Effect of monocular blockade of retinal activity on the development of visual callosal connections in the rat

KEVIN CHANG[1*], RICHARD C VAN SLUYTERS[1] and JAIME F OLAVARRIA[2**]

[1] School of Optometry, University of California, Berkeley, CA, USA
[2] Department of Psychology, University of Washington, Seattle, WA, USA

It has been previously reported that neonatal monocular enucleation in rats and hamsters induces the development of an anomalous band of callosal connections in the middle of area 17 (primary visual cortex) in the hemisphere ipsilateral to the remaining eye. In order to determine whether this effect is due to elimination of retinal activity in one eye, we used the anatomical tracer horseradish peroxidase (HRP) to study the pattern of visual callosal connections in rats in which retinal activity had been blocked by intraocular injections of tetrodotoxin during the first two weeks of life. We found that the callosal pattern in the hemisphere ipsilateral to the eye not treated with tetrodotoxin was not distinguishable from the pattern present in normal rats. In particular, we did not observe the anomalous extra band of callosal connections that occurs in area 17 in the hemisphere ipsilateral to the remaining eye in monocularly enucleated rats. These results indicate that blockade of retinal activity in one eye is not sufficient to cause the marked changes in the pattern of visual callosal connections that are induced by neonatal monocular enucleation.

Key terms: *corpus callosum, interhemispheric commissure, striate cortex, visual cortex.*

INTRODUCTION

Studies of the interhemispheric connections through the corpus callosum have revealed that visual input plays an important role in the development of this pathway in occipital cortex (see Innocenti, 1991, for review). For instance, neonatal disruptions of retinal projections cause marked alterations in the pattern of callosal connections in visual area 17 (striate cortex) (Olavarria *et al*, 1987; Van Sluyters *et al*, 1990; Innocenti, 1991). A particularly striking effect of neonatal monocular enucleation in rats (Olavarria *et al*, 1987) and hamsters (O'Brien and Olavarria, 1995) is the appearance of an anomalous band-like distribution of callosal connections in the middle of area 17 in the hemisphere ipsilateral to the remaining eye.

Identifying what causes the development of this anomalous pattern of callosal connections is difficult because the changes produced by removal of one eye are numerous and not well-understood. For instance, the effects of monocular enucleation on the visual pathway may be due to the elimination of retinal activity in one eye, to the interruption of axonal transport of trophic

* **Present address:** 11506 Manor Drive, Carmel, IN 46033, USA
** **Correspondence to:** Jaime F Olavarria, Department of Psychology, University of Washington, Box 351525, Seattle, Washington 98195-1525, USA. Fax: (1-206) 685-3157. E—mail: jaime@u.washington.edu.

substances along the ascending visual pathway (Matthews, 1985), or to changes in mechanical or chemical cues that guide the development of visual projections. In the present study we tested the hypothesis that the development of anomalies in the visual callosal pattern of monocularly enucleated rats is due to the elimination of retinal activity in one eye. This hypothesis predicts that blockade of retinal activity in one eye by injections of the sodium channel blocker tetrodotoxin (TTX) should yield the same result as removal of one eye, namely, the development of an anomalous band of callosal connections in area 17 in the hemisphere ipsilateral to the non-injected eye. A preliminary report of some of these data has appeared earlier (Chang *et al*, 1987).

MATERIALS AND METHODS

Intravitreal injections of TTX (3 to 16 µg/kg, adjusted according to age) were made in neonatal Long Evans rats anesthetized with fluothane (2% - 5% in a mixture of 70% N_2O and 30% O_2). Our data on the effect of intraocular injections of TTX on the visual callosal pattern come from experiments performed in 15 rats that survived one of the following treatments: a total of 3 injections of TTX during the third and fourth postnatal day (n = 3); a total of 12 injections of TTX, one every 24 h from birth to 12 days of age (n = 7); and a total of 24 TTX injections, one every 12 h from birth to 12 days of age (n = 5). Treatment was not continued beyond 12 days postnatal because development of callosal pathway is virtually complete by this age (Olavarria and Van Sluyters, 1985). Another group of pups (n = 6) underwent monocular enucleation under fluothane anesthesia on the first postnatal day, and 5 additional normally reared rats were used as age-matched controls. At 12 days of age, rats from all three experimental groups (TTX-treated, monocularly enucleated and normally reared) were anesthetized with fluothane and prepared for experiments designed to reveal the distribution of callosal connections. Multiple (12 -15) injections of horseradish peroxidase (HRP, Sigma type VI; 1.5 µl of a 30% solution in 0.9% saline) were delivered into the occipital cortex of one cerebral hemisphere through glass micropipettes. In TTX treated rats, the hemisphere injected with HRP was the one contralateral to the eye not injected with the activity blocker, while in monocularly enucleated rats, HRP was administered to the hemisphere contralateral to the remaining eye. One day later, the rats were deeply anesthetized with pentobarbital (50 mg/kg) and perfused through the heart with 0.9% saline followed by 2% glutaraldehyde in 0.1 M phosphate buffer. In all cases, the hemisphere opposite to the one injected with HRP was flattened (Olavarria *et al*, 1987), frozen, and sectioned into tangential sections (40 µm) which were processed for HRP histochemistry (Mesulam, 1978). Data from our previous studies in both normally-reared and monocularly enucleated rats (Olavarria and Van Sluyters, 1985; Olavarria *et al*, 1987) were also available for comparison.

An additional group of 9 rats (ages 12 - 24 days) were used for recording electrophysiologically from the superior colliculus (SC) to verify that our intraocular injections of TTX effectively blocked retinal activity. In pups placed under fluothane anesthesia, the posterior neocortex was suctioned to expose the surface of the underlying superior colliculus. Tungsten in glass microelectrodes (2-3 M1/2) were used to record from the anteromedial portion of the superior colliculus where binocular units are usually found (Tiao and Blakemore, 1976). After reliable responses were obtained by stimulating the eye contralateral to the recorded SC, volumes of 0.1% TTX solution ranging from 0.04 µl for pups 12 days of age to 0.29 µl for pups 24 days of age were injected into this eye to silence its retinal activity. In order to ensure that each eye was tested separately, the non tested eye was covered with a black occluder. Visual stimuli were presented on a tangent screen located 35 cm in front of the animal. Recording sessions lasted up to 16 hours, at the end of which animals were euthanized by a pentobarbital injection (5O mg/kg) followed by a thoracotomy. All animal procedures were performed according to NIH guidelines (NIH Publication N° 85-23, Revised 1985) and protocols approved by animal care committees at the University of

California, Berkeley, and the University of Washington, Seattle.

RESULTS

Blockade of retinal activity

Prior to the intraocular injections of TTX, visual stimuli presented to either eye elicited vigorous responses from cells recorded in anterior (binocular) portions of the superior colliculus (Tiao and Blakemore, 1976). Typically, responses elicited from the contralateral eye were stronger than those elicited from the ipsilateral eye. Figure 1 shows recordings from the right SC while visual stimuli were presented to the left eye. The top tracing shows a typical pattern of responses elicited by a stroboscopic light stimulus. Cells responded briskly to the "on" phase of the stimulus (indicated by the line segment immediately above the spike tracing), whereas they were virtually silent during the "off" phase of the stimulus. However, as the other tracings show, all activity elicited from the left eye disappeared following an injection of TTX into the left eye (the right eye was covered by a black occluder). The effect of the TTX injection lasted at least 16 hours in this case. Similar results were obtained from the other 8 rats studied electrophysiologically.

It is possible, however, that absence of evoked responses following TTX injections was due to tissue damage or nonspecific depression of neuronal activity at the recording site in the superior colliculus, rather than to blockade of retinal activity. This is unlikely because we were often able to elicit neuronal activity by slightly moving the electrode, indicating that live neurons were present at the recording site. As shown in the last tracing of Figure 1, these responses were not related to the visual stimulus, and were probably due to direct mechanical stimulation of neurons by the moving electrode. In addition, we were frequently able to elicit visually evoked responses by removing the occluder from the ipsilateral right eye and visually stimulating this eye, which was not treated with TTX. Finally, the possibility that injections of TTX

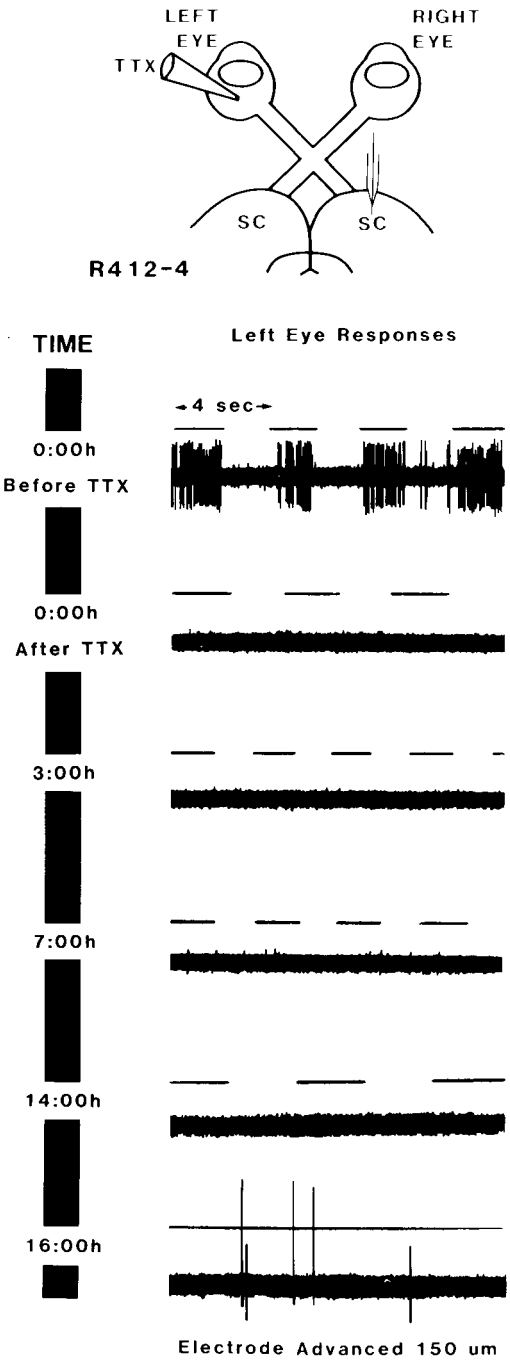

Fig 1. Blockade of retinal activity by intraocular injections of TTX. Diagram illustrates experiment in which electrical activity was recorded from the right superior colliculus (SC) before and after TTX was injected into the left eye. Neural activity was elicited with a stroboscopic light whose "on" state is indicated by the line segments above each tracing. Upper tracing shows spike trains elicited from the contralateral, left eye before TTX was injected into this eye. The following tracings show no evoked activity at different times after the injection (right eye was covered with black occluder). The last tracing shows activity produced by advancing the electrode 150 μm into the colliculus.

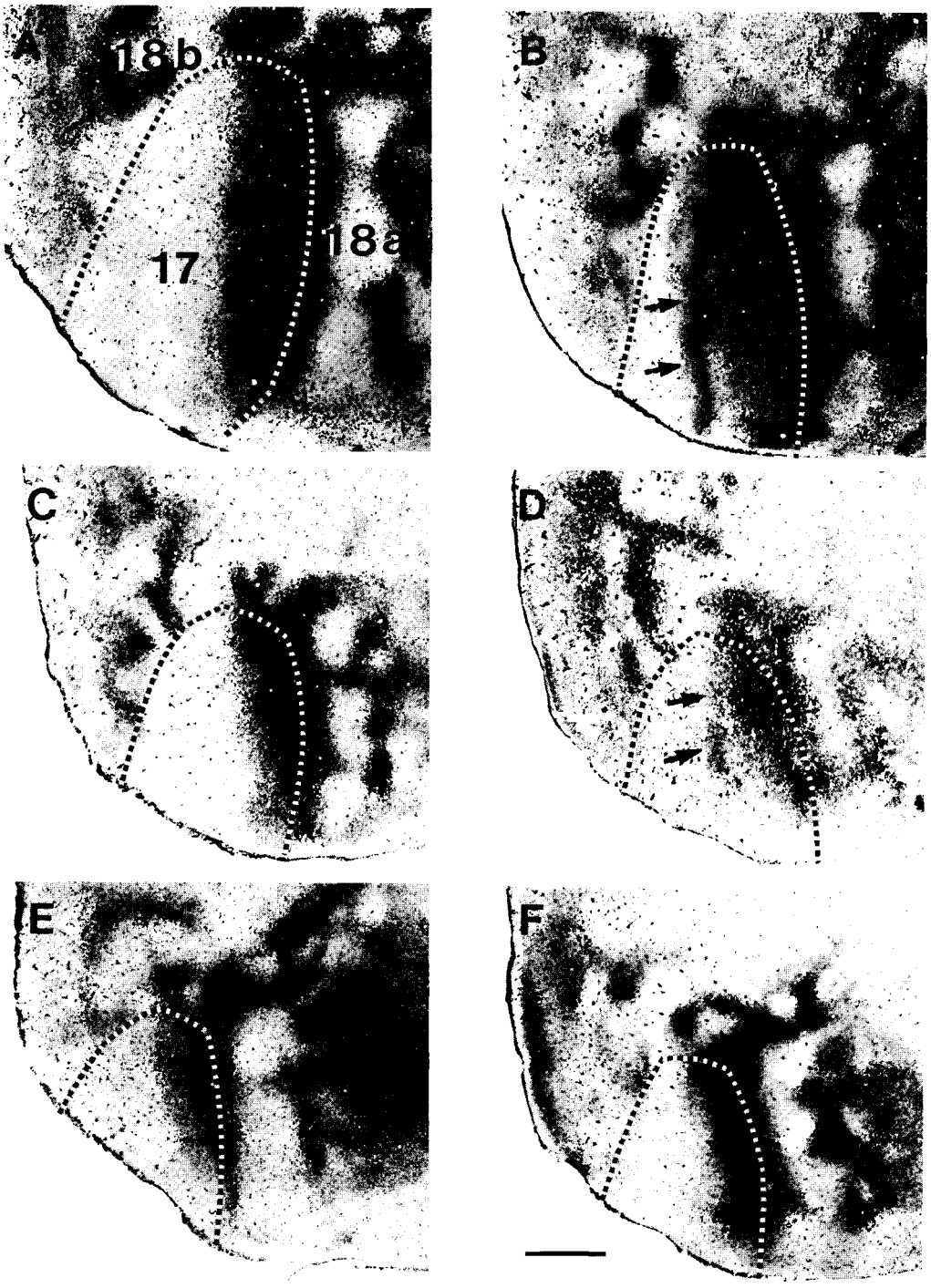

Fig 2. A-F. Low magnification, lightfield views of HRP-reacted tangential sections through layers III-IV showing callosal patterns in occipital cortex. Medial is to the left, posterior is down. Dark areas indicate dense accumulations of retrogradely labeled cells and anterogradely labeled terminations. Dashed lines indicate the borders of area 17, which were determined from adjacent, myelin-stained sections (see Olavarria *et al*, 1987). **A:** normally-reared adult rat. **B:** hemisphere ipsilateral to the remaining eye in adult rat monocularly enucleated at birth; arrows indicate anomalous band of callosal connections in middle portions of area 17. **C:** normally-reared 12-day-old rat. **D:** hemisphere ipsilateral to the remaining eye in 12-day-old rat monocularly enucleated at birth; arrows indicate anomalous band of callosal connections in middle portions of area 17. **E, F:** callosal patterns in the hemisphere ipsilateral to the eye not treated with TTX in rat that received monocular injections of TTX every 24 h from birth until 12 days of age (E), and in rat injected with TTX every 12 h from birth until 12 days of age (F). Scale bar = 1.0 mm. Data in A,B are from Olavarria *et al* (1987), with permission.

caused significant damage of the retina can be ruled out because we never observed anomalies in the callosal pattern that were similar to those induced by removal of one eye (see below). Together, these results indicate that retinal activity was effectively blocked by our TTX injections.

Callosal connections

In normal adult rats, the main feature of the visual callosal pattern is a dense band of callosal connections straddling the lateral border of area 17 (Fig 2A). Examination under high power indicates that both labeled cell bodies and fine labeling indicative of anterogradely transported tracer are found in this callosal band (Cusick and Lund, 1982; Olavarria *et al*, 1987). As shown in Figure 2A, the 17/18a callosal band has well-defined, smooth borders and is about 1.5 mm wide (Olavarria and Van Sluyters, 1985). Other features of the callosal pattern include a ring-like configuration anterolateral to area 17, a region of dense labeling lateral to area 18a, several narrow bands of labeling bridging area 18a at different anteroposterior levels, and one or more labeled regions in area 18b. These results are in close agreement with previous descriptions of the visual callosal pattern in normal-eyed pigmented and albino rats (Cusick and Lund, 1982; Záborszky and Wolff, 1982; Olavarria and Van Sluyters, 1985; Olavarria *et al*, 1988). The pattern in Figure 2C is from a 12 day-old normally-reared rat. Comparison of the pattern in Figure 2C with that in Figure 2A indicates that by the 12th postnatal day the pattern of callosal connections resembles that found in adult rats (Olavarria and Van Sluyters, 1985).

In adult rats monocularly enucleated at birth, an anomalous band of dense callosal labeling (indicated by arrows in Fig 2B) is observed in middle portions of striate cortex in the hemisphere ipsilateral to the remaining eye. This anomalous band runs roughly parallel to the 17/18a callosal band except anteriorly, where these two bands merge (Olavarria et *al*, 1987). Typically, the labeling is continuous along the anomalous callosal band, but periodic fluctuations in density (see Fig 2B and Olavarria *et al*, 1987) give the band a beaded appearance. Similar anomalies have been described in monocularly enucleated hamsters (O'Brien and Olavarria, 1995). As shown previously (Olavarria *et al*, 1987), this anomalous band can be clearly distinguished in 12 day old rats monocularly enucleated at birth (arrows in Fig 2D).

Our results from two TTX-injected rats are illustrated in Figure 2E,F. The pattern in Figure 2E is from a rat that received one intraocular injection of TTX every 24 h from birth to 12 days of age, while that in Figure 2F is from a rat the received one injection of TTX every 12 h from birth to 12 days of age. The patterns in Figure 2E,F are from the hemisphere ipsilateral to the eye that did not receive TTX injections (corresponding to the remaining eye in one-eyed rats). As illustrated in these photomicrographs, many features of the pattern in normally-reared rats can be recognized in TTX-treated rats, including the dense band of callosal labeling at the 17/18a border and the ring-like callosal configurations in extrastriate cortex. However, comparison of the callosal patterns in TTX-injected rats (Fig 2E,F) and one-eyed rats (Fig 2B,D, and Olavarria *et al*, 1987) indicates that the anomalous band of labeling seen in middle portions of striate cortex in one-eyed rats is not observed in TTX-treated rats. In fact, the overall appearance of the callosal patterns in TTX treated rats does not differ significantly from that in normally reared rats. Figure 3 shows similar results from 4 additional rats that received monocular injections of TTX every 12 h from birth until 12 days of age.

DISCUSSION

We found that blocking retinal activity with monocular injections of TTX during the first two weeks of life does not have the same effect on callosal development as removing one eye neonatally. Our failure to replicate the anomalies in the callosal pattern produced by monocular enucleation could have been due to incomplete blockade of retinal activity by our injections of TTX. This is unlikely because our electrophysiological recordings from the superior

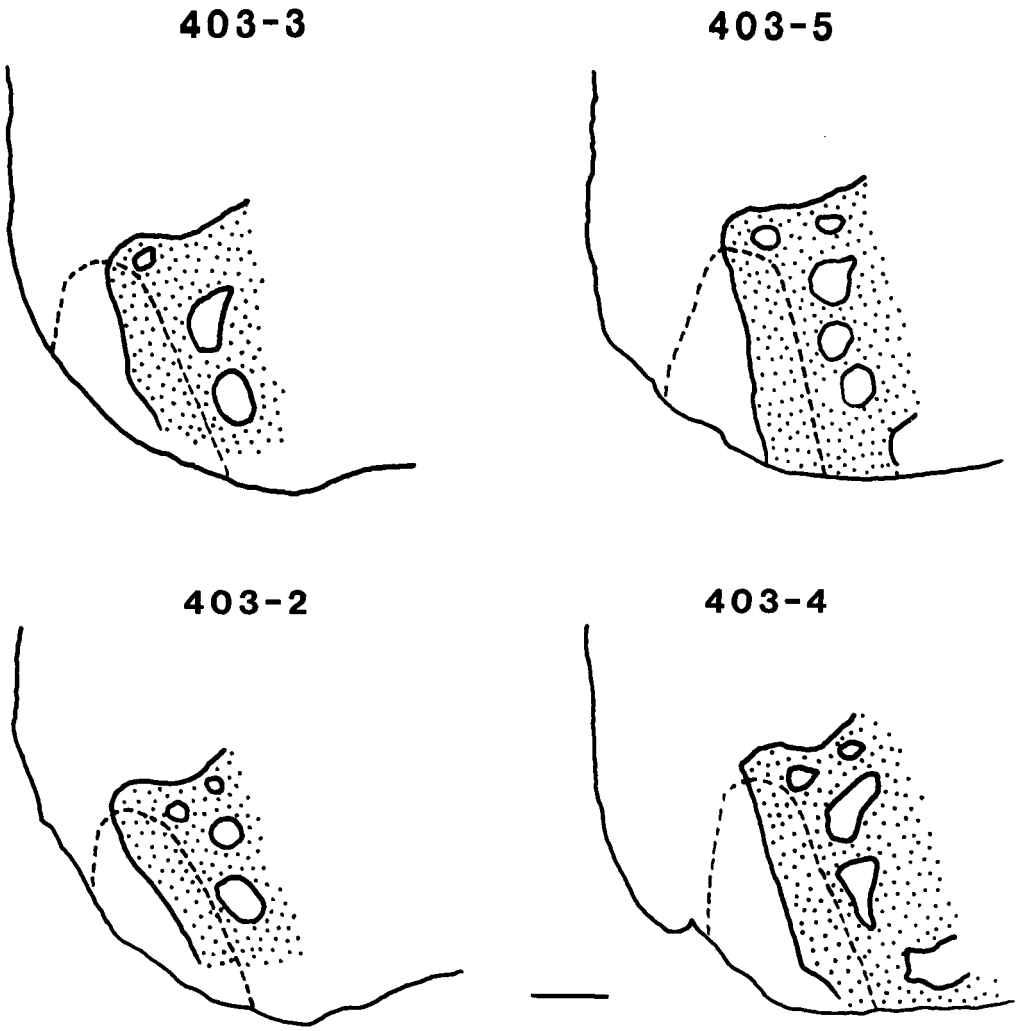

Fig 3. Camera lucida drawings of the visual callosal patterns (stippled areas) in four additional rats treated with monocular injections of TTX every 12 h from birth until 12 days of age. The patterns are from the hemispheres ipsilateral to the eye not injected with TTX. The dashed lines indicate the borders of area 17 drawn from adjacent myelin-stained sections. Medial is to the left, posterior is down. Scale bar = 1.0 mm.

colliculus unequivocally showed that even our smallest dose of TTX was effective in completely blocking retinal activity in rats older than 12 days for a period of at least 12 h. Furthermore, we administered doses of TTX that were equal to or greater in concentration, and more frequent, than those used in previous studies describing the effect of intraocular injections of TTX on the visual pathway (*e.g.*, O'Leary *et al*, 1986; Riccio and Matthews, 1987). To ensure that we measured the effect of continuous activity blockade throughout the time of callosal development (Olavarria and Van Sluyters, 1985), we studied a group of animals (n = 5) that was injected with TTX every 12 h during the first 12 days of postnatal development. Finally, the callosal patterns were quite similar in all 15 of the TTX-injected animals we studied, and none of them differed significantly from the callosal pattern in normally reared animals. Thus, we are confident that our results are not due to incomplete or inconsistent blockade of retinal activity during development. Instead, our findings indicate that blockade of retinal activity in one eye is not sufficient to cause the marked anomalies in the pattern of callosal connections that develop in one-eyed rats. Our results also indicate that injections

of TTX did not appreciably alter the time course of callosal development because, as in normally reared and enucleated rats (Olavarría and Van Sluyters, 1985; Olavarria *et al*, 1987), the callosal patterns appeared mature by postnatal day 12 in rats injected with TTX.

We have previously suggested (Olavarria *et al*, 1987) that the appearance of an anomalous band of callosal connections in area 17 of monocularly enucleated rats is closely linked to the marked expansion of the ipsilateral retino-geniculo-cortical projection that originates from the remaining eye (Lund *et al*, 1973; Jeffery, 1984; Manford *et* al, 1984; Reese, 1986; Yee *et al*, 1987). Indeed, the hypothesis that monocular blockade of retinal activity could be sufficient to induce the development of an anomalous band of callosal connections in striate cortex is consistent with previous reports that injections of TTX into one eye causes the other eye to develop an ipsilateral retino-collicular projection that is more widespread than normal (Fawcett *et al*, 1984). However, it is not known at present whether injections of TTX into one eye alter the pattern of ipsilateral retino-geniculate projections from the non treated eye. Preliminary data from our laboratory (Chang and Van Sluyters, unpublished observations; Chang *et al*, 1987) suggest that this is not the case. Instead, it appears that the ipsilateral retino-geniculate projection from the non treated eye does not undergo changes that are comparable in magnitude to the expansion observed in the retino-geniculate projection of monocularly enucleated rats (Lund *et al*, 1973; Manford *et al*, 1984; Reese, 1986). If confirmed, these results would indicate that monocular blockade of retinal activity by TTX is also insufficient for markedly affecting the development of the retino-geniculate projection in rats.

Our findings therefore suggest that at least some of the changes in the visual pathway brought about by monocular enucleation are not due to the imbalance of afferent ganglion cell activity caused by the destruction of one retina. If it is not the loss of retinal activity, what other mechanisms can explain the effects of monocular eye removal on the visual pathway? It is possible that during development retinal projections from both eyes compete for synaptic space or trophic factors at the level of the dorsal lateral geniculate nucleus. Removal of one eye would eliminate this competition, leading to the development of an abnormally expanded ipsilateral projection from the remaining eye (Lund *et al*, 1973; Manford *et* al, 1984; Reese, 1986). Changes in the topography of this anomalous retinal projection would then be relayed to the visual cortex (Jeffery, 1984; Yee *et al*, 1987), causing, as discussed previously (Olavarria *et al*, 1987; O'Brien and Olavarria, 1995), the development of the anomalous band of callosal connections observed in striate cortex of one-eyed rats.

ACKNOWLEDGMENTS

We thank Psyche Lee and Kathy Yee for technical assistance, and John Fiorillo and August Zitzka for assistance with illustrations. This work was supported by NIH grants EY02193 to RCVS, and EY09343 to JFO, and by an NIH National Eye Institute CORE Facilities Support Grant (EY03176).

REFERENCES

CHANG C-Y, VAN SLUYTERS RC, OLAVARRIA J (1987) Effects of intraocular injections of tetrodotoxin in newborn rats on the development of the visual callosal pattern. Invest Ophthal Vis Sci ARVO Abst Suppl 28: 236

CUSICK CG, LUND RD (1982) Modification of visual callosal projections in rats. J Comp Neurol 212: 385-398

FAWCETT JW, O'LEARY DDM, COWAN WM (1984) Activity and the control of ganglion cell death in the rat retina. Proc Nat Acad Sci USA 81: 5589-5593

INNOCENTI GM (1991) The development of projections from cerebral cortex. Prog Sens Physiol 12: 65-114

JEFFERY G (1984) Transneuronal effects of early eye removal on geniculo-cortical projection cells. Dev Brain Res 13: 257-263

LUND RD, CUNNINGHAM TJ, LUND JS (1973) Modified optic projections after unilateral eye removal in young rats. Brain Behav Evol 8: 51-72

MANFORD M, CAMPBELL G, LIEBERMAN AR (1984) Postnatal development of ipsilateral retino-geniculate projections in normal albino rats and the effects of removal of one eye at birth. Anat Embryol 170: 71-78

MATTHEWS MA (1985) Effects of neonatal intraocular colchicine on synaptogenesis and on the retention of the ipsilateral retinofugal projection within the superior colliculus. Exp Brain Res 60: 465-482

MESULAM MM (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction product with superior

sensitivity for visualizing neural afferents and efferents. J Histochem Cytochem 26: 106-117

O'BRIEN BJ, OLAVARRIA JF (1995) Anomalous pattern of callosal connections develop in visual cortex of monocularly enucleated hamsters. Biol Res, in press.

OLAVARRIA J, VAN SLUYTERS RC (1985) Organization and postnatal development of callosal connections in the visual cortex of the rat. J Comp Neurol 239: 1-26

OLAVARRIA J, MALACH R, VAN SLUYTERS RC (1987) Development of visual callosal connections in neonatally enucleated rats. J Comp Neurol 260: 321-348

OLAVARRIA J, BRAVO H, RUIZ G (1988) The pattern of callosal connections in posterior neocortex of congenitally anophthalmic rats. Anat Embryol 178: 155-159

O'LEARY DDM, CRESPO D, FAWCETT JW, COWAN WM (1986) The effect of intraocular tetrodotoxin on the postnatal reduction in the number of optic nerve axons in the rat. Dev Brain Res 30: 96-103

REESE BE (1986) The topography of expanded uncrossed retinal projections following neonatal enucleation of one eye: Differing effects in dorsal lateral geniculate nucleus and superior colliculus. J Comp Neurol 250: 8-32

RICCIO RV, MATTHEWS MA (1987) Effects of intraocular tetrodotoxin on the postnatal development of the dorsal lateral geniculate nucleus of the rat: a Golgi analysis. J Neurosci Res 17: 440-451

TIAO Y-C, BLAKEMORE C (1976) Functional organization in the superior colliculus of the golden hamster. J Comp Neurol 168: 483-504

VAN SLUYTERS RC, OLAVARRIA J, MALACH R (1990) Development of visual callosal connections. In: BLAKEMORE C (ed) Vision: coding and efficiency. Cambridge: Cambridge University Press. pp 224-233

YEE KT, MURPHY KM, VAN SLUYTERS RC (1987) Expansion of the ipsilateral primary visual pathway in rats monocularly enucleated at birth. Invest Ophthal Vis Sci ARVO Abst Suppl 27: 335

ZABORSZKY L, WOLFF JR (1982) Distribution patterns and individual variations of callosal connections in albino rat. Anat Embryol 165: 213-232