered blink modulations in response to the upper frequency conditions; in particular by the suspension of the otherwise increased blink modulations when the airpuff was preceded by the 16 kHz-tone set at 6 dB SL, the expected range of tinnitus loudness (Fowler, 1938; Graham and Newby, 1962; Henry, 2016; Reed, 1960; Roeser and Price, 1980). Given the specificity of the revealed interference effects unfolding within this particular frequency and loudness range, we inferred tinnitus-matching properties of this particular tone, thus determining the presence of tinnitus at that particular frequency, namely 16 kHz. This finding is in line with previous human and animal studies (Bauer et al., 1999; Day et al., 1989; Janssen et al., 2000; Liu and Chen, 2015; Martel et al., 2019; McCabe and Dey, 1965; Stolzberg et al., 2011; Yang et al., 2007), reporting salicylate-induced tinnitus at the higher frequency range (Cazals, 2000; Eggermont, 2015). Our cross-validation in human samples revealed a comparable interference effect at 8 kHz, corresponding to the frequency range independently determined by audiologists. Within this specific frequency condition, the patients not only exhibited the suspension of the otherwise increased blink modulations but additionally showed an interference as a function of their tinnitus loudness. In conclusion, the present work suggests considerable transferability of our newly established EB-setup to human tinnitus patients. Fig. 6 comparatively depicts the outcome of the pilot test revealed from both humans and NHPs.

4.1 The NHP as promising animal model of tinnitus

Over the last few decades, rodent models were able to gain important insights into the cellular and molecular mechanisms contributing to the plasticity and metaplasticity that induce or drive tinnitus (Haider et al., 2018; Knipper et al., 2020; Shore and Wu, 2019). However, these animal models limited their scope rather to peripheral and brain structures throughout the auditory pathways. Rodents are hardly comparable to humans regarding particular medial prefrontal brain structures (i.e., BA 13b and 14r) (Öngür and Price, 2000; Preuss, 1995), which are undoubtedly vital for the full understanding of tinnitus (Jastreboff, 1990; Leaver et al., 2012, 2011; Rauschecker et al., 2015, 2010; Seydell-Greenwald et al., 2012), and may

potentially expand the treatment possibilities when targeting them with sophisticated neuromodulation devices (Rauschecker et al., 2010). The NHPs possess more established and human-like prefrontal structures than rodents or carnivores, thus an NHP-based tinnitus model promises to capture the disorder more adequately. In addition, the NHP offers ecological validity unparalleled by other animal models not only due to critical similarities to humans in terms of neuroanatomy but also in terms of hearing range and lifespan (Recanzone, 2018). NHPs hold great potential for clinical translation to humans, as demonstrated across multiple fields in biomedical sciences, particularly in aging-related topics (Capitanio and Emborg, 2008). The usage of NHPs permit experimental approaches that can be applied to humans with little modification. In the present work, we were able to apply very similar recording protocols in both NHPs and humans, ensuring between-species translatability. Specifically, we monitored the EB behavior in NHPs as well in humans with non-invasive EMG. By contrast, in rodent studies the ASR is commonly recorded using startle chambers, bearing far less translational value.

4.2 Advantage of tactile reflex-inducing stimuli for tinnitus-verification

Instead of relying on the ASR for tinnitus-verification as in the previous GPIAS studies, we used a tactile airpuff to trigger the blink reflex. In comparison to the ASR, the airpuff-evoked blink reflex is significantly more reliable, with a response rate of virtually 100% (Haerich, 1998). This reliability was confirmed in our findings, as in both humans and NHPs the blink modulation was robust across all frequency conditions, with intensity effects of moderate to large sizes (see Table 2 and 3). GPIAS studies not only bear the problem of having to deal with “none-responder” animals and/or trials during data processing but also often find altered amplitudes of the ASR after tinnitus induction. A reduced ASR amplitude after tinnitus induction has been attributed to hearing loss and an augmented ASR amplitude to hyperacusis, respectively (Chen et al., 2013, 2015; Lobarinas et al., 2013; Longenecker and Galazyuk, 2011), both conditions confounding with tinnitus effects and thus impairing the tinnitus-verification performance. The tactile airpuff as a reflex-inducing stimulus bypasses this confounding factor due to its non-auditory modality. Furthermore, the airpuff is less aversive than the ASR-related sound burst (Lissek

et al., 2005). This aspect is crucial given that tinnitus is often comorbid with hyperacusis (Hayes et al., 2014). The hyperacusis-related painful sensations in response to the loud sound bursts may additionally interfere with the diagnostic power of the verification technique, as stress is known to interact with tinnitus (Alpini and Cesarani, 2006; Kim et al., 2021; Schmitt et al., 2000).

4.3 Reflex inhibition and facilitation

Our newly established EB-setup revealed both facilitation and inhibition of the airpuff-evoked blink reflex, a phenomenon often observed in GIAPS studies as well (Longenecker et al., 2018). As discussed by Longenecker et al., 2018, the reason for this dichotomy is not yet fully understood. Nevertheless, both systematic modulation types reflect most likely a contribution of the encoding of the reflex-preceding event. We decided to treat both types as such, and to study the modulation regardless of its direction. For this, we extracted the absolute values of the differences within a given frequency block between the puff-evoked responses with preceding tones and the puff-evoked responses without preceding tones. To counteract large variabilities across the scores, we additionally performed a logarithmical transformation, a technique previously suggested in analyzing eye-blinking behavior (Ornitz et al., 1986). Indeed, particular modified ratios for correcting this “direction problem” and sophisticated data-processing approaches for generally reducing variability in ASR data have been suggested in the past (Grimsley et al., 2015; Longenecker et al., 2018; Longenecker and Galazyuk, 2012). To the best of our knowledge, our correction formula has not been applied before.

4.4 Future directions

In our work, we chose to induce tinnitus in NHPs via salicylate administration due to its reversibility, reliability, and rapid effect. Salicylate administration as an induction-method models tinnitus fairly well, as the substance enters the cochlear perilymph and cerebrospinal fluid, unfolding the tinnitus-contributing effects at peripheral and the key brain structures involved in tinnitus perception (Stolzberg et al., 2012). However, the degree to which the underlying mechanisms overlap with the ones underlying

tinnitus of other etiologies are still debated (Eggermont, 2016; Lanaia et al., 2021). The cross-validation performed on our tinnitus patients suggests that the EB-setup is capable of also detecting tinnitus of the more typical etiologies, such as noise exposure or perhaps age. In the context of the NHP as an animal model, an investigation of the latter would be uniquely fruitful. Under captivity, some NHPs manage to live long enough to naturally develop age-related hearing loss (Engle et al., 2014, 2013; Gray et al., 2014a, 2014b, 2013; Ng et al., 2015), which potentially may involve tinnitus. Identifying geriatric NHPs with tinnitus might constitute a future milestone with great impetus for tinnitus research and treatment development. Given that our verification technique is non-invasive and relatively free from aversion (Lissek et al., 2005), it may be considered refined especially for sensitive populations such as elderly subjects with hearing issues (Haerich, 1998). This aspect together with the fact that the technique relies solely on a reflex and consequently does not require instrumental conditioning or animal training allows rapid testing of larger colonies.

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Table 1

Tinnitus characteristics.

Patient #	Age (years)	Associated aetiology	Description	Location	Frequency (kHz)	Loudness (dB SL)	Onset (years ago)	Impact (THI scores)
1	69	Chemotherapy	Tone	Both ears	9	2	>10	4
2	50	Unknown	Tone	Both ears, worse in right	8	18	19	14
3	46	Stress	Noise	Inside the head	8	4	5	86
4	39	Loud blast	Tone	Both ears	9	18	19	4
5	55	Loud blast	Crickets	Both ears	10	10	2	48
6	40	Prolonged sound exposure	Tone	Both ears, worse in left	8	12	10	6
7	20	Labyrinthitis	Noise	Left ear	8	10	5	46
8	75	Unknown	Hissing, humming, whooshing	Both ears	8	8	5	4
9	55	Unknown Sound exposure	Tone	Both ears	8	4	?	10
10	27	Sound exposure	tone	Both ears	6	20	>9	6
11	63	Loud blast	tone	Both ears	14	6	30	8

Table 2

ANOVA results from both NHPs (monkey C and S).

	<i>F</i> -value		<i>P</i> -value		Effect size (η^2p)	
	C	S	C	S	C	S
1 kHz						
Recording block	1.31	0.23	0.31	0.80	0.21	0.05
Intensity	21.34	19.53	2.4×10^{-4}	3.5×10^{-4}	0.81	0.80
Interaction	0.31	0.35	0.87	0.84	0.06	0.07
2 kHz						
Recording block	3.96	1.39	0.05	0.29	0.44	0.22
Intensity	50.25	29.22	6×10^{-6}	6.7×10^{-5}	0.91	0.85
Interaction	2.96	0.27	0.06	0.89	0.35	0.52
4 kHz						
Recording block	3.67	0.20	0.08	0.82	0.40	0.04
Intensity	26.74	35.12	9.7×10^{-5}	3.0×10^{-5}	0.84	0.86
Interaction	1.43	0.68	0.26	0.62	0.22	0.12
8 kHz						
Recording block	4.40	0.85	0.04	0.46	0.47	0.15
Intensity	25.57	114.05	1.2×10^{-4}	1.3×10^{-7}	0.84	0.96
Interaction	0.39	0.69	0.81	0.61	0.07	0.12
16 kHz						
Recording block	12.46	1.09	1.9×10^{-3}	0.37	0.71	0.18
Intensity	14.90	26.06	1.0×10^{-3}	1.9×10^{-4}	0.75	0.84
Interaction	5.38	3.12	4.17×10^{-3}	0.04	0.52	0.39

The *p*-values of significant interaction effects are highlighted in bold.

Table 3

ANOVA results from humans.

	<i>F</i> -value	<i>P</i> -value	Effect size (η^2p)
1 kHz			
Group	5.1×10^{-4}	0.98	2.6×10^{-5}
Intensity	29.87	5.1×10^{-8}	0.60
Interaction	0.28	0.76	0.01
2 kHz			
Group	0.57	0.46	0.03
Intensity	13.22	3.9×10^{-5}	0.40
Interaction	0.76	0.47	0.04
4 kHz			
Group	2.10	0.16	0.10
Intensity	21.83	3.9×10^{-7}	0.52
Interaction	0.34	0.72	0.17
8 kHz			
Group	8.68	8.0×10^{-3}	0.30
Intensity	21.37	4.9×10^{-7}	0.52
Interaction	7.29	2.0×10^{-3}	0.27
16 kHz			
Group	1.29	0.27	0.06
Intensity	23.23	2.0×10^{-7}	0.54
Interaction	0.68	0.52	0.03

The *p*-values of significant interaction effects are highlighted in bold.

Fig. 1. Audiograms of both NHPs (C and S) revealed by ABR recordings across session days. S1-S6 indicate days with salicylate administration. Values across the particular Baseline (B) and Follow-up (F) days were identical; thus collapsed here.

Fig.2 Blink modulation scores of both NHPs (monkey C and S) across all conditions of frequency, intensity and recording block. The floating bars represent the range and the within-lines the mean. The blue connected dots represent the individual values of the session days ($N = 6$). Significant interaction effects are indicated.

Fig. 3 Audiograms of both groups (patients and controls) revealed by ABR recordings and behavioral testing. Plotted are the means together with the standard deviations as whiskers.

Fig. 4 Blink modulation scores of both groups (patients and controls) across all conditions of frequency and intensity. The floating bars represent the range and the within-lines the mean. The blue connected dots represent the individual values of the participants ($N = 11$). The significant interaction effect is indicated.

Fig. 5 Relationship between tinnitus loudness and the blink modulation scores in response to the 8 kHz-tone set at 6 dB SL. The dots depict the individual pairs of values revealed by each patient ($N = 11$). Tinnitus loudness was independently determined by audiologists.

Fig. 6 Summary of the blink modulation findings. On top, the means of the blink modulation scores from both NHPs (C and S) are shown across all conditions of frequency, intensity and recording block. Below, the means of the blink modulation scores from both groups (patients and controls) are shown across all conditions of frequency and intensity. The whiskers depict standard error bars. Tinnitus effects are indicated.