

Deficit in acoustic signal-in-noise detection in glycine receptor $\alpha 3$ subunit knockout mice

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Abstract

Hearing is an essential sense for communication in animals and humans. Normal function of the cochlea of higher vertebrates relies on a fine-tuned interplay of afferent and efferent innervation of both inner and outer hair cells. Efferent inhibition is controlled via olivocochlear feedback loops, mediated mainly by acetylcholine, γ -aminobutyric acid (GABA) and glycine, and is one of the first sites affected by synapto- and neuropathy in the development of hearing loss. While the functions of acetylcholine, GABA and other inhibitory transmitters within these feedback loops are at least partially understood, especially the function of glycine still remains elusive. To address this question, we investigated hearing in glycine receptor (GlyR) $\alpha 3$ knockout (KO) and wildtype (WT) mice. We found no differences in pure tone hearing thresholds at 11.3 and 16 kHz between the two groups as assessed by auditory brainstem response (ABR) measurements. Detailed analysis of the ABR waves at 11.3 kHz, however, revealed a latency decrease of wave III and an amplitude increase of wave IV in KO compared to WT animals. GlyR $\alpha 3$ KO animals showed significantly impaired prepulse inhibition of the auditory startle response in a noisy environment, indicating that GlyR $\alpha 3$ -mediated glycinergic inhibition is important for signal-in-noise detection.

Introduction

Possible causes of hearing impairment

Hearing is an essential sense for communication among and survival of nearly all higher vertebrates like birds and mammals. The primary acoustic sensors, the hair cells, are located in the organ of Corti within the cochlea. Based on morphological and functional characteristics, these hair cells fall into two classes. The inner hair cells (IHCs) form a single row with predominantly afferent innervation, while the three rows of outer hair cells (OHCs) with predominantly efferent innervation show electromotile characteristics allowing them to act as cochlear amplifiers (Fettiplace & Hackney, 2006).

Human beings use speech for life-long communication, and hearing impairment therefore often leads to social isolation that finally may result in psychological pathologies like depression. Thus, hearing impairment contains a huge socioeconomic impact. In Europe, the calculated yearly costs of hearing deficits are in the range of 200 billion Euros (Shield, 2006). Sensorineural hearing impairment

may be caused by several factors, including inflammation, genetic predisposition or mechanical insult due to noise trauma (Henderson *et al.*, 2006). Such damage does not always lead to pure tone hearing threshold elevation, but may also result in impaired signal-in-noise detection, impaired speech recognition in cocktail-party situations, or tinnitus (Plack *et al.*, 2014). In this report we focus on the role of the glycinergic system in a behavioural signal-in-noise detection task.

The role of the efferent system in hearing and hearing protection

Efferent innervation fine-tunes acoustic information transmission within the auditory pathway and is involved in the processing of complex audio patterns, including the discrimination of transient sounds in background noise (Guinan, 2006). Neurons within the superior olivary complex (SOC) project back to the cochlea, thereby forming an inhibitory olivocochlear feedback loop. In principle, two different types of efferent projections can be distinguished by means of anatomical and functional characteristics: First, the lateral olivocochlear (LOC) bundle – which contains mostly non-crossing inhibitory and excitatory axons (Groff & Liberman, 2003; Darrow *et al.*, 2007) from the lateral superior olive – forms mainly axodendritic

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synapses with the auditory nerve below the IHC, thereby allowing for direct modulation of the propagation of IHC-generated signals. LOC efferent transmission, which is mediated by the neurotransmitters acetylcholine (ACh), γ -aminobutyric acid (GABA), glycine, dopamine, enkephalins, dynorphins and the calcitonin gene-related peptide (CGRP) (Ruel *et al.*, 2007; Długańczyk *et al.*, 2008; Sewell, 2011), has been associated with protection of the cochlea against acoustic trauma (Darrow *et al.*, 2007). Second, the medial olivocochlear (MOC) bundle contains axons from the contralateral (70%) and ipsilateral (30%) medial superior olive and directly contacts the basolateral region of OHCs via axosomatic synapses, thus inhibiting their electromotility. MOC efferent innervation is especially discussed in the context of protection against noise trauma (Liberman & Gao, 1995; Maison & Liberman, 2000; Maison *et al.*, 2013) and detection of transient sounds in background noise ('MOC unmasking'; Guinan, 2006).

The function of glycine in olivocochlear feedback loops

Glycinergic transmission within the cochlea (Długańczyk *et al.*, 2008; Buerbank *et al.*, 2011) is mediated via glycine receptors (GlyR) that are ligand-gated postsynaptic chloride channels, abundantly expressed in mammalian spinal cord and brainstem (Eulenburg *et al.*, 2005). In the adult CNS, the predominantly postsynaptic localized pentameric receptor channel complex exerts an inhibitory function, consists of two ligand-binding α and three structurally homologous β subunits and is anchored to the cytoskeleton by gephyrin. Up to now, four α subunit isoforms (GlyR α 1-4) and one β polypeptide (GlyR β) are known (Dutertre *et al.*, 2012). GlyRs are also found in the auditory brainstem, where they contribute to lateral inhibition and directional hearing in the SOC (Grothe *et al.*, 2010). In particular, GlyR α 3 transcripts have been described from the cochlear nucleus up to the superior olivary complex (Sato *et al.*, 2000).

GlyR α 3 has been identified as the only ligand-binding subunit in the adult rodent cochlea. The localization of α 3-containing GlyRs within the organ of Corti – at the basolateral end of the OHCs (efferent fibres from the MOC) and the afferent auditory nerve fibres contacting the IHCs (efferent fibres from the LOC) – suggests that they might serve as targets of inner ear efferent innervation (Długańczyk *et al.*, 2008; Buerbank *et al.*, 2011), thus modulating auditory processing in the cochlea. Recent studies, displayed unaltered click- and f-ABR (frequency-specific auditory brainstem responses) hearing thresholds in knockout mice lacking the GlyR α 3 protein (GlyR α 3^{-/-}) as compared to their wildtype littermates (GlyR α 3^{+/+}). However, measurement of otoacoustic emissions (OAEs) and suprathreshold ABR wave I amplitudes indicated a role of α 3-GlyRs in regulating auditory nerve activity and OHC function, which would be consistent with a contribution of α 3-GlyRs to cochlear MOC and LOC efferent innervation (Długańczyk *et al.*, 2016).

We therefore here tested the hypothesis that GlyRs containing the α 3 subunit are able to enhance signal-in-noise detection in a noisy auditory environment. To this end we used C57BL/6J mice with and without a systemic knockout of the GlyR α 3 subunit and tested their performance within a signal-in-noise detection task based on an acoustic startle response (ASR) paradigm.

Materials and methods

Ethics statement and animals

GlyR α 3 wildtype (WT; GlyR α 3^{+/+}) C57BL/6J mice and their homozygous GlyR α 3^{-/-} littermates (Harvey *et al.*, 2004) were bred

and housed in the Emil-Fischer-Zentrum, Erlangen (Germany) in standard type 3 cages at constant temperature, air humidity and a 12 h/12 h light/dark cycle with free access to food and water. The genotype was determined on genomic DNA isolated from tail biopsies using a PCR-based protocol as described (Harvey *et al.*, 2004). All experiments were approved by the animal use and care committee of the state of Bavaria (Regierungspräsidium Mittelfranken, Ansbach, Germany, AZ: 54-2532.1-15/10 and AZ 54-2532.1-38/08).

Overall, 17 adult male mice (8 WT; 9 KO) were used in the experiments; the median age of the animals was 58 days (interquartile range: 46–72 days). None of the animals showed any motor impairment. All animals (8 WT, 9 KO) were assessed with the behavioural ASR signal-in-noise detection paradigm (see Section Signal-in-noise detection determined by ASR). Out of these, 14 animals (8 WT, 6 KO) were tested for their hearing thresholds at the frequencies used for ASR experiments by f-ABR under ketamine anaesthesia.

f-ABR data acquisition

Animals were placed within a sound-attenuated chamber (Industrial Acoustics, Niederkrüchten, Germany) in deep anaesthesia [mixture of ketamine (96 mg/kg), xylazine (4 mg/kg), physiological NaCl solution and atropine (1 mg/kg) at a mixing ratio of 9 : 1 : 8 : 2, initial dose: 0.3 mL s.c.; continuous application at a rate of 0.2–0.3 mL/h]. f-ABRs were measured via subcutaneously placed thin silver wire electrodes (0.25 mm diameter) using a CED micro 1401 data acquisition unit (Cambridge Electronic Division Limited, Cambridge, UK) with a CED 3505 programmable attenuator via a custom-made program (Spike2, CED). Auditory stimuli were presented free-field to one ear at a time at 0.5 cm distance from the animal's pinna via a speaker (SinusLive neo 25S, pro hifi, Kaltenkirchen, Germany). The speaker's frequency response function was corrected to be flat within ± 1 dB. Stimuli presented were clicks (0.1 ms duration) and pure tones (4 ms duration including 1 ms cosine-squared rise and fall times) at 11.3 and 16 kHz, the frequencies of best hearing in GlyR α 3^{+/+} and GlyR α 3^{-/-} mice (Długańczyk *et al.*, 2016), which were used for the ASR paradigm later on (Section Signal-in-noise detection determined by ASR). 300 stimuli were presented with alternating inverted phase with a repetition rate of 10 Hz. Stimulation started at the highest attenuation (17 dB SPL speaker output) and proceeded to lowest attenuation (82 dB SPL speaker output) in 5-dB steps.

Signal-in-noise detection determined by ASR

For behavioural testing, animals were placed into a transparent acrylic tube (length: 8 cm; inner diameter 2.2 cm). This tube was placed 10 cm in front of a speaker (Canton Plus X Series 2, Canton, Weilrod, Germany) onto a Honeywell FSG15N1A piezo force sensor (sensitivity 0.24 mV/g; null shift at 25 °C is ± 1 mV; force range 0–1500 g) to detect startle responses, assembled within a sound-attenuated chamber (Industrial Acoustics) on a low-vibration table (TMC). The front end of the tube was closed with a stainless steel grate (wire mesh width 0.5 mm) allowing acoustic stimulation with no detectable distortion (signal-to-noise ratio at least 70 dB). Sound pressure level was controlled via a Brüel & Kjær (B&K, Naerum, Denmark) Type 2610 measuring amplifier fed with a B&K Type 2669 preamplifier/B&K Type 4190 condenser microphone combination. Stimulus generation and data acquisition were controlled using custom-made MATLAB 2008 programs (Math-Works, Natick, MA, USA). For sound generation, the frequency response function of the speaker was calibrated to produce an output spectrum that was flat within ± 1 dB.

To test the ability of the animals to detect signals in noisy background we utilized the pre-pulse inhibition (PPI) of the ASR (Koch & Schnitzler, 1997) in an experimental design suggested by Ison and Allen (Ison & Allen, 2012). A 90 dB SPL pure tone startle stimulus (12 or 16 kHz, 6 ms length including 2 ms rise and fall ramps) was either presented within a 40 dB SPL white noise background (40 dB noise condition) or without background noise in complete silence (0 dB noise condition). The startle stimulus was either not preceded by a prestimulus (0 dB prestimulus) or preceded (100 ms) by a prestimulus of the same frequency and duration with 40, 50 or 60 dB SPL. Each startle frequency, background condition and prestimulus intensity was repeated 15 times. To ensure the validity of the PPI response of the animals, a standard hearing threshold paradigm (Walter *et al.*, 2012) was used in six animals at 12 and 16 kHz with prepulse intensities ranging from 10 to 70 dB SPL in 10 dB steps. The data at 70 dB SPL had to be discarded (cf. Section Signal-in-noise detection) resulting in control measurements ranging from 10 to 60 dB SPL.

Data evaluation and statistical analysis

To obtain f-ABR-based hearing thresholds (cf. above), the mean f-ABR waves were compared to the mean amplitude 200–100 ms before the stimulus (baseline). Thresholds were defined automatically by a custom-made MATLAB program at the highest attenuation at which the evoked amplitude raised over two standard deviations of the baseline (Walter *et al.*, 2012). Data were discarded at frequencies where this procedure was not possible, for example, at low signal-to-noise ratios. Data were analysed by the two-sided unpaired Student's *t*-test or two-factorial ANOVA with the factors 'group' and 'stimulation frequency', where appropriate. Additionally, the individual wave complexes I/II, III, IV and V of the measurements at 67 dB SPL (ca. 26 dB above hearing threshold at a medium level) with 11.3 kHz stimulation were analysed in detail for wave amplitude and latency in WT and KO animals as described earlier (Tziridis *et al.*, 2015). Click- and f-ABR amplitude analyses were performed by two-factorial ANOVAs and/or two-sided Student's *t*-tests. f-ABR latencies were statistically analysed by non-parametric methods (e.g. Mann–Whitney *U*-test), as normal distribution cannot be assumed. f-ABR wave I corresponds to the activity in the cochlear nerve, wave II reflects the neuronal response in the nucleus cochlearis, wave III that of the superior olivary complex/trapezoid body, wave IV that of the lateral lemniscus and wave V corresponds to the summed activity of the inferior colliculus (Henry, 1979).

The data obtained in the PPI of the ASR paradigm were checked visually via a custom-made MATLAB program. Trials in which the animals moved within 100 ms before the startle stimulus (especially in the 60 dB SPL prestimulus condition, where the prestimulus started to evoke startle responses of its own) were discarded. In the remaining valid trials only peak-to-peak amplitudes of responses within the first 25 ms after startle stimulus onset were used for further analysis. The PPI effect was calculated by dividing the response amplitudes of the valid trials with prepulses (40–60 dB SPL = signal-in-noise, or 10–60 dB SPL = control measurement) by the median of the valid trials without prepulses (0 dB SPL) for each frequency and animal individually. This results in normalized individual trial amplitudes for each frequency and animal. The PPI effect is expressed in per cent change relative to the 0 dB SPL prepulse response amplitude and analysed by two two-factorial ANOVAs independently for the two background conditions (0 dB SPL and 40 dB SPL noise), with the factors 'group' (WT and KO) and the three prestimulus intensities.

Results

f-ABR thresholds and wave analysis

Evaluation of f-ABR thresholds at 11.3 and 16 kHz in both groups (8 WT and 6 KO) by a two-factorial ANOVA is given in Fig. 1. Exemplary f-ABR data of animals from both groups are depicted in Fig. 1A with 11.3 kHz stimulation below and above the hearing threshold and for the highest output presented. Both groups show no difference in mean f-ABR thresholds (two-factorial ANOVA; stimulation frequency \times group; $F_{2,32} = 0.05$, $P = 0.95$) for clicks and the tone frequencies tested (Fig. 1B) with the identical mean threshold values for both groups (two-factorial ANOVA; factor group; $F_{1,32} = 0.76$; $P = 0.39$; Fig. 1B, inset). Moreover, no significant interaction of both factors can be seen. In other words, animals with a knockout of the GlyR α 3 subunit have no obvious deficit in

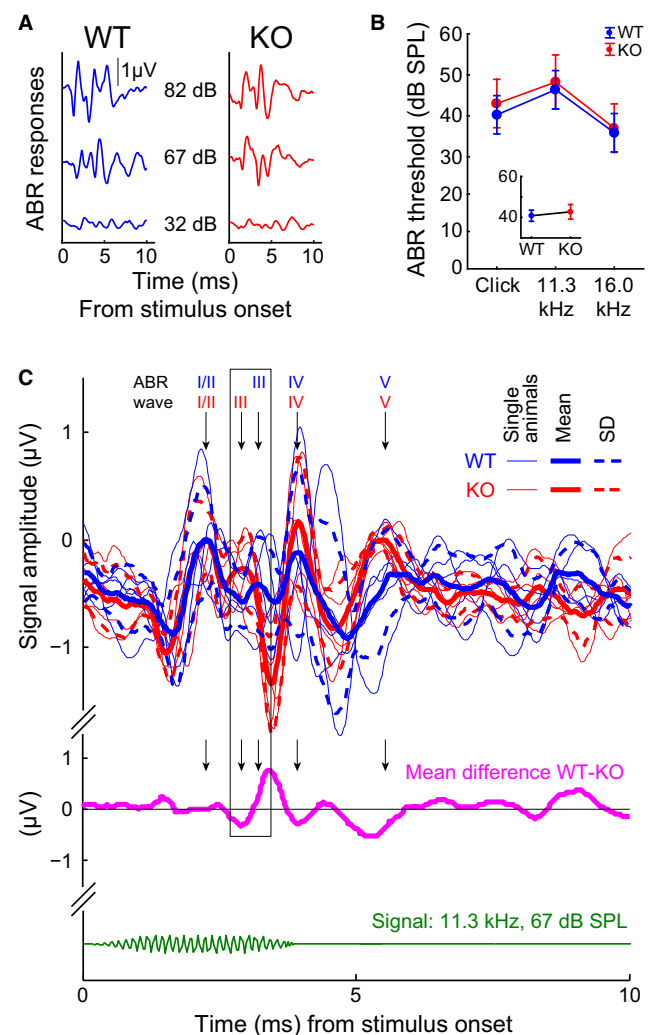


FIG 1. (A) Exemplary f-ABR data from wildtype (WT) (blue) and knockout (KO) (red) animals for different 11.3 kHz stimulus intensities. (B) Interaction of the two-factorial ANOVA (group \times stimulation) of the click- and f-ABR hearing thresholds of both animal groups. The inset depicts the effect of the factor group. (C) f-ABR (single animals: thin lines; mean: fat lines; SD: dashed lines) for 11.3 kHz at 67 dB SPL for both animal groups. Arrows indicate the different wave complexes. Shown below (magenta) is the mean difference between f-ABR data from WT and KO animals. Note that a significant difference in latency was only seen in wave III and a significant difference in amplitude was only seen in wave IV.

f-ABR-based hearing threshold in the frequency range tested, as compared to wildtype animals.

We also investigated the latencies and amplitudes of the different f-ABR waves (Fig. 1C) at a moderate intensity level well above hearing thresholds, (i.e. 67 dB SPL) at the lower of both pure tone frequencies (11.3 kHz). These analyses are depicted in detail in Fig. 2. By *t*-tests (Fig. 2A) we found no differences between WT and KO animals in absolute amplitude values in three of the four identified wave complexes (*t*-tests WT vs. KO; wave I/II ($t_{13} = 0.7$), III ($t_{13} = 0.3$) and V ($t_{13} = 0.4$); always $P > 0.05$). Only for wave IV the *t*-test showed a significantly smaller absolute amplitude in WT as compared to KO animals (*t*-test wave IV; $t_{13} = 8.3$, $P < 0.001$). Latencies (Fig. 2B) of the different wave complexes also did not show any significant differences between WT and KO animals in three out of four f-ABR waves assessed by nonparametric Mann–Whitney *U*-tests (WT vs. KO; wave I/II, IV and V; always $P > 0.05$). These values are well in the range of ‘normal’ latencies as known from the literature (e.g. Kurt *et al.*, 2009). Only for wave III the latency in WT animals was significantly longer than in KO animals (Mann–Whitney *U*-test WT vs. KO; wave III; $P < 0.001$). In other words, the latency of the superior olive/trapezoid body complex (wave III) – which is most probably the source of the glycinergic input to the cochlea – showed a strong deviation from the normal latency range in KO animals.

Signal-in-noise detection

The results for the signal-in-noise detection paradigm (cf. Section Materials and methods) are summarized in Fig. 3. Exemplary data of single trials at 12 kHz startle stimulus frequency and different prepulse intensities of one animal are depicted in Fig. 3A. Please note that prepulses above 60 dB SPL already led to explicit startle responses (given in mV piezo sensor output) in the range of the following startle amplitude to the main startle stimulus, which is still reduced by PPI. To avoid any interactions between prepulse and startle stimulus, we chose to restrict stimulation intensities of the prepulses to 60 dB SPL (while even 40 dB prepulses sometimes led to minor responses). For the 0 dB noise condition, the 8 WT animals showed a significantly stronger PPI effect than the 9 KO animals (Fig. 3B inset ‘0 dB noise’; two-factorial ANOVA; factor group; $F_{1,1890} = 12.87$, $P < 0.001$) and increasing prepulse intensity

resulted in a significantly increasing PPI in both groups ($F_{2,1890} = 12.14$, $P < 0.001$). No interaction of group and prestimulus intensity was found in this condition (two-factorial ANOVA; prestimulus intensity \times group; $F_{2,1890} = 0.78$; $P = 0.46$; Fig. 3B, left panel). Thus, we found a general offset of the startle amplitude between WT and KO animals, but the increase of the PPI effect with prepulse intensity was similar in both groups.

In the 40 dB noise condition – the proper signal-in-noise detection task – again a generally stronger PPI effect in WT compared to KO animals was seen (Fig. 3B inset ‘40 dB noise’; two-factorial ANOVA; factor group; $F_{1,1984} = 49.01$, $P < 0.001$), and an increasing PPI effect with increasing prepulse intensity for both groups was observed ($F_{2,1984} = 49.39$, $P < 0.001$). The major difference in this task lies in the significant interaction of both factors (two-factorial ANOVA; prestimulus intensity \times group; $F_{2,1984} = 6.91$; $P < 0.001$; Fig. 3B, right panel). While the WT animals show a steadily increasing PPI effect with increasing signal-to-noise ratio, the KO animals show no PPI for very low signal-to-noise ratios (0 dB, i.e. 40 dB SPL prestimulus before a 40 dB SPL background noise), but an even increased startle responses (mean \pm SD: $+6.1 \pm 3.4\%$; *t*-test vs. 0 dB noise: $t_{198} = 27.2$, $P < 0.001$). Furthermore, the KO mice do not show a significant improvement of signal-in-noise detection (Tukey *post hoc* test, KO 50 dB vs. KO 60 dB, $P = 0.99$) with further increasing signal-to-noise ratios above 10 dB (i.e. 50 dB SPL prestimulus within 40 dB SPL background noise). As a consequence, they are significantly impaired in their PPI at 60 dB SPL prepulse intensity compared to the WT animals (Tukey *post hoc* test, $P < 0.001$). In other words, while WT and KO animals show similar PPI effects without any background noise, in a signal-in-noise detection task the KO animals are impaired in detecting pure tones within a white noise background.

Discussion and conclusion

In this report we have demonstrated that animals with a knockout of the GlyR α 3 subunit do not show obvious deficits in pure tone hearing thresholds within the frequency range of their best hearing, but are impaired in signal-in-noise detection at those frequencies. The olivocochlear feedback loops are thought to facilitate signal detection in noise by inhibitory transmitter release (Guinan, 2006; Dlugaczkyk *et al.*, 2008; Buerbank *et al.*, 2011; Sewell, 2011). Therefore, it seems likely that the main effect of the GlyR α 3 systemic knockout reported here is based on a deficit in glycinergic transmission within the auditory system. Our finding of changed latencies of f-ABR wave III, which reflects processing within the superior olive, is in line with this interpretation.

Numerous studies have investigated the function of efferent inhibitory neurotransmitters (e.g. ACh, GABA, CGRP, dopamine) which can be found in both the LOC and MOC feedback loops. The MOC has been identified to be crucial for signal-in-noise detection (Winslow & Sachs, 1987; Kawase & Liberman, 1993) and noise protection of the cochlea (Cody & Johnstone, 1982; Liberman & Gao, 1995; Maison *et al.*, 2013). Much less is known about the LOC back-projections to the cochlea, as they cannot be assessed as easily as the MOC (Guinan, 2010): One of the few facts known is that it is much slower than MOC-mediated communication (Sterenborg *et al.*, 2010) and that it may be involved in noise protection of the cochlea (Darrow *et al.*, 2007). In this study, we now provide evidence for a functional role of the glycinergic efference from the olivary complex (LOC and/or MOC) to the cochlea for signal-in-noise detection using a knockout mouse model.

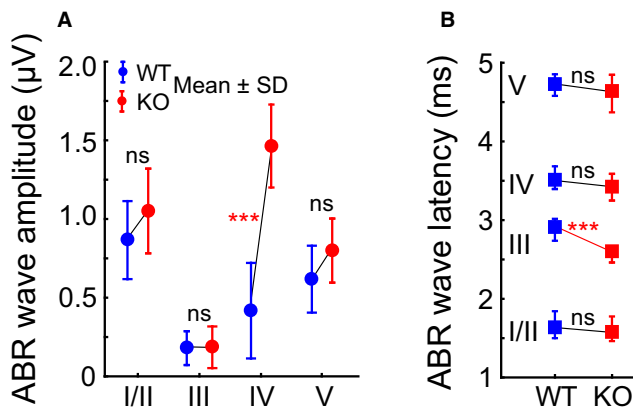


FIG. 2. (A) Mean f-ABR wave amplitudes \pm SD of the different wave complexes of wildtype (WT) (blue) and knockout (KO) (red) animals statistically compared by *t*-tests: ns not significant, *** $P < 0.001$. (B) Median f-ABR wave latencies \pm interquartile range of the different wave complexes statistically compared by Mann–Whitney *U*-tests.

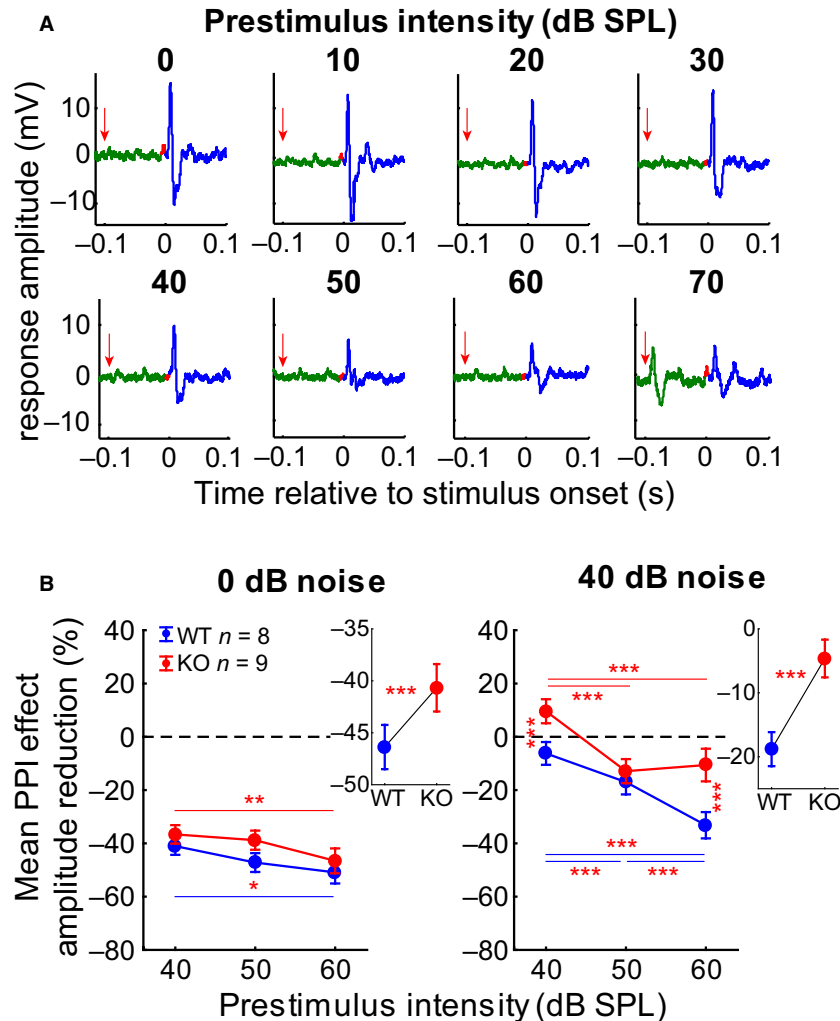


FIG. 3. (A) Exemplary single trial ASR amplitudes as registered by the piezo sensor (cf. Section Materials and methods) to a 90 dB SPL startle stimulus with different prepulse intensities ranging from 0 to 70 dB SPL. The response at the time before the startle stimulus is given in green, during the stimulus in red and afterwards in blue. The time of the prepulse onset is depicted by a red arrow in each panel. (B) Mean PPI effect in the signal-in-noise detection task. Both animal groups are able to detect the signal without background noise (left panel, interaction of the two-factorial ANOVA group \times prepulse intensity), but knockout (KO) animals (red) are impaired in the 40 dB white noise background condition (right panel, interaction of the two-factorial ANOVA group \times prepulse intensity) compared to wildtype (WT) animals (blue). The insets show the effect of the factor group for both background noise intensities. Asterisks give the significance levels of the Tukey *post hoc* tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

In this report, f-ABR-based hearing thresholds in the frequency range of best hearing determined under anaesthesia were in the range of around 40 dB SPL. As we know from earlier work (Walter *et al.*, 2012), the behaviourally determined thresholds in awake animals usually are 10–15 dB better, that is, all intensities used in the ASR-based signal detection task were well above hearing threshold. This is further supported by the observation of minor responses of the animals to the 40 dB SPL prestimuli (cf. Fig. 3A, small response after red arrow). In the signal detection task, the KO mice showed quite a normal – while slightly decreased – PPI response when tested without any background noise (cf. Fig. 3B, left panel). But with the 40 dB white noise background, they were impaired especially in the ‘hardest’ detection task (40 dB prestimulus) and in the ‘easiest’ one (60 dB prestimulus), while they did not show any significant impairment in the 50 dB prestimulus task compared to WT animals. In particular, the enhanced dynamic range for signal-in-noise detection observed for WT as compared to their KO littermates resembles the well-known effect of ‘MOC unmasking’ for the detection of transient pure tone stimuli in background noise

(Guinan, 2006). We refrained from using louder prestimuli than 60 dB SPL, as 70 dB SPL tones already produce startle responses to a large degree and therefore may interfere with the PPI effect. Therefore, we are currently not able to judge signal-to-noise ratios above 20 dB.

The systemic knockout of the glycine receptor $\alpha 3$ -subunit affects the whole organism. Hence, we cannot rule out that the changes we observed in the signal-in-noise detection ability of these animals were also a result of a changed afferent innervation and processing throughout the whole auditory pathway. Nevertheless, the very specific latency shift in f-ABR wave III in KO animals corresponds well to the hypothesis of changed processing within the olivocochlear feedback loops. Here, especially the lack of GlyR $\alpha 3$ subunits in the SOC of the KO animals (Sato *et al.*, 2000) might contribute to a reduced inhibition within the SOC and therefore to a reduced response latency. Furthermore, the lack of inhibition in the SOC could lead to the increase of f-ABR wave IV amplitude, which further supports the idea of glycinergic efferent feedback innervation being involved in the observed effect on signal-in-noise detection.

Likewise, we cannot rule out compensatory effects of other glycine receptor subunits or even other inhibitory receptor types. But we can rule out that the described change in the signal detection ability in the KO animals is a result of motor deficits, as the KO has been tested and found to have normal motor function (Harvey *et al.*, 2004). Additionally, the effect seems to be quite specific to the GlyR α 3 subunit, as it is the only GlyR α subunit expressed in the cochlea of adult rodents (Buerbank *et al.*, 2011).

Taken together, the GlyR α 3-subunit dependent efferent feedback from the superior olive to the cochlea seems to play a major role in signal-in-noise detection.

Conflict of interests

The authors declare that they do not have any conflicts of interests.

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References

- Buerbank, S., Becker, K., Becker, C.M., Brandt, N., Engel, J., Knipper, M., Schick, B. & Dlugaiczyk, J. (2011) Developmental regulation of glycine receptors at efferent synapses of the murine cochlea. *Histochem. Cell Biol.*, **136**, 387–398.
- Cody, A.R. & Johnstone, B.M. (1982) Temporary threshold shift modified by binaural acoustic stimulation. *Hear. Res.*, **6**, 199–205.
- Darrow, K.N., Maison, S.F. & Liberman, M.C. (2007) Selective removal of lateral olivocochlear efferents increases vulnerability to acute acoustic injury. *J. Neurophysiol.*, **97**, 1775–1785.
- Dlugaiczyk, J., Singer, W., Schick, B., Iro, H., Becker, K., Becker, C.M., Zimmermann, U., Rohbock, K. *et al.* (2008) Expression of glycine receptors and gephyrin in the rat cochlea. *Histochem. Cell Biol.*, **129**, 513–523.
- Dlugaiczyk, J., Hecker, D., Neubert, C., Buerbank, S., Campanelli, D., Becker, C.-M., Betz, H., Knipper, M. *et al.* (2016) Loss of glycine receptors containing the α 3 subunit compromises auditory nerve activity, but not outer hair cell function. *Hear. Res.*, **337**, 25–34.
- Dutertre, S., Becker, C.M. & Betz, H. (2012) Inhibitory glycine receptors: an update. *J. Biol. Chem.*, **287**, 40216–40223.
- Eulenburg, V., Armsen, W., Betz, H. & Gomeza, J.S. (2005) Glycine transporters: essential regulators of neurotransmission. *Trends Biochem. Sci.*, **30**, 325–333.
- Fettiplace, R. & Hackney, C.M. (2006) The sensory and motor roles of auditory hair cells. *Nat. Rev. Neurosci.*, **7**, 19–29.
- Groff, J.A. & Liberman, M.C. (2003) Modulation of cochlear afferent response by the lateral olivocochlear system: activation via electrical stimulation of the inferior colliculus. *J. Neurophysiol.*, **90**, 3178–3200.
- Grothe, B., Pecka, M. & McAlpine, D. (2010) Mechanisms of sound localization in mammals. *Physiol. Rev.*, **90**, 983–1012.
- Guinan, J.J. Jr (2006) Olivocochlear efferents: anatomy, physiology, function, and the measurement of efferent effects in humans. *Ear Hear.*, **27**, 589–607.
- Guinan, J.J. (2010) Cochlear efferent innervation and function. *Curr. Opin. Otolaryngol. Head Neck Surg.*, **18**, 447–453.
- Harvey, R.J., Depner, U.B., Wasse, H., Ahmadi, S., Heindl, C., Reinold, H., Smart, T.G., Harvey, K. *et al.* (2004) GlyR alpha3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. *Science*, **304**, 884–887.
- Henderson, D., Bielefeld, E.C., Harris, K.C. & Hu, B.H. (2006) The role of oxidative stress in noise-induced hearing loss. *Ear Hear.*, **27**, 1–19.
- Henry, K.R. (1979) Differential changes of auditory nerve and brain stem short latency evoked potentials in the laboratory mouse. *Electroencephalogr. Clin. Neurophysiol.*, **46**, 452–459.
- Ison, J.R. & Allen, P.D. (2012) Deficits in responding to brief noise offsets in Kcna1-/- mice reveal a contribution of this gene to precise temporal processing seen previously only for stimulus onsets. *J. Assoc. Res. Otolaryngol.*, **13**, 351–358.
- Kawase, T. & Liberman, M.C. (1993) Antimasking effects of the olivocochlear reflex. I. Enhancement of compound action potentials to masked tones. *J. Neurophysiol.*, **70**, 2519–2532.
- Koch, M. & Schnitzler, H.-U. (1997) The acoustic startle response in rats: circuits mediating evocation, inhibition and potentiation. *Behav. Brain Res.*, **89**, 35–49.
- Kurt, S., Groszer, M., Fisher, S.E. & Ehret, G. (2009) Modified sound-evoked brainstem potentials in Foxp2 mutant mice. *Brain Res.*, **1289**, 30–36.
- Liberman, M.C. & Gao, W.Y. (1995) Chronic cochlear de-efferentation and susceptibility to permanent acoustic injury. *Hear. Res.*, **90**, 158–168.
- Maison, S.F. & Liberman, M.C. (2000) Predicting vulnerability to acoustic injury with a noninvasive assay of olivocochlear reflex strength. *J. Neurosci.*, **20**, 4701–4707.
- Maison, S.F., Usubuchi, H. & Liberman, M.C. (2013) Efferent feedback minimizes cochlear neuropathy from moderate noise exposure. *J. Neurosci.*, **33**, 5542–5552.
- Plack, C.J., Barker, D. & Prendergast, G. (2014) Perceptual consequences of “hidden” hearing loss. *Trends Hear.*, **18**, 1–11.
- Ruel, J., Wang, J., Rebillard, G., Eybalin, M., Lloyd, R., Pujol, R. & Puel, J.L. (2007) Physiology, pharmacology and plasticity at the inner hair cell synaptic complex. *Hear. Res.*, **227**, 19–27.
- Sato, K., Shiraiishi, S., Nakagawa, H., Kuriyama, H. & Altschuler, R.A. (2000) Diversity and plasticity in amino acid receptor subunits in the rat auditory brain stem. *Hear. Res.*, **147**, 137–144.
- Sewell, W.F. (2011) Pharmacology and neurochemistry of olivocochlear efferents. In Ryugo, D.K. and Fay, R.R. (Eds), *Auditory and Vestibular Efferents*. Springer, New York, pp. 83–101.
- Shield, B. (2006) Evaluation of the social and economic costs of hearing impairment. *Hear-it AISBL*, 1–202.
- Sterenberg, J.C., Pilati, N., Sheridan, C.J., Uchitel, O.D., Forsythe, I.D. & Barnes-Davies, M. (2010) Lateral olivocochlear (LOC) neurons of the mouse LSO receive excitatory and inhibitory synaptic inputs with slower kinetics than LSO principal neurons. *Hear. Res.*, **270**, 119–126.
- Tziridis, K., Ahlf, S., Jeschke, M., Happel, M.F., Ohl, F.W. & Schulze, H. (2015) Noise trauma induced neural plasticity throughout the auditory system of Mongolian gerbils: differences between tinnitus developing and non-developing animals. *Front. Neurol.*, **6**, 22.
- Walter, M., Tziridis, K., Ahlf, S. & Schulze, H. (2012) Context dependent auditory threshold determined by brainstem audiometry and prepulse inhibition in Mongolian Gerbils. *Open J. Acoust.*, **2**, 34–49.
- Winslow, R.L. & Sachs, M.B. (1987) Effect of electrical stimulation of the crossed olivocochlear bundle on auditory nerve response to tones in noise. *J. Neurophysiol.*, **57**, 1002–1021.