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Contrasting effects of strabismic amblyopia on metabolic activity in superficial and deep layers of striate cortex

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Adams DL, Economides JR, Horton JC. Contrasting effects of strabismic amblyopia on metabolic activity in superficial and deep layers of striate cortex. *J Neurophysiol* 113: 3337–3344, 2015. First published March 25, 2015; doi:10.1152/jn.00159.2015.—To probe the mechanism of visual suppression, we have raised macaques with strabismus by disinserting the medial rectus muscle in each eye at 1 mo of age. Typically, this operation produces a comitant, alternating exotropia with normal acuity in each eye. Here we describe an unusual occurrence: the development of severe amblyopia in one eye of a monkey after induction of exotropia. Shortly after surgery, the animal demonstrated a strong fixation preference for the left eye, with apparent suppression of the right eye. Later, behavioral testing showed inability to track or to saccade to targets with the right eye. With the left eye occluded, the animal demonstrated no visually guided behavior. Optokinetic nystagmus was absent in the right eye. Metabolic activity in striate cortex was assessed by processing the tissue for cytochrome oxidase (CO). Amblyopia caused loss of CO in one eye's rows of patches, presumably those serving the blind eye. Layers 4A and 4B showed columns of reduced CO, in register with pale rows of patches in layer 2/3. Layers 4C, 5, and 6 also showed columns of CO activity, but remarkably, comparison with more superficial layers showed a reversal in contrast. In other words, pale CO staining in layers 2/3, 4A, and 4B was aligned with dark CO staining in layers 4C, 5, and 6. No experimental intervention or deprivation paradigm has been reported previously to produce opposite effects on metabolic activity in layers 2/3, 4A, and 4B vs. layers 4C, 5, and 6 within a given eye's columns.

exotropia; suppression; cytochrome oxidase; strabismus; stereopsis; ocular dominance column; blobs

THE IMPACT OF STRABISMUS ON visual perception was first investigated in monkeys by von Noorden and Dowling (1970), who disrupted ocular alignment by operating on the extraocular eye muscles. They noted that amblyopia occurred in animals with esotropia but not in those with exotropia. The absence of amblyopia in exotropic monkeys was attributed to their ability to share fixation, acquiring some visual targets with the right eye and others with the left eye.

Consequently, esotropia became the preferred experimental model for investigating strabismic amblyopia in nonhuman primates. Esotropia has been induced by eye muscle surgery or botulinum toxin injection in more than 30 monkeys, with amblyopia occurring reliably in the majority of cases (Harwerth et al. 1986; Kiorpes 1992; Kiorpes and Boothe 1980; Kiorpes et al. 1989, 1998, 2006; Kiper and Kiorpes 1994; Murphy et al. 1998; Sasaki et al. 1998). In contrast, exotropia has been created by eye muscle surgery on only rare occasions, with just one report of success at generating amblyopia. Harwerth et al. (1983) performed a recession of the medial rectus muscle and a resection of the lateral rectus muscle in one eye to produce an incomitant exotropia in two monkeys. Both animals developed mild amblyopia in the operated eye, with a peak contrast sensitivity at 1 cycles/°, compared with 2.5 cycles/° in the fellow eye.

In our experiments, we induce exotropia rather than esotropia, precisely because it is unlikely to cause amblyopia. Our goal is to elucidate how the visual system engages in suppression to avoid diplopia when the eyes are misaligned. Suppression occurs in its purest form when both eyes have normal acuity. When amblyopia is present, the issue becomes muddled because input from one eye may be degraded because of suppression, low acuity, or both. To produce exotropia, we perform a tenotomy of the medial rectus muscle of each eye in macaques at 1 mo of age (Economides et al. 2007). To date, this procedure has been carried out successfully in six animals, resulting in a relatively comitant, alternating exotropia. Contrast sensitivity function testing has shown no evidence of amblyopia in either eye.

We now report the occurrence of severe amblyopia in a monkey with exotropia. This finding was unexpected, because the animal underwent exactly the same postnatal eye muscle surgery as the previous six animals. Cytochrome oxidase (CO) activity, a reflection of relative tissue metabolism, was examined in the lateral geniculate nucleus and in the primary (striate) visual cortex. It revealed a remarkable pattern of enzyme activity in striate cortex, which has not been described previously in any animal, under any experimental circumstances. CO activity within ocular dominance columns flipped contrast between the upper layers (2/3, 4A, and 4B) and lower layers (4C, 5, and 6) of striate cortex.

METHODS

The subject of this report is a single male *Macaca mulatta* obtained from the California National Primate Research Center, Davis, CA. Experimental procedures were approved by the Institutional Animal Care and Use Committees at the University of California, Davis and the University of California, San Francisco (USCF).

Exotropia was induced by disinsertion of the medial rectus muscle tendon in each eye at the age of 28 days. Anesthesia was provided with ketamine HCl (15 mg/kg im) and by topical application of 1% proparacaine HCl. The released medial rectus muscle retracted into each orbit after the tenotomy.

The infant monkey was observed to have a large exotropia immediately after the eye muscle surgery. Frequent daily observations

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verified that both eyes healed rapidly, with no evidence of edema, infection, or discharge. Photographs were taken 15 days after the surgery with a 100-mm macro lens equipped with a ring-flash. The camera was held ~1 m from the animal, and the position of the corneal light reflex in each eye was used to gauge the magnitude of exotropia.

After the monkey reached maturity, it was transferred from the primate colony at the Davis Primate Center to our laboratory at UCSF. A titanium headpost was implanted under general anesthesia (Adams et al. 2007).

The monkey's eye movements were recorded while head-restrained in a primate chair, following procedures described previously (Economides et al. 2007). In brief, stimuli were rear-projected onto a tangent screen with a digital light projector. Eye movements were monitored at 60 Hz with two infrared video eye tracking cameras (SensoMotoric Instruments, Teltow, Germany). For calibration, the gain and offset were adjusted online to match eye and target locations while the monkey tracked a spot. He was rewarded for accurate fixation with aliquots of slurry, made from biscuit powder, fruit, and juice. This procedure was carried out for the left eye while the right eye was covered. When the left eye was covered, the animal would not track a target with the right eye. Therefore, we used a calibration obtained for the right eye of another animal. The gain of slow phase eye movements generated by an optokinetic stimulus was compared between the left eye and the right eye. The gains were found to be equal, using the "borrowed" calibration for the right eye, implying that the calibration was reasonably accurate.

For histology, the animal was euthanized with intravenous injection of pentobarbital (150 mg/kg) and perfused with normal saline followed by 1% paraformaldehyde in 0.1 M phosphate buffer. Flat-mounts of striate cortex were prepared from each hemisphere, cut tangentially at 60 μm on a freezing microtome, and processed for CO activity (Horton and Hocking 1996; Wong-Riley 1979). Coronal sections of the lateral geniculate nuclei were cut at 50 μm and processed alternately for CO activity or stained with cresyl violet.

RESULTS

Photographs were taken 15 days after eye muscle surgery to document the baby monkey's exotropia. The shutter was triggered whenever the alert, swaddled infant seemed to fixate the camera lens. A total of eight photographs was taken. In three, the monkey was not looking at the camera with either eye. In five, the monkey was fixating the camera with the left eye. This observation suggests that within 2 wk of eye muscle surgery the animal had established a strong left eye fixation preference.

After the mature monkey was transferred to our laboratory, an ophthalmological examination was performed while the animal was under general anesthesia for implantation of a titanium headpost. No ocular abnormality was present that might explain loss of visual acuity in the right eye. The pupils were round, equal, and briskly reactive to light. There was no afferent pupillary defect. The cornea, iris, and lens were normal in each eye. After pupil dilation with 1% cyclopentolate, retinoscopy yielded a refraction of $+1.00 + 0.50 \times 180°$ in the right eye and $+0.75 + 0.50 \times 180°$ in the left eye. On indirect ophthalmoscopy the optic disc and retina were normal in each eye.

Amblyopia demonstrated by eye movement recordings. Figure 1 shows the position of the two eyes while the monkey fixated a 1° diameter spot presented on a tangent screen. Sustained fixation within a window measuring $2 \times 2°$ was rewarded. The animal fixated the spot exclusively with the left eye. In primary position there was a right exotropia of 34°. The exotropia was slightly incomitant, measuring 33° in left gaze and 38° in right gaze. In addition, the right eye became progressively more hypertropic in right gaze. The ocular deviation was slightly variable, evinced by the increased scatter of right eye position clusters compared with left eye position clusters.

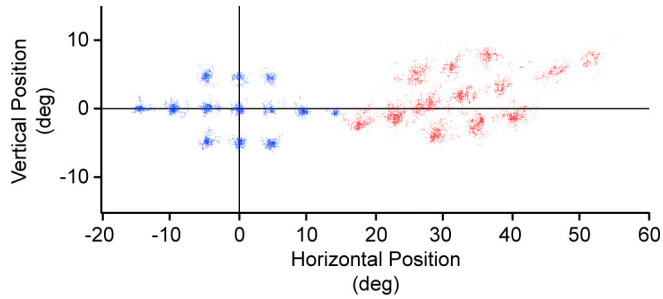

Fig. 1. Magnitude of exotropia at different gaze angles. Position of left eye (blue dots) and right eye (red dots) while the monkey fixated a 1° spot at locations separated by 5° on a tangent screen. The target was always fixated with the left eye. Note increased scatter of points representing eye position for the deviated right eye. The exotropia measured 34° in primary gaze and increased slightly on right gaze. A right hypertropia also became evident on right gaze.

Figure 2*A* shows smooth pursuit of a target oscillating horizontally ±30°. The monkey tracked only with his left eye. When the target moved to the right, requiring adduction of the left eye, the monkey eventually gave up tracking. However, he never acquired the target with the right eye. In contrast, monkeys rendered exotropic by medial rectus muscle tenotomy who retain normal acuity in each eye immediately switch fixation to the other eye (Economides et al. 2007). This maneuver enables them to maintain tracking without having to adduct either eye too far. Presumably, adduction is impaired because of residual weakness of the medial rectus muscle from postnatal tenotomy.

Figure 2*B* shows smooth pursuit of a 1° target moving back and forth sinusoidally ±15°. Within this range, the monkey could maintain fixation on the target continuously with the left eye. The stimulus was presented monocularly by occluding the fellow eye with a filter that transmitted only infrared light, allowing video eye tracking but blocking vision. The monkey pursued faithfully when the right eye was covered. When the target was presented with the left eye occluded the monkey never acquired it with the right eye and made saccades that bore no relationship to the target. These saccades revealed that he could adduct each eye at least 25°, despite the history of medial rectus tenotomy. This was comparable to the magnitude of adduction documented in other exotropic monkeys without amblyopia (Economides et al. 2007).

Figure 2*C* illustrates the monkey's ability to make saccades to a target stepping to different locations. Accurate saccades were made to the target only when the left eye viewed the tangent screen. Even when the target size was increased to 5°, the monkey did not fixate it with the right eye (data not shown).

Figure 2*D* shows eye movements generated by a full-field random dot pattern moving at constant velocity on the tangent screen. With only the left eye viewing, optokinetic nystagmus was evoked with the stimulus moving in either direction. With only the right eye viewing, optokinetic nystagmus was absent.

To further assess visual function in the right eye, the animal was observed in his home cage while wearing an opaque black contact lens in the left eye. He groped his way around his

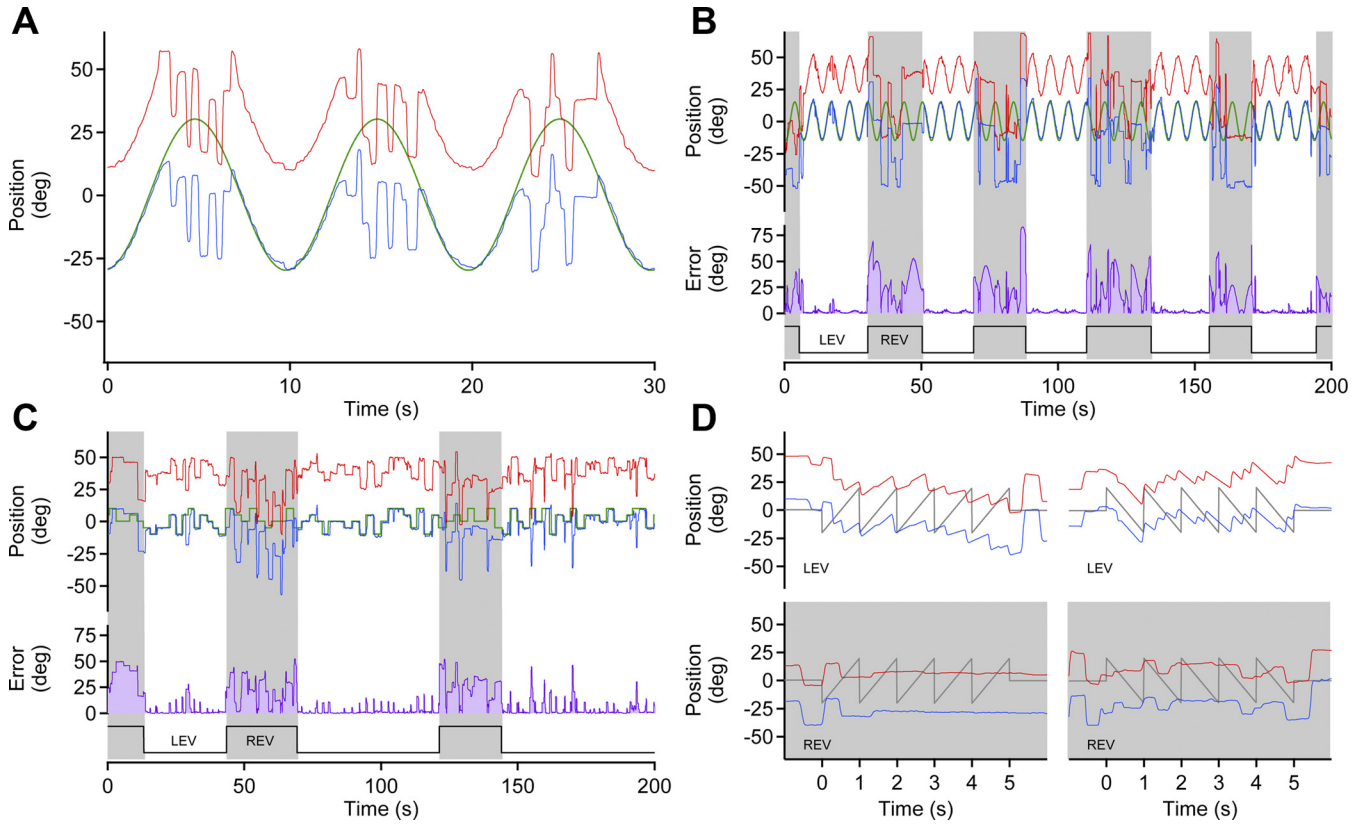

Fig. 2. Eye movement recordings demonstrating amblyopia in the right eye. *A*: smooth pursuit of a 1° target moving horizontally ±30° at 0.1 Hz. The monkey pursued the target (green trace) with the left eye (blue trace) but not the right eye (red trace). The monkey was capable of adducting to at least 25° with either eye, but on this task he gave up at ~15° nasal to primary gaze. Positive values represent right gaze. *B*: horizontal smooth pursuit of a 1° target moving ±15° at 0.15 Hz. The low amplitude allowed the monkey to complete cycles with either eye. With the left eye viewing (LEV), the monkey pursued accurately. With the right eye viewing (REV; gray shading), the target was never acquired. Error represents the moment by moment magnitude of the retinal slip of the target on the currently viewing eye. *C*: horizontal saccades to a 1° target stepping every 2 s to a random location on the horizontal meridian (range = ±15°, 5° steps). As in *B*, the monkey performed the task during left eye viewing, but not during right eye viewing (gray shading). *D*: optokinetic nystagmus generated by a patterned stimulus, represented by the sawtooth gray line (40°/s, noise pattern = 1° elements, randomly distributed, 50% coverage, 50% contrast). The *top traces* show eye movements to the stimulus moving to the right or left, under left eye viewing conditions. The *bottom traces* show the response to the same stimuli, under right eye viewing conditions (gray shading). There is no optokinetic nystagmus with the right eye viewing.

enclosure, ignored food treats offered to him, and showed no evidence of visually guided behavior.

Histological findings in exotropic amblyopia. In the right lateral geniculate nucleus, there was atrophy of the parvocellular and magnocellular laminae receiving input from the amblyopic right eye (Fig. 3). In cresyl violet sections, cells in laminae supplied by the amblyopic eye were smaller and contained less Nissl substance than those in normal eye laminae. They also exhibited weaker CO activity.

In the left lateral geniculate nucleus, there was atrophy of the parvocellular laminae supplied by the amblyopic right eye (Fig. 3). This was evident in both Nissl and CO sections, but the relative degree of atrophy was less pronounced than in the right lateral geniculate nucleus. In the magnocellular laminae, the situation was reversed. Nissl and CO activity were stronger in the amblyopic right eye lamina than in the normal left eye lamina. This unexpected reversal in the magnocellular laminae was present in coronal sections throughout the rostrocaudal extent of the nucleus.

In striate cortex of normal macaques, the CO method reveals rows of patches in all layers except 1, 4A, and 4C. The rows of patches, which are most prominent in layer 2/3, align with the ocular dominance column in layer 4C (Horton and Hubel 1981). In this exotropic monkey, every other row of patches in layer 2/3 exhibited a sharp reduction in the density of CO staining (Figs. 4, *A*, *B*, and *D*). This was true throughout striate cortex, except in the representation of the blind spots and monocular crescents, where ocular dominance columns are absent. Presumably, loss of CO activity occurred in every other row of patches because amblyopia was present in one eye.

For the sake of comparison, Fig. 4, *C* and *E*, show the patches in the upper layers from a monkey with alternating exotropia and intact acuity in both eyes. The rows formed by the patches are more obvious than in normal animals. We attribute this property to loss of CO activity in the binocular border regions, located between each row of patches (Adams et al. 2013). Loss of CO in these regions deepens the "valleys" between rows of patches, causing the rows to stand out more clearly.

In layer 4A, CO activity was reduced in regions aligned with the pale rows of patches in layer 2/3. In layer 4B the same was true: pale rows of CO staining matched pale rows in the upper layers (Fig. 5, *A* and *B*).

In layer 4Cα and 4Cβ there were faint thin CO dark columns alternating with wide pale columns. Surprisingly, the thin dark columns in layer 4C were aligned with the pale rows of CO patches in layer 2/3, 4A, and 4B (Fig. 5, *A* and *C*). In layer 5/6, there was a subtle pattern of dark and pale rows of CO patches.

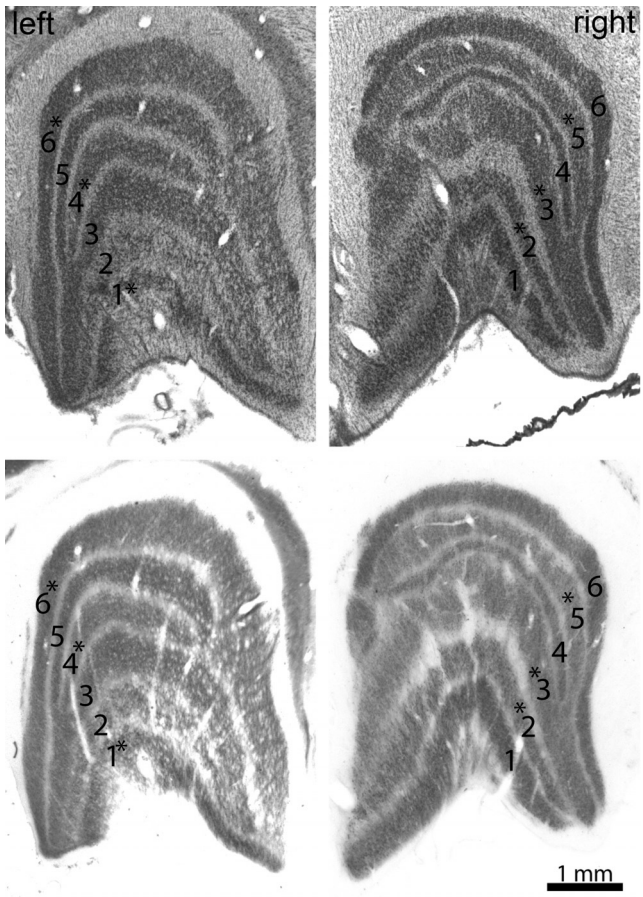

Fig. 3. Lateral geniculate nucleus (LGN) in strabismic amblyopia. *Top*: Nissl-stained sections. *Bottom*: adjacent sections processed for cytochrome oxidase (CO). In the right LGN, there is obvious atrophy in laminae 2, 3, and 5, supplied by the amblyopic right eye (*). In the left, LGN there is atrophy of parvolaminae 4 and 6 supplied by the amblyopic right eye (*) but not magnocellular lamina 1.

As for layer 4C, dark rows in layer 5/6 were in register with pale rows in layer 2/3 (Fig. 5, *A* and *D*). Four regions, two in opercular cortex and two in calcarine cortex, in each hemisphere were examined at high power to compare CO activity in different layers. Blood vessel profiles were used to align the sections precisely. In all eight regions, light rows of CO activity in layers 4B, 4A, and 2/3 flipped to match dark rows in layers 4C and 5/6.

In area V2, the repeating pattern of thin-pale-thick-pale stripes appeared similar to the pattern described in normal animals (Fig. 4). Neither strabismus nor amblyopia had any visible effect on CO activity in V2 stripes.

DISCUSSION

As pointed out by Kiorpes and Boothe (1981), it is rare for surgical exotropia to result in amblyopia. Yet in our monkey, amblyopia was so profound that visual function could not be quantified using standard behavioral methods. The animal did not orient to light stimuli or show any evidence of visually guided behavior when his normal eye was occluded. Loss of optokinetic nystagmus has been reported previously only in monkeys raised with monocular eyelid suture (Sparks et al. 1986). The absence of optokinetic nystagmus in our monkey suggests dense amblyopia, tantamount to the deficit produced by long-term monocular form deprivation. No other explanation could be found for his vision loss, although it is always possible that another unknown factor was responsible. These observations are based on only a single animal, but they are reported to highlight the fact that not every monkey that undergoes early bilateral medial rectus muscle recession will develop an alternating exotropia with normal acuity in each eye.

In humans, amblyopia is comparatively rare in exotropia and the onset of ocular misalignment occurs at a later age than in esotropia (Chia et al. 2007; Mohney 2007; Pascual et al. 2014). Children usually enjoy normal visual function through infancy but then develop an intermittent exotropia that eventually decompensates into a constant exotropia in some individuals (Buck et al. 2012; Choi and Kim 2013; Mohney and Huffaker 2003; Nusz et al. 2006). By the time exotropia becomes constant, the critical period for visual development is over. Hence, the subject is no longer susceptible to amblyopia. The peripheral temporal retina is suppressed in each eye to avoid diplopia, but both foveas remain perceptually active (Cooper and Feldman 1979; Economides et al. 2012; Herzau 1980; Joosse et al. 1999).

The situation is quite different in macaques that undergo eye muscle surgery at the age of 4 wk. The onset of exotropia is abrupt, and it occurs right during the critical period. Nonetheless, most animals seem to compensate in the same manner as humans, by suppressing the peripheral temporal retina in each eye (Adams et al. 2008, 2013). They retain normal visual acuity and the ability to alternate fixation. The fact that amblyopia is rare in monkeys with early exotropia and in humans with late exotropia implies that age at onset of ocular misalignment is not a decisive factor. Rather, the low prevalence of amblyopia appears to be due to the strategy of avoiding suppression of the macula in each eye.

In the monkey described in this article, photographs taken 2 wk after eye muscle surgery revealed a fixation preference for the left eye. Rather than suppressing the temporal retina in each eye and keeping both maculae active perceptually, he appears to have suppressed the right eye completely, resulting in severe amblyopia. It is unclear why this animal compensated in a different fashion than the majority of subjects with exotropia. There was nothing different about his eye muscle surgery or rearing conditions.

In the lateral geniculate nucleus, there was atrophy of laminae supplied by the amblyopic eye, as described previously in primates with strabismus (Barnes et al. 2010; Crawford and von Noorden 1979; Sasaki et al. 1998; von Noorden and Crawford 1992). The effect was most pronounced in the lateral geniculate nucleus ipsilateral to the amblyopic eye (Noorden and Crawford, 1992). Relative loss of Nissl substance and CO activity occurred in all deprived laminae, with one exception. In the magnocellular laminae contralateral to the amblyopic eye, Nissl and CO staining were weaker in the lamina supplied by the normal eye (Fig. 3). For this anomaly, we have no explanation.

Tychsen and Burkhalter (1997) discovered that strabismus disrupts the normal distribution of CO in striate cortex, even in the absence of amblyopia. They described a pattern of light and dark CO columns in layer 4C of a monkey raised with daily monocular occlusion, who ultimately became exotropic. They

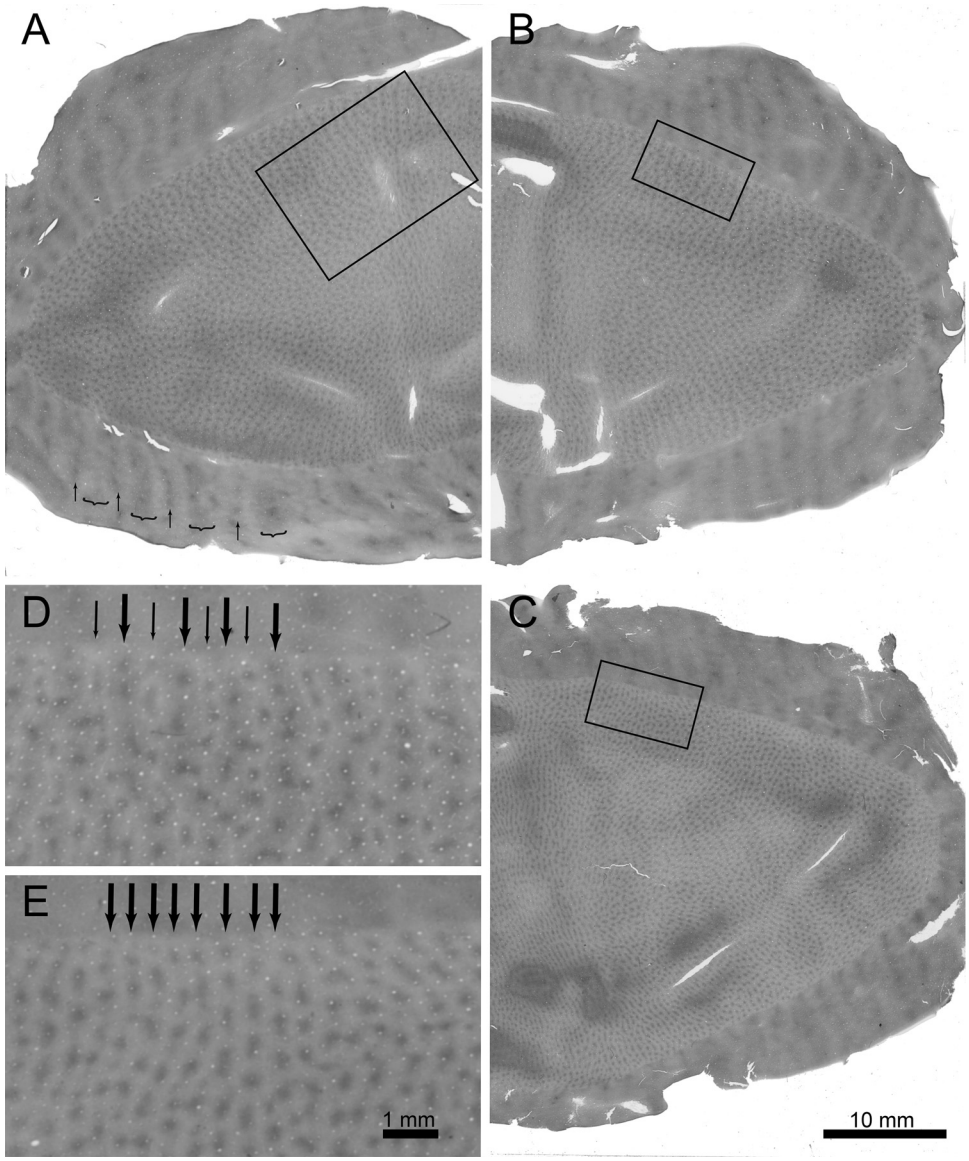

Fig. 4. CO patches in strabismus. *A*: left opercular cortex showing alternating rows of light and dark patches in a single tangential section through layer 2/3. Boxed region is shown in Fig. 5. V2 pale, thin (arrows) and thick (brackets) stripes appear normal, despite strabismus and amblyopia. *B*: right opercular cortex, showing similar pattern of CO activity in layer 2/3. Boxed region is shown in *D*. *C*: right operculum from a different monkey, with alternating exotropia and no amblyopia. The organization of patches into rows is more striking than usual, but there is no light/dark alternation in contrast. *D*: boxed region from *B*, showing rows of light (small arrows) and dark (big arrows) patches. *E*: boxed region from *C*, showing that rows (arrows) of patches have equal contrast. Absence of light and dark rows seen in *D* is due to lack of amblyopia.

suggested that "nasal ocular dominance columns of both hemispheres retain greater CO activity," an idea consistent with suppression of the temporal retina in each eye. Fenstemaker et al. (2001) reported a similar pattern of light and dark columns in layer 4C of two monkeys with surgical esotropia and normal acuity in each eye.

We have described previously two distinct patterns of CO activity in macaques with alternating strabismus (Adams et al. 2013). Both occur in the same animal but in different regions of striate cortex. In cortex representing the central visual fields, where input from neither retina is suppressed, there is robust CO activity within the core zones of the ocular dominance columns (Figs. 4, *C* and *E*, and 6*A*). CO activity is reduced along the border strips between columns, because binocular function is impaired. This produces pale border strips in layer 4C and rows of patches in layer 2/3 that are equal in density but more stripe-like than usual. In cortex representing the peripheral visual fields, where input from the ipsilateral temporal retina is suppressed, the pattern of CO activity is different. There are columns of light and dark CO activity in layer 4C (Fig. 6*B*). They match rows of light and dark patches in the upper layers. The contrast between light and dark rows of patches is relatively subtle (see Fig. 7 in Adams et al. 2013), compared with the robust effect produced by enucleation or eyelid suture (Horton and Hocking 1998).

The monkey in this report had a unique pattern of CO activity, not described previously in either amblyopia or strabismus. There were light and dark rows of CO patches in layers 2/3, 4A, and 4B (Fig. 6*C*). The contrast between light and dark rows was high, resembling the effect of eyelid suture or enucleation (Horton 1984). Remarkably, the CO contrast became inverted between the upper layers (2/3, 4A, and 4B) and deeper layers (4C, 5, and 6). This meant that pale rows of patches in the upper layers were situated over dark columns in 4C and dark rows of patches in 5/6 (Fig. 5).

This bizarre arrangement makes it difficult to be sure which set of columns belonged to the amblyopic eye. We did not inject [^{3}H]proline into one eye to label the ocular dominance columns. The only available clue comes from an anomalous finding described by LeVay et al. (1980) in a monkey raised

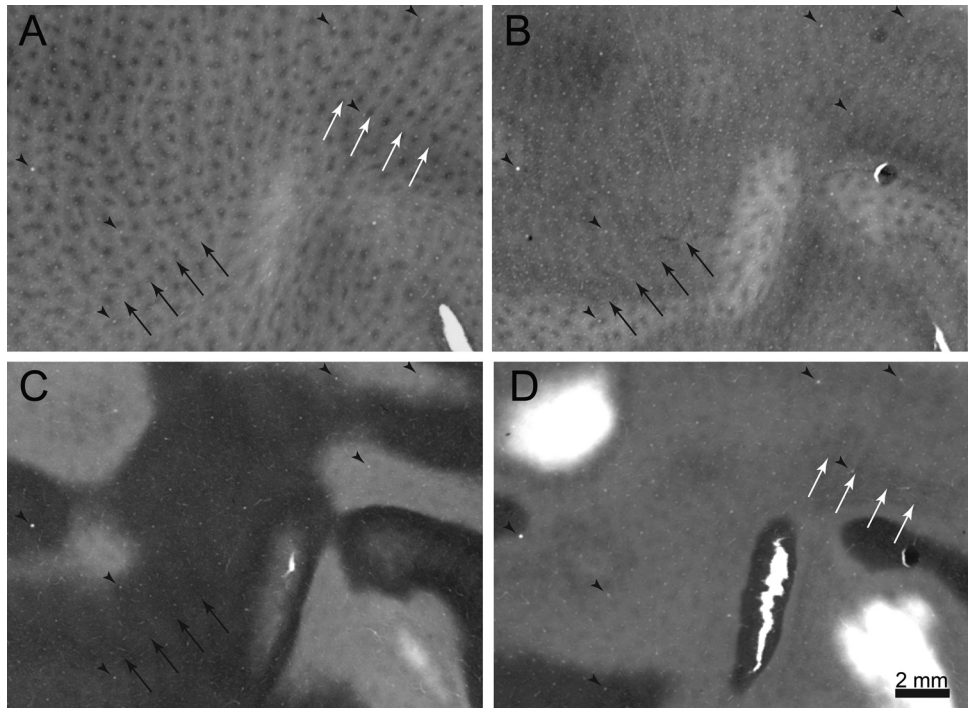

Fig. 5. Contrast of CO activity within ocular dominance columns depends on cortical layer. *A*: layer 2/3 from the boxed region in Fig. 4*A*; light rows of patches are marked with arrows. *B*: layer 4B, showing that pale rows of CO (black arrows) match pale rows in upper layers. Arrowheads denote examples of vessel profiles used for precise section alignment. *C*: layer 4C, showing thin dark rows of CO activity (black arrows), which match pale rows of patches in layer 2/3 (*A*) and 4B (*B*). *D*: layer 5/6, showing rows of dark patches (white arrows), which align with rows of pale patches in layer 2/3 denoted by white arrows in *A*. In *A–D*, the sets of 4 arrows denoting columns are located in precisely the same position to facilitate comparison.

with strabismus starting at at 1 mo of age. The ocular dominance profile recorded at the age of 4 yr was lopsided, suggesting that the deviated eye was amblyopic. In layer 4C, a Liesegang silver stain showed alternating wide pale and thin dark columns, resembling the CO pattern present in our monkey with strabismic amblyopia (see Fig. 36, LeVay et al. 1980). The authors remarked, "an unusual feature of the silver preparations, compared with what was seen in long-term deprived [lid sutured] monkeys, was that the columns for the deprived eye were darker, not paler, than those of the open eye." In other words, strabismus caused an increase in Liesegang staining in the amblyopic eye's columns in 4C, contrary to the effect of eyelid suture. If the same occurs for CO activity in strabismic amblyopia, then the amblyopic eye's columns correspond to the dark stripes in layers 4C and the pale rows of patches in layer 2/3.

Altered CO activity in striate cortex has been reported after eye enucleation, eyelid suture, laser lesions, and tetrodotoxin injection (Horton 1984; Horton and Hocking 1998; Tychsen and Burkhalter 1997; Wong-Riley and Carroll 1984; Wong-Riley 1979). Each manipulation produces loss of CO activity in all cortical layers of the ocular dominance columns serving the affected eye. The contrasting effects in layers 2/3, 4A, and 4B vs. layers 4C, 5, and 6 we now describe in strabismic ambly-

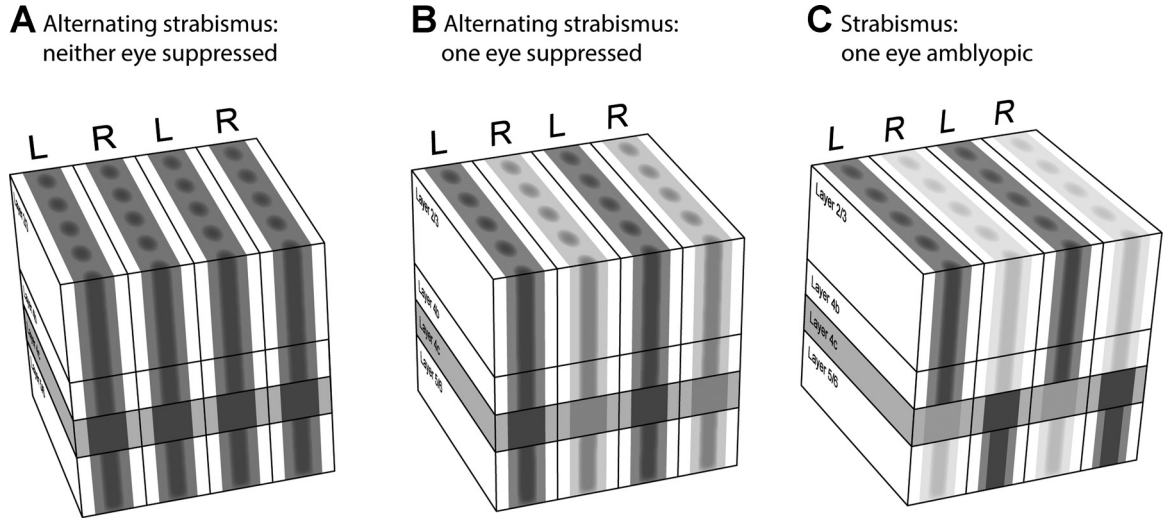

Fig. 6. Schematic illustration of 3 distinct patterns of CO activity that can occur in exotropia. *A*: in regions of cortex where neither eye is suppressed, CO activity is diminished along column borders. As a result, the stripe-like appearance of patches in layer 2/3 is enhanced (see Fig. 4, *C* and *E*) and pale strips are present along the borders of ocular dominance columns in layer 4C. *B*: in regions of cortex where one eye is suppressed, CO activity is reduced in all layers of the suppressed eye's ocular dominance columns (Adams et al. 2013). *C*: in the exotropic animal featured in this report, there appears to be relative loss of CO activity in layers 2/3, 4A, and 4B of the amblyopic right eye's (R) ocular dominance columns and in layers 4C, 5, and 6 of the normal left eye's (L) ocular dominance columns.

opia were wholly unexpected. The explanation is unclear, but ultimately a full understanding of the mechanism of strabismic amblyopia will have to account for the curious reversal in CO activity that we have stumbled upon across cortical layers.

This experiment is difficult to replicate because, as mentioned previously, amblyopia is rare following induction of exotropia. We do not expect to encounter another animal with amblyopia from exotropia in the near future. One could generate amblyopia fairly reliably by induction of esotropia. However, in esotropic amblyopia, changes in CO activity are reported to be consistent across cortical layers (Wong et al. 2005). Perhaps another laboratory by chance will raise a monkey with exotropia and amblyopia, allowing the opportunity to test another animal.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: D.L.A., J.R.E., and J.C.H. conception and design of research; D.L.A., J.R.E., and J.C.H. performed experiments; D.L.A., J.R.E., and J.C.H. analyzed data; D.L.A., J.R.E., and J.C.H. interpreted results of experiments; D.L.A., J.R.E., and J.C.H. prepared figures; D.L.A., J.R.E., and J.C.H. drafted manuscript; D.L.A., J.R.E., and J.C.H. edited and revised manuscript; D.L.A., J.R.E., and J.C.H. approved final version of manuscript.

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