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List of abbreviations

ABR: auditory brainstem-evoked response

ASR: acoustic startle reflex

CF: characteristic frequency

DPOAE: distortion-product otoacoustic emission

MEMR: middle-ear muscle reflex

MOC: medial olivocochlear

SEM: standard error of the mean

Abstract

Hyperacusis, an exaggerated, sometimes painful perception of loudness even for soft sounds, is a poorly understood distressing condition. While the involvement of modified gain of central auditory neurons and the influence of nonauditory brain regions are well-documented, the issue of where in the auditory system these abnormalities arise remains open, particularly when hyperacusis comes without sensorineural hearing loss. Here we used acute intraperitoneal administration of sodium salicylate (150 mg/kg) in rats, enough to produce > 10-dB decrease in acoustic startle threshold with mild hearing loss at low frequencies (< 10 kHz). Anesthesia, necessary for middle-ear-reflex (MEMR) threshold measurements, abolished the olivocochlear efferent reflex but not the MEMR acting on frequencies < 10 kHz, and its mean threshold increased from 55 dB SPL in controls to 80 dB SPL in salicylate-treated animals 60-90 minutes after injection, with an about 3-dB increase in acoustic energy reaching the cochlea. The mean latencies of auditory brainstem-evoked responses (ABR) conspicuously decreased after salicylate, by 0.25 millisecond at 6 kHz at every level, a frequency-dependent effect absent above 12 kHz. A generic model of loudness based upon cross-frequency coincidence detection predicts that with such timing changes, a transient sound may seem as loud at < 40 dB SPL as it does in controls at > 60 dB SPL. Candidate circuits able to act at the same time on the startle reflex, the MEMR and ABRs may be serotoninergic, as salicylate is known to increase brain serotonin and 5-HT neurons participate in MEMR and ABR circuits.

Keywords: hyperacusis; loudness; middle-ear muscle reflex; acoustic startle reflex; auditory brainstem responses

Introduction

Hyperacusis is a disorder of loudness perception defined as decreased tolerance to ordinary environmental sounds that, although not uncomfortably loud to a typical person, are felt as disagreeable or even, unbearably painful ((Baguley, 2003), review in (Knipper et al., 2013)). In loudness recruitment, another framework of hearing hypersensitivity typical of sensorineural hearing loss of cochlear origin, a narrower dynamic range of intensities between increased auditory thresholds and the thresholds of discomfort is reported (Baguley, 2003; Moore, 2002). Whether loudness recruitment and hyperacusis differ is unclear even though the former condition does not usually lead to intolerance at low sound levels (Baguley, 2003).

The concept that intolerance to sound is underpinned by an aberrant increase in central auditory gain is now widely invoked (Auerbach et al., 2014; Hebert et al., 2013; Knipper et al., 2013; Zeng, 2013). On the other hand, the neural bases of this gain abnormality remain unclear, as well as more generally the mechanisms that encode loudness in auditory-brainstem nuclei (Florentine & Heinz, 2009). An increase in central auditory gain may be intrinsic or the result of auditory changes in more peripheral parts of the auditory system, as suggested by some functional-imaging studies (Gu et al., 2010), an issue that it is important to clarify as any treatment that aims to alleviate hyperacusis would be more efficient by directly targeting the mechanism(s) at its origin.

Hyperacusis often occurs together with tinnitus (Eggermont & Roberts, 2015). Their debilitating consequences and the lack of radical cure have fostered intense research using animal models (Eggermont, 2013) in some of which tinnitus is reversibly produced by acute administration of sodium salicylate, the active metabolite of aspirin. With this model, reversible hyperacusis is also induced (Radziwon et al., 2017). One goal of the present work, for exploring the changes in brainstem responses to sound associated with a distorted perception of loudness, was therefore to use a moderate dose of sodium salicylate, 150 mg/kg, enough to provoke acute tinnitus (Lobarinas et al., 2004) and hyperacusis (Radziwon et al., 2017), without inducing more than a mild impairment of cochlear sensitivity. Such an impairment results from blockage by salicylate of the electromotility of outer hair cells, a category of sensory cells that amplify sound-induced vibrations. This amplification also compresses the growth of basilar-membrane motion with stimulus level whereas loss of compression, if severe, leads to loudness recruitment (Moore, 2002) that might interfere with the intrinsic effects of salicylate-triggered hyperacusis.

Here, exaggerated sensitivity to sound after salicylate intake in rats was ascertained by its effect on the acoustic startle reflex (ASR). We asked whether the distorted loudness had peripheral objective correlates in terms of brainstem-evoked potential characteristics and acoustic protective-reflex changes. As their main features (latencies, thresholds, amplitudes) show a large intersubject variability, comparisons between samples with *versus* without hyperacusis may lack power, so we exploited the reversibility of acute salicylate administration to perform within-subject comparisons of peripheral auditory responses. The auditory brainstem responses (ABRs) to different frequencies were used for evaluating auditory thresholds and timing of ABR waves along the brainstem auditory pathways where it is thought that loudness is encoded (Carney, 1994). Being loudness-related, the efferent-

feedback brainstem pathways made of the middle ear muscle reflex (MEMR) and the medial olivocochlear (MOC) efferent reflex were expected to be sensitive to the presence of hyperacusis. We thus tested whether, together with lowering ASR threshold, salicylate injection would transiently affect brainstem responses in a consistent, hyperacusis-dependent manner.

Material & Methods

Subjects

Male Sprague-Dawley rats (Charles River, St. Germain-L'Arbresle) weighing 250-350 g were used for this study, housed (22 ± 1°C, 45 ± 5% humidity) under a 12-h inverted nycthemeral cycle with access to water and food *ad libitum* and background noise levels of less than 50 dB SPL. The experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the national Ethical Committee (APAFIS authorization 12934-20180108 14452517V2). The animals were ascribed to different experiments, behavioral study of ASR (n=18); study of the MEMR (n=10); measurement of the latencies of ABR waves (n=15).

The animals were lightly anesthetized with ketamine (10 mg/kg, i.p., Virbac) and xylazine (20 mg/kg, i.p., Rompun), renewed when needed, the goal being to keep them perfectly motionless during the (noninvasive and painless) measurements that required the stable positioning of an acoustic probe in their ear canal. Their temperature was maintained at 37°C (Microprobe thermometer, BAT-12, WPI) using a homeothermic blanket (Harvard Apparatus) for the duration of the experiment. Before any functional test, the status of the outer and middle ears of every rat was checked under a microscope in order to rule out possible abnormalities.

Treatment

Hyperacusis was induced by acute administration of sodium salicylate (Sigma-Aldrich) dissolved in sterile saline, at time T0. The injected dose was 150 mg/kg intraperitoneally (i.p.) diluted for reaching a volume of 1 ml/100g. This dosage was chosen according to (Radziwon et al., 2017), enough to produce ~~tinnitus, thus likely~~ hyperacusis, while inducing only a minor cochlear dysfunction. Control, sham-treated rats received the same volume i.p. of saline solution only.

Auditory brainstem response recordings

The ABRs were measured using three subdermal electrodes (Medtronic Xomed Inc) (vertex – inverting; ipsilateral mastoid – non-inverting, back - ground) connected to an electrophysiological platform (Otophylab, Echodia®) that controlled both the production of sound stimuli and the averaging process of the sound-induced electroencephalographic voltages between the electrodes. A calibrated earphone sealed in the ear canal delivered the acoustic stimulations. These were tone-bursts made of a short pulse of pure tone at one of four possible frequencies: 6, 10, 12 and 16 kHz (1-ms rise and fall time, 4-ms plateau as in (Le Calvez et al., 1998)) that encompassed the interval of best sensitivity for rats (Heffner et al., 1994) at decreasing intensities from 80 to 0 dB SPL in 10 dB steps. The repetition rate of tone-bursts was 17/s, and 200 electroencephalographic epochs sampled at 44 kHz between -1 and 20 ms post tone-burst onset were collected and synchronously averaged.

Two complete baseline measurements of ABRs were performed: 30 minutes before injection (T-30) and at the time of injection (T0), in control (n = 7) and salicylate-treated (n = 8) animals. Measurements were then made 30, 60 and 90 minutes later (T30, T60 and T90). The hearing threshold at each frequency was defined as the minimum sound level producing one or several identifiable ABR waves labelled in Roman numerals according to (Ruttiger et al., 2017; Willott, 2006). Waves were identified visually, and their latencies defined as the time between stimulus onset and maximum positive deflection.

Middle ear muscle reflex monitoring

The MEMR consists in a sound-triggered contraction of the stapedius muscle that pulls on the stapes, a piston-like ossicle that transmits sound-induced vibrations of the tympano-ossicular system to the fluid-filled inner ear. The MEMR is triggered when the level of a sound presented in either ear exceeds the reflex threshold. The resulting change in stiffness of the ossicular chain attenuates the sound reaching the inner ear-at frequencies below the resonance frequency of the ear (<10 kHz in rats).

For noninvasive detection of the sound-induced increase in stiffness of the stapes in its oval window, standard acoustic-reflectance measurements in the ear canal (Keefe et al., 1992) lack sensitivity as reflectance depends more on eardrum than stapes stiffness. We designed a more sensitive method based upon the detection of otoacoustic emissions generated by sensory cells within the cochlea, which act as a reference sound source (Avan et al., 2000). On their way to the ear canal where they are readily detected (Kemp, 1978), otoacoustic emissions vibrate the stapes. Any increase in its stiffness thus translates into phase and amplitude shifts of

otoacoustic emissions, relative to their highly stable baselines, at frequencies lower than the resonance frequency of the stapes system (Avan et al., 2013). Distortion-product otoacoustic emissions (DPOAEs) generated by a bitonal sound stimulus at neighbor frequencies f1 and f2 are particularly suitable as their frequency, 2f1-f2, can be chosen for optimally detecting the MEMR.

Here, in control (n = 5) and treated (n = 5) rats, the stimulus for DPOAE measurements was made of two tones at f2=8 kHz and f1 = 6.67 kHz (thus, 2f1-f2 = 5.33 kHz) sent to the right ear at 50 dB SPL (below MEMR threshold) with fixed onset-phases. These frequencies fall in the interval of best efficiency of the MEMR in rats. For one DPOAE measurement, a 500-ms epoch of sound was recorded in the ear canal, from which the amplitude and phase of the spectral component at 2f1-f2 were computed using fast Fourier transform. Four epochs were collected in a row to evaluate DPOAE stability and the lack of influence of external noise (Fig.1 for the phase, upper panel, closed symbols; amplitude not shown). The MEMR-eliciting sound was then presented in the left ear by a calibrated earphone sealed in the ear canal to avoid acoustic interferences with DPOAE detection. This elicitor was a bandpass-filtered [0.02 – 20 kHz] white noise, thought to have maximum triggering efficiency, at a level increasing from 40 to 90 dB SPL in 10-dB steps. The DPOAE amplitude (not shown) and phase (Fig.1, upper panel, open symbols) in the presence of the MEMR elicitor were measured, three times in a row.

The MEMR readout was the average phase shift of the DPOAE (2f1-f2) relative to baseline in the absence of MEMR elicitor, as this shift provides the most sensitive and robust response to MEMR operation (Avan et al, 2000). The first shift in phase to be statistically significantly different from baseline (usually > 5°), defined the MEMR threshold (50 dB SPL in Fig.1, upper panel, 90 dB SPL in Fig.1, middle panel). The MEMR-induced DPOAE level shift is a readout of MEMR suprathreshold attenuation, D in dB, in the following manner. On their way to the cochlea, stimulus levels at f1 and f2 are reduced by D dB, and so is the generated DPOAE level as the DPOAE-to-stimulus level satisfies a 1 dB / dB ratio (Avan et al., 2013). On its way back to the external ear canal, the DPOAE undergoes a second attenuation of D dB through the MEMR-stiffened stapes. Hence D = (DPOAE level shift) / 2. While the DPOAE level shift is reliably measured well above MEMR threshold, it becomes small and variable near MEMR threshold whereas the phase shift is still evident and stable. This phase shift, multiplied by the dB / degree proportionality coefficient, independent of MEMR

strength (Avan et al., 2000) and accurately determined at high contralateral-noise levels, gave a reliable estimate of D.

Acoustic startle reflex

While salicylate at doses ≥ 150 mg/kg is known to induce tinnitus in the rat model (Lobarinas et al., 2004), it also induces hyperacusis, revealed in recent studies by a salicylate-induced enhancement of the ASR (Radziwon et al., 2017; Sun et al., 2009).

The startling sound to which control (n = 6) and salicylate-treated (n = 6) rats were exposed was produced in a sound-treated room by a noise generator (Bruel and Kjaer 1405) that emits gaussian noise with constant energy density between 1 Hz and 100 kHz. This signal was then processed through a bandpass filter (Rockland Model 1042F Dual Hi / Lo Filter) with a bandwidth of either [16-32 kHz] ("high-frequency" stimulation) or [5-9 kHz] ("low-frequency" stimulation). The signal, at levels adjusted by an attenuator (Tucker-Davis-Technologies PA5), was sent to a calibrated loudspeaker (KEF R100, with a 6-dB bandpass of [58 Hz – 48 kHz]) via a Nuprime STA-6 power amplifier. Sound calibration was done with a reference sound-level meter (Bruel and Kjaer 2240, ½ inch microphone 4188) at the same position relative to the loudspeaker as the tested rat. This microphone allowed the voltage necessary for obtaining a given sound pressure level to be measured up to 16 kHz, the range of sensitivity of the sound-level meter. At higher frequencies, this voltage-to-SPL relationship still held as the frequency response of the whole setup remained flat within a few dB up to 32 kHz.

For all tests, four stimulation levels were available from 60-90 dB SPL in 10-dB steps. Stimulations were brief 500-ms sound bursts, presented with an interval of 30 seconds minimum between two stimulations to let the animal calm down before hearing the next stimulus. For startle recording, a force sensor probe (AD instrument Powerlab 26T) was connected to the steel-wire system from which the cage hung. The probe readout was amplified such that quiet rat movements (walking, grooming) were all visible as small shifts from baseline. A startle showed as a sound-synchronous peak exceeding the largest deflection produced by natural motion of the tested rat. The first tested level was chosen at random. Then, a bracketing procedure was used whereby when a startle occurred, the next sound level was 10 dB softer, whereas in the absence of startle, the next sound was 10 dB louder. The startle threshold was defined as the smallest stimulation level for which the sensor detected a startle, three times in a row. The step size was chosen for allowing a short procedure, to be

repeated every 30 min without inducing habituation. Its shortcoming was a possible overestimation of some ASR thresholds by several dB as for example, in a rat with an 85-dB true threshold, the procedure would have registered 90 dB SPL as the lowest level giving an ASR. The strength of the ASR at 70 and 80 dB SPL, estimated by the maximum amplitude of the signal from the force sensor, was also analyzed.

Data analysis

Statistical analyses were performed using GraphPad Prism v6. Parametric tests were used if Shapiro-Wilk and Bartlett tests had confirmed normality and homoscedasticity of the populations from which the compared samples came. A p value of < 0.05 was considered significant. Firstly, comparisons were made between controls and salicylate-injected rats at the beginning of every experiment (T0), using unpaired t-tests, in order to check whether the two groups of animals were similar in their auditory characteristics (ASR thresholds, DPOAEs, ABR thresholds, MEMR properties, ABR latencies). Next, the outcomes of auditory tests were compared at T-30 and T0 using paired t-tests in order to check the stability of detection setups, in the absence of treatment.

Last, the effect of injections, whether of saline or salicylate, were tested with an analysis of variance (ANOVA) for repeated measures, with time and when applicable, sound level (for ABR wave latency against sound level plots) as independent variables. Whenever a significant difference was detected, post-hoc tests were performed as paired t-tests with the Bonferroni correction for multiple comparisons, a conservative procedure at the cost of increasing the probability of false negatives, a negligible disadvantage for a post-hoc test. The focus of post-hoc tests was to identify significant differences between each of the mean auditory characteristics under test at the times following injection, from T30 to T90, and the corresponding pre-injection value at T0.

Results

Auditory sensitivity after salicylate administration

The ABR thresholds of the control and salicylate-treated rats at the four tested frequencies are displayed in Fig.2A, the lowest ones being obtained at 10 and 12 kHz in agreement with (Heffner et al., 1994). These thresholds are normal for stimuli delivered to a closed ear canal, slightly less sensitive than in free field when the pinna resonance would bring the best thresholds of detection near 0 dB SPL. Measurements at T-30 and T0 ascertained the absence of significant change in ABR thresholds. At T0, control and treated groups showed no

statistically significant difference. Thresholds in the control group (Fig.2B-E, open circles) remained stable between T0 and T90. In treated rats, a progressive change in ABR thresholds occurred following salicylate administration (Fig. 2B-E, solid squares), already clear at T60 and increasing at T90 to 8.5, 10.0, 17.5 and 18.3 dB at 6, 10, 12 and 16 kHz respectively, relative to the T-30 measurements. At 6 kHz, the ANOVA for repeated measurements showed a significant effect of time ($F_{4,52} = 3.85$, $p < 0.01$, total variance explained $\eta^2 = 0.11$). The post-hoc test lacked power for confirming the trend that seems to single out the T60-T90 ABR thresholds at 6 kHz ($p>0.05$). At 10 kHz, the ANOVA was highly significant ($F_{4,52} = 12.19$, $p < 0.0001$, $\eta^2 = 0.24$; for post-hoc test outcomes see Fig.2C). At 12 and 16 kHz, the ANOVAs were even more significant ($F_{4,52} = 25.96$ and 15.77 respectively, $p < 0.0001$, $\eta^2 = 0.30$ and 0.37 respectively; for post-hoc test outcomes see Fig.2D-E). These hearing threshold elevations are similar in (moderate) size and (weak) frequency dependence to those of previous publications, e.g. (Castaneda et al., 2019).

Acoustic startle reflex study

In control rats, the strength of ASR measured at 70 and 80 dB SPL remained stable throughout, for both noise bands [5-9 kHz] (Fig.3, open circles) and [16-32 kHz] (almost identical plots, not shown). For the lower-frequency noise band [5-9 kHz], ASR was never detected at 70 dB SPL and remained absent at 80 dB in half the cases. The same features held for the salicylate sample at T0 (Fig.3, solid squares). Conversely, at T90, half the treated rats responded already at 70 dB SPL, thus their ASR thresholds were at least 10 dB lower than many controls. Salicylate treatment started acting on the ASR at T60 (Fig.3). The average strength of the ASR was significantly larger at T90 relative to T60 and T0 for the 80-dB noise (ANOVA for repeated measurements, $F_{2,17} = 19.87$, $p< 0.001$, $\eta^2= 0.44$; see Fig.3 for post-hoc test outcomes), but not for the 70-dB noise owing to the dispersion of data ($F_{2,17} = 2.67$, $p= 0.10$). At 80 dB SPL, ASR strength reached 4 times that of control rats (Fig.3). In contrast, for the higher-frequency noise band [16-32 kHz], ASR strengths in treated and control rats remained similar (e.g., at T90, 3.02 ± 1.13 arbitrary units in treated vs 3.16 ± 1.58 in controls; $p >0.05$).

Middle ear muscle reflex monitoring

The main undesired side effect of salicylate specifically targets outer hair cells, sources of DPOAEs, and a decrease in DPOAE amplitude after salicylate injection would hamper MEMR monitoring. The average DPOAE reference amplitude (i.e., without contralateral

MEMR activating noise) is plotted against time in Fig.2E, showing no statistically significant change, only a slight descending trend at T90 despite the 8.5-dB ABR threshold elevation in treated rats (ANOVA for repeated measurements, $F_{4,32}$ = 0.24, p=0.92). This confirms the validity of MEMR measurements with DPOAEs.

In every tested control rat, the first significant shift in phase of the 2f1-f2 DPOAE (f2 = 8 kHz) that defines the threshold of MEMR activation occurred at 50 or 60 dB SPL in physiological conditions (e.g. 50 dB SPL for the example of Fig.1, upper plot at T0). The phase shift then increased monotonically with the level of contralateral stimulation (Fig.1, upper plot). On average, the MEMR threshold remained stable around 55 dB SPL in the control group during the whole recording session (Fig.4A, open circles). It rose up to 80 dB SPL on average for rats treated with salicylate at T60 and T90 (ANOVA for repeated measures, $F_{4,40}$ =4.37, p<0.01, η^2= 0.11; see the outcome of post-hoc tests in Fig.4B, solid squares), while the two groups had non-significantly different MEMR thresholds before T60. Pilot experiments had shown that the MEMR is abolished for 15 minutes by injection of an intravenous bolus of vecuronium bromide in deeply anaesthetized and ventilated rats (500µl, 1mg/ml; no phase shift, no visible MEMR effect -Fig.1, lower panel; suppl. movie 2 with curare as against suppl. movie 1 without). This precludes the contribution to the phase shift of other factors than muscle contraction.

The phase-to-level-shift proportionality coefficient, obtained from MEMR data at 90-dBSPL contralateral stimulation, was 0.113 dB / degree (linear regression, n = 10; R = 0.77, p<0.01). The MEMR-induced decrease in sound level reaching the cochlea remained stable all along, in control rats, and this for every contralateral-noise level (Fig.4C), whereas the overall shape of the plot of MEMR attenuation against elicitor level tended to start changing at T30 in salicylate-treated rats (Fig.4D; ANOVA for repeated measures with time : $F_{4,24}$ = 8.17, p< 0.001, η^2= 0.13). However, post-hoc tests would suffer from the fact that comparisons among measurement times of the strength of the MEMR at a given elicitor level may be confounded by the already described gradual increase in reflex threshold, as strength comparisons would involve different stimulus levels above MEMR threshold. At the highest tested level of 90 dB SPL (i.e., 35 dB above average MEMR threshold at T0 but only 10 dB at T90), the MEMR phase shift dropped from 25° at T0 to 18° at T30, 16° at T60 and 13°, half its initial efficiency, at T90. Due to acoustic crosstalk between left and right ears, the noise produced in the right ear by elicitor levels > 90 dB SPL prevented any valid measurement of DPOAE phase.

Suprathreshold ABR waves after treatment

The averaged ABR waveforms are shown in Fig.5 for control and treated rats (top and bottom panels respectively), in response to 6 and 16 kHz tonebursts (left and right panels respectively). The waveforms at T0 (Fig.5, black bold lines, averages; black thin lines, averages ± one SD) and their repetition at T90 (grey bold lines, averages) are plotted together for comparison. The SDs at T90 being similar to those at T0, the T90 averages ± one SD are omitted for the sake of clarity. Average deflections in chronological order give an approximate idea of where individual waves I to IV fall, given that in some individual ears, morphological variability, particularly beyond wave II, is such that they may lead or lag the average deflections by 0.1 to 0.2 ms. Later waves V and VI (at 16 kHz) are also visible. In controls, regardless of frequency, ABRs at T90 remain stable, within one SD of the T0 average all along. It is not the case for salicylate-treated rats. At 6 kHz, although the deflection corresponding to wave I, slightly decreased in amplitude, culminates at the same post-stimulus time, the following deflections increasingly lead those at T0, from wave II on. The average lead at T90 relative to T0 is about 0.3 ms for wave IV and at post-stimulus onset times > 1.5 ms, the T90 average trace wanders far outside the ± one SD interval at T0. The mean amplitudes of late deflections tend to increase at T90 as compared to T0. At 16 kHz, the reading is compounded by the existence of the 18.75 dB threshold elevation that translates into a reduction by half of wave-I amplitudes and a slight change in morphology of successive waves. Yet the average ABR mostly remains close to its T0 version from 2 to 6 ms post-stimulus onset. Although wave III and IV morphologies are blurred by the averaging procedure, wave-II associated deflections and the deflections beyond wave IV neither lead nor lag their T0 version.

The diagrams that represent, at a given frequency, individual latency against stimulus level are displayed in Fig.6A-J. At frequencies > 6 kHz, only plots of waves I and IV are shown as wave III was difficult to separate from the sloping late part of wave II in some animals. The latency-intensity plots confirm the trends of average ABRs, with a high stability of wave I latencies, except at 16 kHz, T60 and T90, at low stimulus levels at which latencies are abnormally prolonged as a result of the >15-dB ABR-threshold elevation.

At 6 kHz, a two-way ANOVA (time × level) revealed statistically significant changes in latencies of ABR waves III and IV (significant effect of time for wave III, $F_{3,18} = 5.71$, $p<0.01$, $\eta^2= 0.13$; for wave IV, $F_{3,21} = 5.46$, $p<0.01$, $\eta^2= 0.13$). Post-hoc analyses indicate that

at T60 compared with T0, wave-III and IV mean latencies were significantly reduced by approximately the same amount, about 0.25 ms, at all stimulus levels. Wave latencies at T90 and T60 were so similar that their plots are almost indistinguishable. As for wave amplitudes, Fig. 5 (lower panels) suggests that for salicylate-treated rats, wave-IV amplitude tends to increase at T90 despite the tendency for wave I to be slightly smaller at T90 than T0. This trend is not visible for earlier and later ABR waves and does not exist at 16 kHz either. Actually, the amplitude comparisons at 80 dB are not significant (paired t-tests, df = 6; t = 0.95 and 1.29 respectively, $p>0.05$). ANOVAs for repeated measurements with stimulus level as factor could not be done to improve the statistical power, as the amplitude readings at stimulus levels < 70 dB SPL lose accuracy owing to the smaller wave size.

At 10 kHz (ANOVA, significant effect of time for wave IV latency, $F_{3,27}=4.20$, $p < 0.05$, $\eta^2= 0.07$), the mean latency of wave IV was reduced by a similar amount to 6 kHz at levels > 50 dB SPL from T60 on, compared to T0 (Fig.6F). The latency-shortening effect at 12 kHz was restricted to a non-significant trend (ANOVA, $F_{3,27}=4.20$, $p > 0.05$). At 16 kHz, no shortening of wave-IV latency was visible.

Discussion

Ninety minutes after a single i.p. injection of salicylate at 150 mg/kg, rats displayed a rich pattern of changes in their cochlear and lower-brainstem responses, some of them suggestive of a change in processing of sound intensity. As expected since salicylate interacts with cochlear outer-hair-cell activity, a mild loss in auditory sensitivity was observed, ranging from 8.5 to 18.75 dB between 6 and 16 kHz. The MEMR underwent a large threshold elevation of 25 dB on average and a concomitant loss in efficiency, because of which the attenuating effect of the MEMR on incoming sounds fell to hardly 1 dB when triggered by a 90 dB SPL contralateral noise. Whether the medial olivocochlear efferent reflex was affected could not be determined as anesthesia abolished it. Last, the latencies of brainstem-generated ABR waves were shortened, only at low frequencies, from wave II (from the cochlear nucleus) on, by 0.25 ms on average. Such latency reductions, seldom described, match those reported recently in a tinnitus rat model induced by salicylate (oral, 350 mg/kg) (Castaneda et al., 2019). In awake rats at T90, a behavioral consequence of salicylate treatment is the change in ASR triggered by a low-frequency [5-9 kHz] noise band, but not a high frequency band, with a decrease in its threshold, often exceeding 10 dB, and a sharp increase in its

strength at a given inductor level. The ASR is a trusted marker of hyperacusis (Chen et al., 2014; Sun et al., 2009). In humans, increased startle responses may not correlate with reported hyperacusis yet definitely come with reduced loudness discomfort levels (Knudson & Melcher, 2016). All these data identify ASR as pinpointing loudness-encoding disorders. In line with previous models of salicylate-induced hyperacusis, the present situation was thus representative of distorted loudness perception.

A first possible explanation for this distortion might be a mild impairment of cochlear outer-hair-cell mechanics by salicylate despite the <20 dB increase in auditory thresholds. By the compression that they exert on the basilar membrane, outer hair cells extend the dynamic range of the cochlea to about 120 dB, so that when they dysfunction, the interval between the smallest audible and the largest tolerable sound levels is reduced (Moore, 2002). However, typical sensorineural hearing losses of cochlear origin do not affect loudness discomfort levels, which on average depend little on the degree of hearing loss (Sheldrake et al., 2015). In contrast, they may decrease, sometimes down to 40 dB HL regardless of hearing sensitivity in subjects who complain of hyperacusis (Sheldrake et al., 2015). In humans with various degrees of hearing loss, the slope of loudness functions, known to correspond well with basilar-membrane growth functions when directly measured, increases only from 0.60 in normal-hearing subjects to 1.0 for a 40-dB hearing loss (Hellman & Meiselman, 1990) rising to > 2.0 on average only when hearing loss exceeds 60 dB. A theoretical interpolation of these authors predicts normal loudness slopes for a <20 dB hearing loss.

The observation of a sharp increase in MEMR threshold offers another possible explanation of the increased sensitivity to low-frequency noise experienced by salicylate-treated rats, since the MEMR is classically thought to attenuate low-frequency sound transmission to the cochlea, thereby affording protection against overstimulation and masking (Liberman & Guinan, 1998). Here, the animals had to be lightly anesthetized for keeping them motionless during MEMR assays, which might have affected the MEMR. However, the finding of MEMR thresholds around 55 dB SPL in close agreement with the best published thresholds in rats, 57 dB SPL using the maximally sensitive but invasive electromyography for reflex detection (Murata et al., 1986), suggests little inhibition of the reflex by anesthesia. For comparison, MEMR thresholds estimated by immittance measurements are hardly better than 77 dB SPL in rats (Pilz et al., 1997) and 65-80 dB SPL in awake humans (Metz, 1952). Yet anesthesia might have affected MEMR strength so that the finding of a 4-dB average efficiency in control rats, decreased to 1 dB after salicylate, might be an underestimation of

what happens in awake rats. If so, the salicylate-induced MEMR threshold elevation might have more detrimental effects on the loudness experienced by awake rats than what we suggest. However, the MEMR attenuation of sound transmission to the cochlea measured in awake humans using DPOAEs hardly exceeds a few dB even for intense sound elicitors (Avan et al., 2000). Thus, the salicylate-induced MEMR threshold elevation, however interesting by itself, falls short of accounting for the hyperacusis.

In synergy with the MEMR, which only acts upon low frequencies, the MOC reflex also wired in the lower brainstem (cochlear nucleus and superior olivary complex) controls cochlear responses at higher frequencies (Liberman & Guinan, 1998). Thus, it is a shortcoming of the present study that the rats had to be anesthetized. With MEMR-blocking curare, the effect of contralateral noise on DPOAE responses is restricted to the MOC effect, and its absence suggests full inhibition by anesthesia, already well-acknowledged (Guitton et al., 2004). In awake rats, nonetheless, ASR thresholds were measured in response to high-frequency noise, for which the MOC reflex is expected to be active, but not the MEMR. The absence of salicylate-induced change in ASR suggests that either the MOC reflex is insensitive to salicylate or the ASR is not influenced by the MOC reflex.

Another conspicuous effect of salicylate intake on lower-brainstem sound-evoked responses at all tested stimulus levels at 6 kHz is the 0.25 ms decrease in latency of waves III and IV, despite unchanged wave-I latency, a marker of stable cochlear and distal cochlear-nerve processing. The shortening effect of salicylate, slightly less strong at 10 kHz, weaker at 12 kHz and absent at 16 kHz, operates in the same frequency interval where the MEMR is effective. Shortened time intervals between consecutive ABR waves have seldom attracted the attention as, usually, auditory diseases result in delays, such as compressive, vascular or demyelinating conditions. It has been shown that activation of corticofugal projections to the auditory brainstem may well shorten the latency of collicular neurons (by several tenths of a millisecond), which would translate into shortened ABR wave latencies (Peng et al., 2017). More directly relevant to the present issue, in a mouse model with salicylate-induced tinnitus, shorter ABR wave-IV latencies at high levels at frequencies $\leq$ 20 kHz have been measured (Lowe & Walton, 2015). At lower levels, the increased ABR wave-I latencies due to a 24-dB hearing loss hid the true amount of ABR latency decrease specific of retrocochlear changes. Recently, in salicylate-treated rats, it has been shown that reduced late-ABR-wave latencies at 80 dB SPL in the 8-16 kHz range, but not at 32 kHz, come with reduced latencies of middle and late latency responses and with behavioral tinnitus (Castaneda et al., 2019).

Could this shortening of ABR wave latencies directly influence loudness? The idea that loudness is simply a measure of the overall rate of spikes in the auditory nerve is challenged by the observation of objective signs of exaggerated loudness in cases with a sharp decrease in auditory-nerve activity (Cai et al., 2009; Relkin & Doucet, 1997). Another shortcoming of the rate model is that the growths with sound level of loudness and of the rate of spikes in distal auditory pathways show discrepancies (Delgutte, 1996). A physiological model that circumvents both difficulties incorporates cross-frequency coincidence detectors (a natural task for neurons) that fire only when the sound-induced spikes from different frequency channels converging on the detector are synchronous enough (Carney, 1994). The timing of spikes, reflected by the latencies of far-field ABRs, is critical in this model as it predicts loudness encoding as follows. Even spectrally simple tones produce excitation patterns that spread toward the basal cochlea. The triggered spikes travel mainly through channels represented by neurons whose characteristic frequency (CF) is the tone's frequency f, but also through higher-CF channels connected to the more basal cochlea. Spikes in the CF=f channel lag spikes from more basal regions owing to the intracochlear level-dependent delay (Fig.7, right cartoon). The onset timing of neurons with CF = f is fully reflected by the latency of ABR waves at f, a response which they largely dominate relative to more basal neurons also contributing to the ABR. The timing difference between channels at different CFs inferred from ABRs is represented in Fig.7 (middle, solid triangles). The growth of loudness at increasing sound levels is informed by the fact that coincidence detectors, possibly in the cochlear nucleus, fire at increasing rates as cross-frequency delays drop with the decrease in cochlear baso-apical travel time (Cai et al., 2009; Joris, 2009). Of note, this cue is valid only for sounds with varying envelopes, as continuous high-frequency tones cannot induce neuronal phase-locking due to refractory periods. Here, in salicylate-treated animals, the 0.25-ms decrease in ABR waves III-IV latencies observed at lower but not higher frequencies reveals a powerful facilitating mechanism for the build-up of coincidences already at levels probably several tens of dB lower than those at which they should happen (Fig.7 left). The fact that this mechanism is already well-established at 40 dB SPL matches the report by hyperacusic patients that their impression of excessive loudness occurs even for soft sounds, the reason why they feel permanently uncomfortable.

The last issue is how salicylate administration might produce the complete auditory phenotype with combined actions upon ASR, MEMR threshold and ABR-wave timing. This combination of apparently unconnected findings is not as perplexing as it seems. Patients with

the Williams-Beuren syndrome (due to a chromosomic microdeletion) show hyperreactivity to sound (Baguley, 2003), often summarized as hyperacusis, even though their characteristically decreased degree of fear means that other aspects of sound may be judged pleasant, e.g., music. Patients with normal auditory thresholds and middle-ear function have strongly increased MEMR thresholds (Attias et al., 2008) and lowered uncomfortable loudness levels. The ABRs in Williams-Beuren patients have not been described, and anyway, contrary to rats, are not recordable in the low-frequency range of sensitivity. In the Williams-Beuren model of *Gtf2ird1* null mice, the phenotype matches the human one and ABR-wave IV latency at low frequency is about 0.3 ms shorter than normal (Canales et al., 2015) (their Fig.4a).

Among the numerous effects of salicylate, interference with excitatory and inhibitory circuits of the central auditory system have been consistently reported. Its down-regulation of GABA-mediated inhibition is often invoked for explaining enhanced ASR and tinnitus, in terms of increase in gain of the central auditory system, e.g. (Sun et al., 2009). Salicylate also decreases the firing of some glycinergic, inhibitory interneurons in the cochlear nucleus (Zugaib et al., 2016). However, how a release of inhibition could lead to inhibition of the MEMR is unclear.

A third hypothesis is suggested by the *Gtf2ird1* null mice with a serotonin increase in several brain regions (Attias et al., 2008). Increased serotoninergic activity may explain the low innate degree of social fear in the Williams-Beuren syndrome (Proulx et al., 2010). Salicylate increases 5-HT concentration in the inferior colliculus and auditory cortex, which possibly plays a part in tinnitus generation (Liu et al., 2003). The auditory phenotype induced by serotonin activation and the one observed here have common features. Firstly, stapedial motoneurons that innervate the stapedius muscle do receive serotoninergic terminations (Mukerji et al., 2010; Thompson et al., 1998) from different parts of the auditory system, from cochlear nucleus to cortex. The MEMR is conspicuously rich in modulating influences (Mukerji et al., 2010; Vacher et al., 1989), most of which are poorly documented. Serotoninergic circuitry in the auditory brainstem suffers from similar lack of precise knowledge, yet serotonin is known to act in an excitatory or inhibitory manner depending on which receptors (5-HT1 to 7) are involved, with the additional complexity that these receptors modulate the release of many neurotransmitters, some excitatory (glutamate) and others, inhibitory (GABA). A recent work on the effect on ABRs of agonists and antagonists of specific serotonergic receptors reports the possibility of registering reduced latencies (by

several tenths of a millisecond) for late ABR waves, more pronounced at lower stimulus frequencies (Papesh & Hurley, 2016), a pattern reminiscent of the one observed here.

In conclusion, the present work describes three concomitant changes in response to lower-frequency sounds after acute salicylate administration, a 4-fold increase in ASR strength at 80 dB SPL indicative of hyperacusis, a 25-dB elevation of the MEMR threshold and a frequency-dependent shortening of the latencies of ABR waves generated beyond the entrance to the cochlear nucleus. The use of a physiological model of loudness-encoding that incorporates cross-frequency timing cues predicts hyperacusis even at low sound levels, which the increased MEMR threshold cannot explain. In this hypothesized explanatory framework, the key mechanism that leads to hyperacusis is an acute change, not in central neuronal gain (currently seen as prominent, even though peripheral synaptopathy has been mentioned as a possible kindler of hyperacusis, e.g. (Hickox & Liberman, 2014)), but in the function of lower-brainstem circuits that generate the loudness percept. Here, we propose that sounds seem louder because one of the cues that underpin the neural code of loudness is indeed cranked up at most sound levels. The trigger of the auditory changes observed here is nonetheless assumed to be a salicylate-induced change in central pathways, maybe serotonin-driven. In the present model, the ABR changes lead to a temporary distortion of loudness encoding in an otherwise normal subject. Open questions are whether the same brain circuits might act permanently; for what reasons; and how central adaptive plasticity and modulations by non-auditory regions would cope with the distorted loudness percept.

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Declarations of interest: none

Figure legends:

Fig.1: Effect of the middle-ear muscle reflex triggered by a contralateral noise at increasing levels. (upper plot) Phase of a DPOAE produced by two tones at f2=8 kHz and f1 = 6.67 kHz, 50 dB SPL, monitored at a repetition rate of 1 point / s, in the absence (closed circles) then presence of a contralateral noise (open circles) at increasing levels (labels under the plot in dB SPL from 40 to 90), before salicylate injection. The threshold of the MEMR is defined as the first elicitor level for which the phase shifted by more than 5° on average (vertical arrow, 50 dB SPL). (Middle plot) Monitoring of the MEMR in the same rat 90 min after administration of salicylate, showing that MEMR threshold has shifted to 90 dB SPL. (Lower plot) Monitoring of the MEMR 1 min after injection of a bolus of curare. Supplementary movies: views through the open middle-ear bulla of the stapedius muscle of a rat before (control.mp4) and just after (curare.mp4) bolus intravenous injection of vecuronium bromide; MEMR-triggering sound onset 1 second after movie start.

Fig.2: Auditory responses show a mild salicylate-induced threshold elevation. (A) ABR thresholds against tested frequency (Mean ± SEM, open circles: control rats, n = 7; solid squares: salicylate-injected rats, n = 8). (B-E) At 6 kHz, ABR thresholds mildly shifted after injection, compared to pre-injection thresholds at T-30, yet no post-hoc test showed up as significant. Clear threshold shifts occurred in salicylate-treated rats at 10, 12 and 16 kHz, only at T90 at 10 kHz (*: $p<0.05$), both at T60 and T90 at 12 and 16 kHz. (**: $0.001<p<0.01$; *** $p<0.001$). (F) Shift in DPOAE amplitude against time in control and salicylate-treated rats (same symbols and n's as in A-E), the amplitude at T0 being taken as reference.

Fig.3: Acoustic startle reflex strength in control and treated rats. The chosen metrics of ASR strength was the amplitude of the sound-induced deflection reading from the strain gauge to which the rat's box hung, in mV. Control (open circles, n=6) and salicylate-treated (closed squares, n=6) rats were exposed to a [5-9 kHz] noise band at 70 (left diagram) and 80 dB SPL (right diagram) at T0, T60 and T90 (**: $0.001<p<0.01$; *** $p<0.001$ for post-hoc tests).

Fig.4. Salicylate injection increases the activation threshold and reduces the strength of the middle-ear muscle reflex. The MEMR was monitored from T-30 to T90 every 30 min (time interval between ticks on horizontal axis) (A-B) The average MEMR threshold unchanged in controls (A, n = 5), was increased at T60 and T90 only, relative to T0, in salicylate-injected rats (B, n=5) (*** $p<0.001$). (C-D) The attenuation in dB due to the

MEMR increased with contralateral sound-eliciting level, tested from 40 to 90 dB SPL in 10-dB steps for every plot (C: controls; D: salicylate-injected, mean ± SEM).

Fig.5. Averaged auditory brainstem response waves in control and salicylate-treated samples at 6 and 16 kHz. The averages, across individuals of each sample, of their ABR responses (black bold lines: controls; gray lines: salicylate-treated) display deflections (solid arrowheads for the control sample) that roughly correspond to waves I – IV in chronological order. Averages ± SD (thin black lines) of controls delineate the area where a majority of individual traces should fall. Salicylate-treated rats closely followed this normative band at 16 kHz at all times and at 6 kHz, T0, but not T90 when ABR-wave related deflections later than wave I lead the normal deflections.

Fig.6. Shortened auditory brainstem response waves III and IV at lower frequencies in salicylate-treated rats– Mean ± SEM ABR latencies of waves I to IV, in ms, against stimulus level in dB SPL at 6 kHz (A-D), and of waves I and IV at 10 kHz (E-F), 12 kHz (G-H) and 16 kHz (I-J). For every plot, control group, left, n=7; salicylate-treated group, right, n=8 (post-hoc tests comparing T90 to T0 for every stimulus level: **p<0.01; ***p<0.001; **** p<0.0001).

Fig.7. Model of loudness encoding based upon cross-frequency coincidence detection and its changes in salicylate treated animals. Assume that hypothetical cross-frequency coincidence detectors (solid triangles), receiving inputs from neural channels connected to the cochlear places with characteristic frequencies (CF) 6 kHz (upper inputs) and 12 kHz (lower inputs), fire only when their inputs (downward-pointing arrows) fall within a single grey box, i.e., sufficiently close in time (here, arbitrary choice of 0.13 ms). Both channels are excited even by a tone as its activation pattern in the cochlea spreads basally (inserts labelled '6 kHz').

The timing of each channel marked by downward-pointing triangles (bars = SEM) is evaluated using ABRs evoked by pure-tone bursts whose frequency is the CF of the channel. As only timing differences matter, timing is inferred from ABR wave IV, the most robust one reflecting latency changes of earlier waves (The coincidence detectors are thought to lie in the cochlear nucleus (e.g. Cai et al, 2008), somewhere between the sources of waves II and III).

At T0 (middle diagrams), approximate coincidences (output 1 from the detector) occur only from 60 dB SPL up because at lower levels, the intracochlear delay (rightmost cartoon) is too large. With salicylate (T90, right diagrams), the faster neural conduction in the CF = 6 kHz neural channel offsets the intracochlear delay, so that inputs to coincidence detectors become

almost simultaneous already at 40 dB SPL, maybe earlier (output 1 from detectors at all sound levels, instead of only above 60 dB at T0).

References

Attias, J., Raveh, E., Ben-Naftali, N.F., Zarchi, O., Gothelf, D. 2008. Hyperactive auditory efferent system and lack of acoustic reflexes in Williams syndrome. J Basic Clin Physiol Pharmacol 19, 193-207.

Auerbach, B.D., Rodrigues, P.V., Salvi, R.J. 2014. Central gain control in tinnitus and hyperacusis. Front Neurol 5, 206.

Avan, P., Buki, B., Petit, C. 2013. Auditory distortions: origins and functions. Physiol Rev 93, 1563-619.

Avan, P., Buki, B., Maat, B., Dordain, M., Wit, H.P. 2000. Middle ear influence on otoacoustic emissions. I: noninvasive investigation of the human transmission apparatus and comparison with model results. Hear Res 140, 189-201.

Baguley, D.M. 2003. Hyperacusis. J R Soc Med 96, 582-5.

Cai, S., Ma, W.L., Young, E.D. 2009. Encoding intensity in ventral cochlear nucleus following acoustic trauma: implications for loudness recruitment. J Assoc Res Otolaryngol 10, 5-22.

Canales, C.P., Wong, A.C., Gunning, P.W., Housley, G.D., Hardeman, E.C., Palmer, S.J. 2015. The role of GTF2IRD1 in the auditory pathology of Williams-Beuren Syndrome. Eur J Hum Genet 23, 774-80.

Carney, L.H. 1994. Spatiotemporal encoding of sound level: models for normal encoding and recruitment of loudness. Hear Res 76, 31-44.

Castaneda, R., Natarajan, S., Jeong, S.Y., Hong, B.N., Kang, T.H. 2019. Electrophysiological changes in auditory evoked potentials in rats with salicylate-induced tinnitus. Brain Res 1715, 235-244.

Chen, G.D., Radziwon, K.E., Kashanian, N., Manohar, S., Salvi, R. 2014. Salicylate-induced auditory perceptual disorders and plastic changes in nonclassical auditory centers in rats. Neural Plast 2014, 658741.

Delgutte, B. 1996. Physiological models for basic auditory percepts. In: Hawkins, H.L., McMullen, T.A., Popper, A.N., Fay, R.R., (Eds.), Auditory computation. Springer, New York. pp. 157-220.

Eggermont, J.J. 2013. Hearing loss, hyperacusis, or tinnitus: what is modeled in animal research? Hear Res 295, 140-9.

Eggermont, J.J., Roberts, L.E. 2015. Tinnitus: animal models and findings in humans. Cell Tissue Res 361, 311-36.

Florentine, M., Heinz, M.G. 2009. Audition: Loudness. In: Goldstein, E.B., (Ed.), Encyclopedia of Perception, Vol. 1. Sage Publications Ltd, London. pp. 145-151.

Gu, J.W., Halpin, C.F., Nam, E.C., Levine, R.A., Melcher, J.R. 2010. Tinnitus, diminished sound-level tolerance, and elevated auditory activity in humans with clinically normal hearing sensitivity. J Neurophysiol 104, 3361-70.

Guitton, M.J., Avan, P., Puel, J.L., Bonfils, P. 2004. Medial olivocochlear efferent activity in awake guinea pigs. Neuroreport 15, 1379-82.

Hebert, S., Fournier, P., Norena, A. 2013. The auditory sensitivity is increased in tinnitus ears. J Neurosci 33, 2356-64.

Heffner, H.E., Heffner, R.S., Contos, C., Ott, T. 1994. Audiogram of the hooded Norway rat. Hear Res 73, 244-7.

Hellman, R.P., Meiselman, C.H. 1990. Loudness relations for individuals and groups in normal and impaired hearing. J Acoust Soc Am 88, 2596-606.

Hickox, A.E., Liberman, M.C. 2014. Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? J Neurophysiol 111, 552-64.

Joris, P.X. 2009. Recruitment of neurons and loudness. Commentary on "Encoding intensity in ventral cochlear nucleus following acoustic trauma: implications for loudness recruitment" by Cai et al. J. Assoc. Res. Otolaryngol. DOI: 10.1007/s10162-008-0142-y. J Assoc Res Otolaryngol 10, 1-4.

Keefe, D.H., Ling, R., Bulen, J.C. 1992. Method to measure acoustic impedance and reflection coefficient. J Acoust Soc Am 91, 470-85.

Kemp, D.T. 1978. Stimulated acoustic emissions from within the human auditory system. J Acoust Soc Am 64, 1386-91.

Knipper, M., Van Dijk, P., Nunes, I., Ruttiger, L., Zimmermann, U. 2013. Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis. Prog Neurobiol 111, 17-33.

Knudson, I.M., Melcher, J.R. 2016. Elevated Acoustic Startle Responses in Humans: Relationship to Reduced Loudness Discomfort Level, but not Self-Report of Hyperacusis. J Assoc Res Otolaryngol 17, 223-35.

Le Calvez, S., Avan, P., Gilain, L., Romand, R. 1998. CD1 hearing-impaired mice. I: Distortion product otoacoustic emission levels, cochlear function and morphology. Hear Res 120, 37-50.

Liberman, M.C., Guinan, J.J., Jr. 1998. Feedback control of the auditory periphery: anti-masking effects of middle ear muscles vs. olivocochlear efferents. J Commun Disord 31, 471-82; quiz 483; 553.

Liu, J.X., Li, X.P., Dong, Y., Han, H.W., Liu, G.Q. 2003. [Study of neurological mechanism of lidocaine's suppression to tinnitus via microdialysis]. Zhonghua Er Bi Yan Hou Ke Za Zhi 38, 440-4.

Lobarinas, E., Sun, W., Cushing, R., Salvi, R. 2004. A novel behavioral paradigm for assessing tinnitus using schedule-induced polydipsia avoidance conditioning (SIP-AC). Hear Res 190, 109-14.

Lowe, A.S., Walton, J.P. 2015. Alterations in peripheral and central components of the auditory brainstem response: a neural assay of tinnitus. PLoS One 10, e0117228.

Metz, O. 1952. Threshold of reflex contractions of muscles of middle ear and recruitment of loudness. AMA Arch Otolaryngol 55, 536-43.

Moore, B.C. 2002. Psychoacoustics of normal and impaired hearing. Br Med Bull 63, 121-34.

Mukerji, S., Windsor, A.M., Lee, D.J. 2010. Auditory brainstem circuits that mediate the middle ear muscle reflex. Trends Amplif 14, 170-91.

Murata, K., Ito, S., Horikawa, J., Minami, S. 1986. The acoustic middle ear muscle reflex in albino rats. Hear Res 23, 169-83.

Papesh, M.A., Hurley, L.M. 2016. Modulation of auditory brainstem responses by serotonin and specific serotonin receptors. Hear Res 332, 121-136.

Peng, K., Peng, Y.J., Wang, J., Yang, M.J., Fu, Z.Y., Tang, J., Chen, Q.C. 2017. Latency modulation of collicular neurons induced by electric stimulation of the auditory cortex in Hipposideros pratti: In vivo intracellular recording. PLoS One 12, e0184097.

Pilz, P.K., Ostwald, J., Kreiter, A., Schnitzler, H.U. 1997. Effect of the middle ear reflex on sound transmission to the inner ear of rat. Hear Res 105, 171-82.

Proulx, E., Young, E.J., Osborne, L.R., Lambe, E.K. 2010. Enhanced prefrontal serotonin 5-HT(1A) currents in a mouse model of Williams-Beuren syndrome with low innate anxiety. J Neurodev Disord 2, 99-108.

Radziwon, K., Holfoth, D., Lindner, J., Kaier-Green, Z., Bowler, R., Urban, M., Salvi, R. 2017. Salicylate-induced hyperacusis in rats: Dose- and frequency-dependent effects. Hear Res 350, 133-138.

Relkin, E.M., Doucet, J.R. 1997. Is loudness simply proportional to the auditory nerve spike count? J Acoust Soc Am 101, 2735-40.

Ruttiger, L., Zimmermann, U., Knipper, M. 2017. Biomarkers for Hearing Dysfunction: Facts and Outlook. ORL J Otorhinolaryngol Relat Spec 79, 93-111.

Sheldrake, J., Diehl, P.U., Schaette, R. 2015. Audiometric characteristics of hyperacusis patients. Front Neurol 6, 105.

Sun, W., Lu, J., Stolzberg, D., Gray, L., Deng, A., Lobarinas, E., Salvi, R.J. 2009. Salicylate increases the gain of the central auditory system. Neuroscience 159, 325-34.

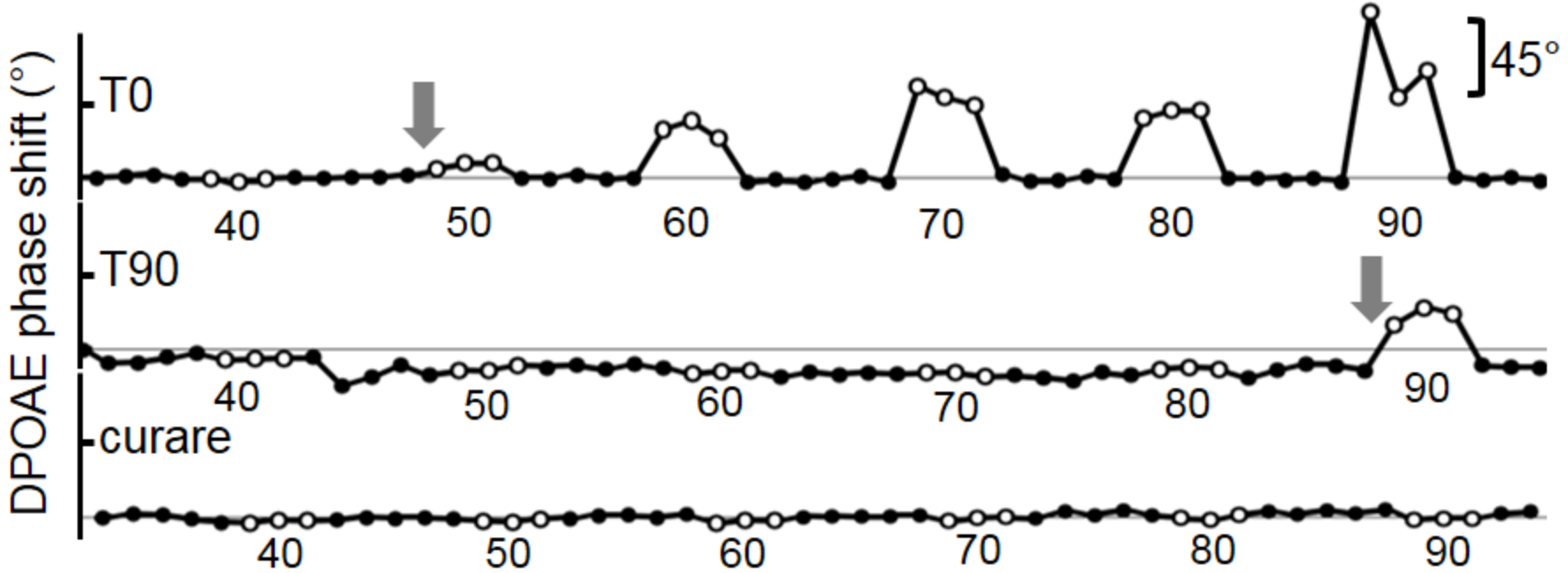
Thompson, A.M., Thompson, G.C., Britton, B.H. 1998. Serotoninergic innervation of stapedial and tensor tympani motoneurons. Brain Res 787, 175-8.

Vacher, S.R., Guinan, J.J., Jr., Kobler, J.B. 1989. Intracellularly labeled stapedius-motoneuron cell bodies in the cat are spatially organized according to their physiologic responses. J Comp Neurol 289, 401-15.

Willott, J.F. 2006. Measurement of the auditory brainstem response (ABR) to study auditory sensitivity in mice. Curr Protoc Neurosci Chapter 8, Unit8 21B.

Zeng, F.G. 2013. An active loudness model suggesting tinnitus as increased central noise and hyperacusis as increased nonlinear gain. Hear Res 295, 172-9.

Zugaib, J., Ceballos, C.C., Leao, R.M. 2016. High doses of salicylate reduces glycinergic inhibition in the dorsal cochlear nucleus of the rat. Hear Res 332, 188-198.


DPOAE phase shift (°)
T0
45°
40
50
60
70
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90
curare
40
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70
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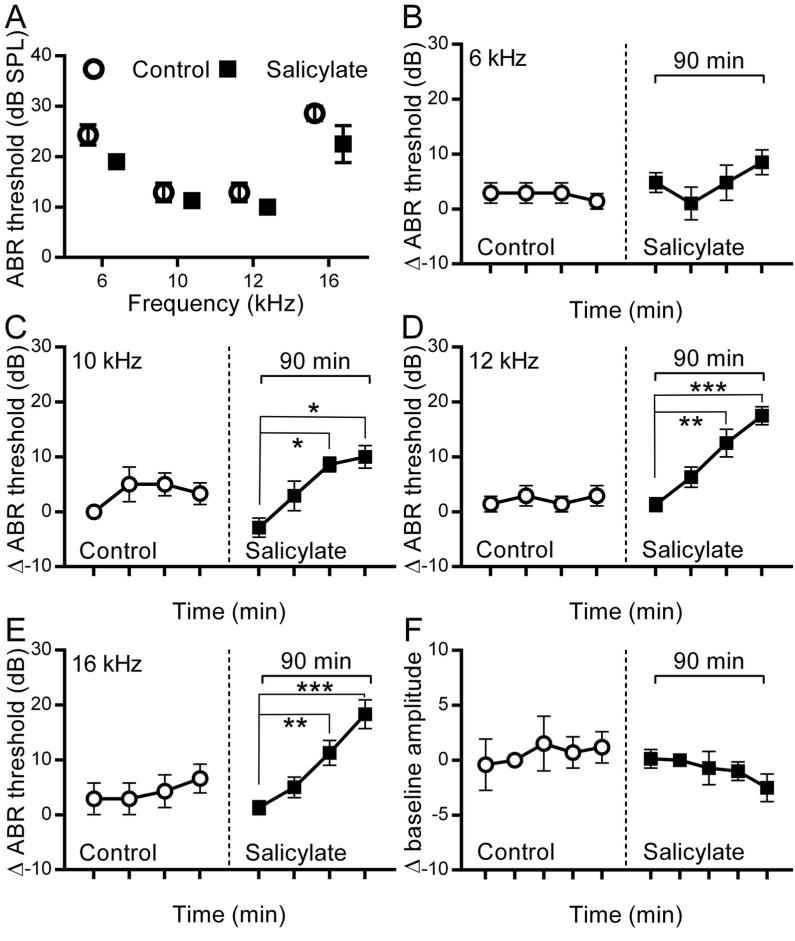
A
ABR threshold (dB SPL)
Control
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Frequency (kHz)
B
6 kHz
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Control
Salicylate
Time (min)
C
10 kHz
90 min
*
*
Δ ABR threshold (dB)
Control
Salicylate
Time (min)
D
12 kHz
90 min
***
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Δ ABR threshold (dB)
Control
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Time (min)
E
16 kHz
90 min
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Δ ABR threshold (dB)
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Salicylate
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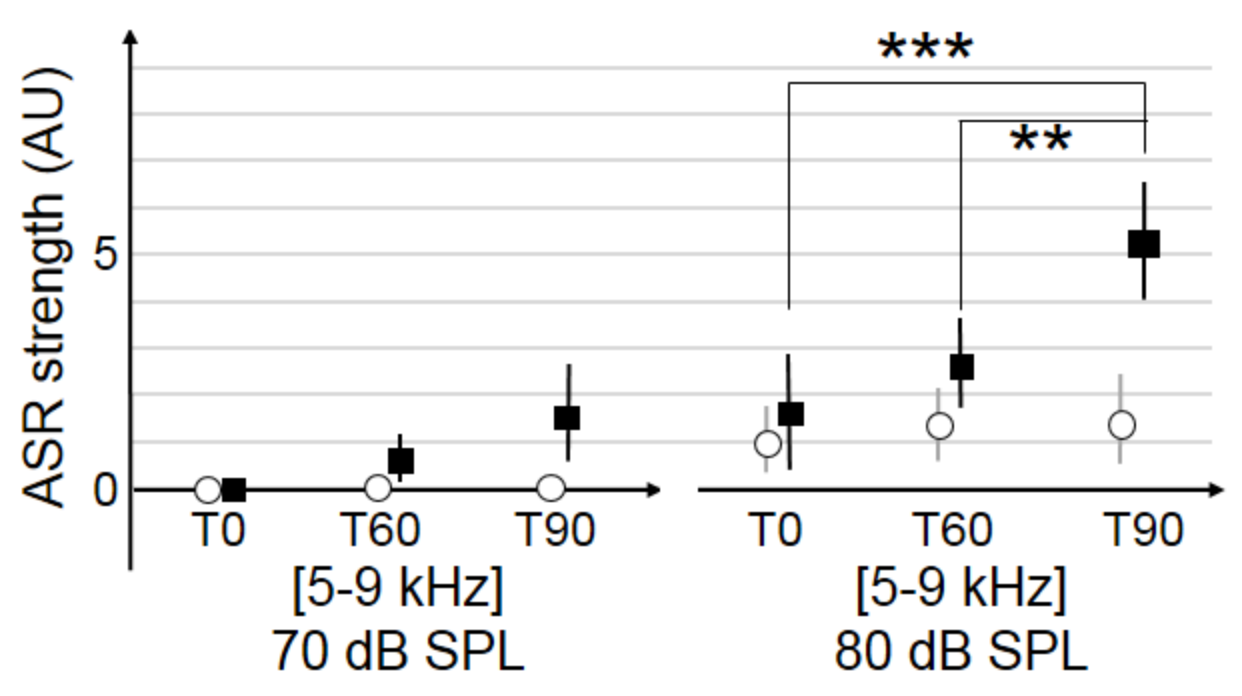
F
90 min
Δ baseline amplitude
Control
Salicylate
Time (min)


ASR strength (AU)
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[5-9 kHz]
70 dB SPL
***
**
T0
T60
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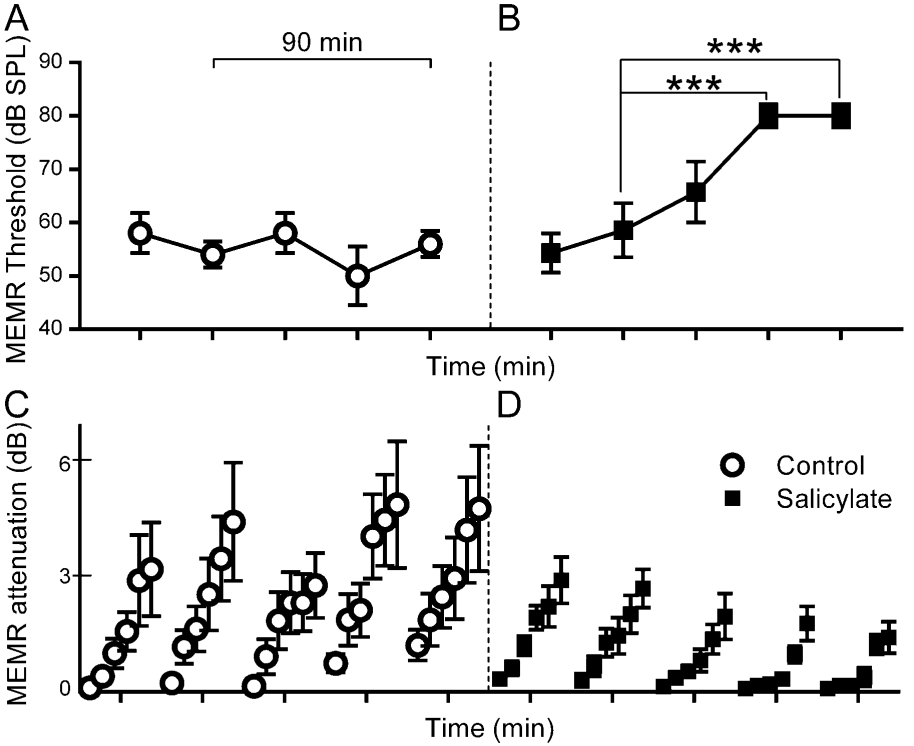
[5-9 kHz]
80 dB SPL


A
B
90 min
***
***
MEMR Threshold (dB SPL)
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80
70
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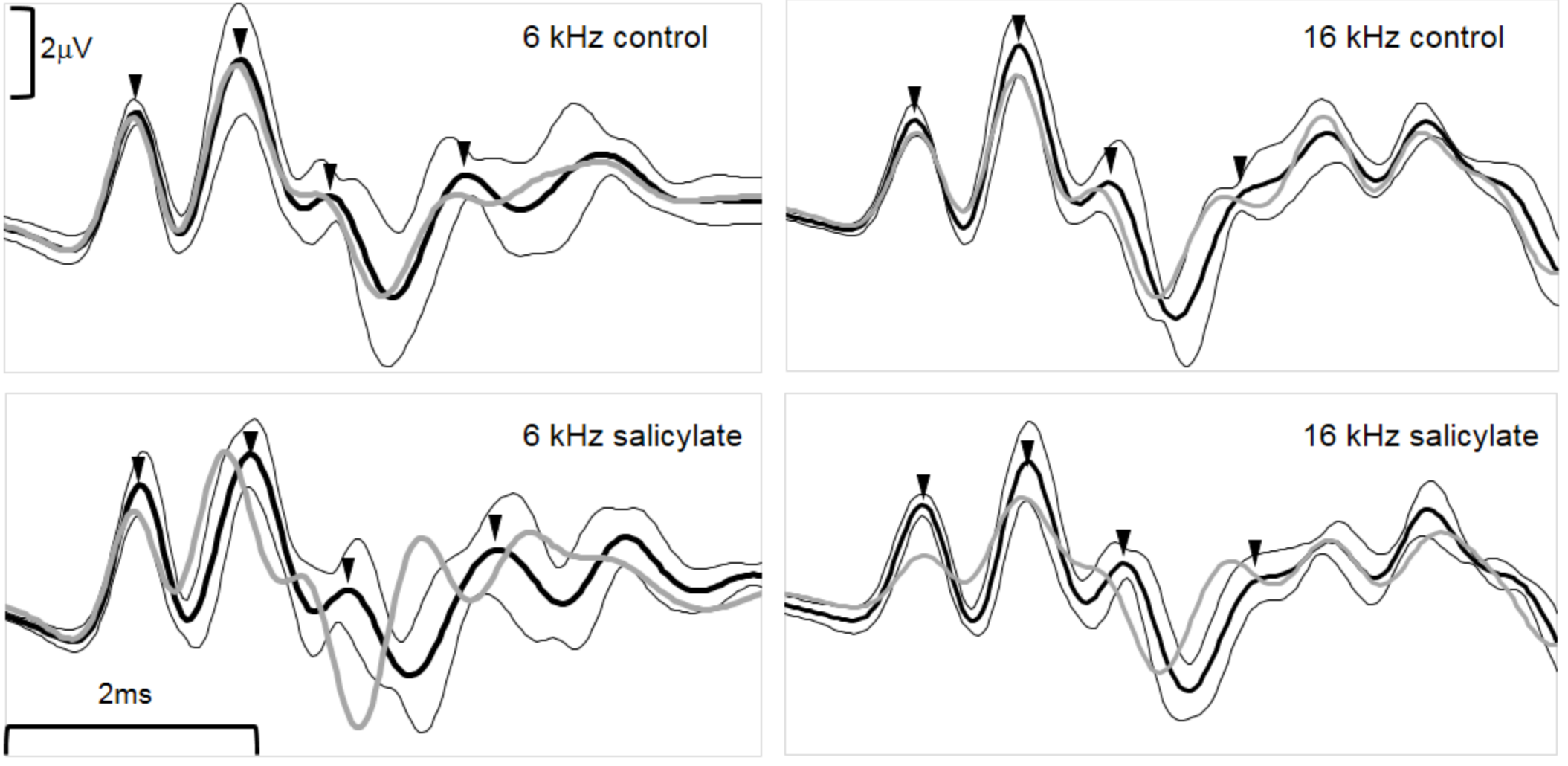
Time (min)
C
D
MEMR attenuation (dB)
6
3
0
Control
Salicylate
Time (min)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6 kHz salicylate
16 kHz salicylate
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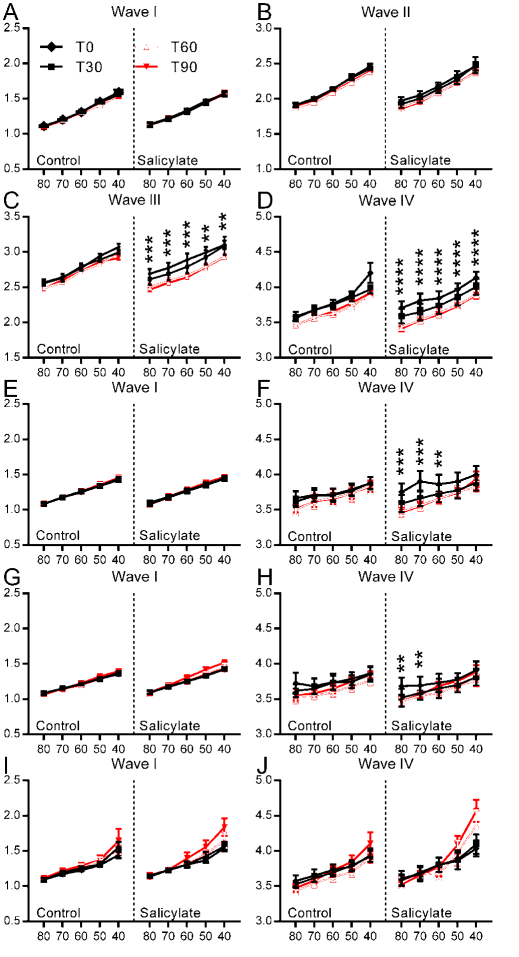
A
Wave I
T0
T30
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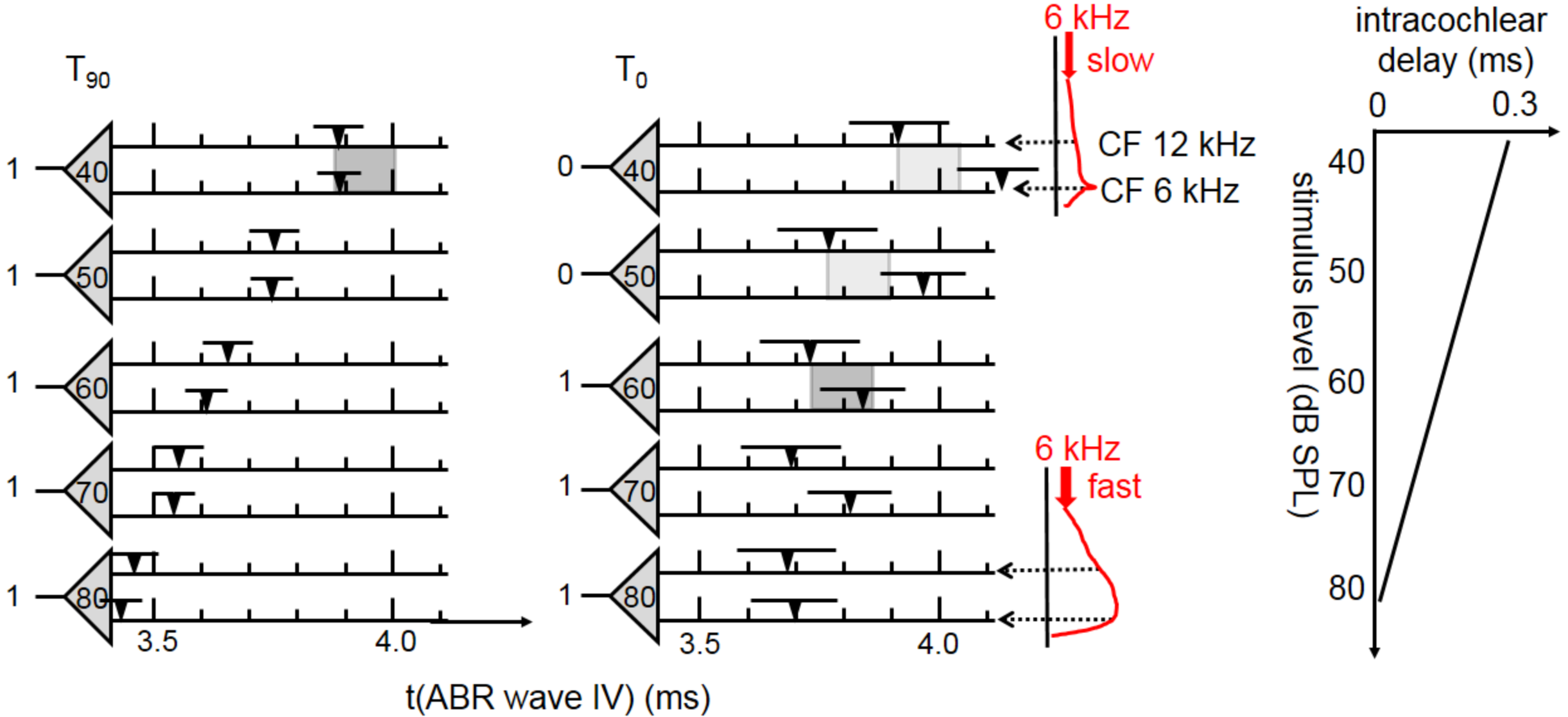
Control
Salicylate
B
Wave II
C
Wave III
D
Wave IV
E
Wave I
F
Wave IV
G
Wave I
H
Wave IV
I
Wave I
J
Wave IV
80 70 60 50 40


T90
T0
6 kHz
slow
CF 12 kHz
CF 6 kHz
6 kHz
fast
intracochlear
delay (ms)
0
0.3
stimulus level (dB SPL)
40
50
60
70
80
1
0
3.5
4.0
t(ABR wave IV) (ms)