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Early discordant binocular vision disrupts signal transfer in the lateral geniculate nucleus

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ABSTRACT **The mammalian lateral geniculate nucleus (LGN) is known to regulate signal transfer from the retina to the brain neocortex in a highly complex manner. Besides inputs from the brainstem, extraretinal inputs via corticogeniculate projections and local inhibitory neurons modulate signal transfer in the LGN. However, very little is known about whether the postnatal development of LGN signal-transfer mechanisms is influenced by early discordant binocular vision. By intraunit comparisons of responses between individual X-LGN cells and their direct retinal inputs, the efficiency of signal transfer was found permanently reduced due to an early interocular misalignment (strabismus). The contrast sensitivity and spatial resolution of cat LGN cells were significantly lower relative to their retinal inputs, and there was substantial decrease in signal-transfer speed. The observed physiological deficits were associated with immature X-retinogeniculate axon arbors. Thus, contrary to previous ideas, conflicting binocular inputs can produce neural deficits in subcortical visual structures.**

Early strabismus, a condition that often leads to spatial vision anomalies in humans (amblyopia), has consistently been shown to produce dramatic physiological deficits in the visual cortex (1–4). Although retinal development is largely unaffected by strabismus (5, 6), it is a matter of long-standing debate whether or not strabismus influences lateral geniculate nucleus (LGN) development (7–10). With respect to our understanding of the neural deficits underlying strabismic amblyopia, this is a critical issue because recent studies of LGN functional organization (11–14) and the development of retinogeniculate connections (15, 16) strongly imply that subcortical deficits might occur in strabismic subjects. Specifically, geniculate signal transfer may be permanently altered by discordant binocular signals because the LGN transfer mechanisms are strongly influenced not only by inhibitory signals from the contralateral eye (17) but also by massive extraretinal inputs coming from the cortex, local inhibitory neurons, the brain stem, and the extraocular muscles (11–14, 18). Moreover, retinogeniculate axon arbor development in the LGN is consistently perturbed after unilateral form deprivation (15, 16), a condition that also leads to amblyopia.

The current controversies concerning the functional status of the LGN in strabismus can be attributed primarily to methodological limitations. All of the previous studies used interanimal or interocular population-based comparisons that are inherently insensitive to subtle abnormalities. In this study, we investigated the effects of strabismus on LGN signal transfer using a highly sensitive experimental paradigm that involved intraunit comparisons of the response properties measured for LGN action potentials (LGN *output*) and the simultaneously recorded S-potentials (LGN *input*). With this method, we could largely avoid many of the potential confounding factors associated with population-based comparisons (e.g., differences in the visual experience of the animals, sampling procedures, and/or receptive field location).

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METHODS

Subjects. Unilateral convergent strabismus was induced in 3-week-old kittens by surgically separating the tendon of the lateral rectus muscle from the globe (tenotomy) (2, 3). All experiments were conducted when the animals were at least 9 months old. The degree of interocular misalignment in individual animals was estimated during the recording experiments by measuring the relative positions of the right and left optic discs after anesthesia and paralysis. All of our experimental animals exhibited convergent misalignment ranging from 9° to 40° (mean, 22.1 ± 13° SD).

Recording Methods and Data Analyses. The general surgical and recording procedures have been described elsewhere (3, 19). Briefly, cats were initially anesthetized with an i.p. injection of sodium pentobarbital (40 mg/kg). A femoral vein was cannulated for subsequent drug infusion, and a tracheotomy was done. Animals were paralyzed with an infusion of pancurionium bromide (0.1–0.2 mg/kg per hr) and artificially ventilated with a mixture of N_2O (59%)/O_2 (39%)/CO_2 (2%). The gas anesthesia was supplemented with a continuous infusion of sodium pentobarbital (1–2 mg/kg per hr). The electrocardiogram and electroencephalogram were used to monitor the animal's physiological state and anesthesia level. Body temperature was maintained at 38°C. Gas-permeable contact lenses were used to optimize conditions of the cornea and optics of the eye. Retinoscopy and an additional refraction using cellular responses were used to correct refractive errors. Extracellular single-cell recordings were made with saline-filled glass micropipettes (electrode impedance, 25–30 MΩ at 100 Hz) in LGN laminae A and A1. Responses were measured for drifting sinusoidal gratings (mean luminance, 21 candelas per m^2; temporal frequency, 3.12 Hz), and response amplitude was defined as the amplitude of the fundamental Fourier component. To determine the preferred stimulus orientation of each cell, orientation response functions were obtained by using a spatial frequency near the high-frequency cut-off of the cell (19). Next, the cells were classified as X- or Y-cells on the basis of a spatial-summation test (20). The spatial frequency-response functions were then measured for the S-potential and action potential at the optimal orientation and analyzed by using a difference-of-Gaussian receptive-field model (21). Temporal frequency-response functions

Abbreviations: LGN, lateral geniculate nucleus; HRP, horseradish peroxidase.

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were obtained for the optimal spatial frequency and used to calculate visual response latencies for S-potentials and LGN cell action potentials. Response properties of LGN cells innervated by the deviating eyes of nine strabismic cats (n = 35) were compared with those of neurons in five normally reared animals (n = 29). We did not use interocular comparisons within the same strabismic animals because the geniculate neurons innervated by the nondeviating eye (n = 32) exhibited similar, but milder, spatial and contrast sensitivity deficits (also see refs. 2 and 3).

Anatomical Methods. Procedures for intracellular injection of horseradish peroxidase (HRP) are described elsewhere (22). Briefly, our recording electrodes were beveled micropipettes (95–100 MΩ at 100 Hz) filled with 5–10% HRP/0.2 M KCl/0.05 M Tris. During the experiments, the micropipettes were lowered through the LGN. When a retinogeniculate axon was encountered, it was first classified as X or Y. After classification, the axon was impaled, and HRP was injected iontophoretically. Arbors of axons, successfully impaled and injected with HRP, were identified in tissue that had been frozen-sectioned at 100 μm and histochemically processed with 3-3′-diaminobenzidine with cobalt intensification. Each recovered axonal arbor was measured as follows: (*i*) the number of boutons within the laminated portion of the LGN was counted. (*ii*) arbor volume was estimated by measuring the area enclosed by the outermost boutons in reconstructions of each section through the arbor, multiplying by the section thickness, and summing over consecutive sections.

RESULTS

Fig. 1 illustrates simultaneously recorded S-potentials and LGN action potentials from an X-cell in a strabismic cat. The waveform and amplitude of the S-potentials, as well as their relationship to the rising phase of the LGN action potentials, were carefully analyzed on-line and/or off-line to ascertain that a given geniculate cell was receiving its retinal inputs from one retinal ganglion cell. Only those neurons that exhibited unitary S-potentials were included in the data analysis for this report. It is well documented that S-potentials recorded from single LGN neurons faithfully reflect the action potential activity of individual retinal ganglion cell inputs (23, 24).

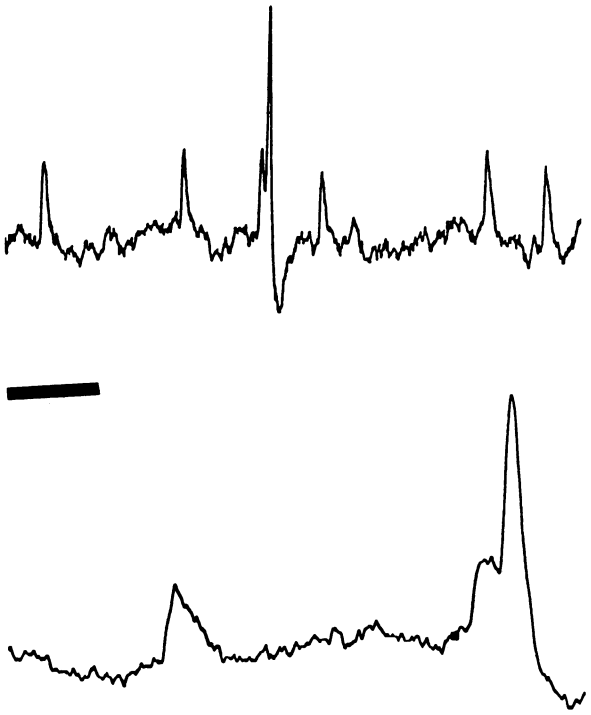

FIG. 1. Examples of simultaneously recorded S-potentials (small waveforms) and LGN action potentials (large waveforms) from an ON-center X-LGN cell in a strabismic cat. [Bar = 10 ms (upper trace) and 2 ms (lower trace).]

Contrast Threshold. Early strabismus clearly altered how spatial contrast information was transferred from retinal ganglion cells to LGN neurons (Fig. 2). Contrast response functions were measured at the cell's optimal orientation and for three spatial frequencies (the peak frequency, one octave lower than the peak, and one octave higher than the peak). From the contrast response functions, the contrast threshold of the cell was found by determining the stimulus contrast at which response amplitude exceeded the criterion level (i.e., two SDs above the average noise). In normal cats (Fig. 2*A*, *Lower*), the minimum stimulus contrast required to produce the criterion response was virtually the same for the LGN action potentials (3.1%) and S-potentials (2.8%). However, in strabismic cats (Fig. 2*A*, *Upper*), the contrast thresholds for

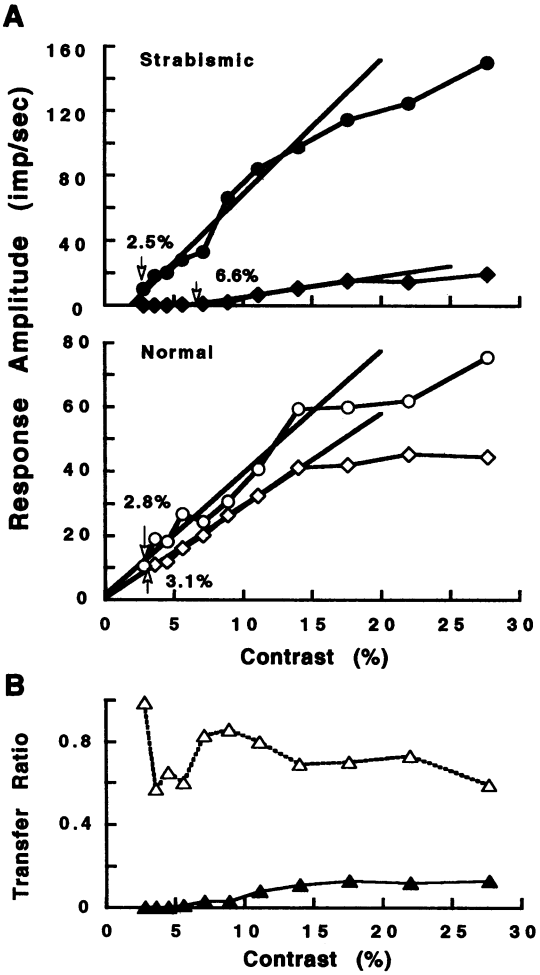

FIG. 2. (*A*) (*Upper*) Effect of stimulus contrast on response amplitude of an ON-center X-LGN cell (◆) and its retinal input (●) in a strabismic cat. (*Lower*) Similar contrast-response functions for an ON-center X-cell (◇) and its retinal input (○) in a normal cat. Stimuli were drifting (3.1 Hz) sinusoidal gratings of high spatial frequency [0.8 cycle per degree (c/d) for strabismic and 1.6 c/d for normal units]. The linear portion of each contrast vs. response function was fit with a linear regression line to measure the contrast gain of the cell [impulse (imp) per sec per percent contrast] and contrast threshold (arrows). (*B*) Transfer ratios as functions of stimulus contrast for the LGN units of *A* for the strabismic (▲) and normal (△) cats. Transfer ratio was defined as firing rate of an LGN cell divided by firing rate of its S-potential.

high spatial frequency stimuli were much higher for LGN neurons (6.6%) than for their retinal ganglion cell inputs (2.5%). The transfer ratio of these neurons in strabismic animals (defined as the firing rate of an LGN cell response divided by that of its S-potential) clearly demonstrated a relative reduction in signal transfer at all stimulus contrasts (Fig. 2*B*).

Fig. 3 summarizes the results of similar comparisons in 26 cells in strabismic and 29 units in normal cats. The mean contrast threshold ratio between LGN responses and their retinal inputs for strabismic cats (0.82 octave ± 0.13 SE) was significantly higher than that for normal controls (0.20 octave ± 0.24 SE: *t* test, $P < 0.05$). Similar comparisons for lower spatial frequencies (i.e., optimal and 1 octave below the peak) were not significantly different between strabismic and normal cats.

Spatial Resolution. Strabismic LGN neurons also exhibited significant reduction in spatial resolution (Fig. 4). The resolution of each cell was determined from spatial frequency–response functions by calculating the spatial frequency at which the firing rate of the cell fell to 10% of its peak amplitude. In some neurons from strabismic cats (Fig. 4 *Lower*), the difference in cut-off spatial frequencies for LGN inputs and outputs was as large as one octave. Furthermore, the mean input–output difference in spatial resolution was statistically significant (0.25 ± 0.05 octaves, *t* test, $P < 0.005$). In normal cats, spatial resolution was unaltered during LGN signal transfer (Fig. 4 *Upper*), the difference in mean resolution between LGN action potentials and S-potentials was negligible (0.03 ± 0.04 octaves). The reduction in spatial resolution of LGN cells in strabismic cats is consistent with a fundamental characteristic of amblyopia—namely, a reduction in contrast sensitivity at high spatial frequencies.

Visual Response Latency. Early strabismus also affected the temporal properties of geniculate neurons; the speed of signal transmission was reduced in many X-LGN cells (Fig. 5). Visual response latencies of LGN X-cells and their retinal inputs in strabismic and normal cats were determined by measuring temporal frequency-response functions and then calculating visual-response latencies on the basis of the relationship between response phase and stimulus temporal frequency (25). Although the range of S-potential input latencies was comparable in strabismic and normal subjects, over a quarter (9/32) of the LGN neurons in the strabismic animals showed output latencies outside the normal range. The prolonged latencies seen in these nine units in our strabismic cats were also associated with elevated contrast

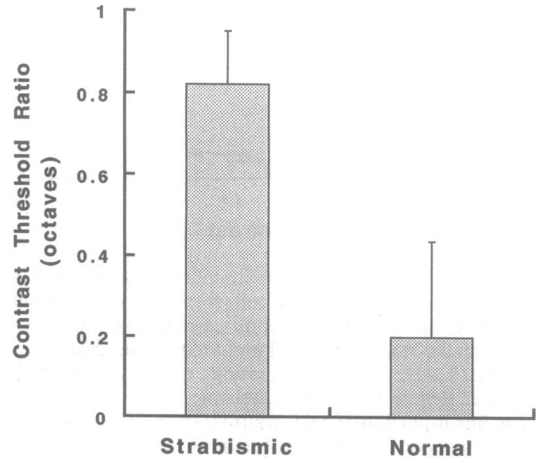

FIG. 3. Contrast threshold ratios (octaves) between the LGN responses and their retinal inputs (means ± SE) for strabismic (n = 26) and normal (n = 29) cats, measured with high spatial frequency gratings (one octave above the peak spatial frequency).

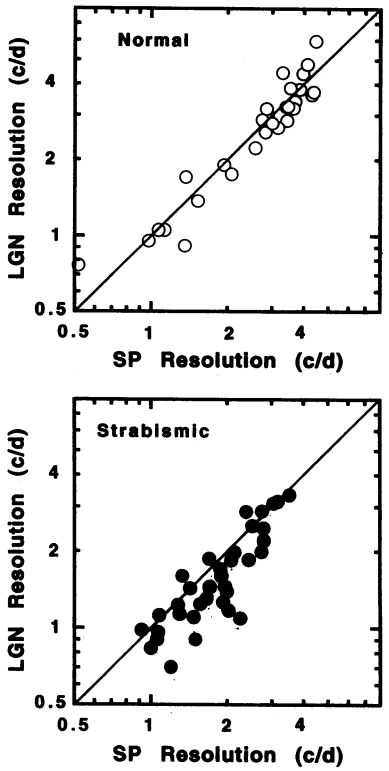

FIG. 4. Comparisons of the spatial resolutions of individual X-LGN cells with those of their retinal inputs [S-potentials (SP)] in strabismic (*Lower*) and normal (*Upper*) cats. c/d, Cycles per degree.

thresholds (14.0% ± 4.3, n = 9) relative to control units (4.3% ± 0.93, n = 29; *t* test, $P < 0.002$).

Retinogeniculate Axons. The observed spatial and temporal filter deficits of LGN neurons in strabismic cats were associated with abnormal retinogeniculate axon arbors (Fig. 6 and Table 1). Measurements of arbor volume, bouton number, and bouton density were made in 18 X-retinal ganglion cell axons recovered from strabismic cats and compared with 13 X-axons from control cats (22). The terminal arbors in strabismic animals were abnormally enlarged, as in normal 3-week-old kittens (15, 16). However, the bouton number per terminal arbor was similar to that in control animals (22). As a result, there was a significant reduction in the density of

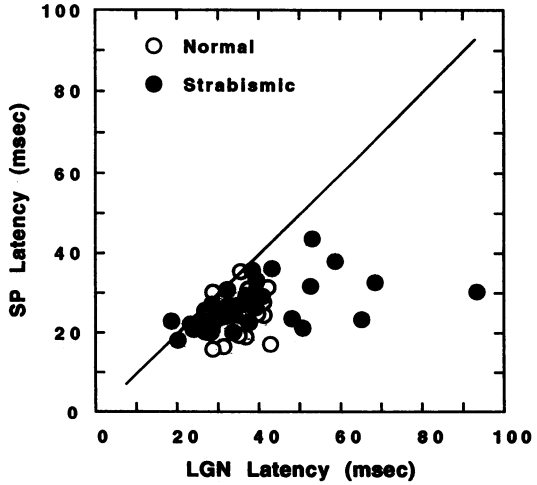

FIG. 5. Effect of early strabismus on speed of LGN signal transfer. Note that the relative visual response latencies of many LGN units in strabismic cats were longer than those of control units. SP, S-potentials.

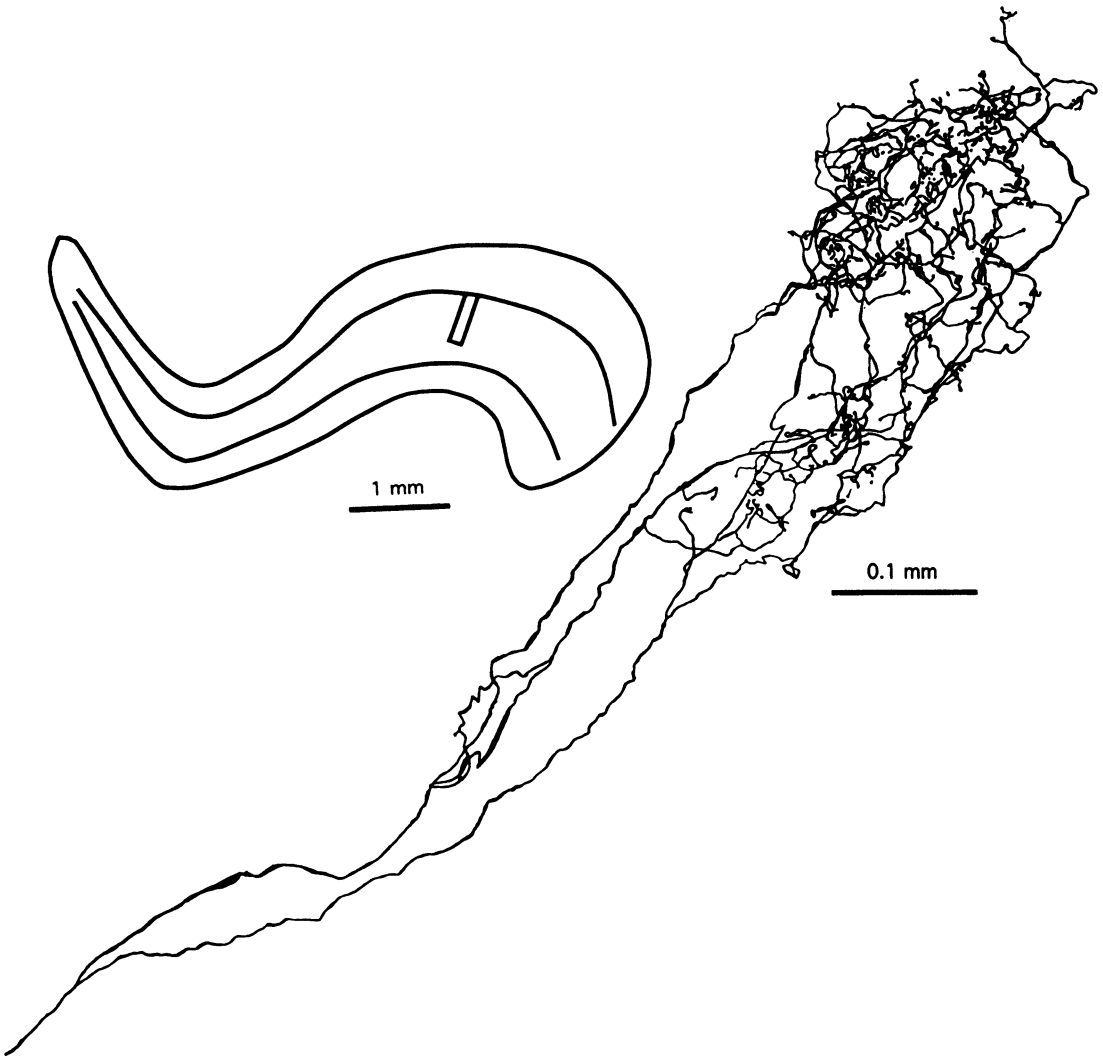

FIG. 6. Representative example of an HRP-labeled retinogeniculate X axon in a strabismic cat. This axon projected ipsilaterally to lamina A1 from the deviating eye. (*Insert*, at left) Drawing in lower magnification of the axon trajectory in the LGN sagittal section.

boutons for individual retinogeniculate axons in our experimental animals (Table 1).

DISCUSSION

This study has demonstrated that early strabismus significantly alters the efficiency of signal transmission from the retina to the cortex. Furthermore, the observed physiological deficits in the LGN are associated with immature X-retinogeniculate axon arbors.

The role of the retinogeniculate terminal abnormalities in determining the functional LGN deficits is a matter of speculation. It is reasonable to assume that the decrease in retinogeniculate bouton density would contribute to the reduction in contrast sensitivity and the increase in LGN response latency. It is also possible, however, that alterations in the modulatory influences of corticogeniculate projections contribute to the observed functional LGN deficits. The responsiveness of cortical neurons in strabismic animals is consistently depressed relative to normal animals (2, 3, 26–28). As a result, the level of tonic corticofugal excitatory influences on LGN neurons may be chronically lowered (29–30) and, therefore, the threshold for retinal activation of LGN neurons in strabismic kittens may not be reached as readily as it is in normal controls (11–14).

Table 1. Mean arbor volume, bouton number, and bouton density (±SE) of X-retinogeniculate axons in cat LGN

Cat	Volume, $mm^3 \times 10^{-3}$	Bouton	
		Number	Density, no. per μm^3
Strabismic	4.09	541	161.5 ± 16.2*
Normal	2.40	584	245.0 ± 22.0*

*Data are significant at $P < 0.005$ by the t test.

Our results provide conclusive evidence that the neural deficits associated with strabismus are not confined to the visual cortex but, instead, extend to the visual thalamus. However, this study does not address the question of the developmental sequence of these abnormalities; it is unclear whether the LGN deficits precede the massive cortical changes or merely reflect the cortical deficits. Both changes could interact with each other in a complex manner during development. Specifically, the observed anomalies in the spatial and temporal filter properties of LGN neurons could be initiated by widespread cortical abnormalities. At the same time, the LGN anomalies could further compound the cortical neural deficits associated with strabismic amblyopia by perturbing the normally precise spatial and temporal convergence of geniculate inputs to cortex.

Finally, our results on subcortical deficits in strabismic cats stand in contrast to the lack of abnormal physiology reported in the primate LGN after monocular form deprivation (31) or optical defocus (32). Because anatomical and

physiological anomalies are relatively well documented for LGN cells and for retinal axon arbors of monocularly deprived cats (8, 15, 16, 33), it is entirely possible that there are fundamental differences between these two species with respect to subcortical deficits (34). On the other hand, besides the obvious differences in the nature of the early visual experience of the experimental animal (monocular form deprivation vs. strabismus), the disagreement in results may be attributed to methodological differences between these studies (i.e., intraunit comparisons in this study vs. the population-based comparisons in other studies; see the introduction). Thus, a comparable study, using intraunit comparisons in the LGN of strabismic monkeys, may be necessary to resolve this issue.

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