Tinnitus is associated with increased extracellular matrix density in the auditory cortex

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Abstract

Most scientist agree that subjective tinnitus is the pathological result of an interaction of damage to the peripheral auditory system and central neuroplastic adaptations. Here we investigate such tinnitus related adaptations in the primary auditory cortex (AC) 13 days after noise trauma induction of tinnitus by quantifying the density of the extracellular matrix (ECM) in the AC of Mongolian gerbils (Meriones unguiculatus). The ECM density has been shown to be relevant for neuroplastic processes and synaptic stability within the cortex. We utilized a mild monaural acoustic noise trauma in 9 gerbils to induce tinnitus and a sham exposure in 3 control animals. Tinnitus was assessed by a behavioral response paradigm. Four of the trauma animals did show tinnitus 13 days after trauma (T), the remaining 5 trauma (NT) and the 3 control animals (C) did not show the percept. The ECM density 13 days after trauma was quantified using immunofluorescence luminance of Wisteria floribunda lectin-fluoresceine-5-isothiocyanate (WFA-FITC) on histological slices of the primary AC, relative to the non-auditory brainstem as a reference area. We found that the WFA-FITC luminance of the AC of NT animals was not significantly different from that of C animals. However, we found a significant increase of luminance in T animals' ACs compared to NT or C animals' cortices. This effect was found exclusively on the AC side contralateral to the trauma ear. These results point to a process of stabilization of synaptic connections in primary AC, which may be involved in the chronic manifestation of tinnitus.

Introduction

Tinnitus – the perception of a sound without external physical source – is commonly believed to be the result of an interaction of a damage to the peripheral auditory system and central neuroplastic adaptations to the new, changed auditory input (Nelson & Chen, 2004). We already put forward a bottom-up model for tinnitus development in which these neuroplastic adaptations – after a mild hearing loss – start in the dorsal cochlear nuclei (DCN) after losing a part of the neuronal information not transmitted from the damaged cochlea anymore (Krauss*et al.* , 2016; Krauss *et al.* , 2017; Schilling *et al.* , 2021). In a nutshell, this Erlangen model of tinnitus development (Schulze *et al.* , 2023) assumes that a neurophysiological mechanism detects a drop in information after a hearing loss by computing the autocorrelation of the signal of the cochlear nerve (Schulze & Tziridis, 2023), as less temporally structured spike trains reach the DCN neurons (Schulze & Tziridis, 2023). By disinhibition of neuronal noise, most probably coming from the somatosensory system (Schilling *et al.* , 2021), a reduced – but still information containing – input signal plus that noise can reach the threshold and activate the DCN neurons, a mechanism known as stochastic resonance. As a consequence, otherwise sub-threshold activity from the cochlea can now be further transmitted along the auditory pathway, thereby optimizing information transmission within the auditory system. This comes at the cost of co-propagation of the noise up to the auditory cortex, where the activity is then perceived as a sound, namely tinnitus. based on that model we were able to predict and explain several tinnitus related effects on hearing loss, e.g., that the hearing thresholds in patients with mild to moderate hearing loss and

tinnitus are better than in matched patients without tinnitus (Gollnast *et al.* , 2017). Furthermore, we were able to develop a new therapeutic approach based on that model which allows a causative reduction of tinnitus loudness in tinnitus patients (Schilling *et al.* , 2020; Tziridis *et al.* , 2022). However, the original model failed to describe the effects of chronic manifestation of tinnitus and made no predictions on tinnitus related plasticity in the auditory cortex. To overcome these drawbacks, in a previous paper, the stochastic resonance model was unified with the predictive coding model of auditory phantom perception (Sedley *et al.* , 2016; Schilling *et al.* , 2023b). in this view, tinnitus might be induced and chronically manifested through an interplay of two feedback loops – the stochastic resonance loop in the brainstem and the predictive coding circuit in the cortex (Sedley *et al.* , 2016; Schilling *et al.* , 2023b).

In the present study we aim to investigate tinnitus related adaptations in the auditory cortex (AC) after an assumed chronic manifestation of the phantom percept. Based on electrophysiological recordings in animals (Engineer *et al.* , 2011; Tziridis *et al.* , 2015) and e.g., imaging methods or EEG recordings in humans (Haab *et al.* , 2009; Schoisswohl *et al.* , 2021), it is already known that tinnitus before and after chronification has different neurophysiological manifestations within the AC of mammals. While before chronification the neurophysiology of the AC seems to undergo profound changes in, e.g., tonotopy (e.g., Eggermont, 2006), the normal processing in the AC seems to be recovered after the new percept is developed, with the addition of the phantom sound activity in the affected frequency range (Ahlf*et al.* , 2012; Langers *et al.* , 2012).

Neuronal plasticity in the cortex can be assessed using several different methods (Ohl & Scheich, 2005; Peters *et al.* , 2017; Irvine, 2018). One of them is the investigation of the density of the extracellular matrix (ECM) by using immunofluorescence luminance of Wisteria floribunda lectin-fluoresceine-5-isothiocyanate (WFA-FITC) on histological slices (e.g., Happel *et al.* , 2014). The higher the density (WFA-FITC luminance) of the ECM, the more stable are the synapses connecting the neurons and therefore the less likely it is that a once formed pattern can be changed by new information (Dityatev*et al.* , 2010; Gundelfinger *et al.* , 2010). With that in mind, we asked, how the ECM density of the primary AC – and indirectly the potential for neuroplasticity of that area – is affected by tinnitus after its chronification in our animal model, the Mongolian gerbil (*Meriones unguiculatus*).

Methods

Animals, Housing and Ethics Statement

12 male Mongolian gerbils, purchased from Janvier (Le Genest-Saint-Isle, France) were housed in standard animal racks (Bio A.S. Vent Light, Zoonlab, Emmendingen, Germany) in groups of 3 to 4 animals with free access to water and food at a room temperature of 20 to 25*C under a 12h/12h dark/light circle. At the beginning of the experiments, the animals were 12 weeks old and had been at least two weeks in the animal facility for habituation. The use and care of the animals was approved by the state of Bavaria (Regierungspräsidium Mittelfranken, Ansbach, Germany, No. 54-2532.1-02/13).

Experimental protocol

The animals were investigated twice with the behavioral paradigm of the gap prepulse inhibition of the acoustic startle response (GPIAS) to evaluate possible behavioral indications of a tinnitus percept and with auditory brainstem response (ABR) audiometry under anesthesia to obtain the hearing thresholds. The respective first measurements were performed five days before a monaural acoustic noise trauma (2 kHz, 115 dB SPL, 75 min; anesthetized) or sham trauma (2 kHz, 65 dB SPL, 75 min; anesthetized). The second ABR measurements were obtained 4 days after that trauma, and GPIAS was obtained immediately before sacrificing the animals (CO_2 euthanasia) 13 days after the trauma. The brains of the sacrificed animals were extracted, cryo-sliced, immunostained and immunohistologically evaluated.

Behavioral measurements

For the GIPAS measurements for tinnitus assessment, a custom-made open-source setup was used as detailed in (Gerum *et al.* , 2019). In short, the animals were placed in an acrylic glass restrainer tube, closed with a wire mesh at the front side and a cap at the back end, and placed on a sensor platform fixed to a vibration-damped table. Movements of the sensor platform were registered using a 3D acceleration sensor. Two loudspeakers were placed at a distance of 10 cm in front of the animal. One presenting the 115 dB SPL startle stimulus (Neo 25 S, SinusLive, noise burst 20ms, flattened with 5 ms $\sin^2$ramps) and the other the 60 dB SPL spectral noise background (CantonPlus XS.2). Spectral noise was centered at 1 to 16 kHz in octave steps with one octave bandwidth, with and without a gap of silence of 50 ms (flanked by 20 ms $\sin^2$ ramps, 10ms complete silence) starting 100 ms before the startle stimulus.

During the two measurements, animals were given 15 min of habituation in darkness in the tube. Prior to the actual measurement, five habituation stimuli were presented to "level" the startle responses. Each stimulus was repeated 30 times (15 with and 15 without gap), summing up to 120 stimuli, which took roughly 30 min.

The complete evaluation of the GPIAS measurements were performed using custom-made Python programs. The GPIAS effect was quantified by calculating the median from the full combinatorial startle amplitudes as a response to gap and no gap pre-stimulus (for details see Schilling*et al.* , 2017). Statistics on the mean GPIAS results were performed with Statistica 14 (TIBCO Software GmbH, Munich, Germany; cf. below).

Brainstem audiometry

For the frequency-specific ABR measurements the animals were anesthetized with a ketamine-xylocaine solution (ketamine 500 mg/kg, xylazine 25 mg/kg). The animals were placed on a remote-controlled heating pad set to 37°C. Individual audiograms of both ears were obtained for stimulation frequencies between 1 and 16 kHz in octave steps for stimulation intensities ranging from 0 to 90 dB SPL in 5 dB steps (6 ms duration with 2 ms sine-square ramps). For each ear, stimulus, and intensity, 300 repetitions were presented. The complete measurement of one ear took around 30 min. Three silver wires were used as electrodes and were placed subcutaneously retroaural above the bulla of the tested ear (recording electrode), central between both ears (reference electrode) and at the basis of the tail (ground electrode). The signal was recorded differentially between recording and reference electrode and filtered (bandpass filter 400 to 2000 Hz) via a Neuroamp 401 amplifier (JHM, Mainaschaff, Germany).

Monaural acoustic noise and sham trauma

Animals were put under deep ketamine-xylocaine anesthesia (cf. above). One ear (pseudorandomly selected) was plugged with foam (3M earplugs 1110, 3M, Neuss, Germany) adding at least 20 dB attenuation in the given frequency range (Stuermer & Scheich, 2000). The animals were placed on a remote-controlled heating pad set to 37degC with the non-plugged ear towards a speaker (CantonPlus XS 2). The acoustic noise trauma (2 kHz, 115 dB SPL, 75 min) or sham trauma (2 kHz, 65 dB SPL, 75 min) was only performed on that ear.

Brain extraction, cryo-slicing and immunofluorescence staining

The sacrificed animals were decapitated and the brains were extracted carefully. They were placed in a 2% paraformaldehyde / 2% sucrose solution on a rocking shaker for 8 hours at 4degC. The tissue was then washed with phosphate-buffered saline (PBS) three times over one minute each, and then transferred into a 10% sucrose solution on a rocking shaker for 8 hours at 4degC. The incubation was finalized with 15% sucrose solution and finally 30% sucrose solution on a rocking shaker for 8 hours each at 4degC, washing was performed with PBS two times for one minute each. After drying, the tissue was shock-frosted in liquid

nitrogen at -196degC and cur on a cryotome in 18 µm thick slices, which were transferred onto object slides and air-dried for 30 min at ambient temperature. Until staining, the slides were stored at -20°C in a fridge,

After defrosting and drying, every 10th object slide was used and fixated with -20°C cold methanol for three minutes and dried for 10 minutes. After that the slices were permeabilized with 2 % horse-serum in 0.1 % Triton X-100 in PBS solution over 2.5 hours. Then the incubation with Wisteria Floribunda Lectin-Fluorescein-5-isothiocyanate (WFA-FITC) started, with WFA-FITC 1:200 in 0.5 % horse-serum/PBS over 5 hours at room temperature. It was stopped with washing with PBS three times over five minutes each. Finally, the slides were mounted with IS Mounting-Medium PI.

Microscopy and densitometry

Immunostained tissue slices were investigated with a BZ 9000 Keyence fluorescence microscope (4x magnification) and single pictures were combined to a full slice picture with the BZ-II software. We used a 1360x1024 pixel resolution, green fluorescence, exposure adjustments: shadow 11, highlight 165, gamma 0.7, exposure time 1/1.1 sec.

The combined slice-pictures were evaluated with ImageJ Fiji (version 1.53) and only the slices containing the primary auditory cortex were evaluated. For marker-intensity reference, always the brainstem of the same slide was used. The appropriate slices and cortical / subcortical areas were identified using anatomical markers based on a Mongolian gerbil brain atlas (plates 28-33 in Radtke-Schuller *et al.* , 2016). The areas of interest ipsi and contralateral to the trauma ear were marked and their luminance was calculated using the inbuilt program function. The luminance values of cortical areas were divided by the reference brainstem area values (luminance ratio). Examples of the brain slices of animals with trauma and behavioral signs of tinnitus, with trauma but without behavioral signs of tinnitus and sham trauma without tinnitus are depicted in **Figure 1** .

Statistics

As mentioned above, all statistics were performed with Statistica 14. The effect of the trauma on the hearing thresholds obtained with the ABR measurements was assessed using the calculated (Schilling *et al.* , 2019) individual and frequency specific hearing loss (HL, post threshold - pre threshold) with a two-factorial ANOVA (factor *ear* and*frequency*).

For the evaluation of the GPIAS results, the means of the effect size of the individual log-normalized gap / no-gap amplitude responses of the different background noise frequencies were analyzed for each animal and each frequency (Schilling *et al.* , 2017). Significant negative effect sizes (Students t-tests) at a given frequency indicate a possible tinnitus percept and an animal with such indication was classified as a tinnitus animal (T group), if no such behavioral sign of tinnitus was found, the animal was classified as non-tinnitus animal (NT group). All animals that received sham-trauma showed no signs of tinnitus and were assigned to the control group C.

The luminance ratio of the ECM of the cortical areas was analyzed using a two-factorial ANOVA (factor *side* (ipsi- / contralateral to trauma) and *animal group* (T, NT, C)). Additionally we investigated the luminance ratios of the two hemispheres separately by one-factorial ANOVAs (with Bonferroni correction for analysis repetition) with the factor *group* only. This was done to find possible side-specific effects that were obscured due to the variance in the comparably few data points of the overall 12 animals.

Results

Hearing loss and tinnitus

As detailed in the Method section, the HL was assessed by individual ABR threshold comparison. HL group effects were analyzed using a two-factorial ANOVA with the factors *ear* (plugged control, trauma, sham trauma) and *frequency* (1 kHz, 2 kHz, 4 kHz, 8 kHz, 16 kHz). The results of the factor *ear* ($F(2, 148)=12.74$, $p<0.001$) revealed a significant higher mean HL (± standard error) in the trauma ears (9.75 dB ± 1.26 dB)

compared to the plugged control ears (2.01 dB ± 1.17 dB, Tukey post-hoc test: p<0.001) and the sham trauma ears (-0.86 dB ± 2.69 dB, Tukey post-hoc test: p=0.001). Plugged control and sham trauma ears did not show a significant difference in HL (Tukey post-hoc test: p=0.59). Neither in the factor *frequency* (F(4, 148)=0.10, p=0.98) nor in the interaction of both factors (F(8, 148)=0.27, p=0.98) we found a significant effect on the HL. In other words, the traumatized ears showed a general significant increase in hearing thresholds after the trauma while neither plugged control nor sham trauma ears were affected by the noise exposure.

Of the nine animals that received a monaural acoustic noise trauma, four developed behavioral signs of a possible tinnitus percept in at least one tested frequency (1 kHz, 2 kHz, 4 kHz, 8 kHz, 16 kHz; T group). This was indicated by a significant decrease in effect size in the GPIAS paradigm 13 d after the trauma (t-tests, p<0.05). Three animals developed a possible tinnitus percept at one frequency (2 kHz (p=0.028 and p=0.007) or 4 kHz (p=0.004)), one animal at two frequencies (4 kHz (p=0.01) and 8 kHz (p=0.001)) and one at three frequencies (2 kHz (p=0.016) and 8 kHz (p=0.001) and 16 kHz (p<0.001)). The other five trauma animals did not show any significant decrease in effect size (NT group). As a side note, we found a strong tendency for a difference in the mean HL of the trauma ears of NT (14.2 ± 3.6 dB) and T animals (7.3 ± 1.6 dB) in a one-factorial ANOVA (F(1, 58)=4.0, p=0.05). None of the three sham noise trauma animals did show a significant decrease in effect size of the behavioral paradigm (sham control group) nor a difference in HL relative to the control ears of NT or T animals (F(2, 102)=2.35, p=0.11).

Cortical ECM density

The luminance ratio of the cortex sides ipsi- and contralateral to the trauma or sham trauma ear was assessed in the T, NT and C groups with a two-factorial ANOVA with the factors *side* and *group* . We found (**Figure 2**) a significant *group* effect (F(2, 114)=7.05, p=0.001) with the mean luminance ration of the T animals' brains (0.85 ± 0.03) being significantly higher than those of the NT (0.71 ± 0.03, Tukey post-hoc tests: p=0.001) or sham control brains (0.72 ± 0.04, Tukey post-hoc tests: p=0.035). Neither in the*cortex side* (F(1, 114)=0.02, p=0.89) nor in the*interaction* analyses (F(2, 114)=1.03, p=0.36) a significant effect could be found in this analysis. So this first analysis points to a general increase in luminance ratio and therefore in ECM density in the auditory cortex of animals with behavioral signs of tinnitus.

When we investigated the two hemispheres separately (and corrected for repeated testing), we found that the described significant ECM density (i.e., luminance ratio) increase in T animals could be found only in the auditory cortex contralateral to the trauma (**Figure 2**): There, the one-factorial ANOVA (F(2, 57)=6.65, p=0.002) revealed a higher ECM density (T: 0.88 ± 0.04; NT: 0.68 ± 0.04; C: 0.71 ± 0.06) with a significant Tukey post-hoc test between T and NT with p=0.002 and a tendency for a higher tinnitus related ECM density between T and C with p=0.08; no difference was found between NT and C with p=0.88. On the cortex side ipsilateral to the trauma, no significant differences (F(2, 57)=1.47, p=0.24) were found between the ECM density of the three animal groups (T: 0.83 ± 0.04; NT: 0.74 ± 0.04; C: 0.72 ± 0.06); also the Tukey post-hoc tests did not show any significant differences.

Discussion

In this report, we have demonstrated that noise-induced tinnitus is associated with an increased ECM density in primary auditory cortex contralateral to the trauma side. This result may point to trauma induced neuroplastic changes in AC, where an ECM density increase may be an indication of tinnitus chronification.

The induction of a mild acoustic noise trauma – here monaurally – is a reliable method of inducing cortical plasticity (e.g., Jeschke *et al.* , 2021) and tinnitus-related behavioral changes in our rodent animal model (e.g., Grimm *et al.* , 2022; Kiefer *et al.* , 2022; Tziridis & Schulze, 2022). To ensure an objective analysis of the data, only automated and parameter-free evaluation methods were used (Schilling *et al.* , 2019) to rule out human evaluation bias (Zaitoun *et al.* , 2016). With a mean HL of nearly 10 dB in the exposed ear (12 dB in the most affected frequency of 2 kHz), the effect of the trauma applied here was comparably mild when compared to other studies (e.g., Tziridis & Schulze, 2022). This fact could also explain the relatively low percentage of animals with behavioral signs of tinnitus (4/9, 44%). These behavioral signs of tinnitus

were determined with a well-established statistical method (Schilling *et al.* , 2017) but are no proof that the animals really perceive a human-like tinnitus percept (Eggermont, 2013). On the other hand, the animals with behavioral signs of tinnitus tend to show less hearing loss compared to animals without these behavioral signs. This is in line with reports of differences in human tinnitus patients compared to non-tinnitus patients with hearing loss (e.g., Gollnast *et al.* , 2017).

Nevertheless, we always combine the behavioral data of possible signs of tinnitus in our animal model with further physiological markers and often find correlations between the tinnitus status of the animals and those markers (e.g., Tziridis *et al.* , 2021), which indicates a causal connection between behavior and physiological changes. In this report, the further physiological marker is the density of the extracellular matrix of the primary auditory cortices of the animals.

The ECM itself occupies between 10% and 20% of the entire brain volume and plays an important role in genesis, plasticity and regeneration of the central nervous system (Chelyshev *et al.* , 2022). Furthermore, it is known that it plays a significant part in physiological as well as pathological conditions (Vargova & Sykova, 2014; Krishnaswamy *et al.* , 2019). The ECM can be divided into different subtypes: perineuronal nets and perisynaptic extracellular matrix (Chelyshev*et al.* , 2022). Specifically for Mongolian Gerbils it has been shown that the intact ECM in its entirety is important for the correct function of the cortical layer dynamics and cross-columnar frequency integration of the auditory cortex (El-Tabbal *et al.* , 2021) but its density modulation (i.e., removing it) can also be used for inducing neuronal plasticity and re-learning (Happel *et al.* , 2014).

With the knowledge of the ECM being that important for neuronal plasticity (or possibly mal-plasticity in the case of tinnitus), we investigated the ECM density of the auditory cortices of Mongolian gerbils 13 d after acoustic noise or sham exposure by using a specific ECM affine marker (WFA-FITC). This marker is a lectin that specifically binds to chondroitin sulfates and thus labels chondroitin sulfate proteoglycans within the ECM (Celio & Blumcke, 1994; Pizzorusso*et al.* , 2002). The fluorescence luminance of the marker is a direct measurement of the ECM density. To correct for possible differences in marker efficacy on the different histological slices, we corrected the luminance by relating it to a reference region in the brainstem. The 13 days after trauma were selected as a possible ECM rearrangement is finalized after that time (Happel *et al.* , 2014) and the neurophysiological changes in the auditory cortex – measured by electrophysiological recordings – leading to a chronification of the tinnitus percept are likely to be completed (Ahlf *et al.* , 2012; Tziridis *et al.* , 2014). We found the clear evidence that animals with behavioral signs of tinnitus have a much more solidified ECM surrounding of their auditory cortex neurons than animals without these signs or healthy control animals. This effect seems to be stronger in the cortical hemisphere contra-lateral to the trauma ear, which can be explained by the functional crossing of excitatory projections within the auditory pathway to the contra-lateral side.

From the described effect of the ECM being more solidified in tinnitus animals compared to control or non-tinnitus animals, several conclusions can be drawn regarding potential mechanistic tinnitus models, respectively. As it is not possible to provide a complete overview of all existing tinnitus models, we here focus on the most common ones.

First, the effect points to the assumption that in contrast to former models, chronic tinnitus is not a result of a re-organization of the tonotopic map after a cochlear damage as claimed by Mühlnickel et al. (Mühlnickel *et al.* , 1998), but is instead characterized by a fixation of the wiring scheme after transient re-wiring in the cortex (Ahlf *et al.* , 2012). Indeed, the hypothesis raised by Mühlnickel et al. has been falsified by several other groups in various recent studies, where huge tinnitus cohorts were investigated with fMRI (Koops*et al.* , 2020; Koops & van Dijk, 2021).

Second, our findings suggest that during tinnitus chronification the responses of the neural system enter a neural attractor, which is further reinforced by stiffening of the ECM in the auditory cortex. In this context, the combined stochastic-resonance-predictive-coding model introduced by Schilling and co-workers might provide an explanation (Schilling *et al.* , 2023b). In a nutshell, the model describes tinnitus as a result of

intrinsically generated neural noise coming from outside the auditory system (see also Zeng, 2013; Koops & Eggermont, 2021) used to compensate for reduced hearing thresholds resulting from decreased cochlear output due to noise-induced cochlear damage (Krauss*et al.* , 2016; Gollnast *et al.* , 2017; Krauss *et al.* , 2017; Krauss *et al.* , 2018; Krauss *et al.* , 2019; Tziridis*et al.* , 2021; Schilling & Krauss, 2022; Schulze *et al.* , 2023). Thus, a feedback loop implemented in the brainstem tunes for the optimal noise level to maximize information transmission along the auditory pathway by means of adaptive stochastic resonance (for details see Krauss *et al.* , 2016; Schilling *et al.* , 2021). This part of the model has already led to several testable hypotheses and was tested in computer simulations (Schilling *et al.* , 2022). It delivered a mechanistic explanation for the little brother of tinnitus, the Zwicker tone (Zwicker, 1964; Schilling *et al.* , 2023a; Schilling *et al.* , 2023c) and explains increased auditory sensitivity after simulated hearing loss in an animal model (Krauss & Tziridis, 2021), and provided individualized treatment approaches for human patients (Schilling *et al.* , 2020; Tziridis *et al.* , 2022). Nevertheless, this part of the model is a pure brainstem model and does not account for the expected and described cortical effects. To that means, it was assumed that the intrinsically generated neural noise is amplified along the auditory pathway via central gain effects and is then transmitted to higher brain structures such as the thalamus and the auditory cortex.

Complementary to this bottom-up information flow, top-down mechanisms seem to play a crucial role in tinnitus development and especially tinnitus manifestation (Sedley *et al.* , 2016; Hullfish *et al.* , 2019). Thus, the brain and especially the cortex is assumed to operate as prediction machines trying to predict the cause of certain signals transmitted via the auditory pathway. Thus, the brain makes a default prediction (predictor for the Bayesian brain formalism), which is silence under normal conditions. The product of predictor (silence) and the top-down neuronal signal (likelihood) are the actual percept.

According to the combined stochastic-resonance-predictive-coding model which unifies the bottom-up and top-down mechanisms (Schilling *et al.* , 2023b), the increased bottom-up neural activity (neural noise) caused by the stochastic resonance effect is mis-interpreted as neuronal signal evoked by a real auditory stimulus. The noise thus leads to increased activity and might also cause a decreased sensory precision – i.e., higher mean and lower variance of the likelihood – as brain states with low activity become very unlikely due to the continuous neural noise. In other words, the neural noise leads to continuous mis-predictions of the cortex with regards to incoming auditory signal. Thus, the brain updates the predictions in a pathological manner and starts to manifest a continuous auditory input as default prediction (updated predictor), or attractor.

On the other hand, it is assumed that certain plastic changes in the cortex could decrease the precision again or even prevent it from happening at all and therefore reset / keep the default prediction to silence (Sedley *et al.* , 2016; Hullfish *et al.* , 2019). The here described results support this idea as in animals without tinnitus the ECM density is not increased, neuronal plasticity is therefore still possible. In tinnitus animals however, the new synaptic weights and the mis-predictions (falsely updated predictor) are consolidated by the solidified ECM.

In conclusion, we assume the tinnitus percept to be caused by increased neural noise, mis-interpreted as auditory input and manifested via an updated predictor (attractor) of higher brain areas. If this mis-prediction is manifested via an increased ECM density there is no possibility for the system anymore to change this wrong predictor, and consequently tinnitus is perceived chronically.

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Figures and figure captions

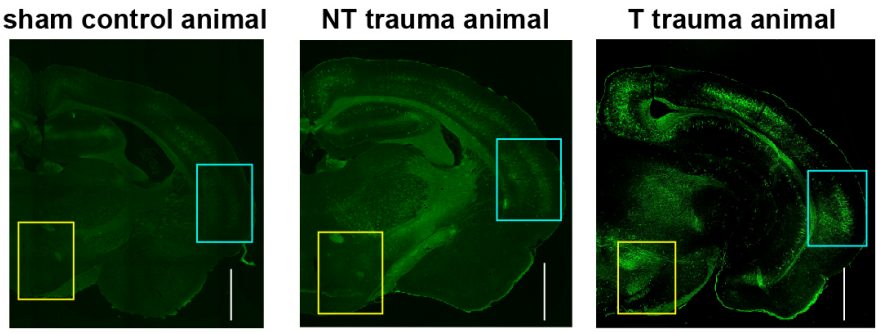

Figure 1 : Exemplary WFA-FITC marked brain slices from a control (**left panel**), non-tinnitus (**center panel**) and tinnitus animal (**right panel**). Rectangles identify the evaluated cortical (turquoise) / brainstem reference (yellow) areas. White scale indicates 1 mm.

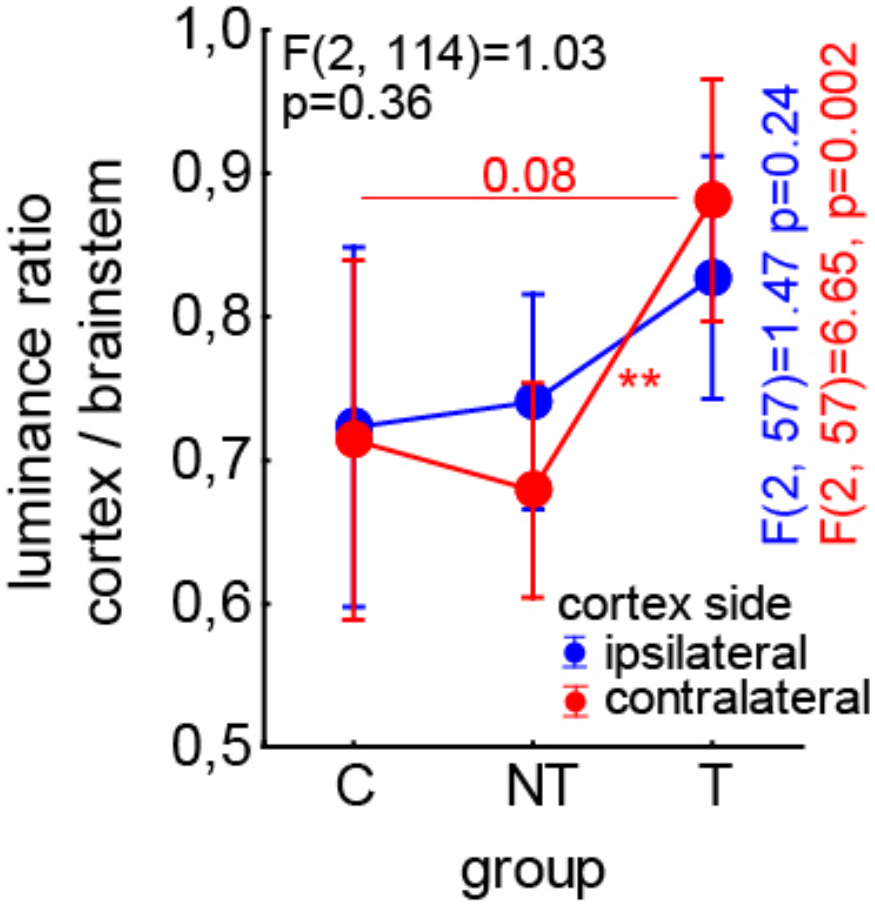

Figure 2 : Interaction plot of the two-factorial ANOVA of the luminance ratio for the factors *animal group* and *cortex side* . The two colored F-statistics give the results of the Bonferroni corrected one-factorial ANOVAs of ipsi- (blue) and contralateral (red) luminance ratios of the three animal groups. Colored asterisks indicate the significance levels of the Tukey post-hoc tests in the one-factorial ANOVAS: ** $p<0.01$.