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RESEARCH NOTE

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Pathological and experimentally induced blindness induces auditory activity in the cat primary visual cortex

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Abstract Early blindness in humans and experimental visual deprivation in animal models are known to induce compensatory somatosensory and/or auditory activation of the visual cortex. An abnormal hydrocephalic cat with extreme malformation of the visual system, born in our breeding colony, rendered a good model system for investigating possible cross-modal compensation in such a pathological case. For comparison, we used normal and neonatally enucleated cats. When introduced to a novel environment, the abnormal cat behaved as if it was completely blind, yet it responded normally to auditory stimuli. As anticipated, single cells in the visual cortex of normal cats responded to visual, but not to auditory stimuli. In the visual cortex of enucleated cats, flashes of light did not elicit field-evoked potentials or single-unit responses. However, several cells did respond to various auditory stimuli. In the remnant visual cortex of the abnormal cat, auditory stimuli evoked field potentials and single-cell responses. Unexpectedly, however, unlike the enucleated cats, in the abnormal cat, flashes of light also elicited field-evoked potentials. Judging by its behavior, it is very likely that this deformed cat had completely lost its ability to perceive images, but had probably retained some sensitivity to light.

Key words Cross-modal neuroplasticity · Enucleation · Visual deprivation · Auditory activation · Hydrocephalus · Walker-Warburg syndrome

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Introduction

Cats visually deprived by eyelid suturing shortly after birth are widely used as a model system for studying intramodal neuroplasticity in the mammalian central nervous system (e.g., Hubel and Wiesel 1970). One consistent finding in such deprived cats is a decrease in the amount of visually driven cells in cortical visual areas (Spear et al. 1983). A similar phenomenon was also described in cats that accidentally developed hydrocephalus following surgical manipulation (Yinon et al. 1990). This finding raises the question of the fate of visually deprived brain areas under experimental or pathological conditions. Studies with visually deprived animals and early blinded humans have shown that primary and/or secondary visual areas in such cases are activated by auditory and/or somatosensory stimuli (e.g., Rauschecker and Korte 1993; Kujala et al. 1995; Sadato et al. 1996; De-Volder et al. 1997).

Recently, a cat with a congenitally malformed visual system was born in our breeding colony. Its right eye appeared normal, albeit extremely small in size and covered by the eyelid. The other eye was markedly larger than the eyes of a normal cat, with a highly opaque cornea. Because this seemingly blind cat did not show any hearing impairment, we hypothesized that visual areas in its brain might in fact be activated by auditory stimuli, compensating for the loss of sight. To test this working assumption, we explored the possibility that the primary visual cortex in this cat could be activated by auditory stimuli. Normal cats served as controls and neonatally enucleated cats were used as a reference for total blindness.

Materials and methods

Animals and surgical procedures

Six normal adult cats, five neonatally enucleated cats, and the abnormal cat were used in this study. Binocular enucleation

and eyelid suturing were performed 5–7 days after birth under deep anesthesia (ketamine hydrochloride 25 mg/kg, i.m.). Postoperative pain was minimized by infiltrating the area around the treated eyes with local anesthetic (lidocaine hydrochloride 2%). Prophylactic antibiotic treatment consisted of synthomycine ointment (5%), gentamycin (16 mg/kg/day, i.m.), and synthomycine (200 mg/kg/day i.m). Following recovery from the anesthesia, the operated kittens were returned to their mothers and kept with them in separate cages until complete weaning. Thereafter and until the recording session (1.5–2 years later), each cat was held individually in a separate cage.

For the electrophysiological recordings, the cats were deeply anesthetized (ketamine HCl 25 mg/kg, rompun 10 mg/kg, i.m.), held in a stereotaxic apparatus by means of hollow ear bars, tracheotomized, and a craniotomy was performed over the visual cortex. Subsequently, the animals were anesthetized by a continuous i.m. injection of thiopentone sodium (3 mg/h), and i.v. infused with a mixture containing gallamine triethiodide (7.5 mg/kg/h), to avoid eye movements while using visual stimuli, atropine (1.25 µg/kg/h), potassium chloride (4.0 mg/kg/h), isoproterenol HCL (isuprel 6 µ g/kg/h), and dextrose-saline solution (2 ml/kg/h). Cats were artificially respirated and body temperature, ECG rate, urine volume, and expiratory CO_2 levels were constantly monitored and kept within the physiological range.

Stimulation and electrophysiological recording procedures

Visual stimuli consisted of 10 µS flashes of light (intensity: 750 000 candlepower) as well as light spots and light bars (410 lumens/m^2) that were produced by a computerized optical stimulation system with a light slit of changeable dimensions ranging between 0.1–10°. The stimuli were projected at various angles, directions, velocities, and duration on a tangent opaque glass screen (placed 2 m in front of the cat with a background luminance of 0.7 lumens/m^2) and presented to the cat binocularly as well as to each eye separately. Auditory stimuli consisted of clicks generated by 0.1 ms square pulses (80 dB peak intensity), broadband white noise (0.5–20 kHz), pure tones ranging between 0.1 and 35 kHz presented in sequential steps of 0.2–1 kHz, and frequency modulated (FM) tones. The latter were swept symmetrically from 0.1 to 30 kHz and backward in three steps from 0.1–1.0 kHz, 1–10 kHz, and 10–30 kHz at constant rates of 9, 90, and 200 kHz/s, respectively. The noise, pure tones, and FM tones were shaped into 200 ms bursts by a trapezoidal waveform with a 15 ms rise and fall time. Routine sound pressure level was 80 dB SPL re 20 µPa, unless stated otherwise. Consecutive stimuli were presented at a rate of 0.5 s^{-1}. Complex sounds such as hand clapping, kissing, and jingling keys were also occasionally used. All auditory stimuli were delivered binaurally through calibrated earphones attached to the outer opening of the hollow ear bars.

Field evoked potentials (FEPs) were recorded by silver-ball electrodes, amplified, filtered, digitized, averaged, and visually monitored. Single-unit activity was extracellularly recorded with epoxylite-coated tungsten microelectrodes advanced perpendicularly to the cortical surface, amplified, filtered, discriminated from background activity, and displayed on-line as dot rasters and/or peri-stimulus time histograms (PSTHs). All the electrophysiological data were stored for off-line analyses on a PC.

Recording sites were determined by means of electrolytic lesions made along the last penetration track. At the end of the experiment, the animals were deeply anesthetized and killed with an overdose of pentobarbitole sodium and perfused through the carotid artery first with 0.9% saline/heparin, followed by 10% neutral buffered formalin. Cresyl-violet-stained coronal sections (frozen or paraffin) were used to assign recording sites. For detailed procedures, see Yaka et al. 1999. All surgical and experimental procedures were performed in accordance with the guidelines issued by the "Tel Aviv University Animal Care and Use Committee".

Results

Behavioral observations

When introduced to a novel environment (a 3.5×3.5 m room with several obstacles on the floor), the abnormal cat behaved like a blind animal (as assessed by three real-time observers and slow playback of a video recording). No startle reflexes could be elicited by approaching its eyes with an object, and no behavioral responses could be detected when it was exposed to flashes of light. When exposed to various auditory stimuli such as finger snapping, hand clapping, jingling keys, or a sudden noise, it showed a clear behavioral reaction, indicating that its hearing did not appear to be impaired. We also noticed that its movements were very sluggish and its posture unstable, reflecting muscle fatigue. These, as well as some additional symptoms that were disclosed after killing the animal (see below), suggested that this cat was hydrocephalic and suffered from a disorder that, in many respects, resembled the Walker-Warburg syndrome found in humans. In addition, the cat occasionally performed circular movements resembling those of vestibular impairment. Although this cat was reared and maintained under optimal conditions, its physical condition deteriorated with time and, at the age of about eight months, we decided upon euthanasia. Just prior to this process, we conducted the electrophysiological experiments.

Morphology

The eyes of this cat were asymmetrical, with the right eye being microphthalmic, situated deep in the orbit, and with an eyelid that was almost completely closed. The left eye was extremely large (buphthalmic), protruding from the orbit, with an opaque cornea (Fig. 1A, B). Microscopic inspection of the buphthalmic-eye sections disclosed a scarred, vascularized cornea with the iris pigment epithelium adherent to its posterior surface. The retina showed extensive loss of ganglion cells and nerve-fiber layer, and the optic nerve was exceedingly atrophied. The microphthalmic eye was extremely small, but otherwise looked normal, with only mild loss of the retinal ganglion cells (Fig. 1C).

Gross morphology examination of the brain revealed that it was unusually small with almost no discernible sulci (Fig. 1D). The lateral ventricles were abnormally large and the optic chiasm was partially atrophied. A cytoarchitectural examination of the visual cortex revealed diminution of the cellular layers as compared with normal animals, a disorder resembling one of the symptoms of Miller-Dieker syndrome, also called lissencephaly syndrome, found in humans (Van-Allen and Clarren 1983). In the enucleated cats, the most apparent morphological effect of the visual deprivation was the complete degeneration of the optic nerve and chiasm. Gross morphology of the cortex looked normal, and Nissl staining did not show any obvious cytoarchitectural changes in A17 (Yaka et al. 1999).

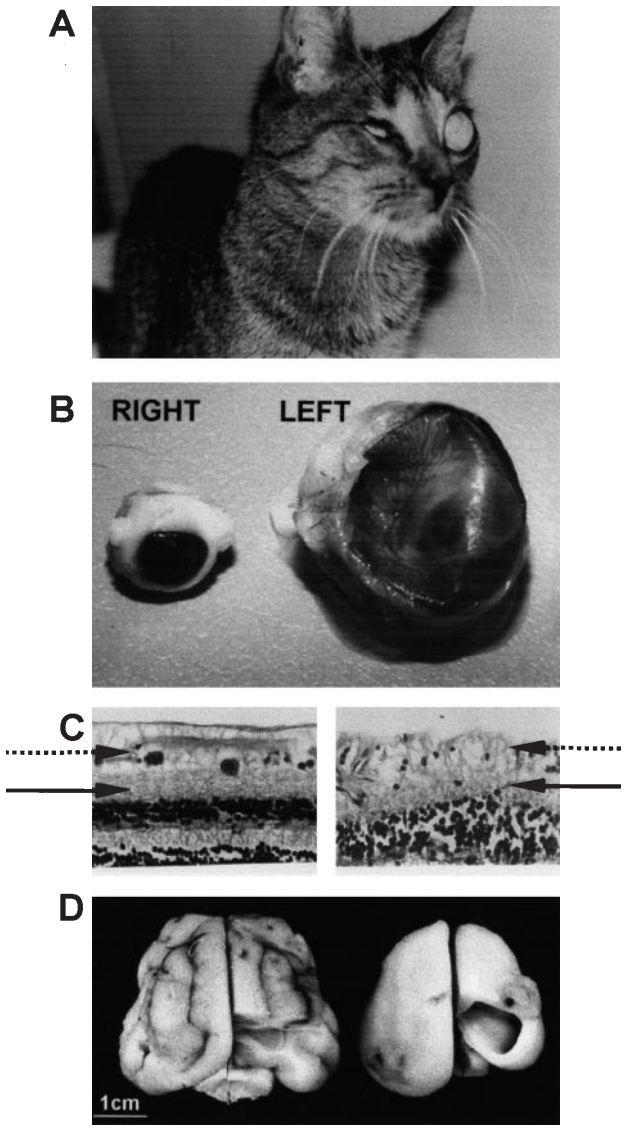

Fig. 1A–D Morphopathology of the abnormal cat. **A** General view of the cat. Note the asymmetrical eyes: the right eye situated deep in the orbit and the large left eye. **B** Isolated eyes of the abnormal cat removed from their orbits after the animal was killed. Note the buphtalmic eye covered with a thick cataract and its large size compared with the left eye. **C** Hematoxylin-eosin-stained cross sections of the retina of both eyes. Note the extensive loss of ganglion cells (*dashed arrow*) and nerve-fiber layer (*solid arrow*) of the right eye as compared with the mild loss of the retinal ganglion cells of the left eye. Magnification ×400. **D** Whole brain of a normal (*left*) and the abnormal (*right*) cat. The right occipital regions were removed in both cases. Note the small size and smooth surface of the abnormal cat's brain and the large lateral ventricle as compared with the normal brain

Electrophysiology

Normal cats

Short flashes of light evoked prominent field potentials in the primary visual cortex (area 17) of normal cats, whereas auditory clicks did not elicit any response (Fig. 2A, B). Out of 75 single cells tested in the primary visual cortex of these control animals, a total of 65 cells vigorously responded to visual stimuli. None of these cells or the remaining ten cells responded to any of the auditory stimuli we used. Response properties of the visually driven cells, such as size of receptive fields, direction and orientation selectivity, and ocular dominance, were similar to those previously described and documented for the primary visual cortex (for a review, see Reid and Alonso 1996).

Abnormal and enucleated cats

As expected, visual stimuli did not evoke either field potentials or single-cells responses in A17 of the enucleated cats. Auditory stimuli also did not elicit FEPs. However, out of 135 cells isolated along 22 penetrations within this region, eight cells clearly responded to at least one of the auditory stimuli we used (Fig. 2C). All these eight cells responded to tone bursts, yet their thresholds to this stimulus, at best frequency, were as high as 50–80 dB SPL. Recordings in the abnormal cat were made only in the right occipital cortex (an area corresponding to the primary visual cortex of normal cats), contralaterally to the buphthalmic eye. Curiously, although this cat behaved as functionally blind, flashes of light did elicit some field potentials (Fig. 2B) in this area, yet none of 32 single cells, isolated along eight penetrations within this area, responded to either flashes of light or moving light bars. Auditory stimuli evoked prominent field potentials in this area and, out of the 32 isolated cells, three clearly responded to auditory stimuli (Fig. 2C).

Discussion

It is apparent from this study that some activation of the primary visual cortex by auditory stimuli had occurred in both the enucleated animals and in the abnormal cat. In the abnormal cat, this was manifested by field potentials and single-cell responses. In the enucleated cats, no auditory FEPs were detected, although auditory single-cell responses were encountered. Statistically, there was no significant difference between the two groups in the proportions of auditory responding cells (*z*-test with Yates correction, P=0.81). The lack of auditory FEPs in the enucleated cats probably reflects a difference in the amount of auditory input that invades area 17 in these cats as compared with the abnormal cat. Possibly, this difference is not manifested statistically by the proportion of auditory-responding cells because of the relatively small sample of cells we had in the abnormal cat. If this is indeed so, then this case supports earlier findings demonstrating that the timing of visual impairment, its degree, and probably its cause have a marked effect on neuronal reorganization (e.g. Yaka et al. 1999 and articles cited therein).

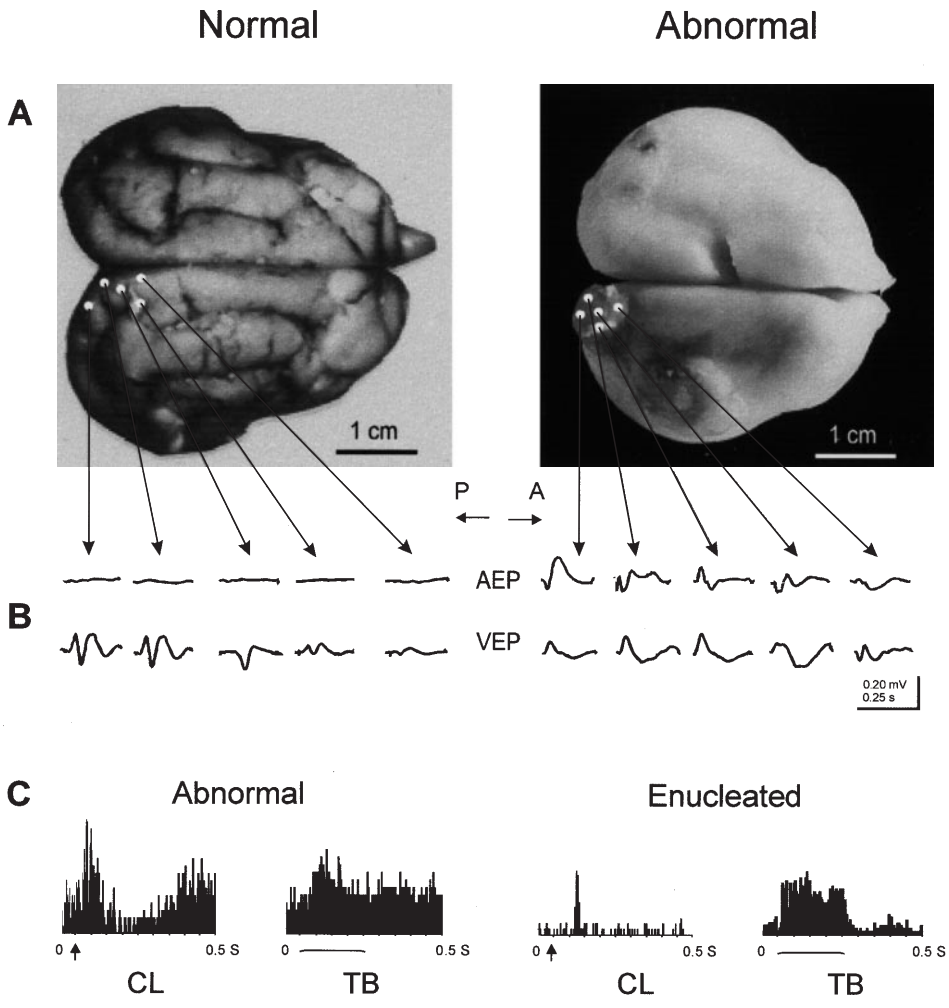

Fig. 2A–C Responses elicited by auditory and visual stimuli in the occipital cortex of a normal cat and the abnormal cat. **A** Top view of the brains. *White dots* designate recording sites of field-evoked potentials (FEP) (*A* anterior, *P* posterior). **B** Averaged field potentials, evoked by a flash of light (*VEP*) and by an auditory click (*AEP*), recorded from the surface of the occipital cortices of the normal and the abnormal cats (seen in **A**). Note the lack of auditory responses in the visual cortex of the normal cat and the clear auditory and visual responses in the abnormal cat. **C** Peristimulus time histograms (PSTHs, 1 ms bin duration; *ordinate* relative number of spikes) illustrating cellular responses in A17 of an enucleated cat and the abnormal cat to click (*CL*) and to a tone burst (*TB*) (presented at the best frequency of the cell). *Arrows* and *horizontal bars* indicate timing of click and tone presentation, respectively

Behaviorally, the abnormal cat acted as if it was completely blind. Unexpectedly, however, in spite of the absence of visually driven cells in its visual cortex, flashes of light elicited FEPs in this area, indicating that it was still receiving some visual input. A reasonable explanation for this seemingly contradictory finding might be the fact that many of the cells in this study were detected by their spontaneous activity (although "searching stimuli" were used as well). Hence, a prominent reduction in the spontaneous firing rates of light-sensitive cells in the deformed visual system of the abnormal cat might explain the existence of FEPs in spite of the "absence" of single-cell responses in our sample.

Studies with congenitally anophthalmic mice, naturally blind mole rats (*Spalax ehrenbergi*), and enucleated hamsters have revealed an extensive presentation of auditory and/or somatosensory input in primary visual targets (Bronchti et al. 1989; Asanuma and Stanfield 1990; Necker et al. 1992; Toldi et al. 1994; Izraeli and Wollberg 1998). The fact that auditory activation of area 17 in the enucleated cats and the abnormal cat was less prominent than in other visually deprived animal models implies that it is not the auditory modality that predominates cross-modal reorganization in visually deprived cats, at least concerning area 17. A very reasonable other putative candidate is obviously the somatosensory system.

The activation of primary visual areas by auditory and/or somatosensory input found in blind-animal models is in accordance with recent findings for blind humans, as revealed by contemporary noninvasive procedures (Veraart et al. 1990; Alho et al. 1993; Kujala et al. 1995; Sadato et al. 1996). Some evidence suggests that such compensatory cross-modal neural reorganization might account for certain superior hearing and/or tactile capabilities reported in blind animals and humans. This is manifested, for instance, by improved auditory spatial tuning in visually deprived cats (Rauschecker and Kniepert 1994) and blind humans (Lessard et al. 1998; Röder et al. 1999) and by superior tactile perceptual abilities of blind subjects (Cohen et al. 1997).

Functional mapping by means of the metabolic tracer 2-DG in the blind mole rat revealed auditory activation of the dorsal lateral geniculate nucleus (dLGN) and the occipital cortex (Bronchti et al. 1989; Heil et al. 1991). In the occipital cortex, also auditory single-cell responses were electrophysiologically detected (Heil et al. 1991). Horseradish-peroxidase (HRP) projection-tracing studies in this rodent demonstrated reciprocal connections be-

tween the dLGN and the occipital cortex, suggesting that in this species auditory input reaches the occipital cortex via the dLGN (Heil et al. 1991; Doron and Wollberg 1994). The major source of auditory input to the dLGN has been shown to be the inferior colliculus, which in addition to all its typical auditory targets also projects into this visual thalamic nucleus (Doron and Wollberg 1994). Similar projections from the inferior colliculus to the LGN have also been demonstrated in the mole *Mogera* (Kudo et al. 1997). It has also been shown that transient somatosensory projections that invade the dLGN of normal mice are consolidated in congenitally anophthalmic adult mice (Asanuma and Stanfield 1990). Electrophysiological experiments in neonatally enucleated Syrian hamsters revealed that their occipital cortex is activated by auditory input and that this "historical" visual structure, similar to the congenitally blind mole rat, retained its reciprocal connections with the dLGN that is typical for sighted forms (Wollberg et al. 1999). The origin of the auditory input to the hamster's visual system has not yet been disclosed. The pathway of the auditory input to the visual cortex of the experimentally or pathologically visually impaired cats is also not yet known. It would thus be interesting to determine whether cross-modal reorganization in experimentally induced blindness or in pathological cases follows a similar pattern to the one found in naturally blind animals.

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