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Cross-modal neuroplasticity in neonatally enucleated hamsters: structure, electrophysiology and behaviour

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Abstract

Potential auditory compensation in neonatally bilaterally enucleated Syrian hamsters was explored anatomically, electrophysiologically and behaviourally. Gross morphology of the visual cortex appeared normal and no obvious cytoarchitectural malformation was discerned. However, enucleation induced a significant increase in the spontaneous firing rate of visual cortex cells. Further, auditory stimuli elicited field potentials and single unit responses in the visual cortex of enucleated, but not normal, animals. About 63% of the cells isolated in the visual cortex of 16 enucleated hamsters responded to at least one type of auditory stimulus. Most of the responses were less vigorous and less time-locked than those of auditory cortex cells, and thresholds were typically higher. Projection tracing with WGA–HRP disclosed reciprocal connections between the visual cortex and the dorsal lateral geniculate nucleus in both intact and enucleated animals. However, in the enucleated animals retrogradely labelled cells were also found in the inferior colliculus, the major midbrain auditory nucleus. Behaviourally determined auditory sensitivity across the hearing range did not differ between enucleated and intact hamsters. Minimum audible angle, as determined by a conditioned suppression task, ranged from around 17 to 22°, with no significant difference between normal and enucleated animals. The two groups also did not differ with regard to the direction of their unconditioned head orientating response to intermittent noise. However, the enucleated animals showed a more vigorous response and were slower to habituate to the noise. These results show that bilateral enucleation of newborn hamsters results in auditory activation of visual targets, in addition to the typical activation of the intact auditory pathway. Behaviourally it appears that enucleated hamsters, compared with their normal littermates, are slower to habituate in their response to an unexpected source of sound.

Introduction

The question of whether specific sensory nuclei possess intrinsic constraints that enable them to process only the input of the original modality has been extensively studied in recent years in congenitally blind as well as in experimentally enucleated animals. In newborn rats, afferents from dorsal column nuclei or the inferior colliculus extend beyond their appropriate terminal targets within the thalamus into adjacent thalamic nuclei. Typically, however, these erroneous projections subsequently degenerate (Asanuma *et al*., 1988). A prominent innervation and arborization of the dorsal lateral geniculate nucleus (dLGN) by ascending somatosensory projections has been demonstrated in adult congenitally blind mice (Asanuma & Stanfield, 1990). In the blind mole rat (*Spalax ehrenbergi*), auditory stimuli activate the dLGN and the occipital cortex, the two major forebrain visual targets (Bronchti *et al*., 1989; Heil *et al*., 1991). The main origin of the auditory input to the occipital cortex in this animal is the dLGN, which receives direct input from the inferior colliculus (IC), the major midbrain auditory nucleus (Doron & Wollberg, 1994). Auditory projections from the IC to the dLGN were also found in the common mole, *Mogera*, a subterranean insectivore with a highly reduced visual system (Kudo *et al*., 1997). Activation of primary and secondary visual areas by auditory and/or somatosensory stimuli has also been demonstrated in neonatally enucleated mice, rats and cats binocularly deprived at birth by eyelid suturing or enucleation (e.g. O'Leary & Stanfield, 1987; Asanuma & Stanfield, 1990; Bronchti *et al*., 1992; Rauschecker *et al*., 1992; Rauschecker & Kniepert, 1994; Toldi *et al*., 1996; Yaka *et al*., 1999; Negyessy *et al*., 2000).

Cross-modal neuronal reorganization as a result of sensory impairment has also been addressed by a different approach in which information-carrying projections of one modality were neonatally rerouted by surgical manipulations to the processing area of another modality (e.g. Frost, 1999; Frost & Metin, 1985; Sur *et al*., 1988; Pallas *et al*., 1990; Roe *et al*., 1992; Pallas & Sur, 1993; Gao & Pallas, 1999). Preliminary experiments with neonatally enucleated Syrian hamsters conducted in our laboratory revealed that auditory stimuli elicited prominent field evoked potentials and single-cell responses in the occipital cortex. The present study was undertaken in order to substantiate this finding, to characterize the response properties of the auditorily activated cells and to compare these properties with those in auditory cortex of intact hamsters. To examine whether enucleated hamsters might possess altered hearing abilities, we behaviourally determined the minimum audible angle

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around midline, the unconditioned orientating response to sound and detection thresholds throughout the hearing range in neonatally enucleated hamsters and their normal littermates.

Hamsters were selected for this study primarily for the reason that they are born at an early stage of neural development (Crossland & Uchwat, 1982) and because previous studies with impaired hamsters form a well established database for comparison (e.g. Smith & Bodemer, 1963; Tiao & Blakemore, 1976; Bennett-Clarke *et al*., 1989; Metin & Frost, 1989). We used enucleated animals rather than eyelid-sutured animals because sutured eyelids still permit penetration of light, therefore representing a less severe impairment. Unlike the studies cited above, in which visual projections were routed into auditory deprived targets (cf. Frost, 1999; Frost & Metin, 1985; Roe *et al*., 1992; Pallas & Sur, 1993), we did the converse experiments, inducing auditory activation of visual targets deprived of their normal input. Furthermore, whereas in the previous studies retinal projections were rerouted to auditory thalamic targets by deafferenting the ascending auditory pathway to the thalamus and by also ablating retinal targets, we left the entire auditory system intact. By doing so, we induced an auditory activation of visual targets without affecting the typical auditory system. We believe that this approach more accurately simulates some congenital or accidental blindness.

Preliminary results of this study were presented earlier in abstract form (Heffner *et al*., 1994; Wollberg *et al*., 1999).

Materials and methods

Animals

All animals used in this study were from a single breeding colony at Tel Aviv University. Bilateral enucleation was performed in newborn Syrian hamsters no later than 12 h after birth. Animals were anaesthetized by hypothermia (in an ice-water bath) and small slits were made in the skin/eyelids that overlay the eyes. The eyes were then carefully removed and the skin flaps glued with tissue glue (medical cyanoacrylate). The entire surgical procedure was performed under a dissecting microscope assuring a complete removal of the eyes. The operated animals (referred hereafter, interchangeably, as 'enucleated' or 'blind' animals) were then re-warmed and returned to their mothers until weaning. A complete enucleation was finally confirmed, after killing the animals, by the total degeneration of the optic nerve and optic chiasm (see Results section). In the electrophysiological experiments this was further corroborated by the lack of visual evoked potentials. Intact pups, reared under identical conditions, served as controls. Four to six animals were housed per cage and kept at a constant temperature of 24 °C and under a constant light/dark regime (14/10 h). Their diet consisted of concentrated rat chow and water *ad libitum*. Experiments were performed with animals of both sexes that had reached full maturation and body weight. Mean body weight (± SD) of the normal and enucleated animals was 159.2 ± 15.2 and 160.2 ± 22.3, respectively, with no significant difference between the two groups (*t*-test, $P = 0.85$). Except for the blindness and some more intensive motor activity of the enucleated hamsters, no difference in the health of the two groups could be observed.

Electrophysiology

Animal preparation

All surgical procedures and electrophysiological recording sessions were conducted under deep anaesthesia (ketamine hydrochloride 150–200 mg/kg i.m. and xylazine hydrochloride 2.5–7 mg/kg i.m.). Supplemental doses of ketamine (50–70 mg/kg) were administered as needed. Anaesthetized animals were placed in a stereotaxic apparatus and held in place by means of a specially designed nontraumatic head holder leaving the ears unobstructed. A craniotomy was performed over the left visual and/or auditory cortices leaving the dura intact. Paraffin oil was administered over the exposed surface of the brain to prevent dehydration. A local anaesthetic (lidocaine hydrochloride 2%) was applied to wound edges. Heart rate, ECG and EEG were monitored continuously and body temperature was maintained at ≈ 37 °C using a DC temperature-controlled heating pad (Frederick Haer & Co., Brunswick, ME 04011, USA). Recording sessions were conducted within a suspended double-walled sound attenuation chamber (IAC model 1204; Industrial Acoustics Co., New York, USA) with its inner walls covered with sound-damping foam to reduce echoes. A similar anaesthesia regime was used during recordings.

Recording and stimulation

Field evoked potentials (FEPs) and single and multiunit responses were recorded extracellularly by means of glass-coated platinum–iridium microelectrodes (impedance 0.5–2.0 MΩ at 1 kHz) that were advanced through the dura by a calibrated micromanipulator that was remotely controlled by a stepping motor (4.5 μm resolution). FEPs were amplified, band-passed filtered (0.02–5.0 kHz; LN Neurolog System, Digitimer Research Instrumentation, England), visually monitored, digitized and averaged (sample period 100 μs; RC Electronics, USA). Cellular activity was amplified, band-passed filtered (0.5–10 kHz; LN Neurolog System), auditorily and visually monitored, discriminated from background activity by a window discriminator (type 121, WPI Instruments, USA), digitized and displayed on-line as dot rasters. All electrophysiological data were stored for off-line analyses on a personal computer.

Visual stimuli consisted of a light flash (750 000 candlepower) produced by a photostimulator (type PS3, Grass Instruments, USA). As this stimulus was used primarily for determining the boundaries of visual cortex and for detecting visually responsive cells in intact animals, no attempts were made to determine the shape and size of visual receptive fields or other response properties of the visually driven cells. Auditory stimuli consisted of 0.1-ms clicks generated by a programmable pulse generator (Master-8, A.M.P.I., Israel), broadband noise (0.02–20 kHz; noise generator type 1405, Brüel and Kjaer, Naerum, Denmark) and pure tones rastered over a range of 0.25–31 kHz in sequential linear steps of 0.25–2.0 kHz (voltage-controlled oscillator type 136, Wavetek, San Diego, USA). The noise and pure tones were shaped into 200-ms bursts by a trapezoidal waveform with 15-ms rise and fall times. Stimuli, presented at a rate of 0.3–0.5 stimuli per second, were fed into a custom-made power amplifier via a manually controlled attenuator (Hewlett-Packard 350D) and delivered under free-field conditions through a three-way loudspeaker (type AR 2ax, Acoustic Research International, England), located ≈ 1.5 m in front of the animal (unless specified differently). Sound pressure levels were calibrated and monitored by a 0.5-inch condenser microphone (type 4134, Brüel and Kjaer) connected to a sound level meter (type 2209, Brüel and Kjaer) located above the animal's head. Standard sound pressure levels of all auditory stimuli was 80 dB SPL (unless specified differently). Frequency response of the entire sound delivery system was nearly flat (± 5 dB) from 0.5 to ≈ 15 kHz, falling off 20 dB at 30 kHz.

Boundaries of the visual cortex in normal animals were determined by visual field evoked potentials and used as guidelines in the experiments with the enucleated hamsters. The boundaries of the auditory cortex in intact and enucleated hamsters were determined in

each experiment using auditory field evoked potentials before initiating the recording of single cell activity. To disclose possible somatosensory activation of the visual cortex in enucleated hamsters, and to roughly determine the borderline between the visual cortex and the adjacent somatosensory areas, we also stimulated electrically the facial whisker pad area with bipolar fine stainless-steel needle electrodes (0.05-ms square pulses, ≈ 10 V).

At the end of the electrophysiological experiments, animals were given an overdose of Nembutal (100–140 mg/kg i.p.) and perfused through the heart with 0.9% saline solution followed by 10% neutral buffered formalin. The brains were then carefully removed and checked for possible gross morphological changes in the enucleated animals. Spatial localization of electrode penetration sites was referenced to the midline and the interaural lines and to the bregma and lambda sutures. Assignment of recording depths was based on the calibrated microelectrode driver and on reference electrical lesions (10–20 μA, 10 s) made at the end of the experiment and identified in Nissl-stained coronal frozen sections.

Histology and projection-tracing procedures

Pertinent thalamic and cortical structures in the normal and enucleated hamsters were identified in Nissl-stained sections using stereotaxic atlases of the golden hamster as guides (Smith & Bodemer, 1963; Knigge & Joseph, 1968). Topological, morphological, cytoarchitectural, fibroarchitectural and electrophysiological criteria were applied as well.

Wheat-germ agglutinin conjugated to horseradish peroxidase (WGA–HRP) (Sigma type VI) was applied into the primary visual cortices and/or the lateral geniculate nuclei (LGN) of seven normal and 13 neonatally enucleated hamsters. Application of the tracer, performed under deep anaesthesia (Avertine, 1 mL/100 g body weight, i.p), was accomplished using a glass micropipette with its tip (diameter 100–200 μm) filled with WGA–HRP flakes (Mori *et al*., 1981). A small hole was drilled in the bone, the dura was gently removed, and the electrode was advanced into the visual cortex. Visual field evoked potentials, elicited in normal hamsters, defined the occipital area within which the tracer was applied. To reach the dLGN the micropipette was advanced through the visual cortex using as guidelines the stereotaxic coordinates and our own serial histological sections. After the pipette was inserted into the desired area it was held in place with dental cement until perfusion (24–72 h later). The animals were then anaesthetized with fluothane and perfused through the heart with buffered saline containing 2% heparin, followed by 1.2% glutaraldehyde and 1% paraformaldehyde in 0.1 M neutral buffered phosphate and, finally, with 5% glycerol in cold 0.1 M neutral buffered phosphate. The micropipette was withdrawn, the brain was removed from the skull, immersed overnight in neutral buffered phosphate containing 20% glycerol at 4 °C, and then embedded in gelatin. Frozen frontal sections (50 μm) were cut with a sliding microtome. Alternate sections were processed with tetramethylbenzidine (TMB) (Mesulam, 1982) mounted and dried at 4 °C for 12 h. The sections were then dipped in 2.5% ammonium molybdate [to prevent the HRP reaction product from fading (Fujii & Kusama, 1984)], dehydrated, cleared and covered. The remaining sections were stained alternately for Nissl with Merck's Cresyl violet acetate or for myelin with Merck's haematoxylin. The volume of the LGN in normal (n = 4) and enucleated (n = 4) hamsters was computed by measuring its area in each section (SigmaScan Pro image analysis software, Jandel Scientific/SPSS Inc., USA) and multiplying by the thickness.

Behavioural experiments

Animals

A total of 15 normal and 11 enucleated Syrian hamsters were used in these tests. They were maintained on a 12-h light/dark cycle and individually housed in solid-bottomed cages with corncob bedding. Free access to standard hamster chow was provided, with occasional supplements of fruit and vegetable treats. Water was available only in the test sessions and the animals' weights were monitored daily.

Behavioural apparatus

Testing was conducted in a carpeted, double-walled chamber (IAC model 1204; 2.55 × 2.75 × 2.05 m, Industrial Acoustics Co., New York, USA), the walls and ceiling of which were lined with acoustic foam. The equipment for behavioural and stimulus control was located outside the chamber and the animals were observed over closed-circuit television.

The animals were tested in a wire cage (35 × 21 × 24 cm) constructed of half-inch (1.27 cm) wire mesh mounted on a camera tripod ≈ 1 m above the chamber floor (Heffner *et al*., 2001). A water spout, consisting of 15-gauge brass tubing with a small brass oval 'lick plate' at the top, came up through the floor in the front of the cage. The spout was adjusted to a level that permitted the hamster to drink comfortably with its head facing the perimeter bar, at 0° incidence. Thus the height of the spout did not interfere with the sound reaching the animal's ears when it was drinking from the spout. A contact circuit, connected between the spout and cage floor, detected when an animal made contact with the spout and activated a 25-mL syringe pump to dispense a trickle of water. The flow rate was adjusted so that an animal could obtain adequate water in a single test session lasting 30–60 min. Requiring the animals to keep their mouths on the water spout in order to receive water served to fix their heads in the sound field.

Mild shock, used in the conditioned suppression/avoidance experiments and also to sensitize the hamsters in the unconditioned experiment, was delivered using a shock generator, connected between the water spout and cage ground. Note that the animal could avoid or escape the shock by breaking contact with the spout. Except as noted, testing was conducted in dim light from two 25-W incandescent bulbs to permit monitoring the animals over closed-circuit television.

Acoustical apparatus and sound measurement

The sounds were presented through loudspeakers (Motorola piezoelectric KSN1005A, Motorola Corp., Chicago, IL, USA) mounted at ear level on a perimeter bar (102 cm radius) centred on the position occupied by an animal's head while it was drinking from the spout. Depending on the type of task, pure tones or broadband noise were generated using a signal generator (Zonic A & D 3525, Zonic Corp., Tokyo, Japan), attenuated (Hewlett Packard 350D, Hewlett Packard, Loveland, CO, USA), pulsed (Coulbourn S53-21, Coulbourn, Lehigh Valley, PA, USA) shaped with a rise/decay gate (Coulbourn S84-04), bandpass filtered (Krohn-Hite 3550, Krohn-Hite, Avon, MA, USA) and amplified (Crown D75, Crown International, Inc., Elkart, IN, USA) to the desired level. The electrical signal going to the loudspeakers was constantly monitored on an oscilloscope (B & K Precision 1476 A, Dynascan Corp., Japan).

The sound pressure level (SPL re 20 μPa) was measured daily using a 1/4-in (0.64-cm) microphone (Brüel & Kjaer type 4135, Brüel & Kjaer, Naerum Denmark), preamplifier (Brüel & Kjaer type 2618) and microphone amplifier (Brüel & Kjaer type 2608). The output of the measuring amplifier was then routed to a spectrum analyser

(Zonic A & D 3525) where the acoustic signal was visually inspected and verified. Sound measurements were taken by placing the microphone in the position occupied by an animal's head when it was drinking and pointing it directly toward the loudspeaker (0° incidence). Care was taken to produce a homogeneous sound field (± 1 dB) in the area occupied by the animal's head and ears and that there was no significant distortion when producing pure tones. (For additional details of the test procedure, tone generation and sound measurement see Heffner *et al*., 2001).

Habituation of unconditioned orientation to sound

The time course of habituation of the orientation response to noise was determined for eight enucleated and seven normal hamsters. A thirsty animal was placed in the test cage and allowed to drink from the water spout, an action that centred its head between the two loudspeakers. The loudspeakers were hidden from view by loosely draped fabric. Within the first three minutes after entering the test cage, a brief shock (0.003 mA, 144 V, 300 ms duration), completely unassociated with the auditory stimulus, was administered to the hamster via the spout to sensitize it to the test environment. The shock was used only to increase the hamster's overall level of arousal and was not associated with the sounds in this test. At random intervals after the shock, the orientation stimulus, which consisted of two 100-ms broadband noise bursts (2.5–45 kHz, 85 dB SPL, 0.1 ms rise/decay, 900 ms interburst interval), was presented. This orientation stimulus was presented twice per session (with at least 5 min between presentations) for nine consecutive daily sessions.

The response of the hamsters during and following the presentation of the noise burst was recorded by a video camera located in front of the animal. A light-emitting diode (LED), located above and behind the animals (out of their sight), was turned on during stimulus presentation to indicate stimulus onset. The left–right sequence of stimulus presentation was determined by a quasi-random schedule (Gellermann, 1933).

The video taped responses were analysed frame-by-frame by two independent observers who were unaware of which speaker had emitted the stimulus. For each sound onset (indicated by the LED) the first directional movement of the head within 1 s was coded for direction of the orientating response (either left or right). The observed direction of head turning was also compared to the actual location of the active speaker to examine the appropriateness of the orientation, either toward or away from the correct hemifield. It should be noted that hamsters do not show precise head orientation to sound; this is not surprising because they have relatively broad visual fields. Of interest here was the persistence of the orientation response and we recorded the approximate magnitude of the response (with 0 being no observable directional response, 1 a barely detectable head or pinna turn, 2 a distinct head turn and 3 a whole-body turn). The two observers showed better than 90% agreement (interobserver reliability) and their ratings were averaged for the subsequent analysis. Observed direction of head turning was also compared to the actual location of the active speaker. The animals were 4–6 months old when tested.

Detection thresholds

Auditory sensitivity to pure tones was determined using a conditioned suppression/avoidance procedure for five enucleated and five normal hamsters. For this test the hamster was trained to drink from the spout during silence, but to break contact with the spout whenever it detected a pure tone (four pulses of 400 ms duration, 10 ms rise/decay, 100 ms interpulse interval), thus avoiding the shock that began at the end of the tone. The loudspeaker was placed directly ahead and facing the hamster when it drank from the spout. After the hamsters learned to respond reliably to moderately loud sounds, the intensity of the pure tones was decreased (in 5-dB steps) until the animals no longer responded above chance ($P > 0.01$, binomial distribution).

Testing was conducted in blocks of 6–8 tone trials (accompanied by ≈30–40 silent trials) at fixed intensities, ranging from at least 10 dB above threshold to 10 dB below threshold. Breaking contact with the spout was classified as a 'hit' if the trial contained a tone or as a 'false alarm' if the trial consisted of silence. The performance of an animal at a particular intensity was determined by the formula: Performance = Hit rate – (False Alarm rate × Hit rate). Threshold was then defined as the intensity at which detection performance equalled 50% (Heffner & Heffner, 1995). Testing was considered complete at a particular frequency when the thresholds obtained in at least three different sessions were within 3 dB of each other, with the final threshold being the average of these three thresholds. In order to sample the entire useful hearing range of the hamsters, thresholds were obtained for low (125 Hz), middle (2 and 8 kHz) and high (40 kHz) frequencies. The animals were 12–14 months old when tested.

Sound localization acuity

The minimum audible angle (sound localization threshold) was also determined using the conditioned suppression/avoidance procedure. Eight enucleated and 12 normal hamsters were tested. The animals were first accustomed to drinking from the spout while a broadband noise burst (2.5–45 kHz, 100 ms duration, 0.1 ms rise/decay, 60 dB SPL) was emitted every 3.3 s from a speaker to the right. They were then trained to break contact whenever a single noise burst was emitted from a speaker to the left, to avoid the shock at the end of trials containing left signals. To prevent the hamsters from responding on the basis of slight intensity differences and/or small differences in speaker quality, the intensity of the signal was randomly varied over a range of 3.5 dB and three pairs of matched loudspeakers were used. Noise localization thresholds were determined by gradually reducing the angular separation between the left and right loudspeakers around midline (in blocks of 6–8 left trials at each angle), until the animal could no longer distinguish left from right noise bursts ($P > 0.01$, binomial distribution). Daily testing continued until performance no longer improved at any angle. The mean of the three trial blocks with the highest scores was then calculated to represent the best performance for each animal. If none of the trial blocks showed performance above chance, all scores were included in the average. Threshold was defined as the angle that was discriminated half the time, after correcting for false alarms (see formula above). Angular separations tested were 180, 120, 90, 60, 45, 30, 20, 15 and 10° centred on midline. The animals were 5–10 months old when tested. For details and rationale of the behavioural procedure, see Heffner & Heffner (1995).

All surgical and experimental procedures were performed in accordance with the standards for care and use of laboratory animals as approved by the Tel Aviv University and University of Toledo Institutional Animal Care and Use Committees.

Results

Morphology

The most apparent change in the gross morphology of the enucleated hamster brain was the complete degeneration of the optic nerve and optic chiasm (Fig. 1). In addition, the calculated total volume of the

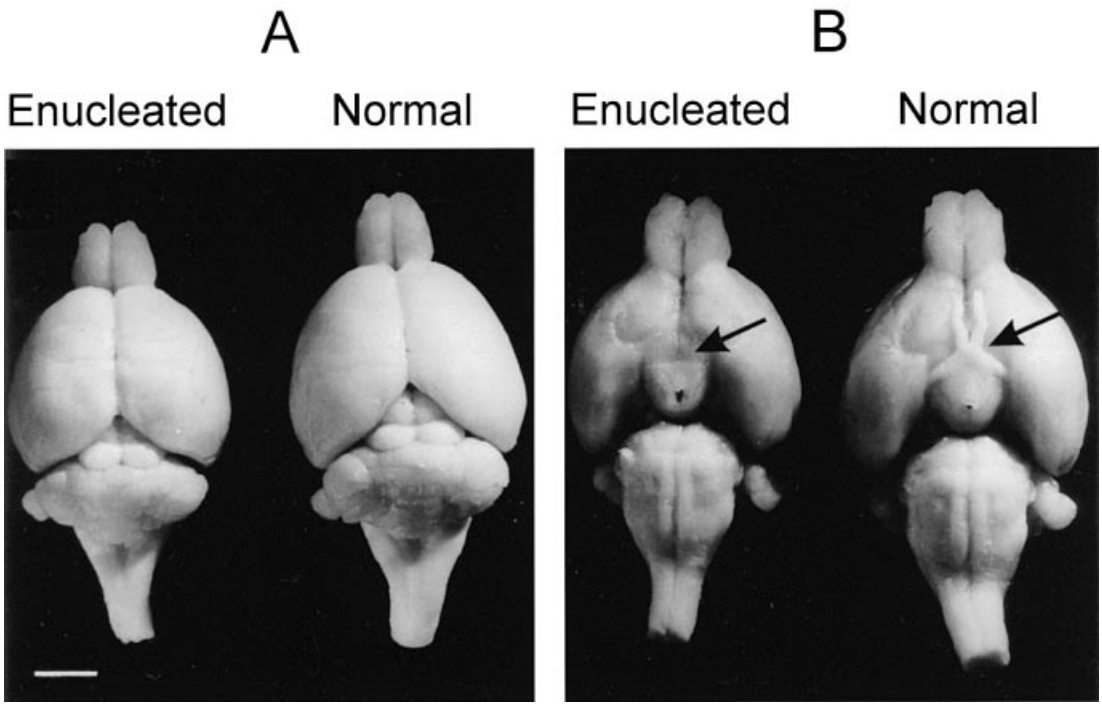

FIG. 1. Whole brains of enucleated and normal hamsters viewed from (A) the dorsal and (B) the ventral aspects. Note that the dorsal aspects of the enucleated and normal animals look very much alike whereas the optic nerve and chiasm of the enucleated animal is completely degenerated (arrows in B). The slight difference in size between the brains of normal and enucleated hamsters, noticeable in this figure, reflects differences in body size of different individuals, including their brains, rather than an effect of enucleation on brain size. Scale bar, 4.0 mm.

lateral geniculate nucleus of the enucleated hamsters was ≈11% smaller than in normal hamsters. No other obvious and consistent gross morphological, histological or fibroarchitectural differences between the normal and the enucleated animals' brains were detected (Figs 2–4). Although no attempt was made to quantify slight histological or fibroarchitectural differences that might exist, by conventional criteria, the major visual centres were still present after enucleation.

Electrophysiology

General overview

Electrophysiological data were collected from 30 normal and 16 enucleated animals. Boundaries of the auditory and visual cortices were determined by surveying the temporal and occipital cortices of intact and neonatally enucleated hamsters for field potentials evoked by auditory clicks and flashes of light. Occasionally, pure tones and white noise were employed as well. To determine the caudal border of the cortical somatosensory vibrissae representation, we electrically stimulated the facial whisker pad. Until the approximate boundaries of the auditory and visual cortices were established, each experiment was initiated by exposing and scanning the entire occipital, temporal and medial areas of the hemisphere dorsal to the rhinal fissure. In all other experiments either the visual or the auditory area was exposed. Single and multiunit responses within each area were regularly recorded from sites where field evoked potentials were most prominent. More peripheral areas were scanned as well. Although most of the cells in our sample were detected by their spontaneous firing rate, search stimuli were also occasionally employed.

Boundaries of the visual and auditory cortices

In the cortex of normal animals, two distinct foci (determined by maximal field evoked potentials) were detected, one visual and one auditory (Fig. 5). The centre of the auditory activated field (referred to hereafter as auditory cortex) lies ≈6 mm lateral to the midline and ≈3–4 mm rostral to the interaural line. The centre of the visually activated field (referred to hereafter as visual cortex) lies ≈3 mm lateral to the midline and ≈2 mm rostral to the interaural line.

As anticipated, flashes of light did not elicit any response in the blind animals, further corroborating the visual impairment. Electrical stimulation of the facial whisker pad evoked field potentials in the somatosensory cortex of both normal and enucleated hamsters but not in their visual cortices. However, in blind animals, auditory clicks evoked field potentials and single unit responses not only in auditory cortex but also in occipital areas that were activated by a visual stroboscopic stimulus in normal hamsters (Fig. 5) (see also Tiao & Blakemore, 1976). Auditory evoked potentials recorded from the surface of the visual cortex varied in their shape but typically they were positive–negative or triphasic. Occasionally, waveforms were even more complex. As the electrode moved towards deeper layers, the polarity of the evoked potentials reversed and turned into a negative or negative–positive waveform with maximum values at depths ranging between 360 and 550 μm, coinciding with layers III–IV. These field evoked potentials were used mainly to delineate the boundaries of the visual and auditory activated areas and the properties of these responses were not explored in detail. Visual stimuli other than a light flash or other sites of somatosensory stimulation were also not explored.

Spontaneous firing rate

Spontaneous firing rates of single cells in the visual cortex and auditory cortex of normal and blind hamsters are summarized in Fig. 6. The possibility that all four groups have similar firing rates was statistically rejected (one way ANOVA, $P < 0.001$). Applying Bonferroni's pairwise *t*-test revealed that in normal hamsters

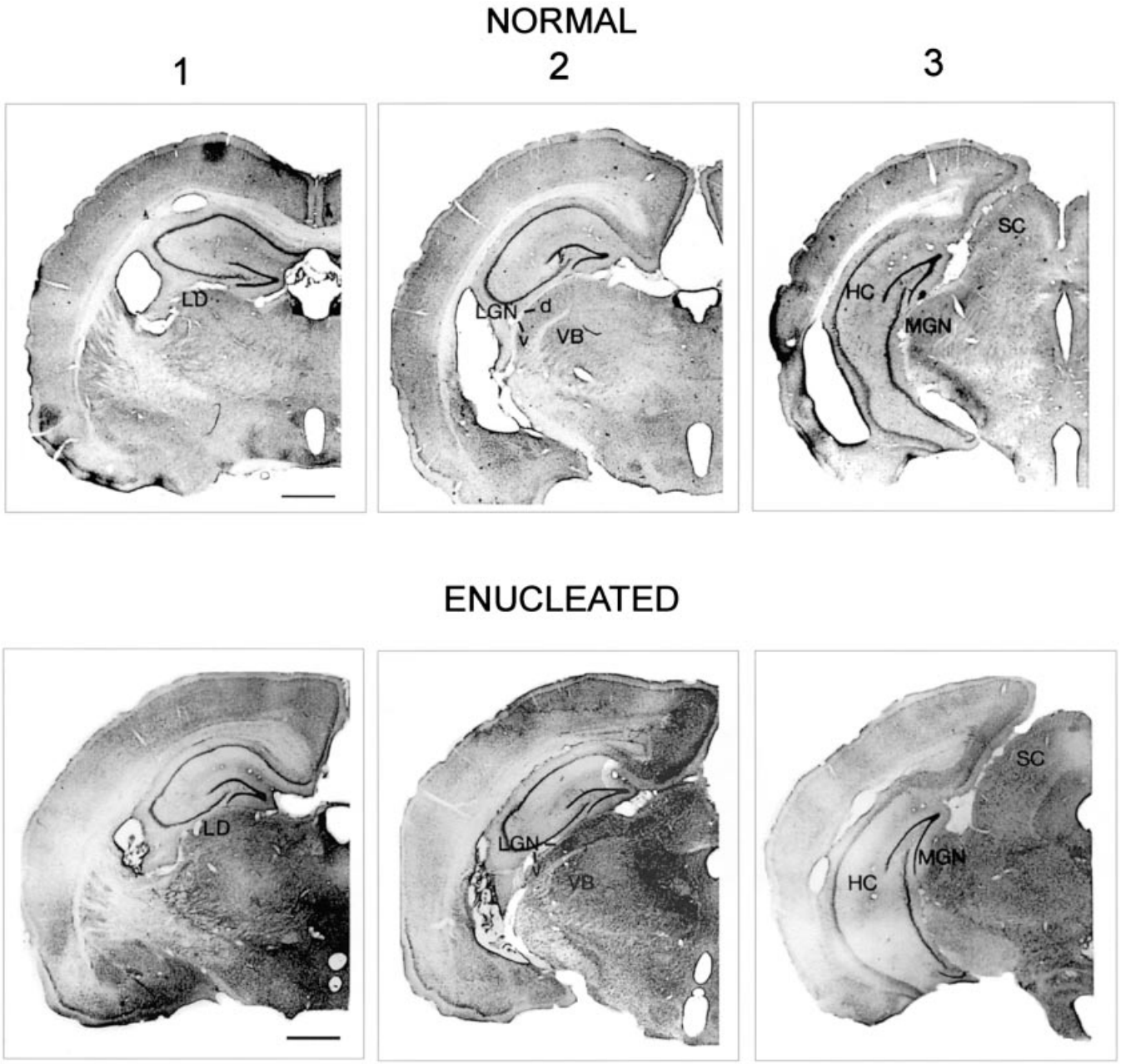

FIG. 2. Sequential Nissl body-stained frontal sections through the brains of a normal and a blind hamster at the pertinent thalamic regions (not at exactly comparable levels). Distance between sections is ≈ 1.0 mm in the normal hamster and ≈ 0.6 mm in the blind hamster. Abbreviations: HC, hippocampus; LD, lateral dorsal thalamic nucleus; LGN, lateral geniculate nucleus (v, ventral, d, dorsal); MGN, medial geniculate nucleus; SC, superior colliculus; VB, ventrobasal thalamic nucleus. Scale bars, 0.8 mm.

spontaneous firing rate of cells in the visual cortex was significantly higher than that of cells in the auditory cortex ($P < 0.008$) and that cells in the visual cortex of enucleated hamsters have a significantly higher spontaneous firing rate than that of cells in the auditory cortex of either normal or enucleated hamsters ($P < 0.001$). Most intriguing was the finding that the spontaneous firing rate of cells in visual cortex of enucleated animals was significantly higher than that of cells in the visual cortex of normal animals ($P < 0.001$). There was no significant difference in the spontaneous firing rate between the auditory cortex of normal and enucleated animals or between visual cortex of normal hamsters and the auditory cortex of blind animals.

Responsiveness and response patterns

In normal hamsters > 90% of the cells in auditory cortex responded to at least one of the three auditory stimuli that were used (clicks, tone bursts and noise bursts). As seen in Fig. 7, almost all of the responding cells were driven by all three stimuli. Similar proportions were found among all of the 428 multiunits (not shown). Although it appears that the responsiveness of auditory cortical cells in enucleated hamsters to each of the three auditory stimuli was slightly lower than in normal hamsters, this difference was not reliable (Fisher's exact test, $P > 0.9$). Responses of auditory cortical cells to

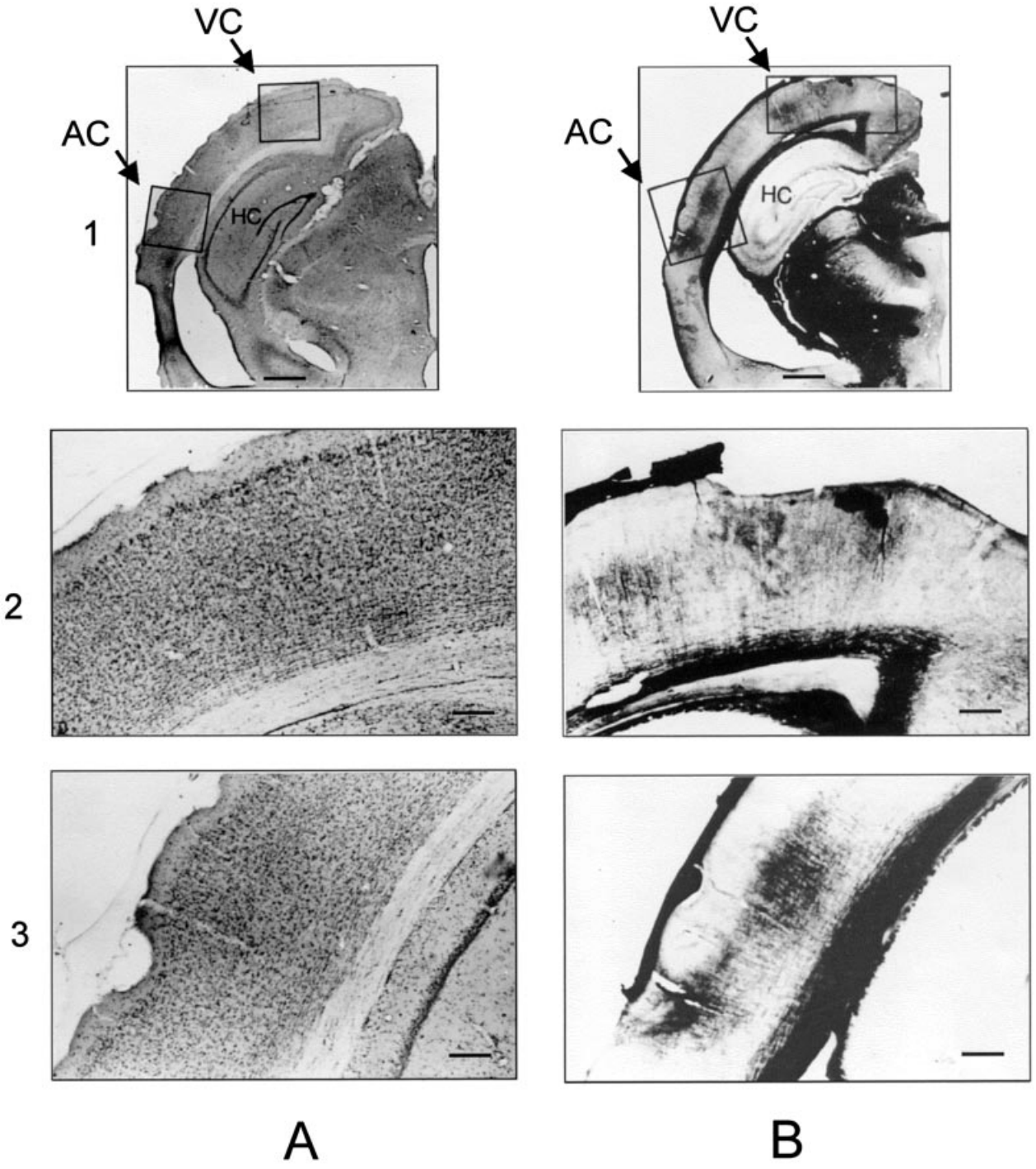

FIG. 3. Nissl body (A1) and myelin (B1) -stained frontal sections through the visual and auditory cortices (VC and AC, respectively) in a normal hamster. A2, A3 and B2, B3: enlargements of the visual and auditory cortices, respectively. Scale bars, 0.8 mm (A1 and B1); 0.15 mm (A2 and B2); 0.2 mm (A3 and B3).

auditory stimuli were less abundant as the electrode was placed farther from the centre of the auditory field. Very few cells located in the area between the auditory and the visual fields responded to auditory stimulation. No bimodal cells were detected in the auditory or the visual areas of normal hamsters.

In the visual cortex of the normal hamsters, 57 cells out of 69 (83%) responded vigorously to flashes of light but not to any of the auditory stimuli that were used. In the blind hamsters, however, 79 out of 126 cells (63%) responded to at least one of the three types of auditory stimuli (Fig. 7). Most of these cells were isolated at different cortical depths in the approximate area of maximum auditory field evoked potentials. It is also apparent that all three auditory stimuli were effective in driving auditory responsive cells in visual cortex, with the click driving a relatively smaller number of cells than tone bursts or noise bursts (χ^2 test, $P < 0.01$ and $P < 0.029$, respectively). Each one of the auditory stimuli was significantly less effective in

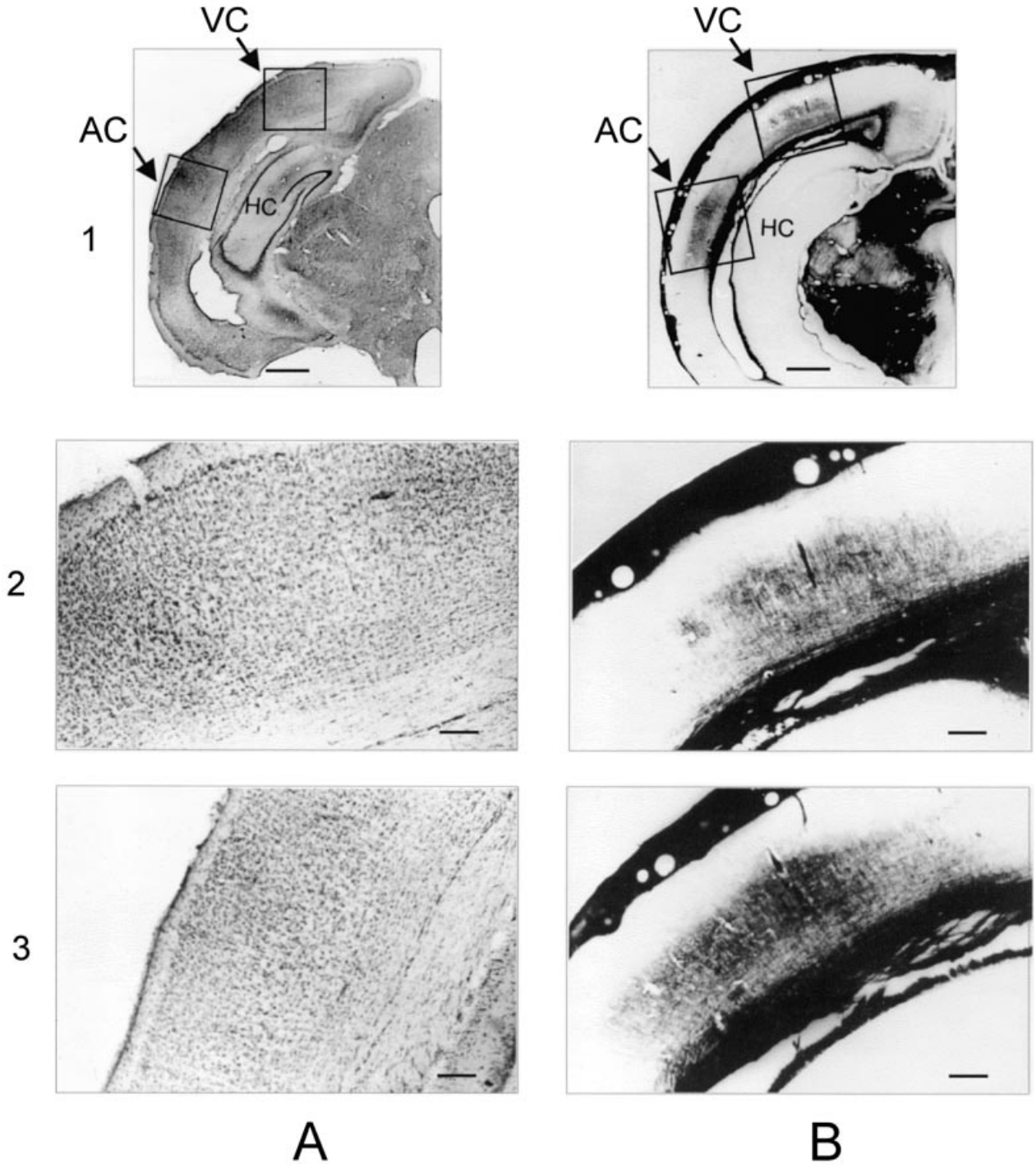

FIG. 4. Nissl body (A1) and myelin (B1) stained frontal sections through the visual and auditory cortices (VC and AC, respectively) in an enucleated hamster. Scale:. A2, A3 and B2, B3: enlargements of the visual and auditory cortices, respectively. Scale bars, 0.8 mm (A1 and B1); 0.15 mm (A2 and B2); 0.2 mm (A3 and B3).

eliciting a response in the visual area of blind hamsters as compared with the auditory cortex of either normal or blind animals (χ^2 test, $P < 0.001$).

Responses of cells in the auditory cortex to auditory stimuli in normal hamsters are illustrated in Fig. 8A. Generally, responses were very vigorous, repeatable and highly time-locked to the stimulus. Response patterns elicited by the auditory click consisted, in most cases, of a short excitatory component often followed by some suppression of the firing rate ('inhibition'). Responses to tone and noise bursts were also quite simple, consisting of a short excitation at the onset of the stimulus followed occasionally by some suppression and some excitation or rebound at the end of the stimulus. In some of these cases, the discharge rate of the onset excitation as a function of sound intensity was 'nonmonotonic', manifested by an increase in

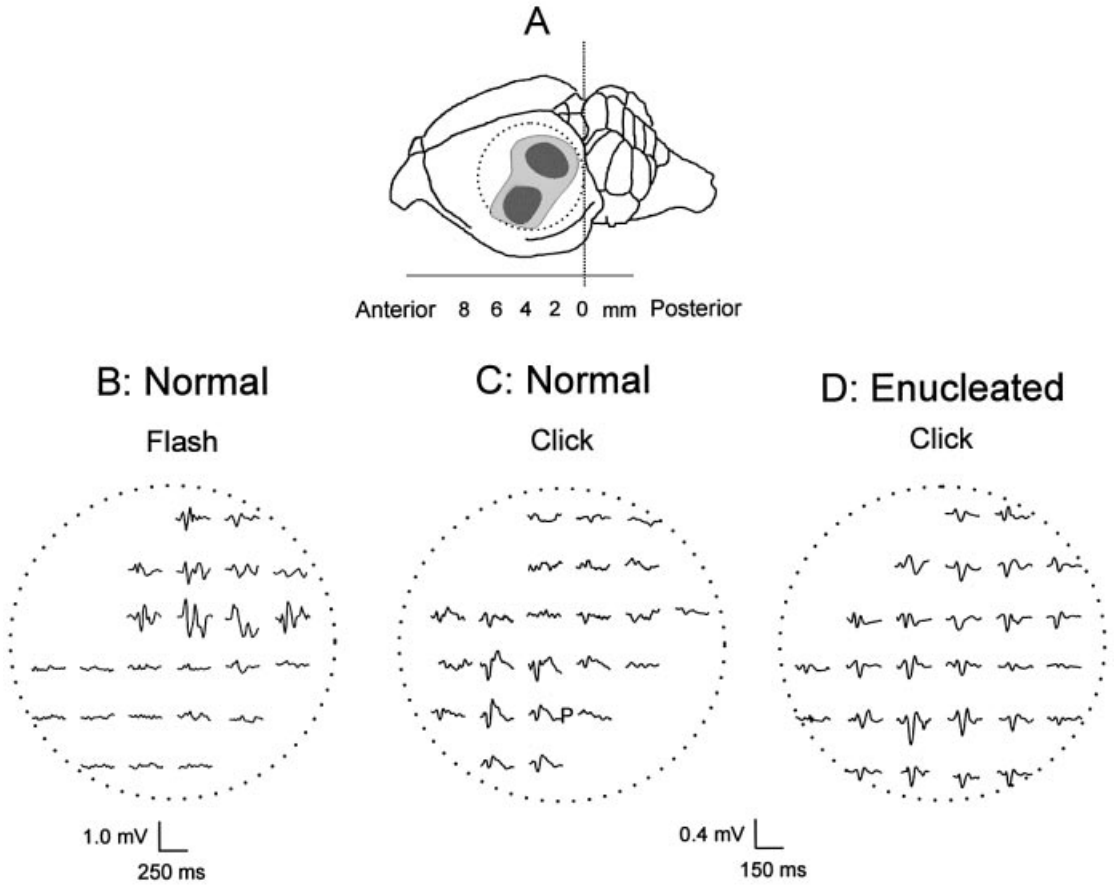

FIG. 5. (A) A dorso-lateral schematic view of a hamster's brain illustrating (B and C, respectively) averaged visual and auditory field-evoked potentials in the cortex of a normal hamster and (D) auditory evoked field potentials elicited in the cortex of a blind hamster. Circles represent the exposed cortical areas where recordings were made. Stimuli were delivered in all cases at the beginning of the sweep. Note the existence of a distinct visual and auditory area in the normal animal and the expansion of the auditory responsive area into the visual cortex in the blind animal. Dashed vertical line in A designates the interaural line. Light grey area in A represents the entire auditory responsive area in blind hamsters and the dark grey areas designate regions with maximal auditory responses based on pooled results from all the experiments.

spike count function up to a maximum value and subsequently a decrease again. The changes in the onset excitation were accompanied by a more pronounced suppressive component and a slight increase in the end of the excitatory component as sound intensity increased. In a few cases, slight changes in response latency and/or response pattern, as a function of tone frequency, were noticed. Sustained excitation throughout the presentation of the tone or noise bursts was rare. Auditory response patterns of auditory cortex cells in the blind hamsters were very much like those encountered in the normal animals (not shown). Response patterns elicited in visual cortex of enucleated hamsters by the auditory stimuli (Fig. 8B) were somewhat different from those encountered in the auditory cortex of normal and blind hamsters. Generally, they were longer, less vigorous, less time-locked with the stimulus, less stable and less repeatable to consecutive stimuli at the repetition rate we used. Hence, apart from response strength, it was not possible to evaluate consistent changes in patterns as a function of stimulus intensity. We did not notice marked changes in response patterns as a function of tone frequency (see also 'Tuning properties', below).

Latencies

Response latencies to clicks in the auditory cortices of intact and blind hamsters and in the visual cortex of enucleated hamsters are presented in Fig. 9. The differences in the mean values among the treatment groups are greater than would be expected by chance (one-way ANOVA with pairwise multiple comparison procedures, Bonferroni's *t*-test, $P < 0.001$). Latencies of auditory cortical cells of normal hamsters did not differ from those of auditory cortical cells in enucleated hamsters. On the other hand, latencies of auditory activated cells in the visual cortex of enucleated animals were significantly longer than those of auditory cortex cells ($P < 0.001$). It is also apparent that the latencies to auditory stimuli in the visual cortex are distributed over a much broader range than cells in auditory cortex suggesting different sources of the auditory input to this area.

Tuning properties

In order to evaluate the sensitivity of the auditory activated cells in the visual cortex of blind hamsters to pure tones we compared the tuning properties of these cells with the tuning properties of cells in the auditory cortex of normal hamsters. Response areas and excitatory characteristic frequencies (CFs) were determined by recording single- and multi-unit activity while gradually reducing the sound pressure level of the entire frequency range, in 10-dB steps, from ≈90 dB SPL to below the cell's lowest threshold. Near threshold step size was decreased to 2–5 dB.

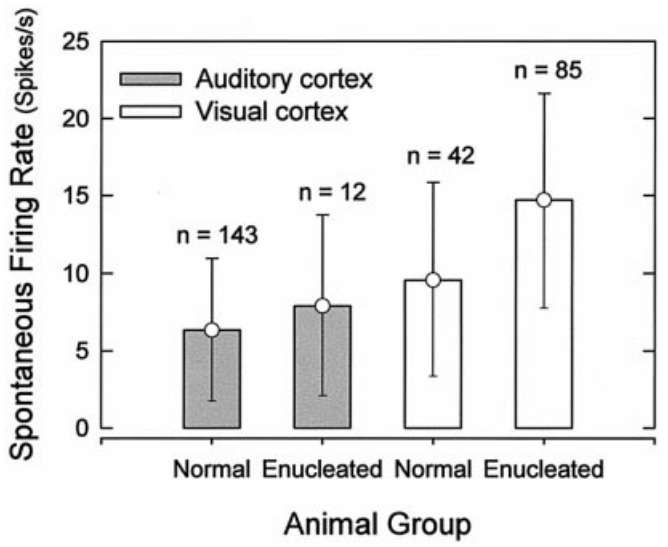

FIG. 6. Spontaneous firing rates of cells in the auditory and visual cortices of normal and blind hamsters (mean ± SD). A detailed statistical comparison of the four groups is given in the Results section, indicating a significantly higher spontaneous firing rate of cells in visual cortex of blind hamsters than that of cells in the visual cortex of normal hamsters ($P < 0.001$).

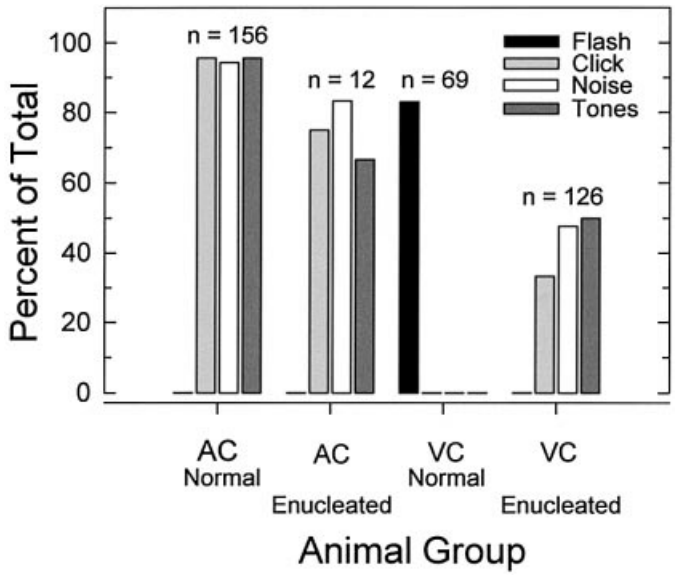

FIG. 7. Responsiveness of single cells to a flash of light and to various auditory stimuli in the auditory and visual cortices of normal and blind hamsters. AC, auditory cortex; VC, visual cortex; n, total number of cells in each group. The ordinate represents the number of responding cells to each stimulus, within each group, relative to the total number of cells in the group (n).

A total of 96 tuning curves of single units, single and double peaked, were obtained from the auditory cortex of normal hamsters. The commonly used Q10dB measure (CF divided by response bandwidth at 10 dB above the lowest threshold) was applied as a criterion for evaluating the sharpness of tuning curves. Approximately 65% of these 96 cells were narrowly tuned (Q10dB > 2) with CFs ranging between 1.0 and 21 kHz (mean ± SD 8.3 ± 3.05 kHz). At intensities higher than 90 dB SPL most cells responded to the entire frequency range we used (0.5–31.5 kHz). Figure 10 summarizes the distribution of threshold intensities as a function of CF. Most of the CFs ranged between 4 and 16 kHz, matching the behavioural audiogram fairly well. Thresholds at best frequencies ranged between ≈5 and 80 dB SPL (mean ± SD 33.5 ± 16.2 dB) with ≈68% of the cells possessing thresholds below 40 dB SPL.

Although many of the cells in the visual cortex of enucleated hamsters responded to pure tones, the response areas of most of them were very broad with no distinct CFs and with high thresholds throughout the entire effective frequency range. Hence, constructing accurate tuning curves and determining best frequencies for these cells was not possible. Out of 63 responding cells we were able to obtain only 16 reliable tuning curves, none of which were narrowly tuned (Q10dB > 2). CFs ranged between 0.5 and ≈21 kHz (8.8 ± 5.6 kHz) with thresholds not lower than 32 dB SPL (mean ± SD 58.8 ± 14.8 dB); 75% of the cells had minimal thresholds higher than 50 dB SPL. The characteristic frequencies of auditory activated cells in the visual cortex of enucleated hamsters and those of cells in the auditory cortex of normal hamsters centred at ≈8.0 kHz with no statistical difference between the two groups (*t*-test, $P = 0.62$). However, mean threshold intensity at the characteristic frequency of cells in the visual cortex of enucleated hamsters was significantly higher that that of auditory cortex cells in normal animals (*t*-test, $P < 0.001$).

The absence in our samples of cells with CFs lower than 0.5 kHz and higher than 21 kHz is probably because most of our unit recordings were made at the centres of the auditory and visual cortices where field evoked potentials were maximal, and where the midrange of the audiogram was represented. As shown below, there was no difference between normal and enucleated hamsters in the hearing range or sensitivity. No systematic attempts were made to disclose possible tonotopicity or any other spatial organization of the cells. However, we noticed that cells that were isolated along the same penetration tended to have similar CFs suggesting possible columnar organization of the auditory projections to visual cortex.

Projection tracing

In order to disclose the origin of the auditory input into the visual cortex, the projection tracer WGA–HRP was applied into the visual cortices of 13 enucleated hamsters where auditory field potentials and single cell responses were recorded. Seven normal specimens were used as controls. As anticipated, the ipsilateral dLGN of normal hamsters was heavily labelled, retrogradely and anterogradely. Heavy retrograde and anterograde labelling of the dLGN was also found in the blind hamsters, indicating that in spite of the early severe damage to the visual system the typical thalamo-cortical connections of this visual nucleus did not degenerate (Fig. 11). In both normal and blind hamsters anterograde labelling was found in the ipsilateral ventral lateral geniculate nucleus (vLGN) as well as retrograde and anterograde labelling in the lateral dorsal thalamic nucleus (LD), probably reflecting spread of tracer into the medial aspect of the visual cortex (Tiao & Blakemore, 1976). Anterogradely and retrogradely labelled cells were also found in the contralateral primary visual cortex. Sporadic retrogradely HRP-labelled cells were also found in the ipsilateral primary auditory cortex of normal and blind hamsters, primarily in layer VI.

Given the apparently typical dLGN–visual cortex connections, we hypothesized that auditory projections invade the dLGN and that the visual thalamo-cortical pathway thereby conveys auditory information as it does in the enucleated mole rat. To examine this possibility we injected WGA–HRP into the dLGN of six enucleated hamsters and one normal hamster. In some of the enucleated animals the tracer

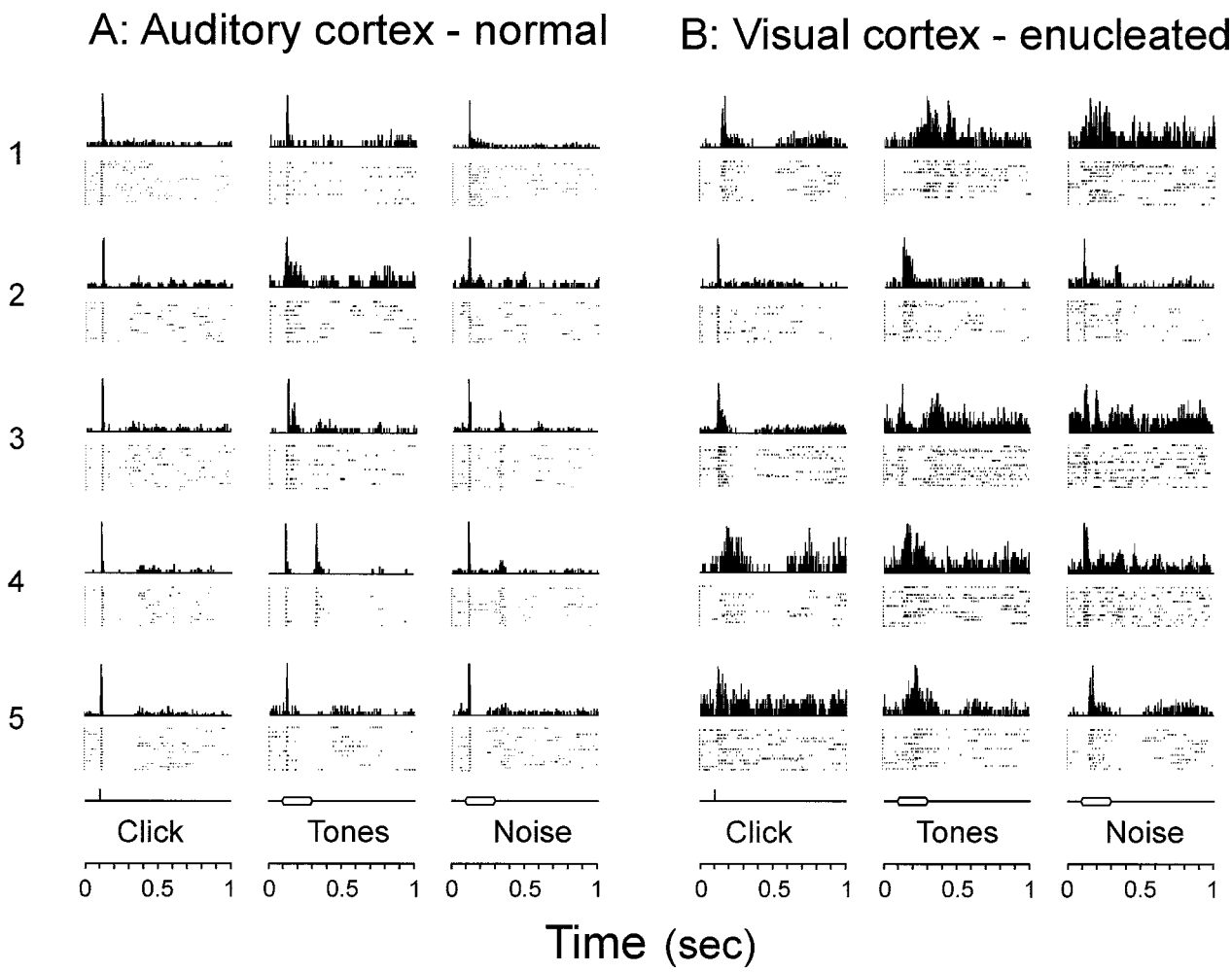

FIG. 8. Dot rasters and corresponding peri-stimulus time histograms (PSTHs) illustrating responses of five different cells in the auditory cortex of (A1–5) normal hamsters and (B1–5) of five different cells in the visual cortex of blind hamsters elicited by clicks, tone bursts (at a frequency around the lowest threshold) and noise bursts. Sound pressure level of all stimuli was 80 dB SPL. The ordinate in the dot raster presentations represents 10 or 15 consecutive repetitions of the same stimulus, 1.2 s apart. Bin duration of PSTHs is 5 ms and the firing rate for each cell is normalized relative to the maximum spike count function (spikes/ s). A short vertical bar at the bottom of the dot rasters indicates the timing of the click, tone and noise burst by the stimulus envelope.

spread into the vLGN. In both normal and blind hamsters anterograde and retrograde labelling was disclosed in the visual cortex, corroborating the results obtained in the experiments in which the tracer was applied to the visual cortex (Fig. 12). In the blind animals we also found retrograde and anterograde labelling in the ipsilateral LD and in the lateral posterior thalamic nucleus (LP). (Some label was also observed in the ipsilateral LP of the normal animal but not in the LD, but definite conclusions concerning the dLGN–LD–LP connectivity pattern cannot be derived from this single case.) Most interesting was that in the blind animals retrogradely labelled cells were also found in the inferior colliculus (IC), the major midbrain auditory nucleus. These cells were found mainly ipsilateral (Fig. 13A) to the application site but also contralateral to it (Fig. 13B). Having only one single case in which the tracer was applied into the LGN of a normal hamster, these findings should be further corroborated.

Behaviour

Unconditioned orientation to sound

Two aspects of the unconditioned orientating reflex to the stimulus were examined: whether the animal correctly orientated in the direction of the sound source (i.e. to the left or right) and how quickly its response habituated. With regard to direction of orientation, both the normal and the enucleated hamsters made few errors. Overall, the directional responses of the normal animals were in the correct direction 95.3% of the time whilst those of the enucleated hamsters were correct 94% of the time. Thus, the two groups did not differ with regard to the correctness of the direction of orientation (*t*-test, $P > 0.05$).

With regard to habituation, however, the two groups did show a difference. This can be seen in Fig. 14, where the average magnitude of the orientating response is shown for consecutive blocks of three trials each. As can be seen in this figure, both the normal and enucleated animals initially showed the same average response to the noise (first block of three stimulus presentations). However, the performance of the two groups diverged over the next four blocks (blocks 2–5), with the blind hamsters showing a stronger response (Mann–Whitney *U*-test, $P < 0.05$). As can be seen in Fig. 14, all of the normal hamsters had habituated by the fourth block of trials, but the blind hamsters did not habituate until the sixth and final block, at which point the two groups, both having habituated, no longer

differed from each other. In short, the enucleated animals were slower to habituate and showed a more vigorous response than the normal animals (Mann–Whitney *U*-test, $P < 0.05$).

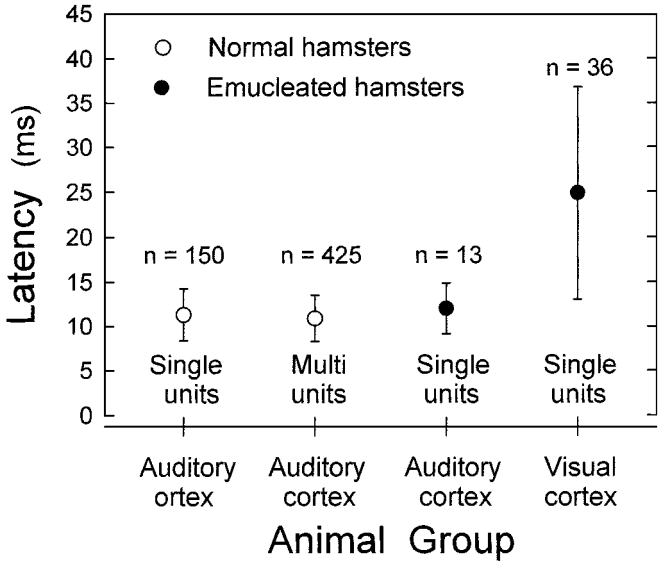

FIG. 9. Latencies to auditory clicks in the auditory cortex of normal and blind hamsters and in the visual cortex of blind hamsters (means ± SD). Note that latencies of auditory activated cells in the visual cortex of blind animals are significantly longer and distributed over a much broader range as compared with those of auditory cortex cells.

Detection thresholds

The absolute thresholds of normal and enucleated animals were assessed to determine whether the two groups differed in auditory sensitivity, to rule out the possibility that the difference in habituation was due to differences in auditory sensitivity, as well as to determine the effect of blindness on absolute thresholds. The results of this test are shown in Fig. 15 where the thresholds of five normal and five enucleated animals are plotted for the four frequencies tested (0.125, 2, 8 and 40 kHz). As can be seen, the absolute thresholds of the normal and blind hamsters did not differ (repeated measures $F_{1,8} = 0.00009$, $P = 0.9924$). It appears, then, that the slower habituation of the blind hamsters was not the result of any increase in auditory sensitivity. The thresholds of both groups are similar to those of a previously published audiogram with one exception. Whereas the previous audiogram showed an average threshold for hamsters at 40 kHz of 29 dB (Heffner *et al*., 2001), the present animals' average threshold was 74 dB. This difference in sensitivity appears to be due to age, as the hamsters were 12–14 months old at testing, whereas the previous hamsters were 3.5 months old. We have previously noted that hamsters undergo a high frequency hearing loss which appears to begin at one year of age (Bitter *et al*., 2000).

Sound localization acuity

Because previous work has indicated that normal and blind animals differ on sound localization ability (Rauschecker & Kniepert, 1994; King & Parsons, 1999), the sound localization test was of special interest. Individual thresholds for localizing a single 100-ms noise burst from loudspeakers centred on midline (left–right discrimination) are illustrated in Fig. 16. Thresholds for 12 normal animals ranged from 17.2 to 21.6°, with a mean (± SD) of 18.8 ± 1.08°;

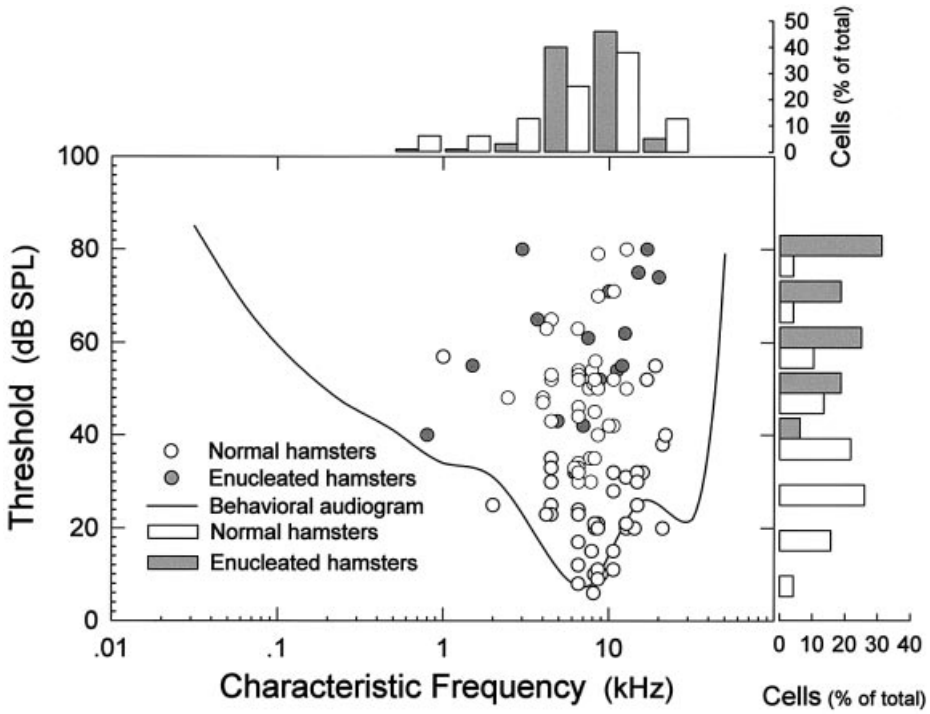

FIG. 10. Distribution of excitatory threshold intensities as a function of characteristic frequency (CF) in the auditory cortex of normal hamsters and the visual cortex of blind hamsters. Solid line is the mean behavioural audiogram of four normal hamsters (see Fig. 15). Bargraph at the right ordinate depicts the relative incidence of the various thresholds. Bargraph at the top represents the relative incidence of the various CFs. Empty and grey circles and bars represent normal and enucleated hamsters, respectively. Note the similarity between the CFs of auditory cortex cells in normal hamsters and those of auditory activated cells in the visual cortex of blind hamsters, and the difference in the thresholds between these two groups.

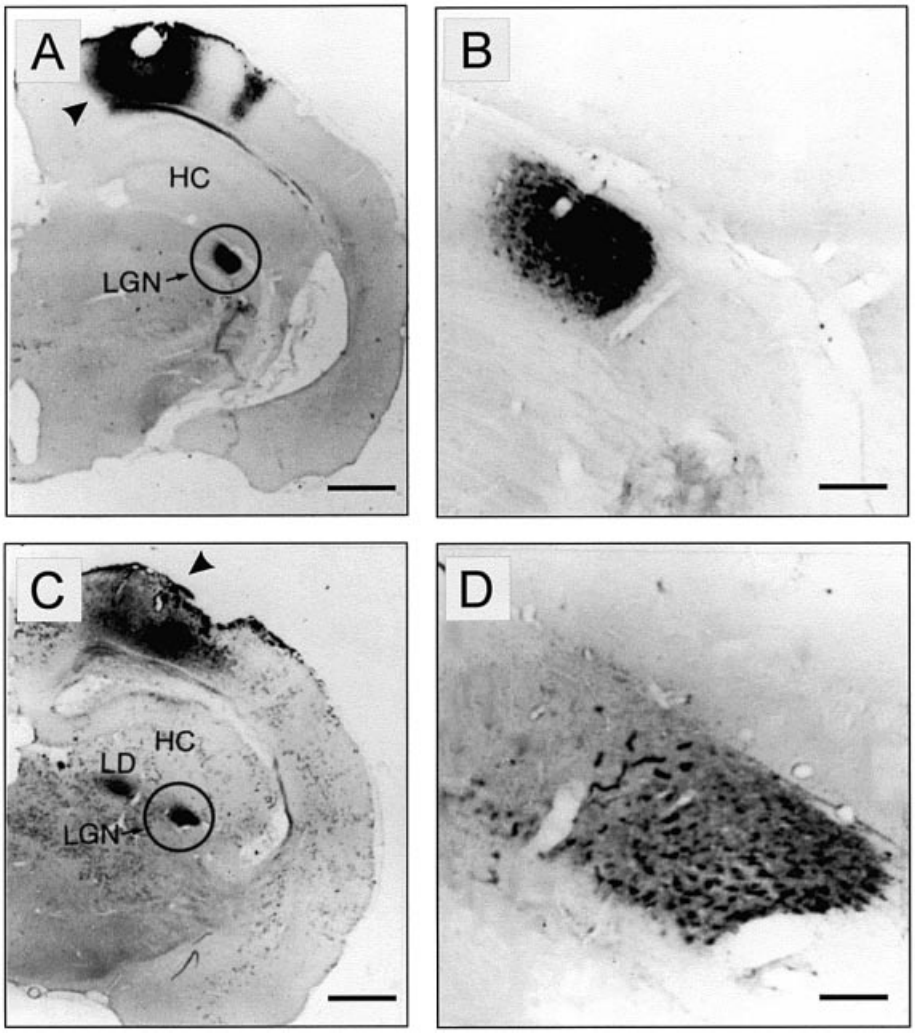

FIG. 11. Retrograde and anterograde labelling in the lateroventral part of the dLGN following WGA–HRP application into the visual cortex of a normal and a blind hamster (A and C, respectively). (B and D) Enlargements of the dLGN labelled areas (circles) in A and C, respectively. Abbreviations: HC, hippocampus; LGN, lateral geniculate nucleus; LD, lateral dorsal thalamus. Arrows indicate application sites. Note that in the enucleated hamster (C) the LD is also labelled, suggesting a spread of tracer into the medial aspect of the visual cortex. Scale bars, 1.0 mm (A and C); 0.18 mm (B); 0.09 mm (D).

thresholds for the eight neonatally enucleated hamsters ranged from 17.4 to 21.6°, with a mean (± SD) of 19.3 ± 1.28°. There was no significant difference between the two groups of hamsters (two-tailed *t*-test, $P = 0.36$). In order to control for any potential effects of the absence of vision during sound localization testing, four of the normal hamsters were re-tested in the dark. Their thresholds obtained in darkness did not differ from those obtained under lighted conditions (two-tailed *t*-test, $P = 0.72$).

The sound localization results were further analysed by recalculating thresholds using a curve fitting procedure (nonlinear model, S curve, Data Desk 6.1, Data Description Inc. Ithaca, New York, USA). Such a procedure takes into account an animal's performance at large angles when calculating threshold, instead of simply basing threshold on the angles just above and below threshold criterion, and has been used by others studying the effect of visual restriction on sound localization (King & Parsons, 1999). As in the previous analysis, the curve fitting procedure also found that the normal and enucleated animals did not differ in localization acuity (two-tailed *t*-test, $P = 0.65$).

Although these results indicate that there was no difference between the normal and enucleated hamsters in azimuthal sound localization acuity, at least for sound sources centred around the midline, their performances at large angles may have differed. The blind animals had, on average, slightly higher scores at 120° ($F_{1,18} = 4.01$, $P < 0.05$) and 180° ($F_{1,18} = 8.66$, $P < 0.01$), suggesting that they had better asymptotic performance (see Fig. 16).

Discussion

In this study we looked for possible auditory activation of the visual cortex in Syrian hamsters that were blinded by bilateral enucleation shortly after birth. We also examined, behaviourally, whether neonatally enucleated hamsters possess superior hearing capabilities. Enucleation (rather than eyelid suturing) was used to induce blindness because eyelid suturing does not cause complete deprivation of light and pattern vision and thus does not represent the most severe impairment of the visual system. Indeed, although cats whose eyelids had been sutured closed and cats that were binocularly

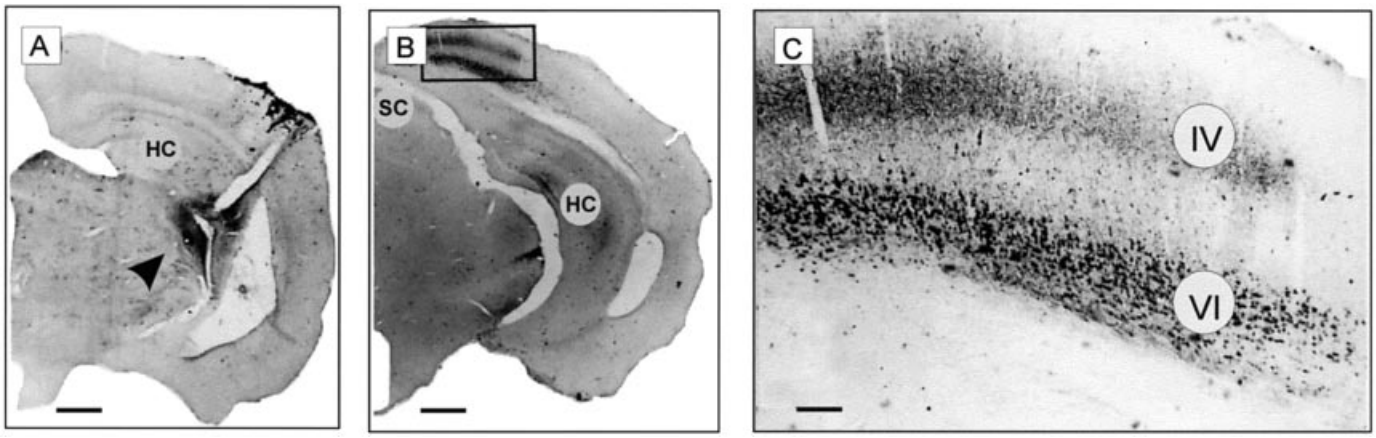

FIG. 12. WGA–HRP anterograde and retrograde labelling in layer VI and layer IV, respectively, in the ipsilateral visual cortex of an enucleated hamster following tracer application into the LGN. (A) Coronal section through the LGN, depicting application site (arrow). (B) Coronal section through the visual cortex. (C) Enlargement of square in B. Scale bars, 1.0 mm (A and B); 0.15 mm (C).

enucleated shortly after birth both demonstrated a significant increase in the number of auditory activated cells in extrastriate visual cortical areas, this effect was much more prominent in the enucleated animals (Yaka *et al*., 1999). Moreover, whereas in the primary visual cortex of eyelid-sutured cats we did not find auditory activated cells, we did find auditory cells in this area in the totally enucleated animals and in a pathologically blind cat (Yaka *et al*., 2000). Enucleation rather than eyelid suturing for inducing total blindness in newborn animals recently received further justification in a study, which showed that visual stimuli presented through the closed eyelids of neonatal ferret kits, between 19 and 32 days old (their eyes normally open around 32 days), can drive neuronal activity in the lateral geniculate nucleus and striate cortex, with many of the cells in visual cortex (but not lateral geniculate) showing orientation selectivity (Krug *et al*., 2001).

Ablating retinal targets and deafferenting the ascending auditory pathway to the thalamus in newborn hamsters and ferrets induces retinal projections to auditory thalamic targets. As a result, visual information is conveyed to the auditory cortex via the intact thalamocortical pathway (cf. Frost, 1984; Frost & Metin, 1985; Sur *et al*., 1988; Pallas *et al*., 1990; Pallas & Sur, 1993, 1994; Roe *et al*., 1993; Gao & Pallas, 1999; Pallas *et al*., 1999). In our experiments all retinofugal projections degenerated but the entire auditory system remained intact. Thus, auditory activation of the visual thalamic targets, and thereby the visual cortex, was in addition to the activation of the typical intact auditory pathway. It should be stressed, in this regard, that the entire occipital area that was activated in normal animals by a flash of light was considered 'visual cortex' including primary and secondary areas (Tiao & Blakemore, 1976). In the absence of detailed cytoarchitectural parcellation of the hamster's visual cortex this appeared to be a reasonable basis for comparisons.

It is also pertinent that, although we could not elicit somatosensory field potentials in this area by electrically stimulating the facial whisker pad, the possibility that other somatosensory stimuli might activate the visual cortex cannot be ruled out. Several earlier findings support this possibility. In neonatally enucleated hamsters the lateral posterior thalamic nucleus (LP), which is reciprocally connected with the visual cortex, is activated by somatosensory stimuli (Mooney & Rhoades, 1983). Somatosensory activation of the lateral visual cortex has been demonstrated in early enucleated rats (Toldi *et al*., 1996; Negyessy *et al*., 2000), and aberrant somatosensory projections to the dLGN were described in rats (O'Leary & Stanfield, 1987) and in mice bilaterally enucleated on the day of birth (Asanuma & Stanfield, 1990). However, it is not clear from these studies whether auditory input, too, activated the abandoned visual structures. Plausibly, the dominant or exclusive modality that replaces an impaired visual modality is species-specific and/or is modulated by the animal's sensory milieu. Responsiveness to somatosensory input, for instance, might be the case in rats, for which the facial vibrissae play a major role in environment exploration.

Our electrophysiological experiments disclosed in the enucleated hamsters an enlarged auditory activated cortex as compared with normal hamsters. This included the typical auditory cortex and parts of the occipital cortex that coincided with the visual cortex as defined by visual evoked responses in normal animals. As in normal hamsters, this area in enucleated hamsters is reciprocally connected with the LGN, suggesting that at least part of the enlarged auditory activated cortex in the enucleated animals represents a reorganized visual cortex. The possibility that parts of the auditory activated visual cortex, especially those that are adjacent to the normal auditory cortex, represent some hypertrophy of the auditory cortex cannot be ruled out. Defining an exact borderline between an auditory activated visual cortex and an expansion of the auditory cortex, if it existed, requires additional fine-grained cytoarchitecture and projection-tracing analyses.

Activation of visual areas in visually deprived animals by another sensory modality is not without precedent. Studies with congenitally anophthalmic mice (Asanuma & Stanfield, 1990), blind mole rats (Bronchti *et al*., 1989; Heil *et al*., 1991; Doron & Wollberg, 1994), the blind mole *Mogera* (Kudo *et al*., 1997), neonatally enucleated rats (Toldi *et al*., 1996) and visually deprived cats (Rauschecker, 1995; Yaka *et al*., 1999) have revealed an extensive somatosensory and/or auditory input to primary visual targets. The activation of primary visual areas by other sensory modalities found in blind animals is also in accordance with recent findings from blind humans as revealed by field evoked potentials and noninvasive brain imaging procedures. During the performance of auditory and/or somatosensory tasks there is an increased metabolic activity, enhanced regional blood flow and event-related responses in the occipital cortex of early blind humans (Veraart *et al*., 1990; Uhl *et al*., 1991; Alho *et al*., 1993; Kujala *et al*., 1995, 2000; Sadato *et al*., 1996, 1998; De Volder *et al*., 1997; Cohen

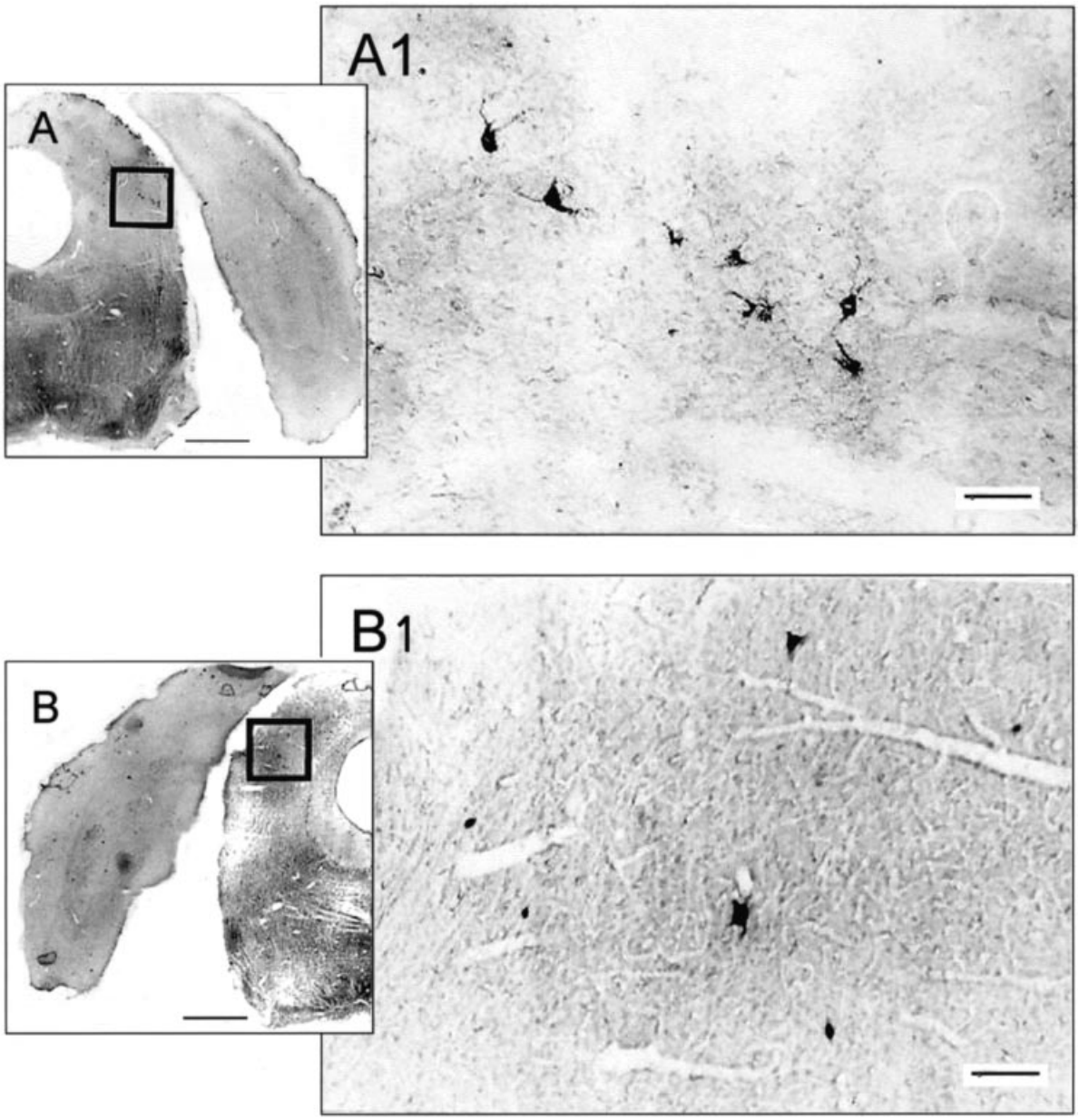

FIG. 13. WGA–HRP retrograde labelling in the inferior colliculus (IC) of a blind hamster following tracer application into the LGN (same animal as in Fig. 11). (A and B) Coronal sections through the ipsilateral and contralateral IC, respectively. (A1 and B1) Enlargements of the IC (squares in A and B). Scale bars, 1.0 mm (A and B); 0.06 mm (A1); 0.09 mm (B1).

et al., 1999; Leclerc *et al*., 2000; Roder *et al*., 2000; Weeks *et al*., 2000). Similarly, it has been shown that in deaf people cortical auditory areas are activated by visual stimuli (Neville *et al*., 1983; Neville, 1990; Roder *et al*., 2000).

Origin of auditory input

What is the origin of the auditory input to the visual cortex of an enucleated hamster? WGA–HRP application into the occipital areas that responded electrophysiologically to auditory stimuli resulted in heavy retrograde and anterograde labelling of the ipsilateral dLGN, the lateral posterior thalamic nucleus (LP) and the lateral dorsal thalamic nucleus (LD). Thus, in spite of the slightly reduced volume of the dLGN in the blind hamsters, the typical LGN–visual cortex reciprocal connection was not profoundly affected. This has also been demonstrated by other investigators with enucleated hamsters (Rhoades & Fish, 1983), congenitally anophthalmic mice (Kaiserman-Abramov *et al*., 1980) and blind mole rats (Heil *et al*., 1991), suggesting great stability of this projection system. Intriguingly, in the blind mole rat, in spite of the millions of years that have elapsed since its ancestors evolved their fossorial lifestyle, the retinal–dLGN projections still exist in newborn animals until the second postnatal week. It is only then that the retinofugal projections to the dLGN gradually degenerate, to be replaced by auditory projections (Bronchti *et al*., 1991).

Some retrograde labelling was also found in the ipsilateral auditory cortex of normal and enucleated hamsters. However, the relatively small amount of this labelling, as compared to that found in the dLGN, suggests that it is not the main origin of auditory input to the

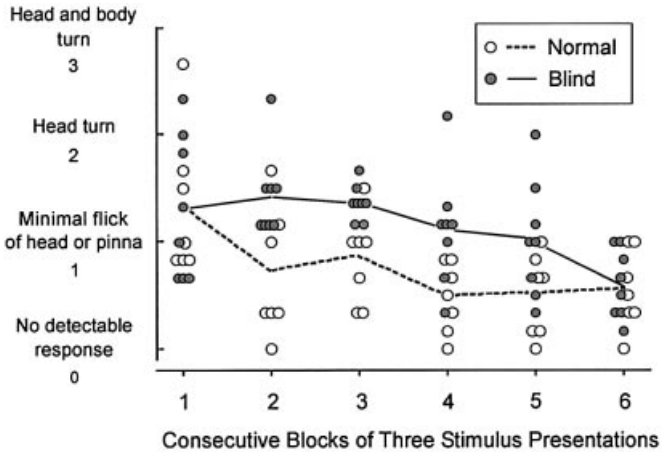

FIG. 14. Magnitude of the orientation response of seven normal (open circles) and eight blind hamsters (filled circles) to a broadband noise stimulus. Although the two groups did not differ in their initial responses, the blind hamsters were slower to habituate than the normal animals and showed a greater response before finally habituating.

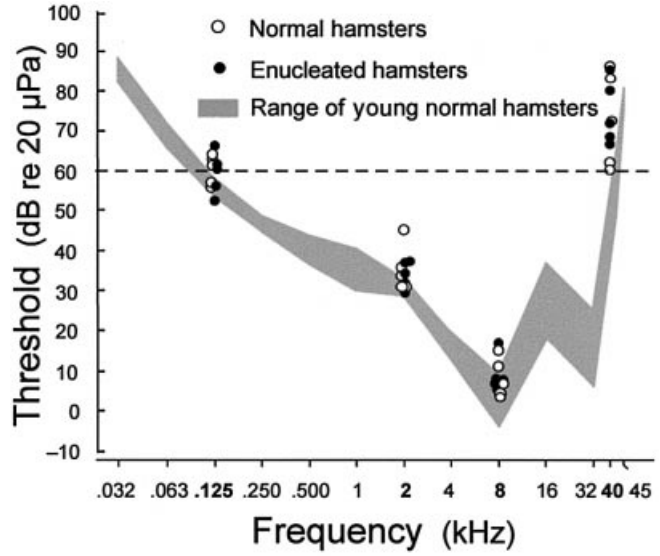

FIG. 15. Absolute thresholds of five normal (open circles) and five blind (filled circles) hamsters at 0.125, 2, 8 and 40 kHz. The two groups did not differ in their auditory sensitivity. However, the animals (which were tested at 12–14 months of age) appear to have a high-frequency hearing loss at 40 kHz as compared with animals tested at 3.5 months of age (shading). A decrease in sensitivity in the midrange of hearing, 16 kHz for hamsters, is common in mammals and is attributed to the directionality of the pinnae as discussed in Heffner *et al.* (2001), from which the normal audiogram is taken.

visual cortex of enucleated hamsters (although this has not yet been determined experimentally). It is thus possible to assume that the auditory input to the visual cortex in this enucleated rodent, as in the blind mole rat, is conveyed primarily by the typical visual thalamo-cortical projections (Bronchti *et al.*, 1989; Heil *et al.*, 1991). However, latencies of auditorily activated cells in the visual cortex varied quite notably as compared with cells in auditory cortex, suggesting more than one origin of the auditory input and probably a more complex subcortical–cortical circuitry.

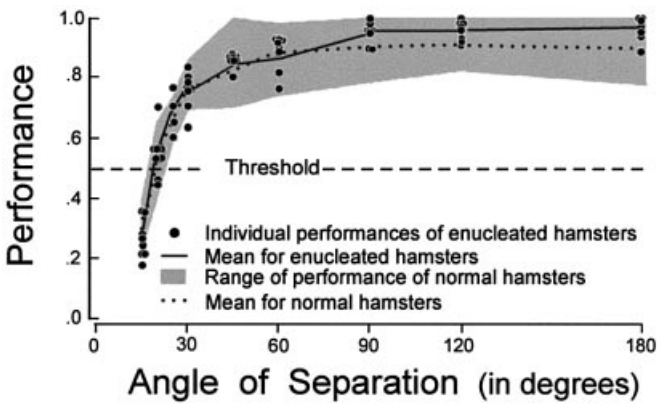

FIG. 16. Sound localization performance of normal (shading) and blind (filled circles) hamsters for a single 100-ms noise burst. Average performance for 12 normal and eight blind hamsters is indicated by a broken and a solid line, respectively. There was no difference in threshold between the two groups. The apparent superiority of the blind hamsters at large angles is significant only at 120 and 180°.

Cross-modal projections from auditory to visual areas may be common early in development. Newborn kittens have transient projections, from layers II/III of the auditory cortex to the ipsilateral and contralateral visual cortex, which degenerate during development (Dehay *et al.*, 1984, 1988; Innocenti & Clarke, 1984). These might persist in the absence of visual input. On the other hand, there are some early reports suggesting that ≈38% of the cells in area 17, 18 and 19 of normal adult cats are bimodal, responding to both visual and auditory stimuli (Murata *et al.*, 1965; Spinelli *et al.*, 1968; Morrel, 1972; Fishman & Michael, 1973). In many of these bimodal cells, the visual and auditory spatial receptive fields coincided, suggesting some synergistic effect of the spatial localization of the two modalities. To the best of our knowledge, there has not been a follow-up of this intriguing finding and of the possible role of these bimodal cells if indeed such exist. The fact that we did not find auditory or bimodal cells in the visual cortex of normal hamsters suggests that either they are species-specific or we did not use the appropriate auditory stimuli or combination of auditory and visual stimuli to reveal such cells. In any event, our finding that in the enucleated hamster, as in the naturally blind mole rat, WGA–HRP application into the dLGN yielded retrograde labelling in the inferior colliculus, the primary midbrain auditory nucleus, suggests that at least part, if not most, of the auditory input to the visual cortex is conveyed by the thalamo-cortical projections (Doron & Wollberg, 1994).

WGA–HRP application into the dLGN of blind mole rats yielded retrograde labelling in the central subdivision of the IC. Application of the tracer into this subdivision of the IC resulted in a very intense bilateral anterograde labelling of fibres, with an ipsilateral dominance, in the dLGN (Doron & Wollberg, 1994). It is thus very likely that most of the auditory input to the dLGN in the blind mole rat originates from either a very dense terminal arborization of the IC–dLGN fibres or a heavy collateralization of IC–medial geniculate nucleus (MGN) projections that invade the dLGN, or both. The retrograde dLGN–IC labelling and the anterograde and retrograde dLGN–visual cortex labelling in the blind hamster were very similar to those seen in the blind mole rat. It is thus reasonable that the pattern of take-over of the visual system by the auditory system in the two visually deprived rodents, one natural and one experimentally induced, is similar. These findings raise the question of whether the

auditory activity of the dLGN by auditory input in neonatally enucleated hamsters, the blind mole rat, and probably in other visually impaired mammals, reflects the existence of transient projections from the IC to the LGN that normally degenerate during maturation but consolidate in visually impaired animals; whether it is a result of ectopic routing of auditory projections into the visually abandoned thalamic nucleus, or whether it is the consequence of uncovering existing 'weak' connections. The fact that dLGN–IC retrograde labelling was found only in the enucleated hamsters favours the first and/or the second alternative. It should be stressed that in this study the label was injected into the dLGN of one normal animal, and if it yielded weak retrograde labelling it could have been missed. It is also possible that in normal animals such connections, if they exist, are ineffective and as such there is probably no uptake of HRP or its uptake is very weak and not noticeable. To the best of our knowledge, there is no published documentation that demonstrates such connections in normal animals. Hence, this entire issue is still open for further investigation.

Electrophysiological response properties

Our electrophysiological experiments revealed that, in early enucleated hamsters, response properties of auditory cortex cells were very similar to those in normal hamsters. In addition, substantial parts of the occipital cortex (coinciding at least partially with area 17 and probably also 18a) also responded to auditory stimuli. This was manifested both by field evoked potentials and by the number of single and multiple units that responded to auditory stimuli. One prominent characteristic of single unit activity in the visual cortex of enucleated hamsters was the relatively high spontaneous firing rate, which was significantly higher than that of cells in visual cortex of normal hamsters and of cells in auditory cortex of normal and blind hamsters. Such an increase in spontaneous activity might reflect a reduction or elimination of ongoing inhibitory activity (disinhibition) in the visually deprived cortex as a result of its rewired circuitry. Alternatively, it might be a result of higher spontaneous activity of the auditory projections that invade the visual system. Whatever the origin of this increased firing rate, it probably affects the thresholds of the enucleated hamsters' visual cortex cells to external stimuli and the shaping of their response properties. It is tempting to hypothesize that this increased spontaneous firing rate is somehow associated with the arousal level of the blind animals and the increased responding of blind hamsters to unexpected noise, as manifested in the resistance to habituation of their unconditioned orienting reflex.

Although an appreciable percentage of our neuronal sample, in the visual cortex of blind hamsters, responded to the clicks, tones and noise bursts, the responses were usually less stable, less repeatable and less time-locked to the stimulus than were auditory cortical cells. Moreover, thresholds were remarkably higher than those of auditory cortical cells, especially for pure tones. Determination of tuning curves of single cells in the enucleated hamsters revealed that in most cases these curves were very broad, with high thresholds over the entire frequency range and with no clear characteristic frequency. Thus, it was not possible to determine whether this modified visual cortex was arranged tonotopically. Judging by these results it seems as if the altered innervation of sensory cortex that results from neonatal blindness appears to be somewhat different from the effect of actually rerouting sensory projections to a different part of cortex in hamsters and ferrets accompanied by ablation of the original targets of the deprived modality. In these studies cellular response properties in visually activated auditory or somatosensory cortex resemble those of cells in primary visual cortex, suggesting that these surgically induced neural pathways might be associated with visual processing (Frost & Metin, 1985; Metin & Frost, 1989; Roe *et al*., 1990, 1992; Frost *et al*., 2000; Sharma *et al*., 2000; von Melchner *et al*., 2000).

Behaviour

Functional significance

There are three issues regarding the potential effect of neonatal blindness on hearing ability. The first is whether the auditory abilities of blind animals are different from those of normal animals and, if so, in what way. Although it is often assumed that blind animals have superior hearing, it is also possible that blindness might degrade their auditory abilities if auditory–visual interaction is necessary for proper auditory development. The second issue is whether any behavioural differences between normal and blind animals represent differences in sensory ability or in performance. For example, superiority on the part of blind animals could be the result of lower sensory thresholds or of better performance, in that the blind animals do not ignore stimuli they detect or that they are more careful, and thus make fewer errors. Finally, if blind animals prove to have superior hearing, is it the result of visual neurons being recruited for processing auditory stimuli? As previously noted, one possible explanation of the auditory responses observed in the visual cortex of the blind hamsters is that it reflects normal auditory input to the cells that has been uncovered by the elimination of visual input. With these issues in mind, the results of the behavioural tests can be considered.

Unconditioned orientation to sound

The results of the unconditioned orientation test showed that the normal and blind hamsters did not differ in their ability to determine whether a sound was to the left or right of midline. However, the blind animals differed significantly from their normal littermates in that they were slower to habituate, continuing to orientate toward a sound source well after the normal hamsters had stopped responding. It appears, then, that while this test did not reveal a difference in sensory ability, it did show that the blind animals were more responsive to the auditory stimuli. Such a result seems reasonable, given that the animals had to rely more heavily on audition for information about the world. Although it is possible that this heightened responsiveness might be due to the recruitment of visual neurons for auditory processing, it could easily be the result of behavioural factors such as attention, motivation, or sensitization.

Absolute sensitivity to sound

The comparison of absolute thresholds at low, middle and high frequencies did not reveal any difference between the normal and enucleated hamsters, indicating that neonatal blindness had no effect on absolute sensitivity, at least not for pure tones. It also indicates that the heightened responsiveness demonstrated in the reflexive orientation test was not due to any change in absolute thresholds. Furthermore, the neurons in visual cortex that responded to auditory stimuli did so only to relatively loud sounds and therefore would not have been expected to improve absolute thresholds. Thus, auditory processing by the visual cortex in neonatally enucleated hamsters does not appear to contribute to behaviourally determined auditory thresholds.

Although at 12–14 months of age the normal and the enucleated hamsters did not differ from each other, both were notably less sensitive at 40 kHz, which is near their high-frequency hearing limit, compared to four hamsters that were tested at 3.5 months of age (Heffner *et al*., 2001). As can be seen in Fig. 14, the thresholds of the four younger animals ranged from 26 to 33 dB (average 29 dB)

whilst the thresholds of the older normal and blind animals ranged from 62 to 85 dB (average 74 dB). This difference appears to be the result of age-related high-frequency hearing loss (i.e. presbycusis) in hamsters (Bitter *et al*., 2000). High-frequency hearing loss begins at ≈12 months, affecting thresholds at frequencies of 32 kHz and higher. By 18 months of age, a hearing loss of 18–50 dB was found at 16 kHz and higher frequencies. Because the normal and blind hamsters in the present study were tested at a younger age for their unconditioned orientation (4–6 months) and sound localization (5–10 months), it is unlikely that they had any high-frequency hearing loss that might have affected the results of these tests.

Sound localization acuity

Previous studies have suggested that visual restriction has an effect on sound localization. In one, cats were trained to orientate to and to approach the source of a sound, with the sound sources consisting of eight loudspeakers spaced at 45° intervals around the animal in a circular arrangement (Rauschecker & Kniepert, 1994). Using a 20-kHz tone pip (40-ms duration), it was found that cats whose eyelids had been sutured at 3 weeks of age orientated on average more accurately and showed less variation in walking to a sound source than did normal cats. In another study, sound localization thresholds for brief noise bursts were determined in ferrets using a two-choice procedure in which the loudspeakers were either centred in front of an animal (i.e. centred on its midline) or off to one side (i.e. centred on 45° left or right of midline) (King & Parsons, 1999). Ferrets whose eyelids had been sutured, either before they naturally opened or as adults, had on average better thresholds than normally sighted animals when the loudspeakers were centred on 45°. However, there was no difference between groups when the loudspeakers were centred on midline.

Given the results from cats and ferrets, it seemed reasonable to expect that the normal and enucleated hamsters might also differ in sound localization ability. However, we found that there was no difference in the left–right sound localization acuity of blind hamsters compared to that of normal littermates, as reflected in minimum audible angle around midline. The only difference between the two groups was a slight superiority in the performance of the blind hamsters at large angles that was statistically significant only at 120 and 180°. In short, we found that the enucleated hamsters had very slightly better performance with no difference in localization acuity.

There are several possible explanations for the absence of a difference between the sound localization acuity of blind and normal hamsters. One is that there is a species difference, such that carnivores, but not rodents, show an effect of restricted vision or blindness on sound localization. Another is that the hamsters might have shown a difference if they had been tested on localization off to one side, instead of around midline, as was necessary to show an effect with the ferrets (King & Parsons, 1999). However, before concluding that the enhancing effect of blindness on sound localization is either species- or task-specific, it is necessary to explore whether the results of the previous studies demonstrated an enhancement in sensory acuity or in performance.

The study using cats found that animals with sutured eyelids orientated more accurately and showed less variability in their accuracy of walking to the source of a sound than did normal cats (Rauschecker & Kniepert, 1994). However, it is unclear whether these results indicate a difference in acuity or in performance, as the animals were reinforced with food if they walked anywhere within ±45° of the sound source. As a result, the animals were under less pressure to perform accurately than is the case in standard psychophysical tests and it is not surprising that both normal and visually restricted cats showed poorer acuity than cats are known to possess (e.g. Heffner & Heffner, 1988). Thus, although this study indicates that visually restricted cats perform with less variation on a sound localization task, suggesting an enhanced performance, it does not demonstrate that such animals have better than normal sound localization acuity.

A similar enhancement of performance may be seen in the study of ferrets (King & Parsons, 1999). That is, the performance of the visually restricted ferrets when localizing off to the side (loudspeakers centred around 45° azimuth) appears to show less variability than that of the normal animals. Indeed, at least one normal animal was unable to perform above threshold (75% correct) when localizing a 40-ms noise burst whereas the visually restricted animals appear to have had little difficulty with this task (see Fig. 5 in King & Parsons, 1999). The fact that the normal animals performed more poorly at large angles suggests that they may have been less well trained than the visually restricted animals. Moreover, the poorer asymptotic performance of the normal ferrets would also have affected the calculation of their thresholds because the curve fitting procedure that was used to calculate threshold is affected by an animal's performance at large angles. Thus, although this study demonstrates that visually restricted ferrets show better asymptotic performance on a sound localization task, it is less convincing in demonstrating that they possess better than normal sound localization acuity.

The results of studies on the auditory abilities of blind humans are not unlike those found in animals. For example, a study of the sound localization ability of normal and blind humans in the azimuthal plane found that half of the blind subjects were better than the normal subjects in localizing sounds off to the side, where monaural locus cues are important (Lessard *et al*., 1998). As the authors point out, their results could be due to neural reorganization resulting from blindness, or to better learning strategies on the part of the blind subjects, as the monaural localization ability of normal subjects improves with practice (Butler, 1987). On the other hand, blind humans were found to be inferior in another monaural localization task, the discrimination of elevation, but only in low signal-to-noise (i.e. noisy) conditions (Zwiers *et al*., 2001). Thus, the issue of superior hearing in the blind is far from clear, as differences seem to occur in relatively restricted conditions or to appear in some, but not in all, subjects.

In conclusion, studies of cats, ferrets, humans and now hamsters have shown that blind individuals can be superior to normals on one or more auditory discriminations. However, it is not clear that such superiority is due to increased auditory acuity as opposed to enhanced performance. This enhanced performance may well result from the greater reliance that animals lacking normal vision must place on hearing and may be due to differences in attentiveness, practice or other factors. It remains to be determined whether this difference is the result of neurons in visual cortex being recruited to play a role in hearing, or of reactive sprouting or atypical stabilization of normally transient connections that degenerate during maturation, or of responsiveness of the neurons to auditory stimuli resulting from the uncovering of existing auditory input.

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Abbreviations

CF, characteristic frequency; dLGN, dorsal lateral geniculate nucleus; FEP, field evoked potential; IC, inferior colliculus; LD, lateral dorsal thalamic nucleus; LGN, lateral geniculate nucleus/nuclei; LP, lateral posterior thalamic nucleus; SPL, sound pressure level; VB, ventrobasal thalamic nucleus; vLGN, ventral lateral geniculate nucleus; WGA–HRP, wheat-germ agglutinin conjugated to horseradish peroxidase.

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