RESEARCH ARTICLE

Congenital Deafness Reduces, But Does Not Eliminate Auditory Responsiveness in Cat Extrastriate Visual Cortex

Rüdiger Land, [a,b*] Jan-Ole Radecke [a,b†] and Andrej Kral [a,b]

[a] *Institute of Audioneurotechnology, Hannover Medical School, Hannover, Germany*

[b] *Department of Experimental Otology, Hannover Medical School, Hannover, Germany*

Abstract—Congenital deafness not only affects the development of the auditory cortex, but also the interrelation between the visual and auditory system. For example, congenital deafness leads to visual modulation of the deaf auditory cortex in the form of cross-modal plasticity. Here we asked, whether congenital deafness additionally affects auditory modulation in the visual cortex. We demonstrate that auditory activity, which is normally present in the lateral suprasylvian visual areas in normal hearing cats, can also be elicited by electrical activation of the auditory system with cochlear implants. We then show that in adult congenitally deaf cats auditory activity in this region was reduced when tested with cochlear implant stimulation. However, the change in this area was small and auditory activity was not completely abolished despite years of congenital deafness. The results document that congenital deafness leads not only to changes in the auditory cortex but also affects auditory modulation of visual areas. However, the results further show a persistence of fundamental cortical sensory functional organization despite congenital deafness. © 2018 The Authors. Published by Elsevier Ltd on behalf of IBRO. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Key words: cochlear implants, deafness, congenital deafness, auditory cortex, visual cortex, plasticity.

INTRODUCTION

Congenital deafness affects the postnatal development of the auditory cortex. The absence of auditory input influences the dynamics and effective connectivity within the auditory cortex (Ponton and Eggermont, 2001; Sharma and Dorman, 2006; Kral et al., 2017, 2000; Clemo et al., 2017; Hunt et al., 2006). However, deafness also affects the development of the normal interrelationship between the visual and auditory system. For example, changes in visual connectivity due to cross-modal plasticity lead to visual activity in deaf auditory cortex areas in deaf humans as well as congenitally and early-deaf cats (Finney et al., 2001; Bavelier and Neville, 2002; Lomber et al., 2010, 2011; Meredith et al., 2011; Meredith and Lomber, 2011).

The absence of auditory input in congenital deafness might not only affect the auditory areas, but visual areas as well. In hearing cats, auditory activity has been demonstrated in the visual areas of the suprasylvian sulcus (Yaka et al., 1999, 2002; Allman and Meredith, 2007). The suprasylvian sulcus (Hubel and Wiesel, 1969; Palmer et al., 1978) lies dorsally to the auditory cortex and adjacent to the auditory dorsal zone DZ (He et al., 1997; He and Hashikawa 1998; Stecker et al., 2005; Lee and Middlebrooks, 2013). The visual lateral suprasylvian areas are located in the banks of the suprasylvian sulcus. They are distinguished into an area on the lateral bank and an area on the medial bank of the suprasylvian sulcus. The lateral bank of the sulcus comprises the anterolateral lateral suprasylvian area and the posterolateral lateral suprasylvian area (ALLS/PLLS). In accordance, the medial bank of the sulcus is divided into the anteromedial lateral suprasylvian area and the posteromedial lateral suprasylvian area (AMLS/PMLS) (see Palmer et al., 1978 for more detail).

Interestingly, neonatal blindness led to an increase in normally present auditory activity in the visual areas of the suprasylvian sulcus (ALLS/PLLS and AMLS/PMLS) in comparison to sighted cats (Yaka et al., 1999). This leads to the question whether congenital deafness also affects auditory activity in the visual areas of the suprasylvian sulcus. If blindness leads to an increase in auditory activity in the visual lateral suprasylvian areas, does deafness lead to a decrease in auditory features in these areas? The present study was designed to investigate this question in the visual areas AMLS/PMLS of congenitally deaf cats.

We compared the presence of auditory activity in AMLS/PMLS between hearing and congenitally deaf cats. For this, both groups were implanted with cochlear

*Correspondence to: R. Land, Institute of Audioneurotechnology, Hannover Medical School, Stadtfelddamm 34, 30625 Hannover, Germany.

E-mail address: land.ruediger@mh-hannover.de (R. Land).

† Current address: Department of Neurophysiology and Pathophysiology, Center of Experimental Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

implants to allow a direct comparison, and auditory activity was elicited electrically. We first tested whether in hearing cats the normally present auditory activity in the lateral suprasylvian visual areas could be elicited by electrical cochlear implant stimulation. We then addressed the same question in the congenitally deaf cats by restoring auditory input with cochlear implants (Heid et al., 1998). We then compared electrically cochlear implant evoked local field potentials (LFPs) in area AMLS/PMLS between the two groups, hearing cats and congenitally deaf cats.

METHODS

Experiments were performed in adult hearing ($n = 3$) and adult congenitally deaf white cats ($n = 5$) (Heid et al., 1998). Hearing cats were between 2 and 3 years old at the time of experiments and their weight ranged from 2.9 to 4.1 kg (one male and two female). Congenitally deaf cats were between 2 and 4 years old at the time of the experiments (thus reflecting 2- to 4-year long-term deafness). Their weight ranged from 2.2 to 3.9 kg (one male and four females).

Experiments were approved by the local state authorities of Lower Saxony (LAVES, Oldenburg, Germany) and were performed in compliance with the guidelines of the European Community for the care and use of laboratory animals (EU VD 86/609/EEC) and the German Animal Welfare Act (TierSchG).

Animals were premedicated with 0.25 mg of atropine intraperitoneally and then anesthetized i.m. with 24.5 mg/kg ketamine hydrochloride (Ketavet; Parker-Davis) and 1 mg/kg xylazine hydrochloride (Rompun 2%; Bayer). The animals were then tracheotomized and artificially ventilated with a Ugo Basile cat ventilator (Ugo Basile, Italy). After tracheotomy anesthesia was switched to isoflurane anesthesia in a 1:2 mixture of O_2/N_2O and maintained during the remaining experiment. Adequacy of anesthesia depth and the animals' physiological state was monitored by means of ECG, heart rate, end-tidal CO_2, muscle tone, and EEG signals. End-tidal CO_2 was maintained at $< 4.5\%$. Core temperature was kept $> 37.5\ °C$ using a homeothermic blanket. Physiological state was additionally monitored by analyzing capillary blood every 12 h for blood gas concentration, pH, bicarbonate concentration, base excess, glycemia, and oxygen saturation. A modified Ringer's solution containing bicarbonate (according to the base excess) was infused intravenously. Depth of isoflurane anesthesia was monitored and adjusted accordingly, during surgical procedures around 1.5% and lowered between 0.9% and 1.3% during electrical cochlear implant stimulation.

Hearing and deaf cats previously underwent hearing screening four weeks after birth by measurement of auditory brainstem responses (ABRs). Hearing status was again confirmed immediately before cochlear implantation during the acute experiment by re-measuring ABR thresholds. For this purpose, a small trepanation was drilled at the vertex and ABR responses were recorded with an epidural silver-ball electrode

(diameter 1 mm) referenced to a silver-wire neck electrode. ABR responses to condensation clicks (50-μs duration) were measured in closed field condition with DT48 speakers (Beyerdynamics, Germany). Recordings were performed using a custom build setup (AudiologyLab, Otoconsult, Germany) and signals were amplified 10,000 times and subsequently averaged (200 repetitions, 33-Hz repetition rate). Click thresholds were < 40 dB SPL_{pe} for hearing cats, reflecting normal click thresholds in young cats (Harrison and Buchwald, 1982). Click thresholds were > 110 dB SPL_{pe} for congenitally deaf cats.

Both congenitally deaf and hearing cats were then implanted bilaterally with a cochlear implant inserted through the round window into the scalae tympani. This involved exposing both bullae and ear canals. Hearing cats were first deafened with intracochlear instillation of 300 μl of 2.5% neomycin sulfate solution over a 5-min period and subsequent rinsing using Ringer's solution. This step was performed to avoid cochleophonic electrical activation of hair cells in hearing cochleae. The custom-made cochlear implants (MED-EL, Austria) had six contacts with a distance between contacts of 1 mm and spanned the higher frequency range of the cochlea after all contacts were inserted into the cochlea.

Then the skull was opened above both sides of the suprasylvian sulcus. The trepanation exposed auditory areas of the dorsal zone (DZ lateral to the suprasylvian sulcus and the upper portion of visual areas PMLS/AMLS and the suprasylvian gyrus medial to the suprasylvian sulcus (Fig. 1A). Our intended target regions of interest were the dorsal zone on the lateral side of the suprasylvian sulcus, and the upper portion of AMLS/PMLS on the medial side of the suprasylvian sulcus. To prevent drying and cooling, the brain was covered with silicon oil during the experiment. During recording with electrodes, the brain was additionally covered and protected with agar and bone wax to dampen brain movements.

To test the functionality of the CIs and to determine the stimulation threshold, we then determined electrical ABR. eABR thresholds were measured between the epidural silver electrode to a reference in the neck (amplification 100,000×, sixth-order band-pass filter 10–10,000 Hz). Electrical brainstem responses were recorded for a biphasic pulse (200 μs/phase) at different current levels with bipolar stimulation between all possible bipolar electrode contact combinations, in order to determine the eABR threshold.

Cochlear stimulation was then performed during the experiment with a triplet of biphasic pulses (200 ms/phase at 500 pulses/s, giving a total stimulation time of 4.4 ms) applied in bipolar configuration between the basal-most electrode and the apical-most electrode of the CI. Previous to intracortical recording the eABR threshold was determined, and electrical cochlea implant stimulation level was performed at 9 levels ranging from -2 to $+6$ dB eABR threshold. Pulse levels were randomized and the interstimulus intervals were 1000 ms. Each electrical stimulus was repeated 30 times. Stimulation was performed 'wide' bipolar between
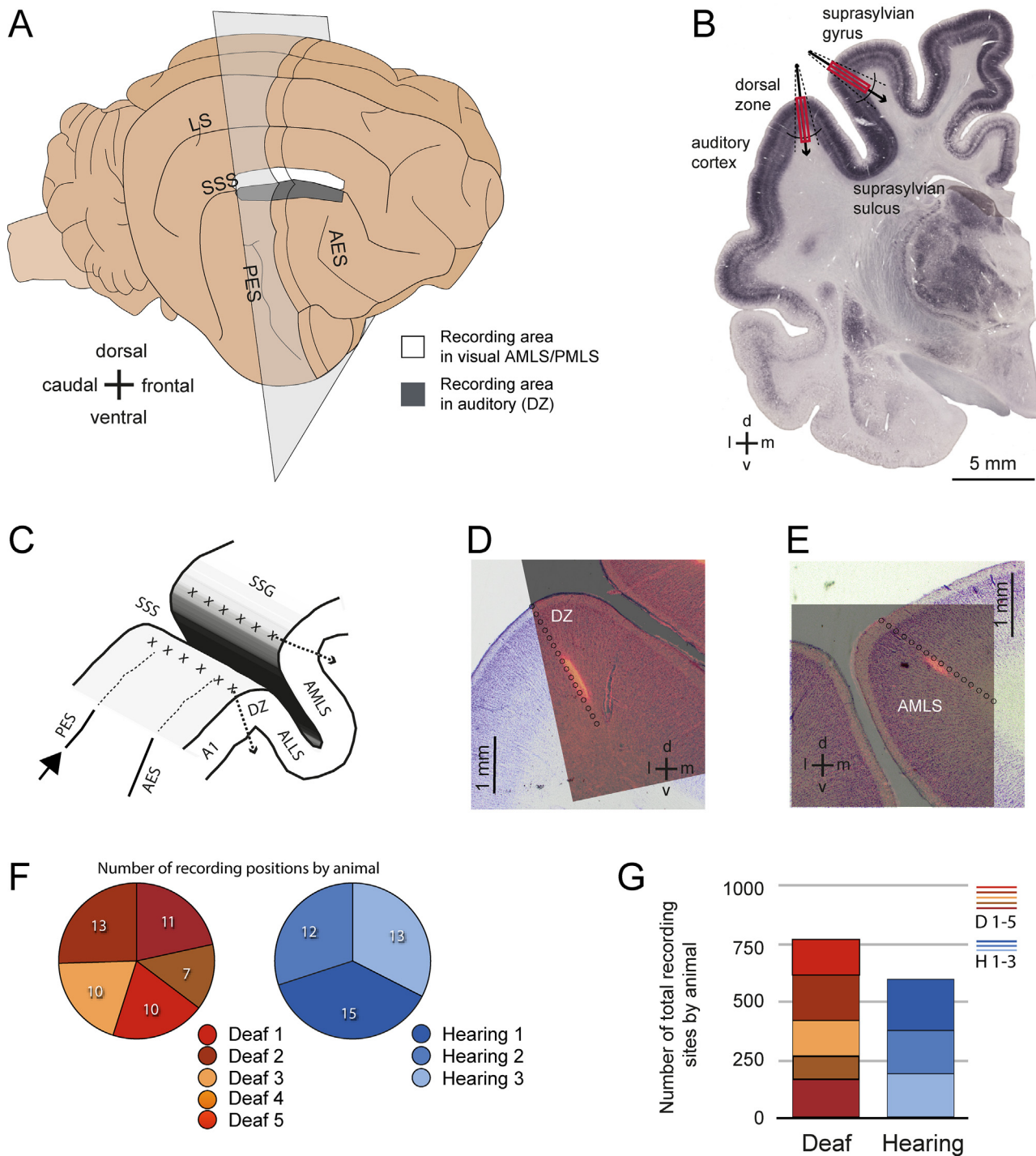
Fig. 1. Dorsal auditory zone DZ and visual lateral suprasylvian areas of the cat brain. (A) Schematic illustration of the cat brain with the regions of interest of the upper portion of visual area PMLS/AMLS (white) and the auditory dorsal zone DZ (gray). (B) Coronal slice at the plane approximately depicted in (A). Slice shows a SMI-32 staining illustrating electrode insertion angles in auditory area DZ and the visual area AMLS/PMLS in the upper portion of the suprasylvian sulcus. Arrows show intended electrode insertion angles with dotted lines depicting estimated variation in actual recordings. The red squares indicate the length of the 16-site multielectrode array electrode. (C) Illustration of mapping procedure along the suprasylvian sulcus. The posterior ectosylvian sulcus (PES) was used as a landmark, and insertion sites were intended to lie between PES and the anterior ectosylvian sulcus (AES) during the experiment. Crosses illustrate electrode positions. (D) Reconstruction of electrode track in area DZ. Nissl-stained image overlayed with fluorescence image of DiI-stained electrode track (red fluorescence). Circles depict electrode sites of the 16-site multielectrode array. (E) Reconstruction of electrode track in area AMLS. Nissl-stained image overlayed with fluorescence image of DiI-stained electrode track (red fluorescence). (F) Pie charts show the number of recording positions along the suprasylvian sulcus for each congenitally deaf (red) and hearing cat (blue). (G) Stacked bar charts for the numbers of respective total recording sites within each animal.

cochlea implant contact 1 and 6, thus stimulating a large portion of the ganglion cells of the auditory nerve.

Neuronal activity was recorded with linear multisite electrode arrays (1 × 16, site distance 150 μm, site area 177 μm², NeuroNexus, USA), which were inserted perpendicular to the cortical curvature to span all cortical layers (Fig. 1B). Electrode shanks for the dorsal auditory zone were inserted with an angle deviating 0 ± 15° from the vertical. For the DZ electrode, the angle was chosen in order to record in area DZ, which lies lateral to the suprasylvian sulcus, but to avoid recording from the adjacent visual area ALLS/PLLS in the lateral bank of the suprasylvian sulcus. The electrode shanks in the lateral suprasylvian gyrus were inserted at a deviation of 45 ± 15° from the vertical. This angle was chosen to avoid recordings too deep in the medial bank for the electrode in AMLS/PMLS. This was intended in order to avoid far-field effects and an overlay of ALLS/PLLS and AMLS/PMLS responses within the banks of the suprasylvian sulcus. The penetration angle was kept constant throughout the experiment. The last penetration in each investigated area was stained using DiI (Invitrogen). Distance to the midline of the suprasylvian sulcus was 0.5 mm on both sides. Electrode shanks were then inserted so that the top most electrode was just at the cortical surface, thus reaching an insertion depth of around 2500 μm. The investigated regions were subsequently mapped along the suprasylvian sulcus, using the posterior ectosylvian sulcus (PES) and the anterior ectosylvian sulcus (AES) as a landmark (Fig. 1C). We tried to ensure equal spacing between recording positions to allow for an unbiased sample of positions along the suprasylvian sulcus (Fig. 1C).

We recorded LFP signals with a Neuralynx system (Neuralynx, USA) using a wide-band filter 1–10,000 Hz and a sampling rate of 30303 Hz and 50,000–100,000× amplification. We recorded LPF activity, as electrical artifacts of CI-stimulation interfere less with LFP responses than with spike extraction, and also it is more sensitive for sampling weak modulation of population activity in the visual AMLS/PMLS elicited by auditory stimuli (Meredith et al., 2009; Haider et al. 2016). The recorded signals were down-sampled to 1000 Hz and digitally filtered between 1 and 250 Hz with a 4th-order butterworth filter using MATLAB (R2013a; The Mathworks Ltd., Natick, MA). To eliminate far-field effects in the evoked LFP we used bipolar derivation of the LFP signal, performed by subtraction of signals on the neighboring channels. This finally yielded in 15 sites for each electrode penetration. Signals were rectified and subsequently corrected for baseline shifts.

For analysis of responses, stimulus repetitions were averaged and overall responses were determined as activity within the first 80 ms post-stimulus exceeding a threshold of 3.5 standard deviations (SDs) above pre-stimulus baseline for a minimum time period of 5 ms. The insertion position of a 16-site electrode shank was defined as responsive, if at least one electrode site was responding to stimulation within the electrode shank. Here, position refers to the insertion site to the 16-site

electrode shank, whereas electrode site refers to the electrode site along one respective 16-site electrode shank. Responses for all electrode sites were calculated for all nine stimulus levels. To extract information on amplitudes and latencies, a semi-automatic approach was used to account for the observed small-signal amplitudes in AMLS/PMLS. Herein, first a time window between 9 and 16 ms was defined based on clearly identifiable activity templates in the data. For reasons of objectiveness, an automatic approach was implemented to preselect recording sites that show onset activity within this time range exceeding a conservative threshold of 3.5 SD above pre-stimulus baseline. The large number and strength of responses in the dorsal auditory zone allowed for a fully automatic approach to select responses in the same time range, without correcting visually for false-positive responses. For sites in the lateral suprasylvian gyrus, the preselected activity was subsequently confirmed visually as single peak within the given time range.

Latencies in AMLS/PMLS were defined as the peak latency of the respective evoked LFP response. Latencies in DZ were defined as the latency at 50% of the maximum evoked LFP response. DZ responses were elongated and considerably larger than in AMLS/PMLS, thus the peak of the LFP responses was in many cases delayed (see Fig. 4A). Therefore, for DZ responses we chose the 50% maximum response latency of the evoked DZ response to provide a better estimate for comparison with the response latency in AMLS/PMLS (compare Fig. 2A and 4A).

Statistical analysis was carried out in IBM SPSS Statistics (version 24.0) and with the Matlab Statistics Toolbox (Matlab 2017a, 2017b, Mathworks). Non-parametric, two-sided Wilcoxon's rank-sum tests were applied. Exact p-values are reported, where possible and r is denoted as an estimator of effect-size, in case of significant results. For testing stimulation–intensity–response relationships we applied a Kruskal–Wallis test, and report chi-square values, degrees of freedom and p-values in the results section. In those cases, where we pooled data from all animals in one group (i.e., more than one data point per animal), it is important to note that statistical results do not generally apply to the overall population, but specifically to the animals studied in the current work.

After the experiment, the animals were transcardially perfused. After thoracotomy, 0.5 ml of heparin (Liquemin; Hoffman-La Roche) was injected into the left ventricle. Then, 2 L of phosphate buffer (0.1 M, pH 7.4) and 2 L of fixative (2.5% glutaraldehyde and 2.0% formaldehyde) were infused transcardially with pressure > 100 mmHg. After 24 h of postfixation in 4% formaldehyde, the brain was excised from the skull, photographed, and a block containing the investigated cortical areas was cryoprotected in 30% sucrose, frozen, and cut in frontal plane in 50-μm sections using a cryotome (Leica). The sections were first photographed in fluorescence mode to reveal the DiI (Keyence, BZ-9000). Subsequently, the sections were alternatively stained with Nissl and antibodies against
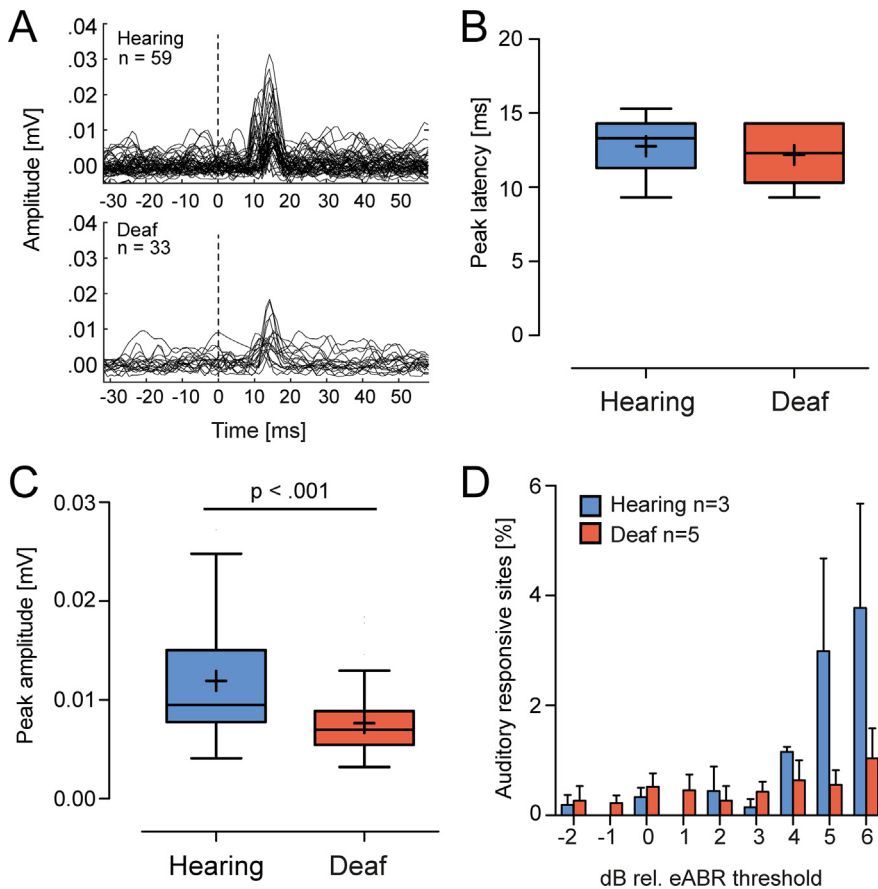
Fig. 2. Differences in auditory responsiveness in visual area AMLS/PMLS between hearing and congenitally deaf cats. (A) Examples of evoked rectified LFP responses in hearing and congenitally deaf cats in visual area AMLS/PMLS. LFP signals were the bipolar derived signals from two neighboring electrode contacts in order to exclude far-field effects from more distant regions. (B) Tukey's boxplots show peak latency of evoked LFP responses in lateral suprasylvian gyrus to a cochlear implant electric pulse. (C) Tukey's boxplots show amplitude of evoked LFP responses in lateral suprasylvian gyrus to a cochlear implant electric pulse. (D) Stimulus level–response relationship depicting number of responsive sites in relation to stimulus intensity for hearing (blue) and congenitally deaf cats (red). Y-axis shows percent of total sites responsive to cochlear implant electrical stimulation in lateral suprasylvian gyrus at increasing intensities. Error bars denote standard error of the mean.

SMI 32 (Mellott et al., 2010). The stained sections were then digitized (Keyence, BZ-9000). In order to reconstruct electrode positions, the DiI-stained penetrations were combined with photographs of Nissl-stained sections (Fig. 1D, E). Using Nissl staining and SMI-32 staining we identified the recording positions being located in the targeted cortical areas DZ and AMLS/PMLS. The areas AMLS and PMLS were not further differentiated due to frontal sectioning. The deepest recording sites were in few penetrations localized in white matter (Fig. 1); however, the exclusion of these sites was in most cases problematic due to bipolar derivation (thus one site in the white matter does not preclude responsiveness). To avoid biasing the results by exclusion based on histology, and due to the fact that bipolar derivation sites within white matter did not reveal any response, these are included in the non-responsive sites.

RESULTS

We studied cochlear implant evoked LFP activity in the visual area AMLS/PMLS of hearing ($n = 3$) and congenitally deaf cats ($n = 5$). We used bipolar cochlear implant stimulation at different intensity levels ranging from -2 dB to $+6$ dB above the eABR threshold. In total this resulted in a sample of 51 multielectrode array penetrations along the suprasylvian sulcus in the congenitally deaf cats and 40 penetrations in the hearing cats (Fig. 1F). This resulted in 765 electrode recording sites in the deaf and 600 recordings sites in the hearing cats (Fig. 1G). These numbers describe simply the total number of recording sites within the brain after insertion of the electrode shanks, without any exclusion of electrodes by location or responsiveness. Before further analyses, bipolar derivation between neighboring electrode sites of the electrode array was performed to limit LFPs to localized population activity and remove far-fields.

We found auditory evoked LFP responses to cochlear implant stimulation in both hearing and congenitally deaf cats (Fig. 2A). Responses to cochlear implant stimulation in AMLS/PMLS had similar latencies in hearing and congenitally deaf cats (Fig. 2B). Responses in hearing cats had mean peak latencies of 12.8 ms (1.9 SD, $n_{sites} = 57$) and in congenitally deaf cats had mean peak latencies of 12.2 ms (1.8 SD, $n_{sites} = 33$), with no significant latency differences between groups (Wilcoxon's rank-sum test, two-sided, asymptotic significance, $Z = -1.453, p = .146$).

However, evoked LFP responses in congenitally deaf cats were fewer and smaller in amplitude than in hearing cats (Fig. 2A, C), with a mean of 12 μV (6 SD, $n_{sites} = 57$) in the hearing cats in comparison to a mean of 8 μV and (4 SD, $n_{sites} = 33$) in the congenitally deaf cats (Wilcoxon's rank-sum test, two-sided, asymptotic significance, $Z = -4.102, p < .001, r = .43$). Auditory evoked responses in AMLS/PMLS of hearing cats were level dependent with a high threshold in relation to the eABR threshold (Fig. 2D). A stimulus level-dependent modulation was not present in congenitally deaf cats. Increasing stimulation level had a significant effect on the number of responses in hearing cats (Kruskal–Wallis, $\chi^2(8) = 19.22, p = .0013$), but not in congenitally deaf cats (Kruskal–Wallis, $\chi^2(8) = 3.94, p = .0.86$). These results indicate a difference in

A

B

C
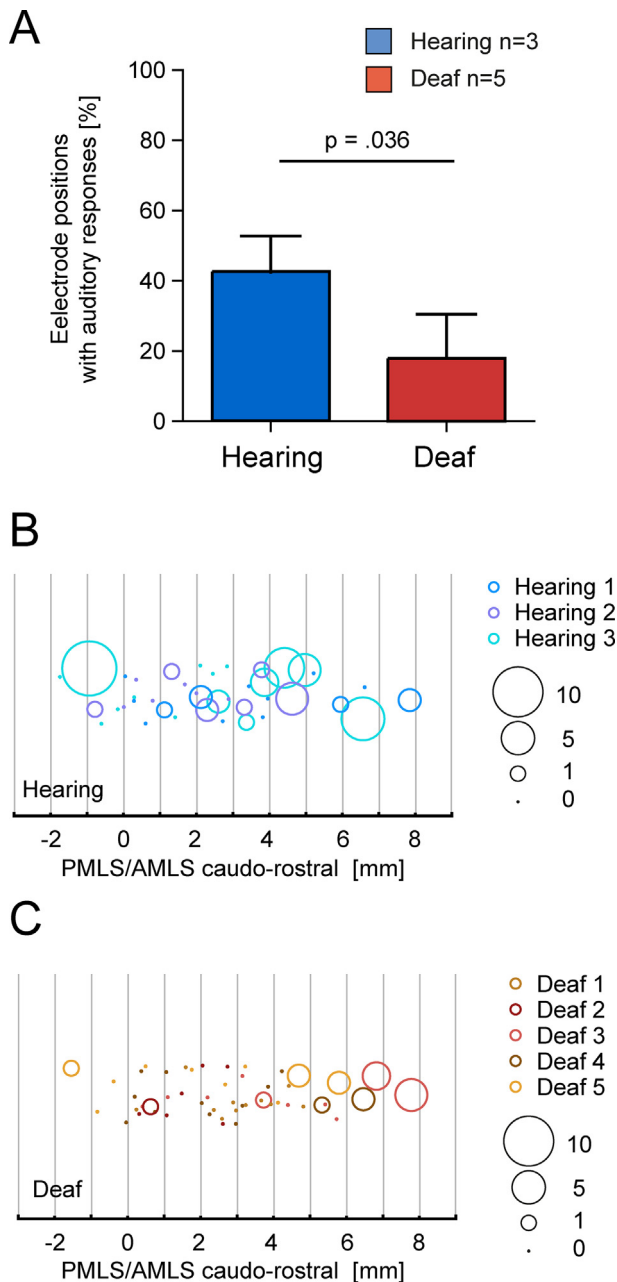
Fig. 3. Congenital deafness reduces, but does not eliminate auditory responsiveness in visual area AMLS/PMLS. (A) The bar chart shows a decrease in number of recording positions within PMLS/AMLS with responses to cochlear implant stimulation in deaf cats (mean; error bars depict standard deviations). (B) Distribution of electrode positions within area PMLS/AMLS, which were responsive to auditory stimulation in hearing cats. Circle size depicts the total number of the electrode sites along the 16-site shank at this recording position which showed significant responses. Recordings were performed in PMLS/AMLS along the caudo-rostral axis along the suprasylvian sulcus. X-axis depicts distance of the posterior–anterior position from the posterior suprasylvian sulcus (PES) used as an anatomical landmark during the recordings. Zero denotes the location of the tip of PES. Circles are randomly scattered in the y-axis direction to enhance visibility. Circle size depicts the total number of the electrode sites along the 16-site shank at this recording position which showed significant responses. Responses are shown for all pooled intensities. (C) Same as in (B) for congenitally deaf cats.

auditory responsiveness in AMLS/PMLS between the animal groups.

When pooling all responsive sites among stimulus intensities in one recording position, in hearing cats 42.4% (10 SD, n_{cats} = 3) of electrode recording positions in AMLS/PMLS showed responses in comparison to 17.9% (12 SD, n_{cats} = 5) in congenitally deaf cats (Fig. 3A). Thus, in AMLS/PMLS of congenitally deaf cats fewer penetration sites were auditory responsive than in hearing cats (Wilcoxon's rank-sum test, two-sided, exact significance, $Z = -2.249$, $p = .036$, $r = .8$). The spatial distribution of responses along the suprasylvian sulcus in AMLS/PMLS was equally spread in hearing cats, and had a tendency to be restricted to rostral positions in congenitally deaf cats (Fig. 3B, C).

In parallel to recording from AMLS/PMLS, we measured auditory evoked LFP responses to cochlear implant stimulation in the dorsal auditory area DZ (Fig. 4A). Between hearing and congenital deaf cats, the latencies and amplitudes of responses in the dorsal auditory zone were similar in absolute terms (Fig. 4B, C). For the highest stimulation level the half-peak response latencies were 12.7 ms (SD 3.2, n_{sites} = 372) in hearing cats and 11.9 ms (SD 2.4, n_{sites} = 477) in congenitally deaf cats (Wilcoxon's rank-sum test, two-sided, asymptotic significance, $Z = -4.887$, $p < .001$, $r = .17$). For the highest stimulation level mean response amplitudes were 52 µV (SD 46, n_{sites} = 372) and 61 µV (SD 56, n_{sites} = 477) in hearing and congenitally deaf cats respectively. Significant differences between groups for both latencies and amplitudes in the dorsal auditory zone were present, but effect size was small (Wilcoxon's rank-sum test, two-sided, asymptotic significance, $Z = -2.275$, $p = .022$, $r = .08$). In the auditory dorsal zone, both hearing and congenitally deaf cats showed a stimulus level-dependent increase in number of responses (Fig. 4D). Increasing stimulation level increased number of responses in hearing cats (Kruskal–Wallis, $\chi^2(8) = 19.88$, $p = .0010$), and also in congenitally deaf cats (Kruskal–Wallis, $\chi^2(8) = 21.61$, $p = .0005$).

DISCUSSION

We observed auditory evoked LFP activity in the visual area AMLS/PMLS in both hearing and congenitally deaf cats. Responses in AMLS/PMLS had similar latencies to responses in the dorsal auditory zone DZ, but had a generally smaller amplitude. Importantly, auditory responses in visual areas AMLS/PMLS of congenitally deaf cats were reduced in number and amplitude in comparison to hearing cats. This indicates the role of auditory experience in the development or maintenance of these responses.

Previous reports described auditory responsive neurons within the visual areas of the suprasylvian sulcus in hearing and blind cats (Allman and Meredith, 2007; Yaka et al., 1999, 2002; Meredith et al., 2009). Here
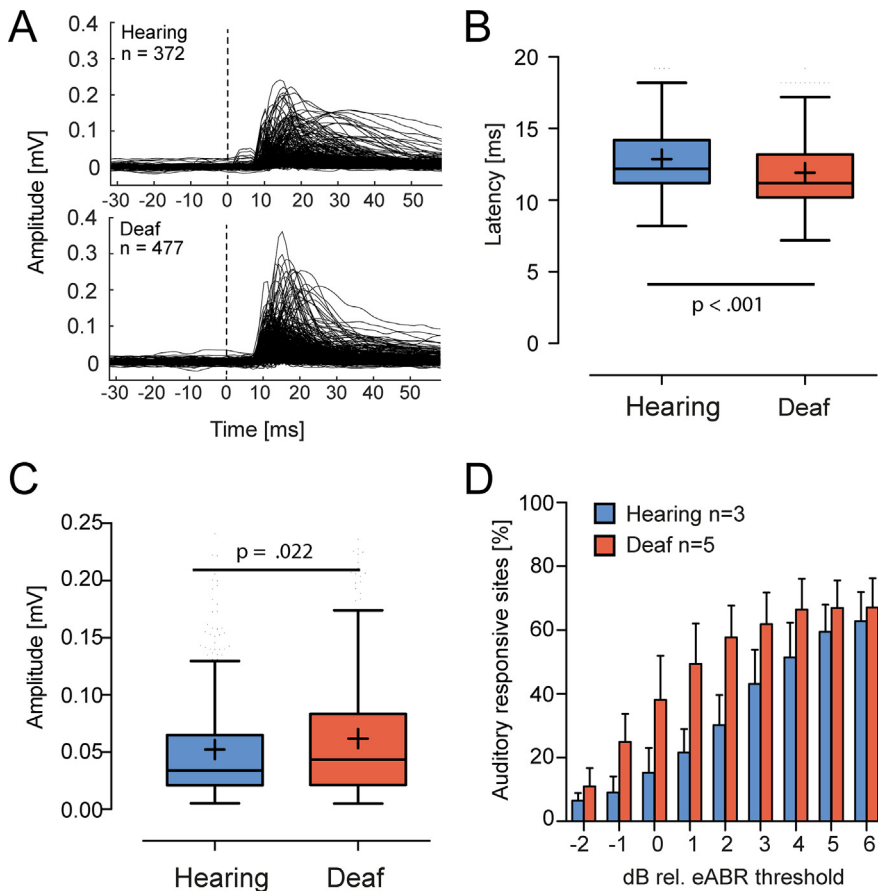
Fig. 4. Auditory response characteristics in the dorsal auditory zone (DZ) of hearing and congenitally deaf cats. Example of evoked rectified LFP responses in hearing and congenitally deaf cats in dorsal auditory zone DZ. (B) Tukey's boxplots show 50%-peak latency of evoked LFP responses in auditory dorsal zone DZ to a cochlear implant electric pulse. (C) Tukey's boxplots show amplitude of evoked LFP responses in auditory dorsal zone to a cochlear implant electric pulse. (D) Stimulus level–response relationship depicting number of responsive sites in relation to stimulus intensity for hearing (blue) and congenitally deaf cats (red). Y-axis shows percent of total sites responsive to cochlear implant electrical stimulation in auditory dorsal zone at increasing intensities. Error bars denote standard error of the mean.

we replicated these findings and described the presence of CI-evoked auditory LFP responses in the visual areas AMLS/PMLS in hearing and deaf cats. Using local field potentials (bipolar derivation) we exploited a sensitive method for sampling responsiveness, since it is a measure of postsynaptic activity irrespective of whether it exceeds the firing thresholds or not. The responses were of small amplitude. Weak auditory responses are consistent with weak anatomical projections from auditory regions to these fields (Barone et al., 2013). Furthermore, in terms of numbers of responsive sites, our results in hearing controls are in line with previous findings in PMLS/AMLS of hearing cats (Yaka et al., 1999, 2002). The small amplitudes of CI-evoked activity in AMLS/ PMLS may further reflect increased thresholds in this area. Peripheral stimulation efficacy, however, cannot be made responsible for the small amplitudes in AMLS/ PMLS for auditory stimulation, since cochlear-implant evoked activity in the auditory dorsal zone DZ was strong in both hearing and congenitally deaf cats (Fig. 4). However, the difference in strength of the CI-evoked responses between auditory DZ and visual AMLS/PMLS

might at least theoretically be influenced by a difference in sensitivity to anesthesia between both areas.

In congenitally deaf cats, auditory responsiveness in visual AMLS/ PMLS was reduced in comparison to hearing cats. Bias due to anesthesia and general brain state changes can be excluded as a reason for this observation, since simultaneous DZ and AMLS/PMLS recordings were performed. Whereas evoked activity in the auditory dorsal zone DZ was similarly strong in both hearing and congenitally deaf cats, in AMLS/ PMLS it was not. This suggests that the observed differences between hearing and deaf cats in AMLS/ PMLS reflect a true difference due to hearing experience.

The auditory inputs that drive auditory responses in AMLS/PMLS can stem from thalamic (Kok and Lomber, 2017) and/or cortico-cortical connections (Barone et al., 2013). Considering the similarity of latencies in AMLS/PMLS and auditory responses in DZ, a common subcortical thalamic origin of input to both regions is possible. However, direct cortico-cortical connections from auditory cortex neurons with fast onset latencies would also be compatible with the observed latencies. For example, tracing studies in hearing animals have shown corresponding connection patterns from the dorsal auditory zone to ALLS (Clemo et al., 2008; Barone et al., 2013). Similar connections toward AMLS/PMLS are weaker (Barone et al., 2013). Interestingly, response latencies for hearing and for deaf in both AMLS/PMLS and DZ in the present study were very similar. This suggests that changes in levels of myelination cannot be made responsible for the present findings in deaf animals. It was further interesting that stimulus intensity had an effect on the number of responsive sites in hearing cats, however it did not in congenitally deaf cats in AMLS/ PMLS.

On the other hand, reciprocal projections from visual to auditory cortex have been shown to originate in PLLS and target the dorsal auditory zone, as well as few originating from ALLS and AMLS/PMLS (congenitally deaf cats: Barone et al., 2013; early deafened cats: Kok et al., 2014). An overlap of cross-modal cortico-cortical or thalamico-cortical connections may exist with a gradient across adjacent sensory regions. From the change in responsiveness one might infer that these connections are modulated in deaf or blind cats in dependence of the type of deprivation, with an increase in the amount of auditory responsive neurons in blind cats (Yaka et al.,

1999, 2002), and a decrease in congenitally deaf cats, as shown in the present study. During visual cross-modal plasticity in auditory regions in deaf cats, a similar process might take place as in blind cats, but in reversed form. A local overlap of cross-modal connections in the border regions may be masked or unmasked when one sensory system is impaired during development. In the normal hearing case these connections are in equilibrium shaped by activity from both modalities.

The question remains whether the auditory responses in the visual areas would serve a functional role in cats. Within the suprasylvian sulcus, visual areas AMLS/PMLS and ALLS/PLLS are regions with large receptive fields, and area AMLS/PMLS has been hypothesized to be related to motion processing (Dreher et al., 1996). The auditory dorsal zone has been related to processes of motion detection and auditory spatial perception (Stecker et al., 2005; Lee and Middlebrooks, 2013). Along the visual representation in the lateral bank of the suprasylvian sulcus (ALLS/PLLS), auditory activation of neurons predominantly occurs in neurons whose receptive fields represent the visual periphery while auditory facilitation occurs in those neurons representing more central visual field locations. These observations demonstrated an auditory functional as well as anatomical gradient within the lateral part of the suprasylvian visual area (Allman and Meredith, 2007). However, a corresponding study in the medial section of the suprasylvian visual area in AMLS/PMLS has so far not been performed. The presence of cross-modal responses may play a role in this interaction and processing. On the other hand, the responses may be a byproduct of a fuzzy overlap of cross-sensory connections in the border areas between distinct sensory regions, with the presence of cross-modal responses in close sensory areas being a developmental byproduct with no functional role. It might be argued that cross-sensory connections are simply developmental remains and were maintained by chance. In that case however, it is not clear how that is compatible with activity dependent mechanisms of plasticity and experience based maturation.

Congenital deafness, and thus the absence of auditory experience during early development appears to affect both sides of the border between visual and auditory areas. On the visual side, it reduces the presence of cross-modal auditory responsiveness in visual lateral suprasylvian areas. On the auditory side, deafness leads to an increase in visual modulation by cross-modal plasticity in the deaf auditory dorsal zone (Land et al., 2016). The observed effects appear to be small for both sides, especially in consideration that the studied deaf cats had a year-long absence of hearing. On the auditory side, congenital deafness appears to lead to modest cross-modal visual reorganization in the auditory dorsal zone (Kok et al., 2014; Land et al., 2016), the auditory dorsal zone itself remains primarily conserved in topology, thalamic-cortical connectivity patterns (Kok and Lomber, 2017) and in terms of basic auditory responsiveness to simple stimuli (Land et al., 2016). Also the cytoarchitectonic characteristics of the dorsal auditory zone are conserved in congenital deafness (Berger et al.,

2017). Although some shift in areal borders in congenital deafness have been described (Wong et al., 2014), areal expansion into adjacent deprived sensory areas does not seem to be a mechanism for cross-modal plasticity (Meredith et al., 2017). In that sense the broad parcellation of cortex is independent of active auditory thalamic input (Rubenstein et al., 1999; Sur and Leamey, 2001),

With respect to clinical intervention with cochlear implants, the question remains how the strength of adaptations in the congenitally deaf brain interferes with the restoration of hearing with cochlear implants. Effects on cross-sensory interplay of congenital, early and late deafness have been also observed in brain imaging in humans (Bottari et al., 2014, 2010; Lee et al., 2007, 2001; Sandmann et al., 2012; Nava et al., 2014). Cortical plasticity as adaptation to congenital or early deafness may influence the clinical outcome of hearing restoration with cochlear implants later in life (Wilson and Dorman, 2008; Kral and Sharma, 2012). Here we here found effects of deafness on auditory responsiveness in visual areas which might preclude normal audio-visual integration after restoration of hearing with cochlear implants. However, we also found that the difference between hearing and congenitally deaf areas were small in the investigated area. It will be a question for further studies, how strength and variance of such adaptive neural effects may be influenced by different individual behavioral compensation strategies during development in congenital deafness.

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