

Postnatal Cortical Development in Congenital Auditory Deprivation

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The study investigates early postnatal development of local field potentials (LFPs) in the primary auditory cortex of hearing and congenitally deaf cats. In hearing cats, LFPs elicited by electrical intracochlear stimulation demonstrated developmental changes in mid-latency range, including reductions in peak and onset latencies of individual waves and a maturation of their shape and latencies during the first 2 months of life. In long latency range (> 80 ms), the P₁/N₁ response appeared after the fourth week of life and further increased in amplitude and decreased in latency, reaching mature shapes between the fourth and sixth months after birth (p.n.). Cortical activated areas became increasingly smaller during the first 3 months of life, reaching mature values at the fourth month p.n. The layer-specific pattern of synaptic activity matured 4 months p.n. In congenitally deaf cats, the developmental pattern was different. The lowest cortical LFP thresholds were significantly smaller than in hearing controls, demonstrating a 'hypersensitivity' to sensory inputs. The development of N_b waves was delayed and altered and the long latency responses became smaller than in controls at the second and third months. The activated areas remained smaller than in controls until the third month, then they increased rapidly and exceeded the activated areas of age-matched controls. From the fourth month on, the activated areas decreased again and smaller synaptic currents were found in deaf cats than in controls. The presented data demonstrate that functional development of the auditory cortex critically depends on auditory experience.

Keywords: auditory cortex, cochlear implant, deafness, deaf white cats, hearing loss

Introduction

In postnatal development the nervous system interacts with the environment, leading to the question of epigenetic influences on postnatal development (Changeux and Danchin, 1976). While early modifications of acoustic environment certainly influence functional properties in the auditory cortex (Harrison *et al.*, 1993; Stanton and Harrison, 1996, 2000; Zhang *et al.*, 2001, 2002; Chang and Merzenich, 2003), it remained unclear whether postnatal development is intrinsically guided by experience or whether it is a consequence of a genetically pre-programmed process that can only be modified by 'unusual' experience. A previous study from our laboratory has shown that cortical circuits are deficient after congenital deprivation (Kral *et al.*, 2000). In the present study we address the question of whether these deficits are a consequence of degeneration or abnormal maturation. This question has gained clinical interest as in congenital deafness treatment by cochlear implants has become available.

The human cochlea functions already during intrauterine periods (Granier-Deferre *et al.*, 1985; Rubel, 1985). This makes

it impossible to precisely monitor auditory input in man: newborns can be deaf but could still have gained prenatal hearing experience. Altricial animals allow a more precise control of environmental inputs. In the cat the external meatus, middle ear and cochlea are not fully developed at birth. Newborn kittens are deaf and the hearing thresholds reach 100 dB SPL first at 10 days after birth (p.n.) (for a review, see Brugge, 1992). Cats reach sexual maturity with 6 months. During these 6 months many developmental changes, corresponding to the human postnatal development, can be traced in their auditory system (Aitkin and Moore, 1975; Brugge *et al.*, 1978, 1981, 1988; Eggermont, 1996).

To investigate postnatal maturation in completely deaf animals, congenitally deaf cats (Mair, 1973) were used in the present study (referred to as deaf cats throughout this paper). These animals do not have any hearing experience (Heid *et al.*, 1998) due to an inherited dysplasia of the organ of Corti. In contrast to pharmacological deafening, the spiral ganglion cells survive in large numbers. In the basalmost halfturn of the cochlea, where cochlear implantation is possible in cats, there is no significant reduction in spiral ganglion cell counts up to the age of 2 years (Heid *et al.*, 1998). Consequently, deaf cats represent the optimal model for cochlear implant studies of congenital deafness, as central input is not significantly affected by a degeneration of the auditory nerve.

Previous studies on deaf cats demonstrated that the gross connectivity of the afferent auditory system shows no significant alterations (Heid *et al.*, 1997) despite significant dystrophic changes in brainstem nuclei (Heid, 1998; Larsen and Kirchhoff, 1992; Saada *et al.*, 1996). In the field A1 of the auditory cortex, a rudimentary cochleotopy could be functionally demonstrated (Hartmann *et al.*, 1997). However, the pattern of cortical activation with electrical stimulation of the auditory nerve demonstrated deprivation-induced deficits in activation of infragranular layers, an increase in latencies of cortical responses and substantial reductions of synaptic activity at latencies > 30 ms post-stimulus (Kral *et al.*, 2000). These deficits were reversible by biologically meaningful chronic electrostimulation through cochlear implants (Klinke *et al.*, 1999). The plasticity in the auditory cortex thereby showed a sensitive period (Kral *et al.*, 2001, 2002).

In the present study cortical responses to cochlear implant stimulation in young congenitally deaf kittens were compared with age-matched cochlear-implanted and electrically stimulated hearing controls.

Materials and Methods

Animals

Ten congenitally deaf and ten hearing cats were used, ages ranging from 1 month p.n. to adult. Deafness of the deaf cats was verified using

auditory-evoked brainstem responses to clicks and tone-pips of intensities up to 125 dB SPL (for details see Heid *et al.*, 1998). Hearing controls were acutely deafened by intrascalar application of neomycin at the beginning of the experiment (details in Hartmann *et al.*, 1984). Complete deafness was verified by the absence of brainstem evoked potentials within 5 min after instillation of neomycin.

Cat Cochlear Implant

The implant consisted of a medical-grade silicone tube with 6 electrical contacts. There were five intrascalar gold contacts: a small ball at the tip (diameter 0.8 mm) and 4 rings, with a distance of 1 mm between the electrodes (Behrendt, 1999; Klinke *et al.*, 1999). An indifferent electrode was located extracochlearly at the region of the neck. The stimulation mode was monopolar, always with the apicalmost electrode.

Experimental Procedure

For the experiments the animals were premedicated with 0.25 mg atropine i.p. and initially anaesthetized with ketamin hydrochloride (Ketavet, Parker-Davis, Germany, 24.5 mg/kg) and propionylpromazine phosphate (Combelen, Bayer, Germany, 2.1 mg/kg). The animals were then tracheotomized and artificially respired with 50% O₂ and 50% N₂O, with the addition of 0.2–1.0% isoflurane (Lilly, Germany) to maintain a controlled depth of anesthesia (Kral *et al.*, 1999). End-tidal CO₂ was monitored and kept below 4%, and the core temperature was kept constant between 38 and 38.5°C using a homeothermic blanket. A modified Ringer's solution containing glucose was infused i.v. Both bullae and ear canals were exposed. The head of the animal was then fixed in a stereotactic holder (Horsley-Clark). In order to record electrically evoked auditory brainstem responses (EABRs), a silver-ball electrode (diameter 1 mm) was attached epidurally at the vertex. The indifferent electrode used for the recordings was positioned medially in the mouth or neck, the indifferent electrode for stimulation was inserted medially in the neck muscles. Trephination was performed above the auditory cortex and the dura was opened. The cortex was photographed for documentation of the recording positions.

For stimulation, charge-balanced pulses (200 μs/phase, repetition rate 2 Hz) were applied to the cochlear implant (monopolar stimulation). Recordings were started ~4 h after the onset of anesthesia. Using an x-y-z micromotor (enabling movements in all three directions with a precision of 1 μm), a silver-ball macroelectrode (diameter 1 mm) was positioned at nine cortical positions on the primary auditory field A1 defined by anatomical landmarks (anterior and posterior ectosylvian sulcus, superior sylvian sulcus, Fig. 1A) to determine the lowest cortical threshold. Signals were preamplified (Tektronix V122, filters 0.01–10 kHz, 6 dB/oct.), amplified at a second stage (Tektronix 5A22N, filters 0.01–10 kHz, 6 dB/oct.) and averaged (50 sweeps, repetition rate 1.97 Hz). These recordings determined the minimal stimulation current required to evoke a cortical response at one or more of the recording positions (threshold current) with a precision of ±1 dB. In order to localize precisely the A1 field functionally and to determine the extent of the cortical activated region, the cortex was mapped using a Ringer-filled glass-microelectrode (Z = 6 MΩ). Field potentials on the cortical surface were recorded at 100–150 cortical positions (Fig. 1B; stimulation 10 dB above the lowest cortical threshold determined with the macroelectrode). Amplitudes of mid-latency responses (peak to baseline) were used to construct cortical activation maps (programmed in MatLab v. 13, © MathWorks).

Brain size increases with age. Therefore, to compare the extent of the primary auditory cortex (field A1) activated by cochlear electrostimulation (**activated area**) between different animals, the determined area of the cortex with responses > 300 μV was normalized using the formula

$$\text{Activated area [r.u.]} = \frac{\text{Area [mm}^2\text{]}}{\text{Weight}^{\frac{2}{3}}[\text{kg}]}$$

This formula reflects the relation of the surface area to the volume in a sphere and approximates the relation of body weight and brain surface (Kral *et al.*, 2002). Developmental changes in activated areas were statistically analyzed using cluster analysis (hierarchical clustering and K-means clustering, Euclidean distance) using SyStat v. 9 (© SPSS Inc.).

After determining the cortical activation map, a region of interest (ROI) of the dimension of 1 mm² was defined at the most activated

region of A1. Here, a regular grid of 2 × 3 positions was defined. The cortex was penetrated at each recording position, with the electrode directed perpendicularly to the cortical surface using a stereotactic system. Local field potentials were recorded every 150–300 μm to a depth of 3600 μm. Electrode location was controlled by an ORIEL x-y-z positioning device (precision 1 μm). To avoid tangential tracks, recordings near the sulci were avoided. One to two penetrations per animal were marked with iontophoretic application of horseradish peroxidase (2 μA, 10 min). The six LFPs recorded at the cortical surface were used to compute a mean LFP in ROI.

After the experiment, the animals were transcardially perfused (details in Heid *et al.*, 1998). The brains were cut in 100 μm thick sections and stained with diaminobenzidine (LaVail and LaVail, 1972; Mesulam, 1976) and by the Nissl method. For determination of cortical layers, the angle of the track to the perpendicular direction with respect to cortical microcolumns was defined as the deviation angle. Histological track reconstruction of stained penetrations was performed (Fig. 1C) and cortical layers were assigned to recording depths. The shrinkage was compensated for (details in Kral *et al.*, 2000).

Current Source Density Analysis

One-dimensional current source densities were computed off-line from the recorded field potentials using custom-made software programmed in MatLab v. 13. The one-dimensional CSD represents the second spatial derivative of the field potentials in the track direction and corresponds to the sum of all synaptic currents near the given recording position (Mitzdorf, 1985). The method eliminates remote generators of LFPs. The amplitudes of the CSD signal are given in mV/mm², as a constant resistivity is assumed and omitted in the computation (Mitzdorf, 1985). The sinks in the CSD reflect inward currents which flow during synchronous activation of groups of synapses (excitatory currents), whereas the sources correspond to outward currents (passive return currents or inhibitory currents). $\phi_{(t)}^d$ represents the potential amplitudes recorded at cortical depth d with latency t (Fig. 1C). Δd represents the distance between the recordings of field potentials in the cortex (150–300 μm, see above). The CSD can then be computed according to formula

$$CSD_t^d = \frac{\phi_t^{d-\Delta d} - 2*\phi_t^d + \phi_t^{d+\Delta d}}{\Delta d^2}$$

The one-dimensional CSD signal represents mainly synaptic currents from vertically oriented cells, predominantly from pyramidal cells (Muller-Preuss and Mitzdorf, 1984). Using the histological track reconstruction, individual current source densities could be assigned to individual cortical layers (Fig. 1C). Sinks were shaded in the figures and quantitatively evaluated. A custom-made software programmed in MatLab identified the peak amplitudes of individual CSD waves automatically and statistic processing was performed on these amplitudes (details in Kral *et al.*, 2000).

The general status of the animals during the experiment was carefully controlled (Kral *et al.*, 1999). It remained stable within the time when data were collected (20–24 h after initiation of anesthesia).

Results

An auditory cortical local field potential (LFP) recorded with a macroelectrode consisted of several waves whose designation is shown in the inset of Figure 2. Largest responses were found in the mid-latency range, with P_a showing highest amplitudes in the primary auditory cortex. Amplitude-intensity functions of P_a waves in deaf cats and hearing controls were saturating, with a dynamic range of ~ 6 dB (Fig. 2). However, the lowest cortical threshold was significantly lower in deaf cats (−30.4 ± 2.3 dB re 3 mApp) than in hearing controls (−25.2 ± 3.6 dB re 3 mApp; two-tailed *t*-test, *P* = 0.006). This result was concluded to be due to a difference at the cortico-thalamic level, as the electrically-evoked brainstem responses did not differ between these animals (deaf: −29.4 ± 4.1 dB; controls: −28.5 ± 4.6 dB; two-tailed *t*-test, *P* = 0.881). Interestingly, the brainstem evoked response

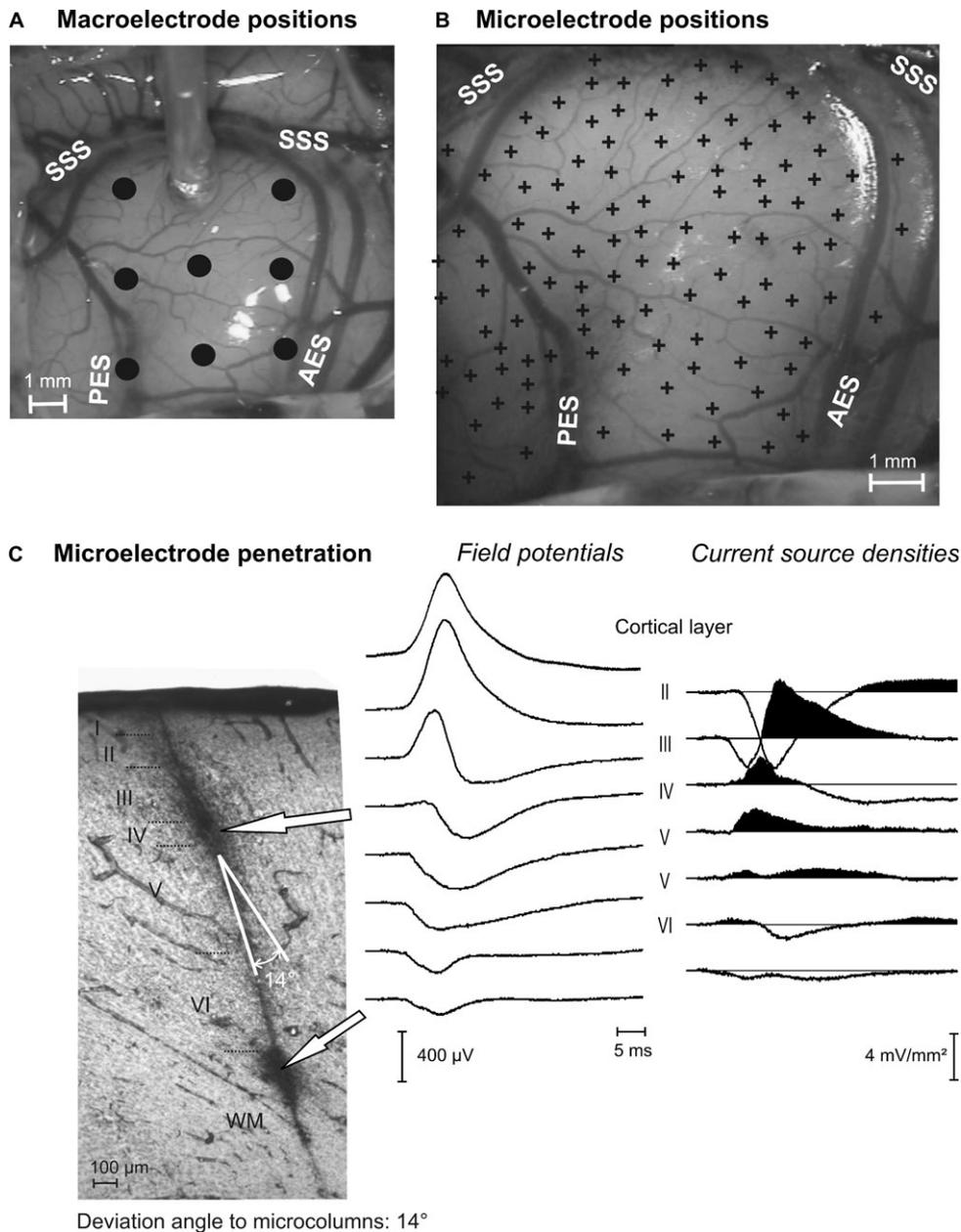


Figure 1. Example of recording positions in the field A1 of cats. (A) Photograph of the auditory cortex; rostral = right; dorsal = top. A silver-ball electrode is positioned underneath the superior sylvian sulcus (SSS). Black dots indicate other recording positions. (B) Microelectrode recording positions (crosses) in the same animal. (C) Histologically reconstructed penetration of a HRP-filled microelectrode with two dye deposits in the auditory cortex (frontal section) with the current source density profile determined with the same electrode (right; sinks are filled). PES = posterior ectosylvian sulcus; AES = anterior ectosylvian sulcus. Determination of cortical layers takes deviation angle into account.

threshold was lower than the cortical LFP threshold in hearing cats (paired *t*-test, $P = 0.012$), but was the same in deaf cats (paired *t*-test, $P = 0.356$). Consequently, the primary auditory cortex in deaf cats was more sensitive to peripheral electrical stimulation.

Afterwards the field A1 was mapped using glass-microelectrodes with stimulation at 10 dB above the lowest cortical threshold determined in the given animal. At this intensity the P_a amplitudes were in saturation in both groups of animals. LFPs recorded with microelectrodes were larger than LFPs recorded with macroelectrodes at the corresponding intensities. Field A1 was defined within the anatomical landmarks (see above) by P_a waves of large amplitudes ($> 150 \mu\text{V}$) and short peak latencies ($< 20 \text{ ms}$). P_a waves were used for computing activation maps as

shown in Figure 3. The area of cortex activated by the electrical stimulus was compared by defining cortical 'activated areas' (see methods). Previous studies have demonstrated large variability of maximum LFP amplitude in different adult animals (hearing and deaf), but a good reproducibility of the activated areas in adult hearing and deaf cats (Kral *et al.* 2002). In the present study, larger activated areas were found in young hearing cats than in adult hearing cats. Cluster analysis determined 2 significantly different clusters in hearing controls: one of animals up to the age of 3 months p.n. and the other as of the age of 4 months (significance tested by two-tailed Wilcoxon-Mann-Whitney test, $P = 0.021$). In deaf cats, cluster analysis identified other significantly different clusters: one of three animals of ~ 3 months of age, and another consisting of animals both above and below 3

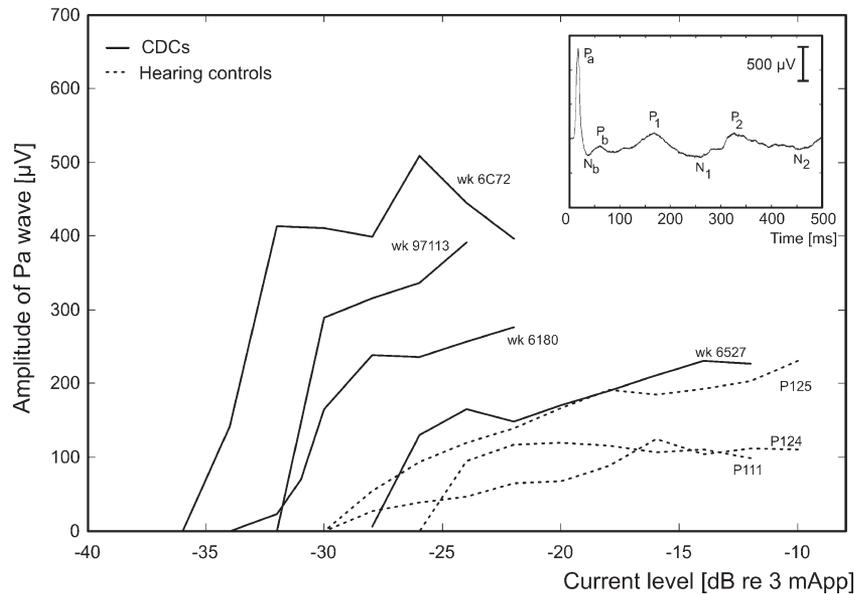


Figure 2. Amplitude-intensity functions of macroelectrode-recorded mid-latency responses (P_a waves). Stimulus: biphasic charge-balanced pulse, 200 $\mu\text{s}/\text{phase}$, monopolar electrode configuration. Designation of the field potential waves used in the present paper is shown in the inset. Animal identification is shown above each function, all animals were > 4 months age.

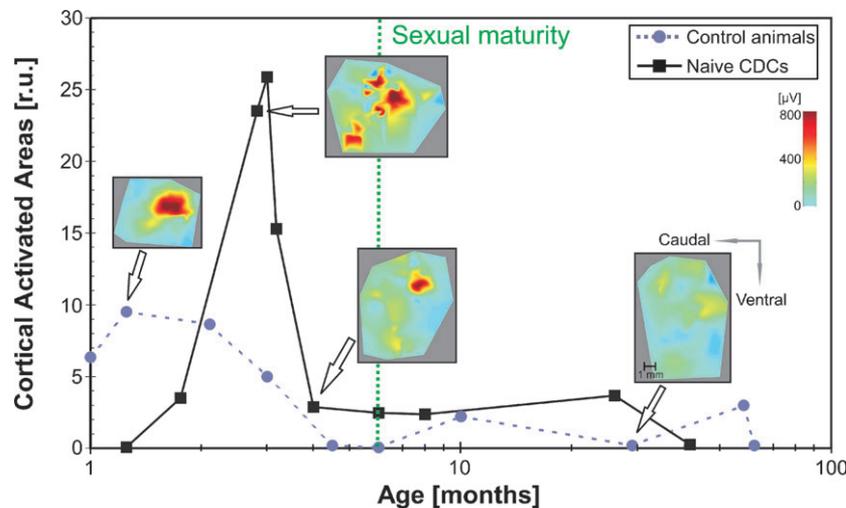


Figure 3. Cortical activated areas as a function of age. Cortical activation maps computed from P_a amplitudes are shown as insets for selected animals. Color code is shown on the right. Cats were considered sexually mature around the sixth month p.n.

months of age (Fig. 3, two-tailed Wilcoxon–Mann–Whitney test, $P=0.017$). The activated areas at the age of ~ 3 months in deaf cats were significantly larger than in any other cluster from all investigated animals. All these differences remained significant even without normalizing the activated areas to brain size.

Thus, an important developmental change takes place at the age of 3–4 months both in hearing controls and in deaf cats. However, the developmental change is significantly different between these two groups of animals.

Within the activated area a region of interest (ROI) comprising the largest LFPs was defined (size of 1 mm^2). Here, in the pattern of a regular grid, six LFPs were recorded at the cortical surface (2×3 positions, 500 μm apart). A mean LFP was computed from these LFPs for each animal, and then the mean LFPs were normalized to maximum amplitude to compensate for the amplitude variability between different age groups (cf. Fig. 3).

In hearing controls, three developmental steps in the morphology of LFPs could be identified (Fig. 4):

1. A decrease in P_a onset latencies within the first 8 weeks of life (Fig. 4A,B).
2. A change in the shape of the N_b – P_b complex. The relative amplitude of the sharp N_b wave decreased and the P_b wave disappeared, giving rise to a broad mature-like N_b wave at the age of 8 weeks (Fig. 4A,B).
3. The P_1 wave was absent in the hearing control at the age of 4 weeks (Fig. 4A). It appeared at 6 weeks p.n. (Fig. 4A) and then successively decreased in latency, showing shortest (mature) latencies at the adult age (6 months, Fig. 4B–D).

The deaf cats did not show all these developmental changes (Fig. 5). At 1.25 months p.n. (Fig. 5A), the shape of mean LFPs from ROI differed only slightly between deaf cats and controls,

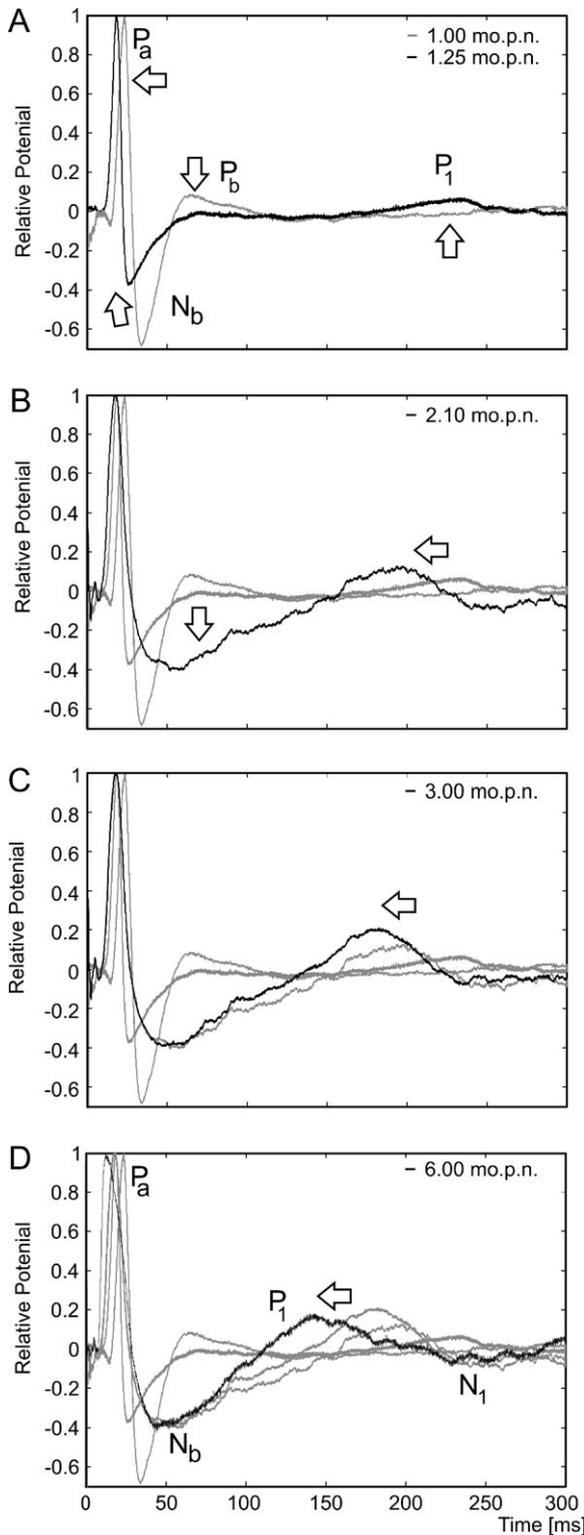


Figure 4. Development of morphology of field potentials in control (hearing) cats. Each LFP represents a normalized mean local field potential of six recordings within the most activated cortical regions (ROI). Youngest investigated animals are shown in the top panel, arrows indicate the direction of change with increasing age. In each panel, local field potentials from younger animals (shown in the panels above) are presented as grey curves.

mainly in the shape of the P_a - N_b complex. The N_b wave was slightly broader in the deaf animal. With 2.1 months p.n. (Fig. 5B), the deaf cat still had an immature, sharp N_b wave, of

comparable shape as the hearing cat at 1.25 months (cf. Fig. 5A). The age-matched hearing cat had already a mature N_b wave. This pronounced difference persisted also at 3 months p.n. (Fig. 5C) and disappeared first at 4.1 months, when the deaf animal had a similarly-shaped N_b wave as the adult control (Fig. 5D). Thus, the development of the N_b wave was delayed in deaf cats. However, it was also incomplete: when grand mean averages of normalized LFPs obtained from all adult animals were compared, a relatively smaller N_b wave in deaf cats showed up (Fig. 6).

Another difference was found in P_1 waves: at 1.25 and 2.1 months (Fig. 5A,B), the deaf cats had a P_1 wave; however, it had a smaller latency than the one of the age-matched controls. From the third month on (Fig. 5C), its relative amplitude diminished, eventually resulting in a delayed and less differentiated P_1 wave in deaf adults (Fig. 6).

The generators of LFPs were revealed using current-source-density analyses. For this purpose, local field potentials were recorded in different cortical layers within the ROI (six penetrations). The penetrations were histologically reconstructed after the experiment and cortical layers were assigned to individual recording depths on Nissl-stained sections. The mean deviation angle of the penetration from the direction of micro-columns was $14 \pm 8^\circ$.

The current source densities showed a developmental sequence corresponding to that of surface-recorded LFPs. In hearing controls, young cats (up to 2 months) had large CSD amplitudes (Fig. 7, top) which decreased at 3–4 months p.n. Additionally a rearrangement of the profiles within the auditory cortex was discernible. Early in development, at the age of 4 weeks, the synaptic activity was concentrated in the superficial (supragranular) cortical layers II and III. At 2–3 months activity had increased in deeper cortical layers (V and VI). From 4 months on, synaptic activity involved all cortical layers, and the earliest sinks showed less difference in peak latency between different layers. The pattern of cortical activation became similar to the one described previously in adult hearing controls: patterned activity was found in all cortical layers, starting with infragranular layers and layer IV and III (thalamic input), succeeded by longer-lasting and large sinks and sources in supragranular layers and succeeded by sinks and sources in infragranular layers (details in Kral *et al.*, 2000; compare Mitzdorf, 1985). Activity extended over the whole 50 ms post stimulus.

In deaf animals, postnatal development was significantly different (Fig. 7, bottom). Very young deaf animals (<6 weeks p.n.) had less synaptic activity in the auditory cortex than age-matched hearing controls (data not normally distributed, mean sink amplitude $317.4 \mu\text{V}/\text{mm}^2$ in deaf versus $2313.3 \mu\text{V}/\text{mm}^2$ in hearing controls, Wilcoxon-Mann-Whitney two-tailed test, $P \ll 0.001$). At 2 months, the amplitudes of CSD peaks did not differ significantly between deaf and hearing groups (mean sink amplitudes of $1318 \mu\text{V}/\text{mm}^2$ in deafs versus $2459.9 \mu\text{V}/\text{mm}^2$ in hearing controls). The sink amplitudes increased with increasing age in deaf cats, peaking at the age ~ third month p.n. The sink amplitudes at this age were significantly larger in deaf cats than in hearing controls ($2804.8 \mu\text{V}/\text{mm}^2$ in deaf versus $1486.6 \mu\text{V}/\text{mm}^2$ in controls, Wilcoxon-Mann-Whitney two-tailed test, $P = 0.02$). The amplitudes at this age in deaf better corresponded to the one in 1-month-old hearing control (non-significant difference). However, infragranular activity was more comparable to age-matched controls. (Note that at 3 months p.n. in deaf cats, the

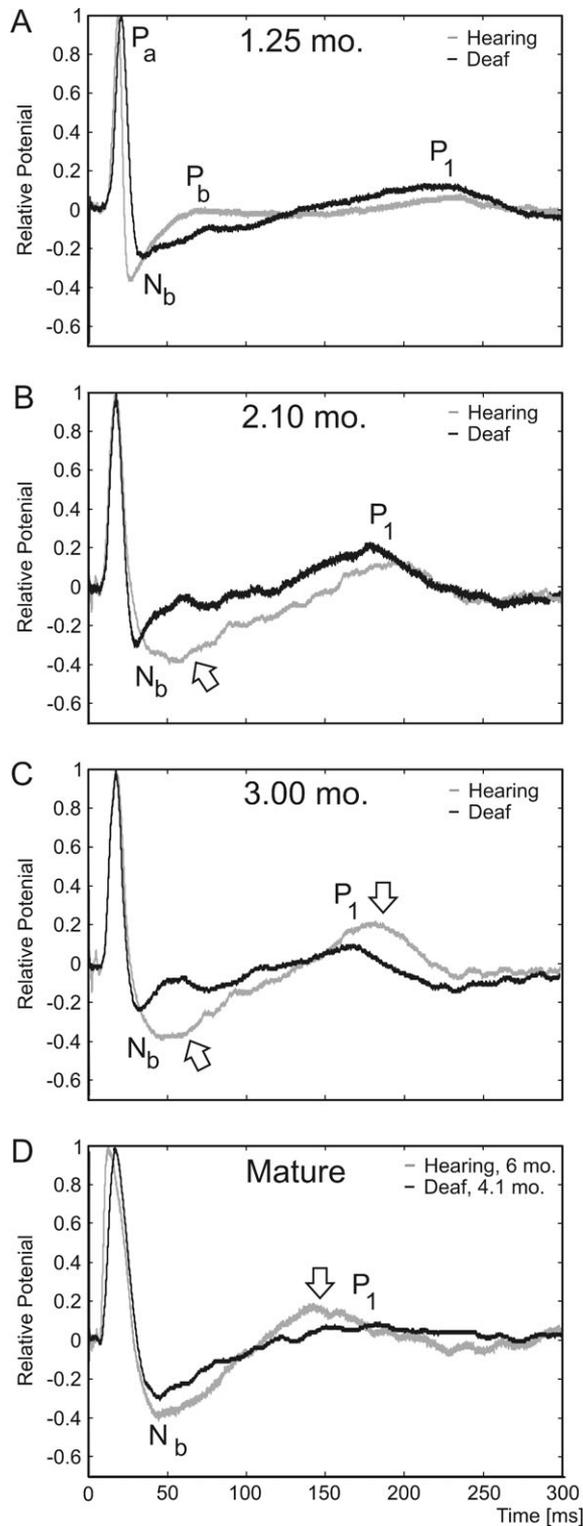


Figure 5. Comparison of morphology of field potentials between deaf cats and controls. Each LFP represents a normalized mean local field potential of six recordings within the most activated cortical regions (ROI). Grey lines show normalized mean LFPs of hearing controls, black lines normalized mean LFPs of deaf cats. Arrows indicate differences between deafs and controls. (A) Comparison of LFPs in animals at 1.25 months p.n. (B) Comparison of LFPs in animals at 2.1 months p.n. (C) Comparison of LFPs in animals at 3 months p.n. (D) Comparison of a 4.1-month-old deaf animal with an adult control (6 months), showing that at 4.1 months the N_b wave in deaf cats approaches the mature shape and P_1 can no longer be differentiated.

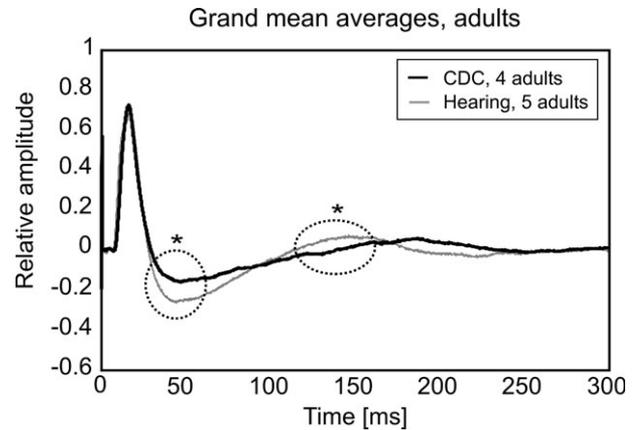


Figure 6. Grand mean averages of normalized mean LFPs from ROI of adult animals show two significant differences: a smaller N_b wave and a delayed, smaller and more 'diffuse' P_1 wave in deaf cats. Statistical comparison by gliding window two-tailed Wilcoxon-Mann-Whitney test at each sampling interval (100 μ s), $\alpha = 5\%$.

activated areas were also significantly larger than at any other age.) Already at 4 months, the deaf animals showed CSD profiles corresponding to adult deaf cats, with all deficits described in an earlier paper (significantly smaller mean sink amplitudes in deaf cats, the present results were within the range published previously, cf. Kral *et al.*, 2000). With respect to mid-latency range, the CSD profiles reached stability at the age of 4 months in both investigated animal groups.

Correspondingly, the mean P_a amplitudes of surface-recorded LFPs in ROI decreased in hearing controls from $908 \pm 98.7 \mu$ V (at 1 month) through $642 \pm 131 \mu$ V (at 2 months) to $543.9 \pm 192.4 \mu$ V (at 3 months). From 4 months on, the range of mean P_a amplitudes was between 240 and 610.5μ V. In deafs, the mean amplitudes in ROI changed from $227.7 \pm 52.7 \mu$ V (1 month) through $256 \pm 92.4 \mu$ V (2 months) to $836.4 \pm 182.3 \mu$ V (3 months). From 4 months on, the mean P_a amplitudes were in the range between 312.9 and 898.4μ V.

Discussion

The present paper demonstrates for the first time the effect of complete absence of auditory experience on the functional development of the auditory cortex. The presented findings support the concept of activity-dependent postnatal development.

The data demonstrate:

1. During early developmental periods (<4 months) in hearing controls, the stimulated cochlear region was represented on a larger area of the auditory cortex than in adult cats. As the overall extent of the cortex devoted to auditory stimuli does not appear to be larger in young hearing animals (on the contrary, the brain grows with age), and since the cochlea does not change significantly after birth in the cat, this finding indicates a larger overlap of cortical representations in young (immature) hearing animals and thus more 'diffuse' cortical neuronal networks.
2. At early ages (<3 months) large synaptic activity was found in hearing animals. It was concentrated in supragranular layers (II, III) and layer IV. Activity further spread to infragranular layers at the age of 2 months, and it was then

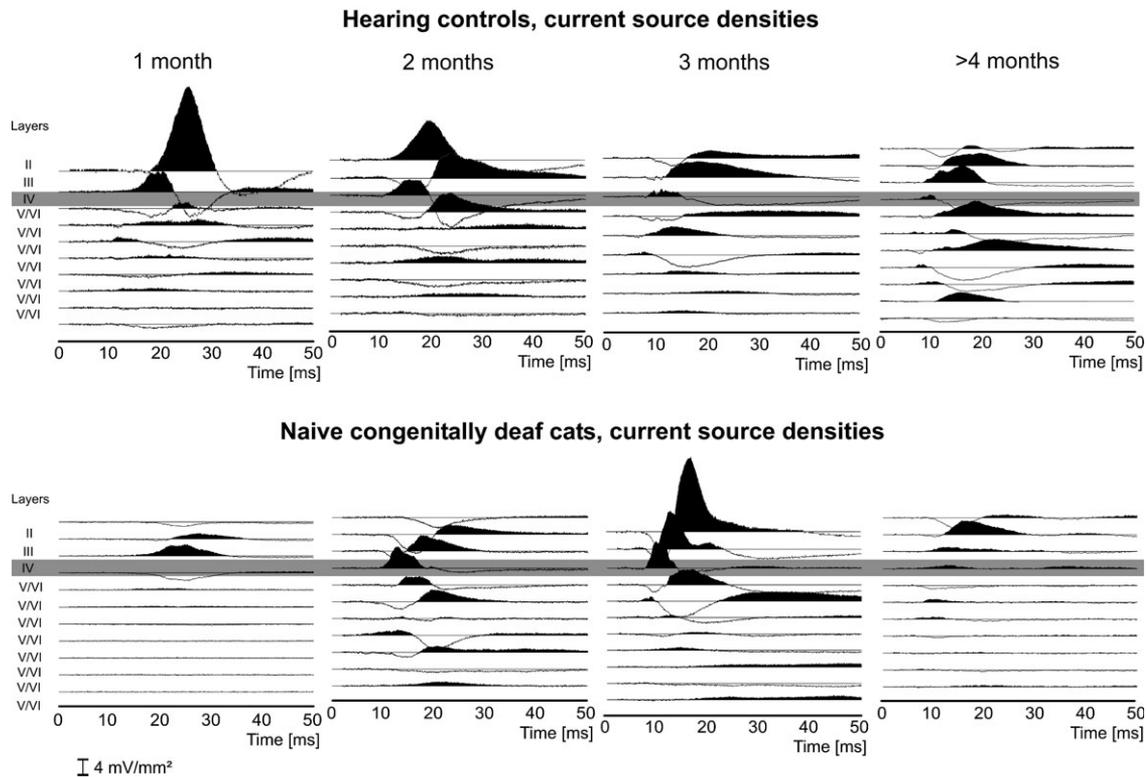


Figure 7. Gross synaptic currents in cortical layers within the ROI as determined by the current source density technique for the first 50 ms after the stimulus; shown are penetrations with largest currents in the given animal. Current sinks are the upward deflections of the signal and were filled black. Penetration of the microelectrode was histologically reconstructed and the current source densities were vertically aligned to match according to individual cortical layers. Layer IV is marked with a grey bar. Top: control cats, bottom: deaf cats.

that surface-recorded LFPs showed a large and broad N_b wave. Long latency responses appeared at 1.25–1.50 months, decreasing in latencies after this age.

3. In hearing animals a mature laminar pattern and mature amplitudes of cortical mid-latency responses were found at the age of 4 months p.n. Functional properties at long latency range (>80 ms) matured later, at 6 months of age, corresponding to sexual maturity.
4. Deaf cats showed a different pattern of development, which demonstrates the shaping influence of hearing experience on the maturation of the auditory system.
5. Young deaf cats (<3 months p.n.) were characterized by small synaptic currents, generating small LFPs at the cortical surface. These LFPs had an immature shape, yet the emergence of long latency responses was found even in the absence of auditory experience. The cortical activated area, and thus the spread of cortical activity, was smaller than in age-matched hearing controls.
6. The age group at ~ 3 months p.n. in deaf cats was characterized by very large activated areas, caused by large synaptic currents in the superficial cortical layers. Some synaptic activity was found also in infragranular layers at this age.
7. At the age of 4 months p.n. the activated areas shrunk to an extent not significantly different from hearing controls. Synaptic activity at this age demonstrated all deficits described for adult deaf cats (Kral *et al.*, 2000): delay of activation in supragranular layers, and near absence of activity at longer latencies and in infragranular layers. Correspondingly, long latency responses almost disap-

peared in surface-recorded LFPs. After 4 months p.n. the cortical activity demonstrated no further developmental changes in deaf cats.

Methodological Considerations

Age-matched hearing cats were deafened by the beginning of the experiment and were stimulated electrically. Deafening prevents a direct electrical stimulation of hair cells (electrophony, Kiang and Moxon, 1972; Popelar *et al.*, 1995). On the other hand, as it takes days to weeks post deafening to cause a degeneration in auditory nerve fibers in pharmacologically deafened animals (e.g. Leake-Jones *et al.*, 1981, 1982; Leake and Hradek, 1988; Leake *et al.*, 1999), it does not constitute a confounding factor for the present experiments.

The activated areas need to take brain growth into consideration by way of normalization of activated areas. However, even without the normalization the same conclusion would be reached in hearing controls, as largest activated areas were found in the ‘smallest’ brains. In the deaf cats significantly larger activated areas were found at ~ 3 months. At this age the activated areas were so large that this conclusion was also reached even without any normalization.

The present study termed the waves of LFPs based on their latencies. Middle latency waves were designated by indices a, b, c, long latency waves by 1, 2, 3. It is difficult to find direct correspondences between cat LFPs and human evoked potentials. Nonetheless, mid-latency responses N_a and P_a could directly correspond to human evoked potential waves N_a and P_a (Eggermont and Ponton, 2002). N_b wave in the present study

overlaps with waves N_b and N_1 in human evoked potentials. P_1 wave in the present study overlaps with wave P_2 described in human evoked potentials (Eggermont and Ponton, 2002).

Normal Postnatal Auditory Development

With electrical stimulation of the auditory nerve in hearing cats, the present study showed an orderly sequence of changes in the shape of surface-recorded LFPs. At 1 month p.n., only mid-latency responses could be recorded in A1. One week later a long latency response was found. Consequently, long latency responses emerged within the fifth week p.n. König *et al.* (1972) reported the appearance of long latency responses one week earlier with acoustic stimulation. The difference may be due to the difference in stimulus (acoustical versus electrical). The early onset response was shown to develop earlier than long latency responses also with single-unit responses ('rebound responses', Eggermont, 1996; cf. Dinse *et al.*, 1997). Additionally, there was a decrease in minimum latency of single-unit responses during the first 6 weeks p.n. (Eggermont, 1996), corresponding to the decrease in P_a latency in the present study. Rebound (long latency) responses matured around the fifth postnatal month with acoustical stimulation (Eggermont, 1996), corresponding to the data presented here.

Hearing controls until the age of 3 months had larger activated areas than older controls in the present study. Similarly, in rodents with acoustical stimulation the lateral spread of excitation was larger in immature animals (Chang and Merzenich, 2003).

The present study revealed a peak in gross synaptic currents (CSDs) and activated areas at the age of 1.0–1.25 months in hearing controls, with a decreasing gradient during the second and third months. Corresponding to this finding, synaptic densities increase in the visual cortex between the first and second months in the cat. After postnatal day 70 a gradual elimination of synapses occurs, with synaptic densities decreasing by 30–40% to reach adult values after the sixth month in the visual cortex of the cat (Cragg, 1975b; Winfield, 1981, 1983; O'Kusky, 1985; for the monkey, see O'Kusky and Colonnier, 1982a,b; Rakic *et al.*, 1986, 1994; Goldman-Rakic, 1987). A pronounced cortical synaptogenesis after birth with a subsequent synaptic elimination reflects a general mechanism of postnatal cortical maturation. The present results demonstrated highest synaptic currents at 1.25 months p.n., which is 2–3 weeks earlier than the synaptic overshoot in the cat visual cortex. This discrepancy may result from each study's particular timetable of brain examination at different postnatal ages; short-lasting peaks in synaptic densities or currents could have been missed in either study. Alternatively, the synaptogenesis could be faster in the auditory cortex than in the visual. Immature synaptic properties with a higher synaptic conductance (Carmignoto and Vicini, 1992) can also be a reason for the earlier peak in synaptic function when compared with synaptic densities.

Developmental CSD data concerning the cat visual cortex have been obtained in brain slices (Friauf and Shatz, 1991). Excitation in the cortical subplate disappeared around birth and shifted to supragranular cortical layers. Stronger activation of infragranular layers first appeared between the third and fourth weeks p.n.; however, it was restricted to shorter latencies and to layer V. Correspondingly, stronger activation of supragranular layers when compared with infragranular layers was also found in the present study in young cats (<2 months). Immunostained neurofilaments in humans, on the other hand, matured first in layer I,

then in infragranular layers and last in supragranular layers (Moore and Guan, 2001). Maturation of neurofilaments thus might not directly follow functional parameters in this aspect, although differences in species have to be considered, too.

Postnatal Development of the Auditory Cortex in Deafness

The cortical thresholds to cochlear electrical stimulation were lower in deaf animals, whereas brainstem evoked potentials did not differ between the two groups. This demonstrates a cortico-thalamic hypersensitivity to peripheral stimulation in deaf cats. A similar decrease in threshold to electrical stimulation has been reported in neonatally-deafened cats when investigated as adults (Raggio and Schreiner, 1999). Disinhibition could have contributed to these findings (see also below), although inhibition is generally not very effective at threshold intensities anyhow. As no developmental change was observed in the lowest cortical threshold in the present study, the difference in threshold seems to be established very early in postnatal development, before the fourth week p.n. From the lack of difference in the thresholds of brainstem evoked responses between the animal groups, it can be concluded that in hearing controls the auditory cortex has specialized to processing of acoustic stimuli and cortical responses to the artificial electrical stimuli have been suppressed. The absence of this effect in deaf cats possibly results from immature cortical networks that were not patterned by auditory experience and thus did not learn to filter out the artificially broad, heavily synchronized activity patterns resulting from cochlear implant stimulation (e.g. Hartmann *et al.*, 1984; Kral *et al.*, 1998). The auditory cortex in deaf cats thus retains a diffuse, immature organization.

Shortest latency responses in CSDs were found in infragranular layers and in layers IV and III in hearing controls (Kral *et al.*, 2000, 2001). These currents contributed to N_a waves in the surface-recorded LFPs (not always discernible). The P_a waves corresponded to large sinks in supragranular layers III and II (Fig. 7). Detailed statistical analysis in adult deaf cats revealed a small but significant increase in latency of the sinks in supragranular layers of deaf cats (Kral *et al.*, 2000). The N_b waves corresponded to a current source located in layer IV (see hearing cats in Fig. 7 top, latency range > 10–20 ms) and also layer III. Sources indicate inhibitory synaptic activity (Mitzdorf, 1985). The immature N_b waves were characterized by a shorter duration, giving them a 'sharp' appearance. Maturation of these waves demonstrated a developmental delay of ~2 months in deaf cats (in hearing cats, immature <2 months; in deaf cats, immature <4 months). Deafness thus possibly leads to a delayed and incomplete development of inhibitory synapses in layer IV and III (compare absence of larger sources in layer IV and III in deaf cats, Fig. 7 bottom). Consistent with this interpretation, layers III and IV are the richest in inhibitory synapses. Inhibition is thought to play a crucial role in relaying activity from layer IV to supragranular layers ('gate theory', Rozas *et al.*, 2001), is affected by deprivation (Bledsoe *et al.*, 1995; Rajan, 1998) and is known to mature later than excitation during development (Gao *et al.*, 1999; cf. Fagiolini and Hensch, 2000).

P_1 waves are thought to represent a rebound from inhibition (Eggermont, 1992), which is under the control of thalamo-cortical loops (Grenier *et al.*, 1998). In the present study, P_1 waves were found as of 1.25 months both in deaf and hearing cats. Their appearance did not depend on auditory experience. However, in contrast to hearing cats, these waves decreased in

amplitude at 2–3 months of age in deaf animals (cf. Klinke *et al.*, 1999; Kral *et al.*, 2001). These long latency responses, found both in multi-unit recordings as well as in LFPs of hearing controls, could be re-induced in deaf cats by early chronic electrical stimulation (Klinke *et al.*, 1999; Kral *et al.*, 2001, 2002).

The smaller synaptic currents in the cortex of young deaf cats (<3 months) show that formation of functional synapses during early postnatal development is slowed down by the absence of auditory experience. Under such condition, synaptic current source densities during development increase slower than in controls, peaking 2 months later — at 3 months p.n. Thus, the functional ‘synaptic overshoot’ found in hearing controls is delayed in deaf cats by 2 months. A larger spread of activity in the auditory cortex was found at the same age as the peak in synaptic activity both in deaf and hearing animals. Few studies allow direct comparison with the present one, and none was performed in the auditory cortex. Morphological data from the visual cortex demonstrate that when visual deprivation was neonatal and long-term, the synaptogenesis was delayed and the synaptic overshoot was amplified (Cragg, 1975a; Winfield, 1981, 1983; O’Kusky, 1985). Thus, synapses are formed slower, but their peak (maximum) number is increased in deprivation. The present study functionally shows the same developmental pattern. A delay in the switch from immature to mature synaptic properties could also contribute to the present finding (Carmignoto and Vicini, 1992; Quinlan *et al.*, 1999a,b).

Subsequently (at 4 months p.n.), a phase of reduction in synaptic currents followed in the deaf cats of the present study. The CSD profiles found at the age of 4 months in deaf cats shared all deficits described in adult deaf cats, including an overall reduction of synaptic currents (Kral *et al.*, 2000). As a result, at this developmental stage more functional synapses were eliminated in deaf cats than in normal hearing controls. It is impossible to assess whether the synapses were eliminated physically, became eliminated functionally (resulting in ‘silent’ or less-conductive synapses) or whether the data represent a consequence of network properties. In the cat visual cortex, deprivation eventually led to adult synaptic densities only slightly under the level of control adults. This was the case mainly for supragranular layers, not for infragranular layers (Winfield, 1981, 1983; O’Kusky, 1985). Therefore, the decreased synaptic currents in deaf adults shown in this and a previous study (Kral *et al.*, 2000) can not be explained solely by increased synaptic elimination. It needs to take changed synaptic conductivities and network properties (e.g. synchronization of neuronal responses, cf. Snyder *et al.*, 1995, 2000, Shepherd *et al.*, 1999) into account.

Infragranular Activity

At 1.0–1.25 months p.n. in both groups of animals, activity was concentrated in supragranular layers. Not before the age of 2 months did larger activity spread to infragranular layers. This developmental process was not influenced by absence of auditory input. However, as of the third month p.n. the activity in infragranular layers began to dissipate in deaf cats, whereas in hearing controls it became more and more pronounced. This process of dissipation in deaf cats is thus the consequence of deafness and represents a degenerative sequence. A reduction in activity in infragranular layers of adult deaf cats has a serious impact on cortical processing. Infragranular layers are origins of corticothalamic (and collicular) feedback projections. In deaf cats, these feedback connections of area A1 do not seem to be

properly activated. Correspondingly, in the primate visual cortex it has been shown that development affects more feedback than feedforward connections (Batardiere *et al.*, 1998, 2002).

The higher-order auditory cortex projects back to A1, mainly to the infragranular layers (review in de Ribaupierre, 1997). A lack of activity in infragranular layers therefore demonstrates also a decreased activity in these feedback projections, reflecting a lack of higher, cognitive modulation of activity in A1 (Raizada and Grossberg, 2003). Such ‘decoupling’ of A1 from modulatory cognitive influences under auditory stimulation could represent the basis for cognitive auditory deficits in late-implanted congenitally deaf humans.

Sensitive Periods

A sensitive period with decreasing developmental plasticity was found during 3–6 months p.n. in deaf cats (Kral *et al.*, 2001, 2002). During this time, plastic adaptations to cochlear implant stimulation showed a decreasing gradient. At the age of 2–3 months a delayed *functional* synaptic overshoot in deaf cats was found in the present study. This age corresponds to the time of ‘early’ implantations in the previous studies (Kral *et al.*, 2001, 2002). Plasticity was high at this implantation age (Kral *et al.*, 2001, 2002). If auditory input is not initiated or resumed until the fourth month, the phase of functional synaptic elimination begins, resulting in functionally deficient cortical circuits, with a reduced synaptic activity when evoked by peripheral stimulation. With the given repertoire of functional synapses the cortex can possibly no longer be reshaped to adequately respond to environmental inputs, corresponding to less adaptations to peripheral inputs found in late implanted animals in previous studies (Kral *et al.*, 2001, 2002; for mechanisms, cf. Carmignoto and Vicini, 1992; Quinlan *et al.*, 1999a,b). Absolute synaptic numbers cannot increase after a sensitive period has expired (somatosensory cortex: Trachtenberg *et al.*, 2002; indirect evidence in visual cortex: O’Kusky and Colonnier, 1982a). This could explain why the auditory cortex of late-implanted animals no longer learns to process peripheral inputs adequately.

Humans

There are several similarities in the development of the cat and human auditory cortex. Morphological studies demonstrated significant developmental changes in the synaptic densities in the neocortex (Huttenlocher and Dabholkar, 1997), in the structure of neurofilaments in cortical neurons (Moore, 2002), very extensive changes in the branching pattern of dendritic trees (Conel, 1939–1967) and changes in cortical myelination (Yakovlev and Lecour, 1967; Paus *et al.*, 1999, Emmorey *et al.*, 2003). In the human auditory cortex, synaptic densities rise steeply during the first 4–6 months after birth in humans, resulting in a maximum synaptic density at the age of 0.5–4.0 years (Huttenlocher and Dabholkar, 1997). Afterward synaptic density decreases from 2 to 4 years of age until adolescence (~15 years, Huttenlocher and Dabholkar, 1997; cf. Conel, 1939–1967). Functional developmental changes in auditory cortical evoked potentials extend to >12 years of age (Ponton *et al.*, 2002; Eggermont and Ponton, 2003) and this development is delayed by prelingual deafness (Ponton *et al.*, 1996, 1999). A sensitive period for developmental changes in evoked potentials has also been found: The latency of P₁ waves matured faster in congenitally deaf subjects if cochlear implantation was performed

before the fourth year p.n. (Sharma *et al.*, 2002a,b,c) and the morphology of later peaks showed few maturational changes if prelingually deaf children were cochlear-implanted late in their teens (Ponton and Eggermont, 2001). The present data correspond well to these findings and possibly demonstrate the neurophysiological substrate of this phenomenon.

Notes

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