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Ocular dominance shift in kitten visual cortex caused by imbalance in retinal electrical activity

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Monocular lid suture during the sensitive period early in the life of a kitten disrupts normal development of inputs from the two eyes to the visual cortex, causing a decrease in the fraction of cortical cells responding to the deprived eye[1]. Such an ocular dominance shift has been assumed to depend on patterned visual experience, because no change in cortical physiology is produced by inequalities between the two eyes in retinal illumination[2] or temporally modulated diffuse light stimulation[3,4]. A higher-level process, involving gating signals from areas outside striate cortex, has been proposed to ensure that sustained changes in synaptic efficacy occur only in response to behaviourally significant visual inputs[5]. To test whether such a process is necessary for ocular dominance plasticity, we treated 4-week-old kittens with visual deprivation and monocular tetrodotoxin (TTX) injections to create an imbalance in the electrical activities of the two retinas in the absence of patterned vision. After 1 week of treatment we determined the ocular dominance distribution of single units in primary visual cortex. In all kittens studied, a significant ocular dominance shift was found. In addition to this physiological change, there was an anatomical change in the lateral geniculate nucleus, where cells were larger in laminae receiving input from the more active eye. Our results indicate that patterned vision is not necessary for visual cortical plasticity, and that an imbalance in spontaneous retinal activity alone can produce a significant ocular dominance shift.

For this study 11 28-34-day-old kittens received intravitreal injections of 5 mM TTX in one eye to block ganglion-cell activity in that eye[6]. Patterned visual input to the other eye was eliminated by suturing the eyelid. Six of the kittens (TTX/lid-suture group) were kept in a normal light cycle, and thus experienced no activity in one eye and diffuse, temporally modulated light in the other. The remaining five kittens (TTX/dark group) were kept in the dark, experiencing no activity in one eye and only spontaneous activity in the other eye. The retinal action potential blockade was maintained for 1 week by repeating the TTX injections. The lid of the injected eye was then sutured and the animals remained or were placed in the dark to prevent vision during recovery of retinal activity. Twenty-four to 48 hours after the retinal blockade began to wear off, the kittens were prepared for electrophysiological recording as described elsewhere[7].

To demonstrate that normal activity had returned to the previously blocked eye, 5-13 single units with central receptive fields (within 15° of the area centralis) were recorded in the lateral geniculate nucleus (LGN) contralateral to the injected eye. In addition, multi-unit responses were assessed every 100 μm through laminae A and A1. No difference in responsiveness was detected between cells driven by the two eyes (see Figs 1, 2), nor between multi-unit recordings in laminae responsive to the two eyes. Briskly responsive ON- and OFF-centre and sustained and transient units were recorded in both laminae.

To determine whether an ocular dominance shift had occurred, we studied cortical area 17 contralateral to the injected eye. This hemisphere was chosen because normal kittens show an ocular dominance bias toward the contralateral eye[6]; thus if recording was in the hemisphere ipsilateral to the TTX-treated eye, an apparent shift in ocular dominance towards the more active eye could be caused, at least in part, by this bias. In the hemisphere contralateral to the injected eye, however, an ocular dominance distribution favouring the more active (ipsilateral) eye must be caused by the effects of deprivation. Horizontal electrode penetrations were used to sample cells across, rather than down, ocular dominance columns; each penetration was long enough to pass through several columns. The intended sample size was 30 cells per animal. Receptive fields of all units studied were within 15° of the area centralis. Ocular dominance was determined for each visually responsive unit using the standard seven-point scale[8]. For both conditions, TTX/lid suture and TTX/dark, ocular dominance histograms were significantly shifted toward the more active, ipsilateral eye (Fig. 3*b*, *c*). These data contrast with histograms from normal kittens of the same age, which show a slight bias in favour of the contralateral eye (Fig. 3*a*), as well as with the more strongly shifted histogram from conventional monocularly lid-sutured kittens (Fig. 3*d*). The degree of shift in each kitten, estimated by a scalar index, indicates that our two groups are significantly different from both normal and conventionally monocularly deprived animals (Mann-Whitney U test, $P < 0.01$), but that the results in the TTX/lid-suture and TTX/dark groups are not significantly different (Mann-Whitney U test, $P > 0.05$) (Fig. 4*a*).

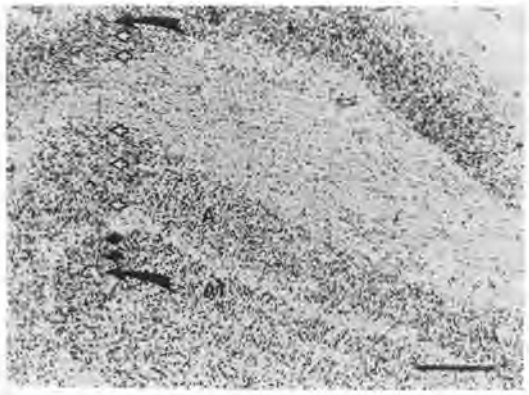

Fig. 1 Coronal Nissl-stained section through an LGN contralateral to the TTX-injected eye. The section shown is 2.88 mm caudal to the rostral pole of the LGN. An electrode track is shown with electrolytic marking lesions indicated by curved arrows. Filled arrows, locations of single units responding to the untreated ipsilateral eye; open arrows, single units responding to the previously blocked contralateral eye. Receptive fields of the units indicated by the top two arrows were at elevation 30°, azimuth 9°; those of the units indicated by the lower five arrows ranged between elevation 6°, azimuth 4° and elevation 4°, azimuth 2°. Scale bar, 0.5 mm.

In 7 of the original 11 kittens, cross-sectional areas of cells in the LGN were measured in Nissl-stained sections. (LGN cell sizes are known to be smaller in the laminae responding to the deprived eye in kittens with monocular lid suture[1,9] or monocular TTX blockade[10].) As expected, cell sizes were smaller in laminae responding to the TTX-treated eye in both TTX/lid-suture and TTX/dark animals. These morphological results, like the physiological ones, are intermediate between those from normal and monocularly deprived animals (Fig. 4*b*).

Although unlikely, the changes in cortical physiology observed could have been caused by damage to the TTX-treated eye that was not detected in our geniculate recordings, rather than by the activity blockade. If such toxicity was responsible for the ocular dominance shifts, the effects should be permanent. If, however, differential activity were responsible, then the shifts should be reversible, as are those produced by conventional monocular deprivation in kittens of the same age[11]. To test the reversibility of our treatment, two additional kittens were studied. These kittens experienced 1 week of the TTX/lid-suture treatment starting at 28 or 32 days old. After the TTX blockade wore off, the kittens remained in a normal light cycle for 9 or 11 days with the sutured eyelid remaining closed. Thus, these kittens experienced a week of no activity in the TTX-treated eye combined with diffuse light vision through a closed lid in

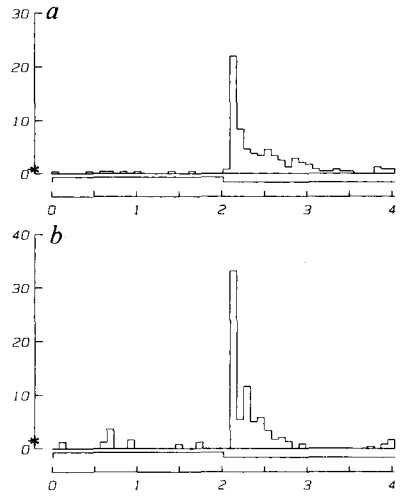

Fig. 2 Post-stimulus histograms showing examples of the flash response of single OFF-centre units driven by the untreated ipsilateral eye (*a*) and by the previously blocked contralateral eye (*b*) in the same LGN. Stimulus intensity was one log unit above threshold. *y*-axis, spikes per s; *x*-axis s. Asterisk, level of spontaneous activity; stimulus time course, line above the *x*-axis (ON, up; OFF, down).

the other eye, followed by a period of conventional monocular deprivation with patterned vision in the previously TTX-treated eye. The first week of treatment presumably produced an ocular dominance shift toward the lid-sutured eye, as occurred in TTX/lid-suture animals. If TTX injections did not cause permanent damage, this shift should have been reversed during the period of monocular deprivation, resulting in a cortical bias in favour of the eye that had been blocked during the first part of the treatment and had experienced patterned vision during the second part. Single-unit recordings from area 17 of the two kittens showed that such a reversal had occurred. Ocular dominance histograms from both kittens were strongly shifted toward the eye that was first blocked and then had patterned vision. The degree of shift in these kittens (contralateral bias indices 86 and 99; see Fig. 4 legend) was comparable to that seen in conventional reverse lid-sutured kittens of the same age[11]. Thus TTX injections do not cause lasting damage to the ability of the treated eye to compete for cortical dominance.

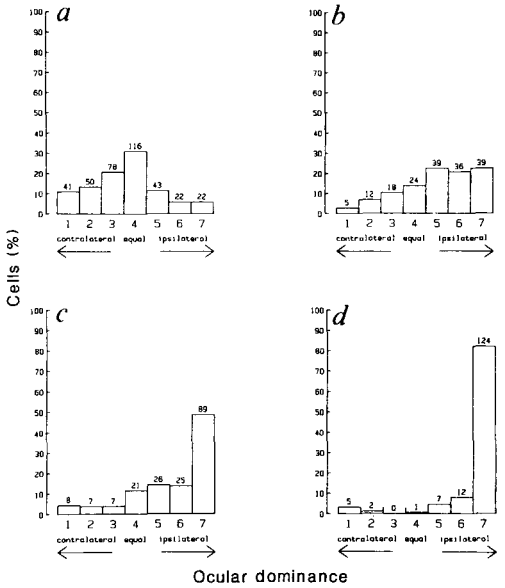

Fig. 3 Ocular dominance histograms[8] compiled from single unit responses in area 17. An ocular dominance of 1 indicates a cell driven only by the contralateral eye; 7 a cell driven only by the ipsilateral eye; and 4 a cell driven equally by the two eyes. In each deprivation condition, the contralateral eye is less active. *a*, 372 visually responsive units from six normal kittens, 36–51 days old: 26 visually unresponsive units recorded[6]. *b*, 173 visually responsive units from five TTX/dark kittens, 37–44 days old: 12 visually unresponsive units recorded. *c*, 183 visually responsive units from six TTX/lid-suture kittens, 36–40 days old: 19 visually unresponsive units recorded. *d*, 151 units from four kittens monocularly deprived by lid suture for 3.5–6 days, followed by 0–2 days recovery in darkness; 29–34 days old[9,15,16].

The similarity of three of the present findings to those from conventional monocular deprivation experiments indicates that the ocular dominance shifts seen in TTX/lid-suture and TTX/dark animals were caused by the differences between activities of the two eyes, rather than by damage to the retinal ganglion cells of the injected eye. (1) LGN responses to stimulation of the two eyes were equally good even in the most shifted animals in which few or no cortical cells responded to the injected eye. (2) LGN cell sizes were smaller in laminae responding to the injected eye. (3) The ocular dominance shifts produced were readily reversible.

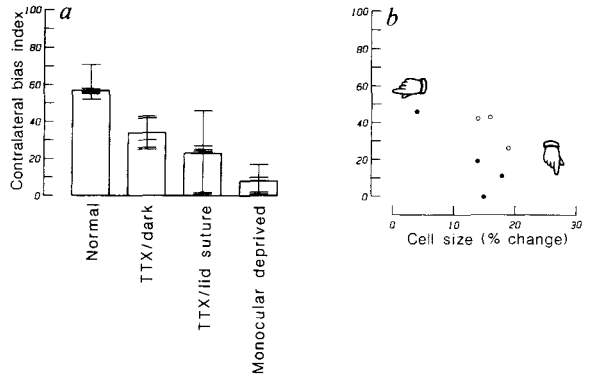

Fig. 4 *a*, Contralateral bias indices showing the range of findings among individual animals from Fig 3. *b*, Scatter plot of per cent change in geniculate cell size versus contralateral bias index. *a*, The index provides a single number reflecting the degree of ocular dominance shift for each animal. The index is calculated according to the equation

$$100[(1-7)+2/3(2\text{-}6)+1/3(3\text{-}5)+n]/2n$$

Italicized numbers, number of units in each ocular dominance group; *n*, total number of visually responsive units. This index is 100 if all cells are driven exclusively by the contralateral eye and 0 if all cells are driven exclusively by the ipsilateral eye. The greater variability among TTX/lid-suture animals is unexplained; it may be caused by differences among the animals of this group in the amount of diffuse-light stimulation of the retina of their sutured eyes. *b*, Cells (75 per lamina) were measured in both LGNs of each animal. Measurements were made in the latero-medial middle third of coronal sections taken ~2.5 mm caudal to the rostral pole of the nucleus (the area corresponding to the central 5–10° of the visual field)[26]. All cell bodies with visible nucleoli were traced in the camera lucida under ×63 or ×100 oil-immersion objectives at final magnifications of ×1,160–1,840. The areas of these tracings were then measured using a Summagraphics Bitpad Planimeter. The standard errors ranged from 4 to 6% of the mean for individual laminae. Per cent change was calculated as $1-\sum$ mean cell sizes from laminae responding to the injected eye $\div \sum$ mean cell sizes from laminae responding to the more active eye. Open circles, data from TTX/lid-suture animals; filled circles, data from TTX/dark animals. Upper finger, region of data from normal animals[6]; lower finger, region of data from monocularly deprived animals[9]. The spread of these control data along the ordinate is shown in *a*; the spread along the abscissa is ~4% for normal animals and 7% for monocularly deprived animals[9]. Although there is no strong correlation between the change in cell size and the degree of ocular dominance shift in our animals, the extents of both morphological and physiological changes are intermediate between data from normal and conventional monocularly deprived animals. The morphological results for TTX/dark animals (average percentage change = 16) are very similar to those previously reported for older TTX/dark kittens (average percentage change = 17)[10].

We draw three conclusions from the results of these experiments. First, the significant changes in ocular dominance in both TTX/lid-suture and TTX/dark animals show that patterned vision is not necessary to produce changes in cortical physiology. Second, the results from the TTX/dark group indicate that an imbalance in spontaneous activity alone is enough to cause an ocular dominance shift. Third, the similar results produced by TTX/dark and TTX/lid-suture treatments suggest that the vision that occurs through a closed eyelid has little additional effect on ocular dominance.

It is generally believed that cortical plasticity is dependent on cortical cells being driven by their geniculate inputs. Silencing all cortical activity through intra-cortical TTX injections prevents ocular dominance shifts in monocularly deprived animals[12]. Because geniculate[13] and cortical[14] spontaneous activities are dependent on levels of spontaneous activity in the retina, spontaneous activity must drive cortical cells, however weakly. Thus the present results are consistent with a model of plasticity requiring that cortical cells be driven.

Our conclusion that ocular dominance shifts can be produced in the absence of patterned vision does not necessarily refute the central gating hypothesis[5]. Although shifts in our animals did occur without behaviourally significant visual input to either eye, these shifts were not as large as those seen in conventionally monocularly deprived animals[9,15,16], although the differences in retinal activities were extreme. Perhaps the central gate is always slightly open, allowing some degree of shift to occur. Behaviourally significant information could further open the gate, causing the larger ocular dominance shifts seen in conventionally monocularly deprived animals. We do not know why ocular dominance shifts were produced by the present conditions but not by other rearing conditions with different inputs to the two eyes in the absence of patterned vision[2-4]. The actual retinal activities produced by any of these rearing conditions are, however, unknown (except that TTX does block all activity[6]). Therefore, either a large quantitative difference or some temporally modulated qualitative difference between the activities of the two retinas may be necessary to produce ocular dominance shifts.

Our present finding that imbalances in spontaneous activity alone can cause changes in connections to cortical cells, together with earlier work on the effects of spontaneous activity blockade on the development of ocular dominance columns[6,17], is consistent with the hypothesis that spontaneous electrical activity plays a part in the normal fetal development of the visual system[18]. Activity-dependent rearrangements of ocular dominance columns, which occur postnatally in the cat[19], begin 3–6 weeks prenatally in the monkey[20,21]. If the development of ocular dominance columns follows similar principles in the two species[22], it is not surprising that spontaneous activity plays this part in the cat because in the monkey *in utero*, vision is occluded, and the only possible electrical activity is spontaneous. Observations that retinogeniculate axons make functionally effective binocular connections prenatally[23], some of which are lost before birth[23,24], suggest that spontaneous activity is also involved in the refinement of these connections[24,25].

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