Loss of Binocular Responses and Reduced Retinal Convergence During the Period of Retinogeniculate Axon Segregation

Jokūbas Žiburkus and William Guido

Department of Cell Biology and Anatomy, Louisiana State Health Sciences Center, New Orleans, Louisiana

Submitted 21 December 2004; accepted in final form 1 August 2006

Žiburkus, Jokūbas and William Guido. Loss of binocular responses and reduced retinal convergence during the period of retinogeniculate axon segregation. J Neurophysiol 96: 2775–2784, 2006. First published August 16, 2006; doi:10.1152/jn.01321.2004. In the developing mammalian visual system, axon terminals from the two eyes overlap in the dorsal lateral geniculate nucleus (LGN) but then undergo a period of refinement and segregate to form distinct eye-specific domains. We report on the changes in synaptic transmission that occur in rodent LGN during the period of retinogeniculate axon segregation by using anterograde labeling techniques in conjunction with an in vitro preparation where large segments of each optic nerve are preserved. Anterograde labeling of retinal projections in early postnatal day (P) rats with cholera toxin β subunit indicated an age-related recession in uncrossed retinal projections. Between P2 and P5 uncrossed projections occupied as much as 50% of the LGN and overlapped substantially with crossed projections. Between the first and second postnatal week uncrossed projections receded, so by P14 they assumed an adult-like profile occupying 15–20% of LGN and showed little or no overlap with crossed projections. The postsynaptic responses of LGN cells evoked by the separate stimulation of each optic nerve indicated that before P14, many relay cells were binocularly innervated and received at least four to six inputs from each eye. However, these features of retinogeniculate connectivity were transient and their attrition occurred in concert with a retraction of retinal arbors into nonoverlapping, eye-specific regions. By P18 cells were monocularly innervated and received input from one to three retinal ganglion cells. These results provide a better understanding of the underlying changes in synaptic circuitry that occur during the anatomical segregation of retinal inputs into eye-specific territories.

INTRODUCTION

The mammalian retinogeniculate pathway serves as an important model for demonstrating how topographic maps develop from a diffusely organized network of cells into one containing precise patterns of synaptic connections and orderly representations of visual space (Shatz 1996). During perinatal life retinal axons from the two eyes share common terminal space in the dorsal lateral geniculate nucleus (LGN) but then segregate to form well-defined eye-specific territories (Godefment et al. 1984; Jaubert-Miazza et al. 2005; Jeffery 1984; Linden et al. 1981; Naska et al. 2004; Shatz 1983). Although the anatomical rearrangement of retinogeniculate projections has been well documented, the functional changes that accompany this remodeling have not been fully realized. For example, at perinatal ages in the cat, the majority of LGN cells receive input from the two eyes (Shatz and Kirkwood 1984). Because these experiments involved extracellular recordings, the actual pattern of retinal convergence was not assessed quantitatively. More recently, in vitro intracellular recordings in postnatal rodents show an age-related reduction in retinal convergence (Chen and Regehr 2000; Lo et al. 2002). However, these studies did not examine the extent to which the projections from each eye contribute to changes in retinal convergence or how such changes correlate with the anatomical segregation of retinal projections into eye-specific territories of LGN.

To address these unresolved issues, we examined the changes in synaptic transmission that occur in the LGN of the rat during the period of retinogeniculate axon segregation. Developing retinogeniculate projections were visualized by labeling axons and their terminal fields with the anterograde tracer cholera toxin subunit β (CTB) (Angelucci et al. 1996; Jaubert-Miazza et al. 2005; Naska et al. 2004; Torborg and Feller 2004). To study synaptic responses we used an in vitro isolated brain stem preparation in which large segments of each optic nerve are spared and the intrinsic circuitry of the LGN is left intact (Žiburkus et al. 2003). This preparation proved especially useful because it allowed us to record the intracellular postsynaptic responses of LGN cells evoked by the separate and independent stimulation of each optic nerve. We found that the retraction of retinal terminal fields into eye-specific domains was accompanied by a loss of binocular responsiveness and a reduction in retinal convergence.

METHODS

Visualization of retinogeniculate projections

We made intravitreal eye injections of the subunit β cholera toxin (CTB) in 24 Long–Evans hooded rat pups at the following postnatal (P) ages: P2 (n = 1), P3 (n = 1), P5 (n = 2), P7 (n = 2), P8 (n = 1), P9 (n = 1), P10 (n = 1), P11 (n = 1), P13 (n = 1), P14 (n = 2), P15 (n = 1), P17 (n = 3), P18 (n = 3), P19 (n = 1), P21 (n = 2), and P23 (n = 1). CTB has proved to be a reliable and sensitive anterograde tracer of retinal projections, labeling even the thinnest terminal branches in great detail (Angelucci et al. 1996; Torborg and Feller 2004). In 10 animals, CTB was visualized using peroxidase-based immunocytochemistry, whereas in 14 others, we used CTB conjugated to fluorescent Alexa dyes. Both tracers yielded similar results (Fig. 1).

Pups were anesthetized by deep hypothermia (<P13) or with isofluorane (≥P13). Before natural eye opening (<P14), fused eyelids were surgically separated to visualize the eye. The sclera and cornea were pierced with small-gauge needle and excess vitreous was drained to reduce the amount of intraocular pressure and to prevent potential

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
spillover of the injected tracer. In 10 animals a micropipette containing 2–8 μl of 1% CTB (List Biological) dissolved in distilled water was injected into one eye. In 5 animals 0.1–0.2% solution of CTB conjugated to Alexa Fluor 594 (red, Molecular Probes) or 488 (green, Molecular Probes) was injected into one eye. In 9 animals, each eye was injected with a different fluorescent conjugate so the terminal fields from both eyes could be visualized simultaneously in a single section of the LGN (Fig. 1C). After eye injections, pups were allowed to recover, returned to their home cage, and given a 2-day survival period. Rats were then deeply anesthetized with halothane and transectedally perfused with phosphate-buffered saline (1.8%, 25–50 ml) followed by a 4% paraformaldehyde in 0.1 M phosphate buffer solution (PB; pH 7.4, 100–250 ml).

For peroxidase-based reactions, the brains were removed from the skull, immersion-fixed overnight, and then cryoprotected in 30% sucrose solution for 24–72 h at 4°C. Brains were then blocked and sectioned at 60 μm in the coronal plane using a freezing microtome. Free-floating sections of tissue were treated overnight in a solution containing goat anti-CTB antibody (1:4,000; List Biological Labs). The binding of the primary antibody was visualized first by incubating tissue in biotinylated rabbit anti-goat IgG (1:200; Vector Labs) for 1 h and then reacting with an avidin–biotin–peroxidase complex (ABC Elite Kit; 1:100; Vector Labs) processed with 3,3′-diaminobenzidine tetrachloride (DAB). Labeled projections were viewed using brightfield microscopy (Nikon Labophot 2) and photographed digitally (Kodak CD-290).

For animals injected with fluorescent conjugates of CTB, the brains were postfixed overnight and sectioned at 60 μm in the coronal plane using a vibratome. Sections containing the LGN were mounted in ProLong (Molecular Probes) and imaged with epifluorescence microscopy. Images of LGN were acquired with a Photometrix Coolsnap camera attached to a fluorescence microscope (Nikon Eclipse). Fluorescent images of labeled sections were acquired and digitized separately using the following filter settings: Alexa 488: Exciter 465–495, DM 505, BA 515–555; Alexa 594: Exciter 528–553, DM 565, BA 600–660. In all labeled material the boundaries of LGN were delineated so as not to include the optic tract, intrageniculate leaflet, and ventral geniculate nucleus. Labeled axons of passage, which tended to be located in more caudal sections of LGN, were also excluded from the analysis. To determine the spatial extent of labeled retinal projections in the LGN, gray-scale images were converted into binary high-contrast black and white images. Background signal was subtracted and images were normalized (0–255) using Metamorph or Photoshop software. A threshold procedure was used that allowed for the specification of a level that corresponded to a location in the gray-scale histogram where there was a clear distinction between signal and residual background (Angelucci et al. 1996; Jaubert-Miazza et al. 2005; Muir-Robinson et al. 2002; Torborg and Feller 2004). Typically threshold levels corresponded to a value of 30–50 on the grayscale–normalized range of 0–255.

To measure the spatial extent of crossed and uncrossed retinal projections, the total number of pixels corresponding to each defined area of LGN was measured. In fluorescent material in which both uncrossed and crossed projections were labeled, the degree of overlap between the two was also assessed. For this the separate fluorescent images representing crossed and uncrossed terminal fields were superimposed and the pixels that contained both red and green signals (represented as yellow in Fig. 1) were counted.

In all cases, the spatial extent of uncrossed, crossed, and overlapping projections were computed by summing their area across five successive sections through the middle of LGN and then expressing the labeled regions as a percentage of the total area of LGN.

Surgical preparation of the isolated brain stem and in vitro recording

A detailed surgical account and illustration of the isolated brain stem was previously published (Lo et al. 2002; see Fig. 1 of Ziburkus et al. 2003). Long–Evans hooded rat pups from P3 to P21 were anesthetized with halothane and killed by decapitation. The brain was removed and placed in a solution of artificial cerebrospinal fluid (ACSF, in mM: 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1.0 MgSO₄, 26 NaHCO₃, 10 dextrose, 2 CaCl₂, saturated with 95% O₂-5% CO₂, pH = 7.4). During the excision, the optic nerves were cut just behind the disk and dissected from the base of the skull, sparing at least 4–6 mm of each nerve. Using a ventral approach, the brain was cut in half along the midline axis, while taking precautions to leave the optic chiasm intact. The hemisected brain was glued to a silver plate and placed into the well of a temperature-controlled recording chamber. The lateral surface of the thalamus and midbrain were exposed by removing the forebrain. The isolated brain stem was kept submerged and perfused continuously (4–5 ml/min) with warmed (31–33°C) ACSF. Recordings began 1–3 h after incubation. Electrode penetrations were made along the dorsolateral surface of the LGN and restricted to depths ≤150 μm (Fig. 2).

Intracellular recordings were done with pipettes made of borosilicate glass (WPI) filled with either 4 M potassium acetate (KAC) or a 2% solution of biocytin dissolved in 2 M KAC. Sharp-tipped electrodes were filled to a final tip resistance of 70–90 MΩ. All intracellular recordings were done in bridge mode with an Axoclamp 2B amplifier using techniques described previously (Guido et al. 1997; Lo et al. 2002; Ziburkus et al. 2003). Neuronal activity was displayed on a storage oscilloscope, digitized at 10 kHZ, and stored directly on a computer using Heka Pulse software.

During intracellular recording, some LGN cells were filled with biocytin (Fig. 2) by passing alternating positive and negative current pulses (±1 nA, 30 ms, 100–300 pulses) through the recording electrode. Tissue containing biocytin-filled cells was placed in a fixative solution of 4% paraformaldehyde dissolved in 0.1 M phosphate buffer for 72 h. The LGN was sectioned at 400 μm in the coronal plane and processed using a standard avidin–biotin–horse-radish peroxidase reaction processed with DAB (Guido et al. 1997; Lo et al. 2002; Ziburkus et al. 2003).

FIG. 1. Pattern of retinogeniculate projections in the developing rat revealed by the anterograde transport of chola toxin subunit β (CTB) and visualized by a peroxidase immunocytochemistry (A and B) or fluorescence (C). A: low (left) and high (right) power photomicrographs of coronal sections through the right lateral geniculate nucleus (LGN) of a postnatal day (P) 2 rat. Shown are the terminal fields belonging to the uncrossed retinogeniculate projection (ipsilateral eye) in the LGN after a right eye injection of CTB. Terminal fields of uncrossed inputs occupy much of the LGN. Asterisk depicts the approximate location of the optic chiasm. Scale bars = 20 μm. B: coronal sections of the dorsolateral thalamus at P3, P7, P13, and P19 showing uncrossed retinal projections in the LGN after a unilateral eye injection of CTB. Labeling is also apparent in the intrageniculate leaflet (IGL) and the ventral geniculate nucleus (VGL). Scale bar = 500 μm. C: coronal sections of the right LGN at P7 and P17. Alexa Fluor 488 (green) conjugated to CTB was injected into the left eye and reveals the terminal fields of crossed projections, whereas Alexa Fluor 594 (red) was injected into the right eye and reveals uncrossed projections. Left panels: green and red fluorescence labeling from the same section of LGN. Right panels: superimposed fluorescence pattern, along with the corresponding pseudocolored image depicting the distribution of green and red labeled pixels. Regions of overlap are colored yellow. Scale bar = 100 μm. D: spatial extent of retinal projections in the developing rat LGN. Left: scatterplot showing the total percentage area in LGN occupied by the crossed (contralateral eye, green squares), uncrossed (ipsilateral eye, red circles), and overlapping (yellow diamonds) projections at various postnatal ages (n = 24). Each point represents an estimate of spatial extent for an individual animal. Measurements are based on 5 successive sections taken through the middle of the LGN. Both uncrossed and overlapping projections reseed with age. Right: summary graph of means and SEs for percentage area occupied by crossed (green), uncrossed (red), and overlapping (yellow) projections at P0–P7, P8–P14, and P15–P21. Between P0–P7 and P8–P14 uncrossed and overlapping projections show significant decreases.

J Neurophysiol • VOL 96 • NOVEMBER 2006 • www.jn.org
Horikawa and Armstrong 1988). Labeled cells were photographed and drawn using camera lucida.

To evoke synaptic activity in LGN, single square-wave pulses (0.1–0.3 ms, 0.1–5.0 mA) were delivered at a rate of 0.20–1.0 Hz through a pair of low-impedance (0.2–0.5 MΩ), thin-gauged Ir wires whose exposed tips (0.1 mm) were placed on each optic nerve. The stimulating electrodes were placed at least 3–6 mm from the optic chiasm and the two nerves were separated by about 5 mm. In previously documented control experiments, this arrangement eliminated the possibility that current spread, even at maximal levels, inadvertently led to the concurrent stimulation of both optic nerves (Shatz and Kirkwood 1984; Ziburkus et al. 2003). To examine retinal convergence, we first determined the minimum intensity needed to evoke a postsynaptic response. Minimal stimulation produced responses that ranged in amplitude from 1 to 10 mV. We then adjusted the current intensity in 1, 2, 5, or 10% increments above this initial value until an excitatory postsynaptic potential (EPSP) of maximal amplitude was consistently reached (Lo et al. 2002; Ziburkus et al. 2003). EPSP amplitude by stimulus intensity plots were constructed and used to estimate the number of retinal inputs converging onto a single LGN cell (Bartlett and Smith 1999; Chen and Regehr 2000; Jaubert-Miazza et al. 2005; Lo et al. 2002; Lu and Constantine-Paton 2004). Trial-to-trial variability is relatively small and individual inputs can be readily distinguished from one another if the amplitude of the evoked response differs by ≥1 mV (Bartlett and Smith 1999; Jaubert-Miazza et al. 2005; Lo et al. 2002; Lu and Constantine-Paton 2004). Thus as shown on the amplitude by stimulus-intensity plots of Fig. 4, a change of ≥1 mV was used to distinguish one step (or input) from the previous one.

In some instances the γ-aminobutyric acid type A (GABA_A) antagonist bicuculline (10–25 μM) or the GABA_B antagonist 2-hydroxysaclofen (100 μM) were bath applied to block inhibitory activity (Figs. 3 and 4).

**RESULTS**

**Retinogeniculate axon segregation in the developing rat**

Using peroxidase-based immunocytochemistry or fluorescence to reveal the axonal transport of CTB in LGN, we were able to visualize retinal projections (Fig. 1, A–C) and obtain estimates of the area occupied by their terminal fields (Fig. 1D). Figure 1, A and B shows the labeling pattern in the LGN ipsilateral to the injected eye at different postnatal ages (e.g., P2, P3, P7, P13, and P19) accomplished with the peroxidase-based immunocytochemistry. At P2–P3 projections originating from the ipsilateral retina (uncrossed inputs) were widespread and diffusely organized, occupying as much as 50% of the total area of LGN. The high-power view in Fig. 1A indicates that terminal fields were uniformly distributed and composed of a rich plexus of thinly branched and beaded axons. This diffuse projection pattern remained with age, so by about P10–P14, uncrossed inputs were limited to a small “ipsilateral patch” that resided in the dorsal medial region of the LGN and occupied about 15–20% of the nucleus (Fig. 1D). A similar pattern was observed with fluorescence labeling and representative examples at P7 and P17 are shown in Fig. 1C. In these cases, because each eye was injected with a different fluorescent conjugate, the terminal fields from crossed (contralateral eye) and uncrossed (ipsilateral eye) projections could be visualized simultaneously in a single section of LGN. This allowed us to assess the extent to which inputs from the two eyes share common (overlapping) terminal space in LGN.

Estimates of the spatial extent of retinal terminal fields for crossed and uncrossed projections as well as the degree of overlap between the two projections are shown in Fig. 1D. As shown in the individual data (Fig. 1D, left), crossed projections failed to show significant changes with age. By contrast, uncrossed projections (r = 0.844, P < 0.001) as well as the degree of overlap between crossed and uncrossed projections (r = 0.674, P < 0.046) showed a significant recession with age. To summarize across different ages, we computed the mean values of spatial extent between P2 and P7, P8 and P14, and P15 and P23 (Fig. 1D, right). Pairwise comparisons indicate a significant attrition for uncrossed projections between P2 and P7 (x = 42.5%) and P8 and P14.
(\bar{x} = 20.8\%, \text{Kruskal–Wallis, } P < 0.001), but only a modest (statistically insignificant) decline thereafter (P15–P23, \bar{x} = 16.7\%). Overlapping terminal fields showed significant losses between P2 and P7 (\bar{x} = 21.9\%) and P8 and P14 (\bar{x} = 4.0\%, \text{Kruskal–Wallis, } P < 0.001). Overall, these changes are similar to those reported previously for pigmented rodents in which CTB was used as an anterograde tracer (Demas et al. 2006; Jaubert-Miazza et al. 2005; Muir-Robinson et al. 2002; Naska et al. 2004; Torborg and Feller 2004).
A  
**Binocular Cells**

Contralateral ON stimulation  
Ipsilateral ON stimulation

**Figure:**

- **Graphs:** Two sets of graphs showing EPSP amplitude vs. stimulus intensity. Each set contains multiple traces and data points indicating neuronal responses to different stimulus intensities.

- **Legend:**
  - EPSP Amplitude (mV)
  - Stimulus Intensity (% of 1mA)

B  
**Monocular Cells**

Contralateral ON Stimulation

**Figure:**

- **Graphs:** Two sets of graphs showing EPSP amplitude vs. stimulus intensity. Each set contains multiple traces and data points indicating neuronal responses to different stimulus intensities.

- **Legend:**
  - EPSP Amplitude (mV)
  - Stimulus Intensity (% of 1mA)
Loss of binocular responsiveness during the period of retinogeniculate axon segregation

To explore this remodeling on a functional level, we examined the synaptic responses of developing LGN cells (n = 285) evoked by the separate stimulation of each optic nerve. All recordings were restricted to the dorsolateral region of LGN, which in the mature rat corresponds to an area that receives a crossed projection (Reese 1988; see also Fig. 1). We confirmed the location of 27 recorded cells by filling them with biocytin during intracellular recording (Fig. 2, A and C). In all cases, labeled cells resided well within the boundaries of LGN representing crossed projections. At all ages, labeled cells had relatively large somata and multipolar dendritic arbors consistent with those of relay cells (Webster and Rowe 1984; Ziurkus et al. 2003). A comparison of labeled cells in a section prepared from an isolated brain stem preparation at P18, with that of a section in which uncrossed terminal fields were labeled with CTB (Fig. 2, A vs. B), provided further evidence that recordings were done in dorsolateral regions of LGN.

Representative EPSPs evoked by optic nerve stimulation are shown in Fig. 3A. At early postnatal ages, separate and distinct EPSPs were frequently activated by stimulating the contralateral as well as the ipsilateral optic nerve. Between P0 and P7, when uncrossed inputs project diffusely throughout the LGN, the majority of responses (64%) were binocular (Fig. 3B). A high incidence of binocular responses was also evident between P8 and P14 (64.5%). However, between P15 and P21, excitatory binocular responses were encountered far less frequently (17.4%, chi square, P < 0.001). A day-by-day inspection of the encounter rate (Fig. 3B) revealed that after P17, binocular responses were extremely rare (3/43, 6.9%; see also Grieve 2005). Indeed, at P18, P20, and P21 all encountered cells were monocular and responded only to stimulation of the contralateral optic nerve.

Loss of retinal convergence during the period of retinogeniculate axon segregation

Another transient feature in the development of retinogeniculate transmission was the prevalence of synaptic responses that reflected the convergence of multiple retinal ganglion cell inputs onto a single LGN cell. To estimate the number of retinal inputs converging onto a single relay cell, we activated the optic nerves at various levels of stimulus intensity and measured the amplitude of the evoked EPSP. Representative examples of these responses along with their corresponding amplitude by stimulus intensity plots are shown in Fig. 4. Individual inputs were distinguished from each other if the amplitude of the evoked response was ≥1 mV and this relation was taken to reflect the successive recruitment of active inputs innervating a single LGN cell (Jaubert-Miazza et al. 2005; Lo et al. 2002). At early ages, a progressive increase in stimulus intensity typically produced a multiple stepwise increase in EPSP amplitude (Fig. 4A). However, at late postnatal ages the same stimulus protocol evoked either a single step or just a few steps (Fig. 4B). The summary plot in Fig. 5A reveals that between P0 and P7 LGN cells, on average, received six inputs from the contralateral eye and four from the ipsilateral eye. Between P8 and P14, the number of inputs a cell received decreased, typically to three to four inputs from the contralateral eye and only one to two from the ipsilateral eye. By P15–21, the degree of retinal convergence declined even further so that LGN cells, like their adult counterparts, received far fewer (one to three) and exclusively monocular inputs.

Estimates of retinal convergence for 72 cells tested at different stimulus intensities are summarized in Fig. 5B. Overall, there was a significant reduction in retinal convergence with age, whether total inputs (r = 0.679, P < 0.001) or only those from each eye were considered separately (contralateral inputs: r = 0.582, P < 0.001; ipsilateral inputs: r = 0.461, P < 0.001). However, the most significant attrition was among inputs originating from the ipsilateral eye (Fig. 5A). Pairwise comparisons indicate between P0–P7 and P15–P21 ipsilateral inputs showed a fourfold decrease (Kruskal–Wallis, P < 0.02) and by P18 inputs from the ipsilateral eye were completely eliminated (Fig. 5B). Finally, whereas binocular cells (x = 7.46) tended to receive more inputs than monocular cells (x = 3.37, Mann–Whitney U test, P < 0.001), both groups showed a decrease in retinal convergence with age (Fig. 5C; monocular cells: r = 0.466, P < 0.001; binocular cells: r = 0.461, P < 0.001).

Discussion

Our results indicate the refinement of retinal terminal fields into eye-specific nonoverlapping territories was accompanied by a loss of binocular responsiveness and a reduction in retinal convergence. At early postnatal ages when uncrossed retinal axons (ipsilateral eye) projected diffusely throughout most of the LGN and shared ample territory with crossed (contralateral eye) projections, the majority of encountered relay cells received as many as four to six inputs from each eye. A high incidence of binocular responses but about a twofold reduction in retinal convergence were also evident at the time of natural eye opening (P14). At this age, uncrossed retinal axons showed substantial recession and occupied about 15–20% of the LGN. A few days thereafter, a further decline in excitatory retinal convergence and a total elimination of binocular responsiveness occurred. Most notable was the eventual elimination of synaptic connections made with the ipsilateral eye. Given the innervation pattern of crossed and uncrossed projections, this result may simply reflect a recording bias (i.e., sampling the dorsolateral rather than ventromedial regions of LGN). However, our CTB experiments revealed that uncrossed projections showed far more retraction than crossed ones. Thus the functional loss of retinal connections...
with the ipsilateral eye, at least in the dorsolateral sector of
LGN, is consistent with the anatomical retraction of their
retinal axonal arbors.

An important aspect of the present findings is that functional
changes in connectivity continue after the anatomical segrega-
tion of retinal inputs is nearly complete. This apparent discrep-
ancy has been observed in other species. In the cat a high
incidence of binocular responses still exists at birth (Shatz and
Kirkwood 1984), even though retinal projections have sorted
into eye-specific layers of the LGN (Shatz 1983). Similarly in
the mouse, a high degree of excitatory retinal convergence is
evident at the time (P14) of natural eye opening (Chen and
Regehr 2000) when retinal axons have finished segregating
into eye-specific territories (Jaubert-Miazza et al. 2005; Muir-
Robinson et al. 2002). Interestingly, the continued loss of
functional inputs after anatomical segregation seems consistent
with the time course of receptive field maturation (Daniels et
al. 1978; Tavazoie and Reid 2000). Thus it seems that antero-
grade labeling techniques that rely on bulk filling of retinal
axons fall short in revealing the precise patterns of synaptic
connectivity of developing relay cells. Perhaps single axon
labeling done in conjunction with an ultrastructural analysis is
needed to resolve this issue.

Overall, the synaptic remodeling we observed in the rat is
consistent with the refinement of connections reported in other
species. At perinatal ages in the cat, LGN cells begin to lose
binocular responsiveness (Shatz and Kirkwood 1984) and in
postnatal mice, relay cells exhibit a severalfold reduction in
excitatory retinal convergence (Chen and Regehr 2000; Jaub-
ert-Miazza et al. 2005). However, it should be noted that our
present estimates of convergence in the rat (see also Lo et al.
2002; Lu and Constantine-Paton 2004) seem conservative
compared with those previously reported in the mouse LGN
(Chen and Regehr 2000). In part, this may reflect a difference
in species (but see Jaubert-Miazza et al. 2005), type of in vitro
recording preparation (thalamic slice vs. isolated brain stem),

FIG. 5. Summary graphs showing the number of retinal inputs LGN cells receive as a
function of age. A: plots of means and SEs for the total number of inputs (gray), contralateral
inputs (white), and ipsilateral inputs (black) for LGN cells at P0–P7, P8–P14, and P15–
P21. Bar depicting the total number of cells is based on responses evoked by optic tract (OT)
(n = 20) and optic nerve ON (n = 54) stimulation. B: scatterplot showing the number of
inputs binocular and monocular cells receive at different ages. Each point represents a sin-
gle cell. Filled gray circles correspond to cells in which responses were evoked by OT stim-
ulation. Crosses and unfilled circles depict cells in which binocular (crosses) and monoca-
lar (unfilled circles) responses were evoked by ON stimulation. In each instance, there is a
decrease in retinal convergence with age. C: graph showing the average number of inputs
(and SEs) for binocular and monocular cells.
recording technique (voltage-clamp vs. current-clamp) or the criterion used to distinguish individual inputs. Nonetheless, our estimates of retinal convergence in the mature LGN (one to three retinal inputs/geniculate cell) are in accord with those reported previously in cat (Mastronarde 1987; Usrey et al. 1999), rodent (Chen and Regehr 2000; Lo et al. 2002), and ferret (Tavazoie and Reid 2000). The functional loss of retinal inputs during retinogeniculate axon segregation may also help explain the postnatal maturation in LGN receptive field structure. Immature fields are large and irregularly shaped and lack distinct on- and off-subregions, whereas mature ones are much smaller and have well-defined concentric center-surround organization (Daniels et al. 1978; Tavazoie and Reid 2000; Tootle and Friedlander 1989).

Finally, it is important to consider the underlying mechanisms responsible for the remodeling of retinogeniculate connections. The establishment of orderly connections in LGN has been attributed to the coordinated firing patterns of spontaneously active retinal ganglion cells (Wong 1999). When early retinal activity is altered or completely eliminated, retinogeniculate projections fail to segregate properly (e.g., Penn et al. 1998; Stellwagen and Shatz 2002; but see Huberman et al. 2003). The spontaneous activity of retinal ganglion cells is also sufficient to generate action potentials in LGN cells (Mooney et al. 1996). A proposed mechanism for activity-dependent remodeling is based on a Hebbian form of synaptic plasticity in which the degree of correlated firing between pre- and postsynaptic elements leads to either a long-term potentiation (LTP) or a depression (LTD) in synaptic strength (Bear and Malenka 1994; Shatz 1996). Long-term modifications in synaptic strength appear to underlie the refinement of sensory connections in the visual cortex (Kirkwood et al. 1996) and may also embody the synaptic rearrangements occurring during retinogeniculate axon segregation (Torborg and Feller 2005). Indeed, we noted a potential bias in retinogeniculate connectivity at early postnatal ages (Fig. 5; P0–P7 and P8–P14) that would seem to favor a Hebbian form of synapse consolidation and elimination. In the dorsolateral regions of LGN, developing cells, although binocular, seemed to receive more inputs from the contralateral rather than the ipsilateral eye. Certainly more definitive estimates would require additional experimentation, particularly at very early ages (e.g., P0–P4). Nonetheless, such a relation would suggest that postsynaptic activity in these regions of LGN is tightly coupled to retinal activity (Mooney et al. 1996) and also dominated by the activity of contralateral eye inputs (Weliky and Katz 1999), thus placing contralateral inputs at a competitive advantage over ipsilateral ones. The synaptic responses in LGN are also subject to long-term modification (Mooney et al. 1993). However, future studies will be needed to elucidate the exact nature and polarity of synaptic plasticity that prevails during early postnatal visual development.

**ACKNOWLEDGMENTS**

We thank E. Green and K. Bui for expert technical assistance. Present address of J. Ziburkus: Dept of Engineering Science and Mechanics, The Pennsylvania State University, University Park, PA 16802 (E-mail: jziburku@gmu.edu).

**GRANTS**

This work was supported by National Eye Institute Grant EY-12716 and the Whitehall Foundation.

**REFERENCES**


Shatz CJ. Emergence of order in visual system development. *Proc Natl Acad Sci USA* 93: 602–608, 1996.


