

# Recruitment of the Auditory Cortex in Congenitally Deaf Cats by Long-Term Cochlear Electrostimulation

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In congenitally deaf cats, the central auditory system is deprived of acoustic input because of degeneration of the organ of Corti before the onset of hearing. Primary auditory afferents survive and can be stimulated electrically. By means of an intracochlear implant and an accompanying sound processor, congenitally deaf kittens were exposed to sounds and conditioned to respond to tones. After months of exposure to meaningful stimuli, the cortical activity in chronically implanted cats produced field potentials of higher amplitudes, expanded in area, developed long latency responses indicative of intracortical information processing, and showed more synaptic efficacy than in naïve, unstimulated deaf cats. The activity established by auditory experience resembles activity in hearing animals.

Self-organization and plasticity are the outstanding features of the cerebral cortex (1). In all sensory modalities, these processes depend on external stimuli. In ontogeny, critical periods seem to exist during which an external influence is required to trigger the subsequent steps of central development (2). In cases of sensory deprivation, however, this process is arrested. This is of particular im-

portance in congenitally deaf patients, whose deafness can now be treated by cochlear implants. When adults who are congenitally or prelingually deaf receive cochlear implants, the results are disappointing (3). They never gain language competence and often request that the implant be removed. In contrast, early cochlear implantation in congenitally or prelingually deafened children can lead to nearly perfect acoustic communication and language competence (3). However, the neuronal bases underlying this achievement have not yet been explored.

Deprivation studies have revealed the deficits in the central nervous system in cases of sensory deprivation and the limitations in repair mechanisms (4). Studies in the auditory system

are faced with the additional difficulty that bone conduction leads to auditory sensation even if the ear canals are blocked. Thus, properly controlled experiments on auditory deprivation have not yet been carried out.

Congenitally deaf cats (CDC) provide the opportunity to study the maturation of central auditory structures that have never received specific sensory input. In this strain of animals, the organ of Corti degenerates before the onset of hearing function (5, 6). The auditory cortex of these CDCs is naïve with respect to auditory experience. Because the auditory nerve fibers survive nearly completely in young CDCs (5), the auditory pathways of these animals can be activated by electrical stimulation through a cochlear implant (7).

The recruitment of the auditory cortex by electrically induced hearing experience was studied in six congenitally deaf kittens (8). At an age of 3 to 4 months, the animals were unilaterally implanted with intracochlear electrodes and chronically stimulated (9). Complete deafness of all experimental animals was previously verified by the absence of acoustically evoked brainstem responses [clicks > 120 dB peak equivalent (p.e.)] at the age of 4 weeks (5). Control data were collected from five naïve, previously unstimulated CDCs and two normal hearing cats acutely deafened by intracochlear application of neomycin (10). These control animals received intracochlear electrodes at the beginning of the acute experiment. One of the chronically implanted CDCs was used for behavioral studies only.

After an 8- to 10-day recovery period, the cochlear implant of the chronic cats was connected to a sound processor worn in a jacket. This customized processor applied single-channel compressed analog coding strategies as used in VIENNA-type human implants

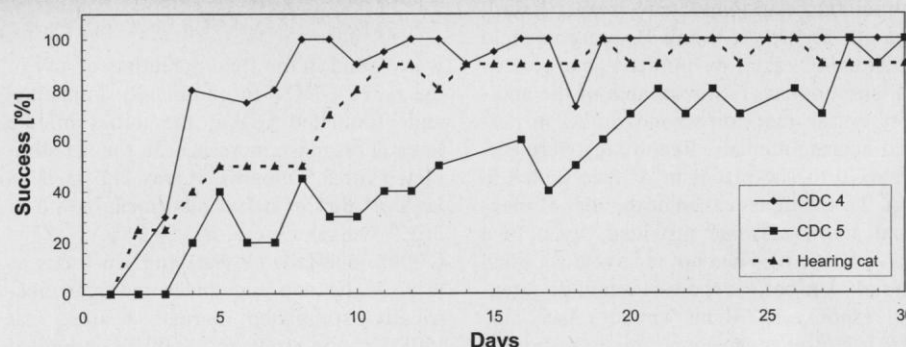
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**Fig. 1. (Top)** Video sequence (left to right) of the response of an implanted deaf cat to conditioned stimulus. Both cats were implanted and conditioned; the implant was active only in the left-hand animal (see text). **(Bottom)** Learning curves of two implanted deaf cats [best (◆) and worst (■) example] and one normal hearing cat.



(11) and provided a time code of the acoustic signal, stimulating a large proportion of the basal fibers (12). Cochleotopic representation is not provided with this coding strategy.

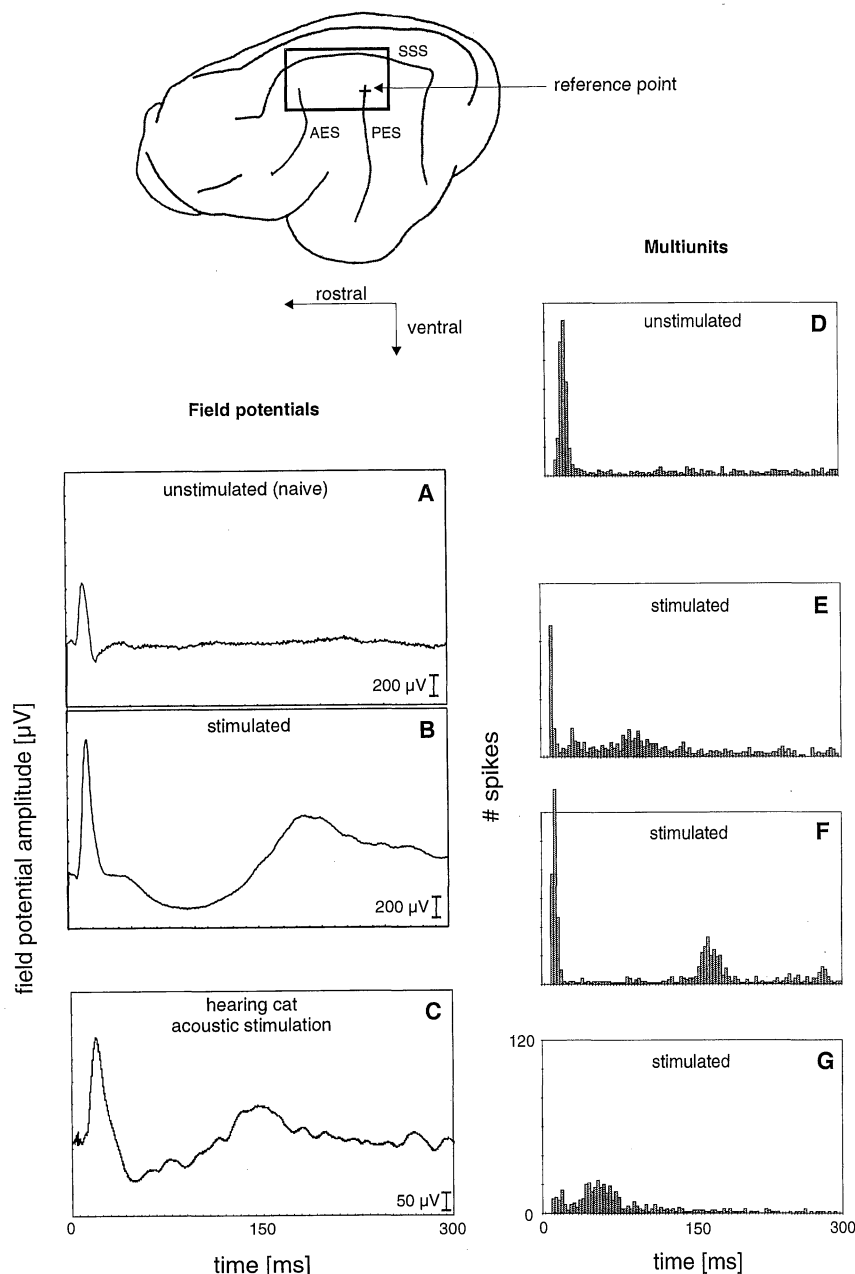
The sound processor was continuously active day and night; the animals could freely move. They received acoustic inputs from their environment and self-produced sounds as would a normal hearing kitten under enriched laboratory conditions. To reinforce the use of the auditory channel, we used a conditioning procedure in which tones (437-Hz sinusoid) served as stimuli for the dispensing of food pellets (13).

In all animals of the experimental group, sound stimuli evoked pinna reflexes immediately upon activation of the sound processor. This indicates that the acoustic brainstem reflexes were functional even without previous auditory experience. In fact, these pinna reflexes were used to adjust the strength of the stimulation current (11).

Within 1 to 3 weeks, the cats reliably responded to the conditioned tones. Learning curves of two CDCs and one hearing cat are shown in Fig. 1. Also, outside the conditioning situation, the implanted cats reacted to sounds. They actively searched for sound sources, learned to respond to human calls, and could be awakened by sound. The top panel of Fig. 1 shows the behavior of implanted cats. To demonstrate the impact of the conditioned stimulus, we took this sequence while cats were eating. Only in the left-hand cat was the sound processor active. Upon the stimulus, the "hearing" cat leaves the food to receive the reward expected, a particularly loved food pellet.

The behavioral observations were confirmed by electrophysiological data (14). Individual cats were investigated after 1, 2, or 5.5 months of auditory experience. Field potentials from the contralateral auditory cortical surface, their intracortical depth profiles, and single-unit and multiunit activity were recorded with glass microelectrodes. Furthermore, the synaptic currents in the different cortical layers were revealed by means of current source density (CSD) analyses. In one chronic cat, the ipsilateral cortex was also investigated.

Middle latency cortical field potentials increased during the course of chronic stimulation, and long latency responses appeared. Figure 2, A and B, presents field potentials evoked by intracochlear electrical stimulation (14) in an area of the auditory cortex that corresponds to AI in normal hearing animals. Recordings were taken at 70 to 100 places in AI (see sketch in Fig. 2). Examples taken at the loci of maximal amplitudes are provided. Even in a naïve CDC, who had not received electrical stimulation before, there is a middle latency response (<30 ms latency) [see also (10)]. However, no long latency responses



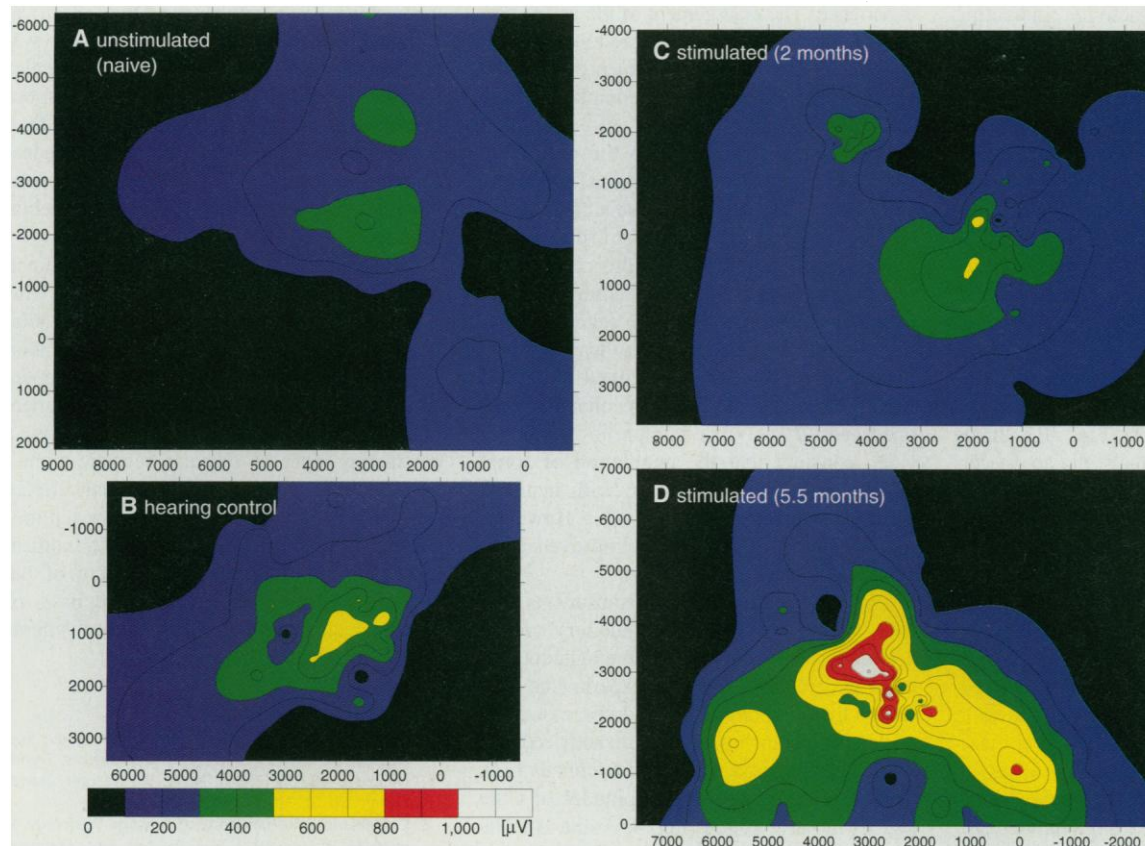
**Fig. 2.** (A and B) Field potential amplitude (maximum response to pulsatile electrical stimuli) (16) of a naïve unstimulated and a chronically stimulated deaf cat. For comparison, an acoustically evoked response (condensation click, 50  $\mu$ s, 80-dB SPL p.e.) in a normal cat is shown in (C). Poststimulus time histograms (D to G) of multiunit recordings in the auditory cortex of naïve and chronically stimulated deaf cats [electrical stimuli (15)]. The first 9 ms are blanked out to suppress stimulus artifacts. A sketch of the investigated cortical area is shown at the top of the figure. SSS, suprasylvian; AES and PES, anterior and posterior ectosylvian sulci.

were found in the field potentials of any of the naïve CDCs. In chronically implanted and stimulated CDCs, the initial middle latency response increased. In the cat stimulated for 5.5 months, it was 2.4 times as large as that of naïve cats (mean  $818.6 \pm 361.9$  versus  $336.5 \pm 54.9$   $\mu$ V;  $P = 0.0002$ , one-tailed  $t$  test) and 1.6 times as large as the amplitude measured in an electrically stimulated normal hearing cat [ $508.5 \pm 154.8$   $\mu$ V;  $P = 0.0015$ , one-tailed

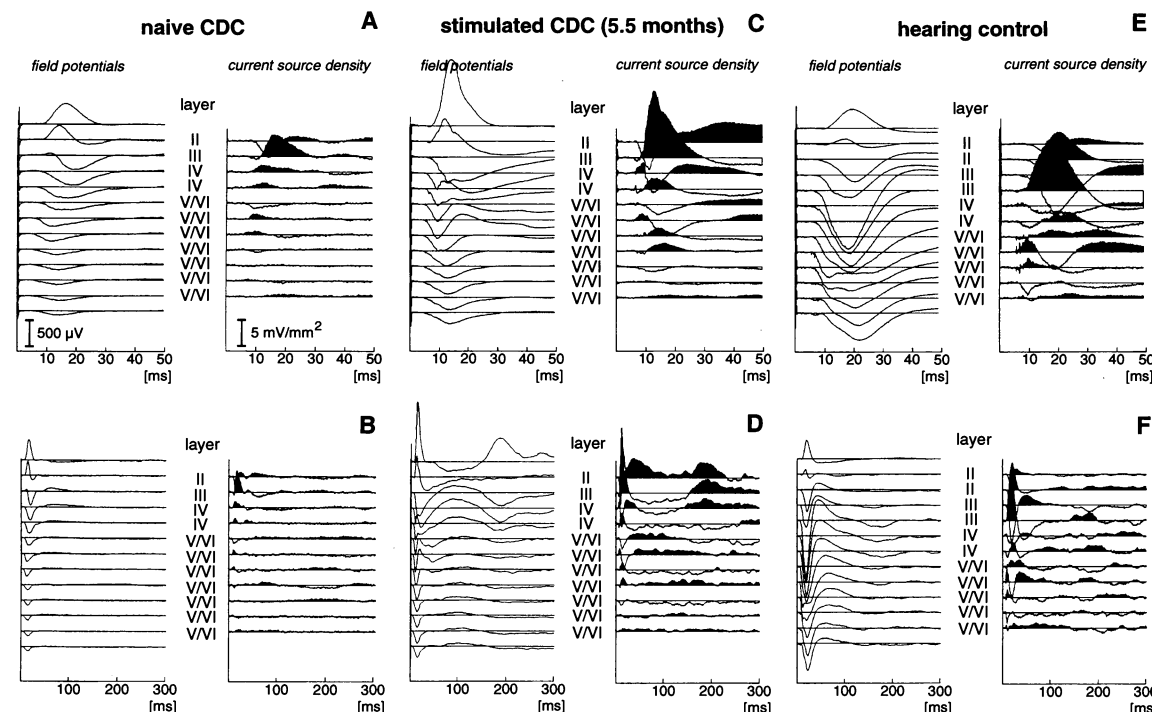
$t$  test; not shown in Fig. 2, see (10)]. In addition, late components near 150 ms appeared in the stimulated CDCs. For comparison, Fig. 2C illustrates the potential recorded in a normal hearing animal stimulated with an acoustic click [80-dB sound pressure level (SPL) p.e.]. In the one chronic cat in which recordings were also made from the ipsilateral cortex, a similar but smaller increase was seen.

The field potential data were supported by

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**Fig. 3.** Color-coded amplitude of the first positive wave of field potentials elicited by intracochlear stimulation (16). For cortical area shown, see Fig. 2. Responses from a naïve CDC (A), from an acutely deafened hearing cat (B), and from two chronically stimulated CDCs (C and D). Identical stimulation in all panels.



**Fig. 4.** CSD analyses in (A and B) a naïve deaf cat, (C and D) a chronically stimulated deaf cat, and (E and F) an acutely deafened hearing control. Scales presented are identical for all subfigures. Same data in top and bottom panels with different time scales. Uppermost recording in field potential is cortical surface; in CSD, it is 300- $\mu$ m depth. Further traces with additional 300- $\mu$ m steps each are shown. Cortical depths are transformed into cortical layers (74). Layers V and VI cannot be differentiated reliably and therefore are marked V/VI. Shaded areas are sinks.

recordings of multiunit activity (Fig. 2, D to G). In naïve, previously unstimulated CDCs, multiunit activity elicited by sinusoidal intracochlear electrical stimulation (15) uniformly consisted of an early response with a peak

latency <30 ms (Fig. 2D). No late responses were ever seen. In chronically stimulated animals, three response types could be differentiated: (i) onset responses similar to those of naïve cats followed by prolonged activity up

to 150 ms (Fig. 2E, 56% of the recordings), (ii) onset responses, followed by a silent interval but late activation (>150 ms, Fig. 2F, 43%), and (iii) rare (1%) type without onset peak (Fig. 2G). Response types i and ii were

also seen in single-unit activity, indicating that this finding is unlikely to result from summation of different types of units. Long latency activity was present after only 1 month of stimulation in all three cats studied.

In addition, the cortical area responding to the electrical intracochlear stimulus (16) expanded with increasing experience of the CDCs (Fig. 3). Color-coded amplitudes of the middle latency components of the field potentials over the auditory cortex are illustrated in a naïve, unstimulated CDC (Fig. 3A), two implanted CDCs with 2 and 5.5 months of auditory experience (Fig. 3, C and D), and one acutely deafened normal cat (10) with identical intracochlear electrical stimulation (Fig. 3B). In the chronically stimulated animals, the active cortical area expanded. The increase in active area (amplitude  $\geq 300 \mu\text{V}$ ) was a factor of 7.3 in the cat stimulated for 5.5 months compared with naïve cats.

The change of neuronal activity in chronically stimulated CDCs was based on an increase of synaptic activity as shown by current source density analyses (14). Field potentials were recorded in depths down to 3600  $\mu\text{m}$  in the cortical area showing maximal field potential amplitudes (Fig. 3). Six to eight tracks were recorded, with an intertrack distance of 500  $\mu\text{m}$ . Data from a naïve CDC, a chronically stimulated CDC, and a normal hearing acutely deafened cat are shown in Fig. 4. The electrical stimulation was identical in all animals (16).

Activity in the naïve CDC occurred 10 to 30 ms after stimulus in layer IV and, particularly, in supragranular layers II and III (Fig. 4A). After 50 ms, synaptic activity was virtually absent (Fig. 4B). In chronically stimulated CDCs, there was an increase in peak synaptic current during the first 50 ms in all layers (Fig. 4C). This effect was significant after 2 months of stimulation (one-tailed Wilcoxon-Mann-Whitney test,  $P < 0.05$ ).

A closer analysis of the temporal sequence of the synaptic activity (17) showed that in naïve cats, synaptic currents were found nearly exclusively with middle latencies and within layers IV, III, and II. In chronically implanted cats, the activity started in layers V, IV, III, and II and then continued also in infragranular layers. Latencies reached and exceeded 300 ms (Fig. 4D). This temporal and local sequence of events resembles the results obtained from electrically (Fig. 4, E and F) or acoustically (17) stimulated normal cats.

Our behavioral and the neurophysiological data indicate that the auditory cortex of chronically implanted animals is used for the processing of acoustic stimuli. In these animals, functional maturation took place between the third and eighth month of life. The changes in neuronal activity occurred soon after the initiation of chronic stimulation. Long latency responses were already observed after 1 month of hearing

experience. The enlargement of the active cortical area took 2 months or more to develop. It is assumed that these developments were possible because the animals were implanted at an early age.

The changes described in the cortex are supposed to comprise maturational processes and neural plasticity at all levels of the auditory pathway. Larger areas of the central nucleus in the inferior colliculus can be activated after part-time chronic stimulation of neonatally deafened cats with regular stimulus patterns (18). Reductions in wave IV amplitude of the electrically evoked brainstem response are also reported in neonatally deafened cats (19). When interpreting these results, consider that the population of spiral ganglia is extremely reduced with neonatal pharmacological deafening (19). However, chronic electrical stimulation improves nerve survival in these animals (20).

We assume that the major adjustments seen here took place within the auditory cortex. 1,1'-diocetadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate-staining experiments have shown that the number of dendrites, as well as of dendritic branches, are significantly reduced in CDCs compared with normal animals (21). This is reflected in small amplitudes of CSD signals in naïve CDCs (Fig. 4, A and B). The increase of CSD signals after auditory experience (Fig. 4, C and D) suggests an increase in gross synaptic currents. This may indicate an increase in synaptic efficacy or sprouting or both. For further clarification, morphological studies are necessary. Late components in CSD signals (up to 300 ms) indicate long latency synaptic activity within the auditory cortex, which is not present in naïve animals. This long latency synaptic activity is indicative of intracortical information processing. It is probably the source of late components in the evoked potentials developing in cochlear implanted children (22). As the observed effects took time to develop, it is also unlikely that a lack of inhibition from the unstimulated fibers of the auditory nerve is responsible for the larger field potentials (23).

Major differences in the organization of the auditory cortex in the stimulated CDCs compared with normal hearing cats are to be expected. This is due to the activity pattern in the auditory nerve, which is largely different from the pattern present in normal hearing cats (24). No cochleotopic differentiation is possible with a single-channel stimulating device. The information available is based on the time structure of the discharge train. Thus, central evaluation strategies developed by the implanted cats most likely differ from those of normal cats. An indication may be seen in Fig. 3. The cortical area activated in an acutely deafened normal cat is smaller than that activated after long-term stimulation in an implanted CDC. Although stimuli

were physically identical, the actual electrical stimulus was "inadequate" for the auditory system of the acutely deafened animal relying also on cochleotopic information. Nevertheless, our implanted animals learned to make use of their auditory system and showed adequate responses to acoustic stimuli. The present experiments therefore reveal both maturation and cerebral plasticity.

The results show that without afferent input, the auditory cortex remains rudimentary. This deficiency can be overcome by reafferentation of the deprived auditory channel by substitution of the missing cochlear activity. This model comprises all factors related to early treatment of deafness in infants. After implantation, a continuous input of relevant acoustic stimuli mimics normal conditions. The animals display exploratory behavior and are attentive and motivated, factors known to strengthen cortical plasticity (25). A similar recruitment of the auditory cortex is likely to be the basis of hearing acquisition in prelingually deaf infants after early cochlear implantation.

## References and Notes

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5. Kittens are born with the inner ear immature. In normal animals, functional maturation starts between postnatal weeks 2 and 3, after which adult thresholds are approached [R. Romand, *Development of Auditory and Vestibular Systems 2* (Elsevier, Amsterdam, 1992)]. During the first year of life in the CDC, nearly 100% of the basal auditory afferents are present [S. Heid, T. K. Jähn-Siebert, R. Klinke, R. Hartmann, G. Langner, *Hear. Res.* **110**, 191 (1997); S. Heid, R. Hartmann, R. Klinke, *ibid.* **115**, 101 (1998)]. In contrast, with neonatal pharmacological deafening, the number of afferents is greatly reduced (19).
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9. The electrode array consisted of four contacts. They were formed by connecting a gold wire to a seven-strand stainless steel wire. The wire formed 200-mm leads to the signal processor and was fed percutaneously between the scapulae. The highly flexible insulated lead wires (0.001 inch; A.M.-Systems, Carlsborg, WA) were embedded in medical grade silicon rubber tube (Dow Corning). The indifferent electrode was formed by

- wrapping a gold wire around the silicon tube about 50 mm from the electrode tip. Electrode impedances were below 6 kilohms at 1 kHz. Impedances were regularly checked to ensure proper function.
10. Additional control data were published in (7) and by J. Popelar, R. Hartmann, J. Syka, and R. Klinke [*Hear. Res.* **92**, 63 (1995)]. Acute deafening of normal hearing cats was performed by intracochlear application of neomycin. Compare also M. W. Raggio and C. E. Schreiner, *J. Neurophysiol.* **72**, 2334 (1994); C. E. Schreiner and M. W. Raggio, *ibid.* **75**, 1283 (1996).
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  13. Experimental cats were conditioned to receive food pellets upon presentation of a 437-Hz tone burst while the cat stayed in a neutral zone. The cat then had to walk to a food dispenser where a pellet was available for a 5-s time window. Getting food was defined as successful trial.
  14. Animals were preanesthetized with ketaminehydrochloride and Combelen (4.5 mg and 2.1 mg/kg body weight, respectively). After tracheotomy, a light isoflurane (0.4 to 0.8%),  $N_2O$  (50%), and  $O_2$  anesthesia was maintained [A. Kral, J. Tillein, R. Hartmann, R. Klinke, *Neuroreport* **10**, 781 (1999)]. The skull over the auditory cortex was opened, and the dura was removed. For precise reconstruction of recording positions and tracks, the surface of the cortex was photographed with a digital camera. The dorsal end of the posterior ectosylvian sulcus was used as reference point at 0.0 (Fig. 2). Recording electrodes were positioned by micromotors, with a precision of 1  $\mu$ m. Electrically evoked field potentials were first recorded by ball silver electrodes, diameter 1 mm. Threshold currents were determined for pulsatile stimuli (charge-balanced, 200  $\mu$ s per phase, repetition rate = 2.1 per second). Current levels were then raised by 10 dB, a value at which intensity functions start to saturate (about 400  $\mu$ A peak-to-peak in most cats). The cortex was then scanned (70 to 100 points) with glass microelectrodes (impedance < 10 megohms). Field potentials were first taken from the surface. Subsequently, depth profiles were taken (300- $\mu$ m steps). The microelectrodes were also used for recording single-unit and multiunit activity after changing filter settings. Quantitative evaluation for statistical analysis was performed with potentials recorded within a region 1.5 mm by 1.0 mm around the maximal response ("hot spot"). Sample tracks were marked by iontophoretic application of HRP. Determination of layers II, III, and IV was thus reliably achieved. Because of variations in width and angle of penetration set by the location of the hot spot, layers V and VI could not be exactly differentiated. Depth profiles were also used for CSD analyses [see U. Mitzdorf, *Physiol. Rev.* **65**, 37 (1985)].
  15. The stimulation was 437 Hz, with a 5-ms duration.
  16. Biphasic charge-balanced pulses of 200  $\mu$ s per phase, with a rate of 2.1 per second and intensity of 10 dB above field potential threshold of the most sensitive point.
  17. Normal hearing cats show shortest latencies in infragranular and granular layers [A. Mitani *et al.*, *J. Comp. Neurol.* **235**, 430 (1985); L. Aitkin, Ed., *The Auditory Cortex* (Chapman & Hall, London, 1990); J. J. Prieto, B. A. Peterson, J. A. Winer, *J. Comp. Neurol.* **344**, 349 (1994)]. These responses are found in a time window of 7 to 9 ms. Thereafter, massive activation of the

supragranular layers occurs only shortly delayed in comparison with the activity in granular layers. This most likely reflects direct activation of these layers from layer IV. Long-lasting activation of infragranular layers is seen next, corresponding well with the functional anatomy of normal auditory cortex. There was almost no activation of infragranular layers in naïve deaf cats. The CSD signals were significantly lower than in normal controls (highest mean sink amplitude = 1117  $\mu$ V/mm<sup>2</sup> in comparison with 1385  $\mu$ V/mm<sup>2</sup>; one-tailed Wilcoxon-Mann-Whitney test,  $P < 0.05$ ). After long-term electrical stimulation, in chronic animals, a pattern similar to normal hearing acutely deafened cats was observed. Sink amplitudes were significantly higher than in naïve CDCs (highest mean value = 1805  $\mu$ V/mm<sup>2</sup>); they were also 30% higher than those of normal controls, but the difference between chronically stimulated deaf and normal cats was nonsignificant.

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26. The authors thank M. Behrendt, N. Krimmel, M. Pramateftakis, C. Fritzsche, K.-F. Winter, and T. Wulf for important technical contributions; M. Kock for breeding the animals; N. Birbaumer and E. Friauf for their helpful comments on an earlier version of the manuscript; and the anonymous reviewers. Supported by the Deutsche Forschungsgemeinschaft (SFB 269).

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# A Chemical Inhibitor of p53 That Protects Mice from the Side Effects of Cancer Therapy

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Chemotherapy and radiation therapy for cancer often have severe side effects that limit their efficacy. Because these effects are in part determined by p53-mediated apoptosis, temporary suppression of p53 has been suggested as a therapeutic strategy to prevent damage of normal tissues during treatment of p53-deficient tumors. To test this possibility, a small molecule was isolated for its ability to reversibly block p53-dependent transcriptional activation and apoptosis. This compound, pifithrin- $\alpha$ , protected mice from the lethal genotoxic stress associated with anticancer treatment without promoting the formation of tumors. Thus, inhibitors of p53 may be useful drugs for reducing the side effects of cancer therapy and other types of stress associated with p53 induction.

p53 functions as a key component of a cellular emergency response mechanism. In response to a variety of stress signals, it induces growth arrest or apoptosis, thereby eliminating damaged and potentially dangerous cells from the organism (1). The p53 gene is lost or mutated in most human tumors (2). Lack of functional p53 is accompanied by high rates of genomic instability, rapid tumor progression, resistance to anticancer therapy, and increased angiogenesis (3). p53 deficiency in

mice is associated with a high frequency of spontaneous cancers (4). On the basis of all these observations, the inactivation of p53 is viewed as an unfavorable event, and much effort has been expended to facilitate anticancer treatment by restoring p53 function.

However, the role of p53 in cancer treatment is not limited to its involvement in killing tumor cells. In mice, the p53 gene is highly expressed in several normal tissues, including lymphoid and hematopoietic organs, intestinal epithelia, and the testis, and it is these tissues that are damaged by anticancer therapy (5, 6). p53-dependent apoptosis occurs in sensitive tissues shortly after gamma irradiation (6, 7), and p53-deficient mice survive higher doses of gamma irradiation than do wild-type animals (8). These data indicate that p53 is a determinant of the toxic side effects of anticancer treatment, and thus may be an appropriate target for therapeutic suppression to reduce the damage to normal

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