ELSEVIER

Developmental Brain Research 107 (1998) 227–231

DEVELOPMENTAL BRAIN RESEARCH

Research report

Specification of retinogeniculate X and Y axon arbors in cats: fundamental differences in developmental programs

P.E. Garraghty [a,*], A. Roe [b,1], M. Sur [b]

[a] *Program in Neural Science, Department of Psychology, Indiana University, Bloomington, IN 47405, USA*
[b] *Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA*

Accepted 9 December 1997

Abstract

Monocular enucleation at E36, followed by intracellular labeling of single, physiologically identified X and Y axons, demonstrates fundamental differences in their termination patterns within the lateral geniculate nucleus (LGN). X axons have arbors that appear normal in their dorsoventral extent, though some are located in inappropriate regions of the LGN. Y axons have arbors that are either abnormally tall, spanning the entire extent of the LGN, or of normal height but located in inappropriate regions of the LGN. These termination patterns resemble patterns seen after monocular enucleation at E44, and reinforce the conclusion that X and Y axons differ fundamentally in the cues that constrain the dorsoventral extents of their arbors. © 1998 Elsevier Science B.V.

Keywords: Retinogeniculate development; Binocular competition; Lateral geniculate nucleus

1. Introduction

The role of binocular interactions in the development of retinogeniculate projections in cats has been extensively studied (see Refs. [6,11] for reviews). We have previously shown that X and Y cell projections to the lateral geniculate nucleus (LGN) are differentially affected when ongoing binocular interactions are eliminated early in development by enucleating one eye. If monocular enucleation is performed on the day of birth (postnatal day 0, or P0), a time when segregation of the afferents from the two eyes is virtually complete [9,10], Y, but no X axons are found to have expanded into geniculate territory denervated by the enucleation (i.e., lamina A1 ipsilateral to the enucleated eye, and laminae A and dorsal lamina C contralateral to the enucleated eye [7,8]). Similarly, if monocular enucleation is performed on embryonic day 44 (E44; the gestation period is 65 days in cats), a time when afferents from the two eyes are maximally overlapped in the LGN [9], X axons are again found to be normally proportioned and appropriately located, while Y axons are either abnormally expanded or inappropriately located [4].

These data suggest that X and Y axons are guided by quite different developmental programs. X axons may be intrinsically programmed to terminate appropriately, whereas Y axons are not. However, it is known that the onsets of the waves of retinal neurogenesis of X and Y cells are temporally separated, with X cells leading by 4 days or so (E21 vs. E25 [15,16]). Thus, it remains possible that the two classes of fibers share a similar developmental capacity, and that the differential response to monocular enucleation performed at P0 or E44 is due simply to timing. In other words, X axons may remain restricted because they are in a more mature state at the time of enucleation. To address this possibility, we have now studied the effects of monocular enucleation at an even earlier date, E36, a time when the ipsilateral retinothalamic projection is just invading the anlage of the LGN [9]. Again, we find no expanded X axonal arbors (though some are completely mislocated), suggesting that timing is not the primary factor governing the differential growth patterns of X and Y axons.

2. Materials and methods

Cesarian sections were performed in timed pregnant cats. On E36, fetuses were exteriorized, monocularly enu-

* Corresponding author. E-mail: pgarragh@indiana.edu
[1] Present address: Section of Neurobiology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510, USA.

cleated, and returned to the womb to await normal term delivery (see Ref. [9] for additional methodological details). Data for the present report were gathered in one cat at adulthood.

Methods of preparation for the terminal recording experiments in the adult cat were identical to those reported previously [8,14]. Briefly, the cat was anesthetized, paralyzed, and artificially ventilated. Stimulating electrodes were cemented in place across the optic chiasm. Glass micropipettes filled with 10% horseradish peroxidase (HRP) were used to record single retinogeniculate axons in the LGN or optic tract. Axons were physiologically classified as X or Y using a standard battery of tests (see Refs. [8,14]). After classification, axons were impaled, and HRP was iontophoresed into them. At the conclusion of the experiment, the cat was given a lethal overdose of sodium pentobarbital and perfused transcardially with saline followed by fixatives. The brain was removed, and the separated hemispheres were sectioned in the parasagittal plane at 100 μm on a freezing microtome. Sections through the LGNs were treated with 3-3′-diaminobenzidine, and the reaction was intensified with cobaltous chloride [1]. Well-filled axonal arbors were reconstructed at 670 $\times$ using a microscope with an attached drawing tube.

3. Results

3.1. Functional classification

We recorded 29 axons in the E36 enucleated cat. Of these, 11 were classified as Y and 18 as X. These classifications were unambiguous. All Y axons had short optic chiasm (OX) latencies, phasic center responses, relatively weak center-surround antagonism, and nonlinear responses to a drifting grating. In contrast, all X axons had longer OX latencies, tonic center responses, strong center-surround antagonism, and linear responses to drifting gratings. We also note that the receptive field locations for all of the recorded axons were in the appropriate visual hemifield. Thus, we found no evidence of a disruption of the normal decussation pattern of nasal and temporal retinal fibers after the early elimination of binocular interaction. Six Y and 8 X arbors were sufficiently well-filled for morphological analysis.

3.2. Axon arbor location

The normal cat LGN is a laminated structure, with the different layers receiving inputs from the ipsilateral or contralateral retina (see Ref. [6] for review). After early monocular enucleation, the pattern of lamination in the LGN is very different from that found in normal animals [3,5]. Rather than the normal pattern of lamination, early monocular enucleation results in LGNs that consist of only two layers, one a large magnocellular layer, and one a small parvocellular layer [5]. Consequently, with respect to the present data, the characterization of terminations as ‘appropriately’ or ‘inappropriately’ located rests on the assumption that the relative dorsoventral *position* of axonal arborizations within the magnocellular layer can be interpreted with respect to the normal pattern of termination.

3.3. Arbor morphology

Fig. 1 presents the camera lucida reconstruction of an ipsilaterally projecting Y axon that we interpret as having an abnormally located arborization. This axon had an optic chiasm (OX) latency of 0.6 ms, and a receptive field center

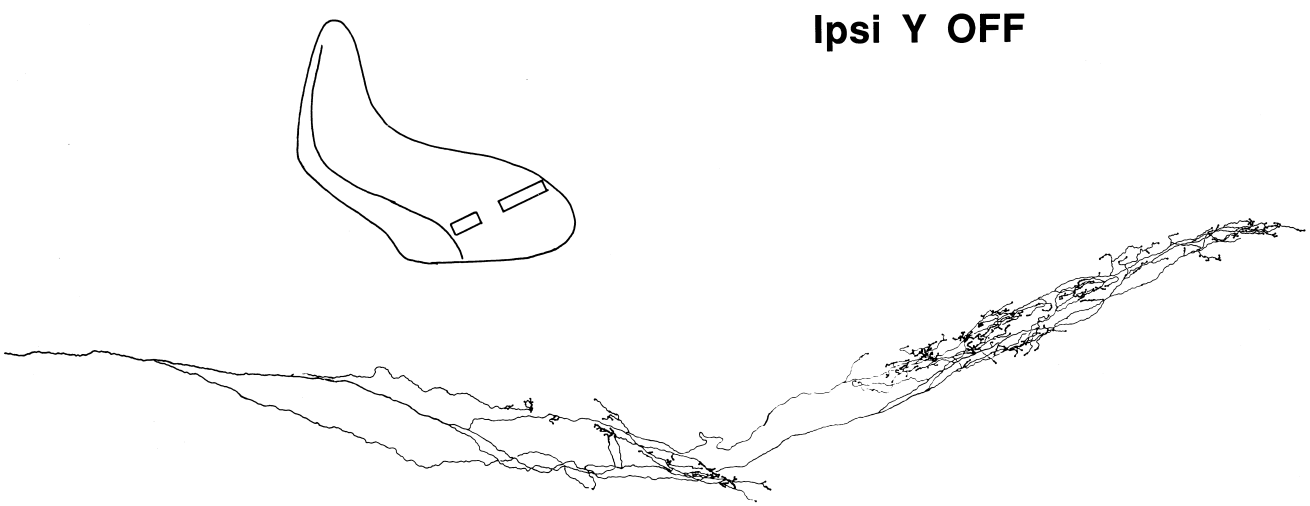

Fig. 1. An ipsilaterally projecting OFF-center Y axon. This axon had an OX latency of 0.6 ms and a receptive field center diameter of 2.2° centered at an eccentricity of 15°. This axon has two distinct arborizations. We have previously argued that the magnocellular layer that develops after prenatal monocular enucleation is a composite of the A, A1, and dorsal C laminae [5]. Thus, this pattern of termination is characteristic of contralaterally projecting Y axons (e.g., Ref. [14]), but is completely inappropriate for ipsilaterally projecting Y axons. Scale bar: 250 μm.

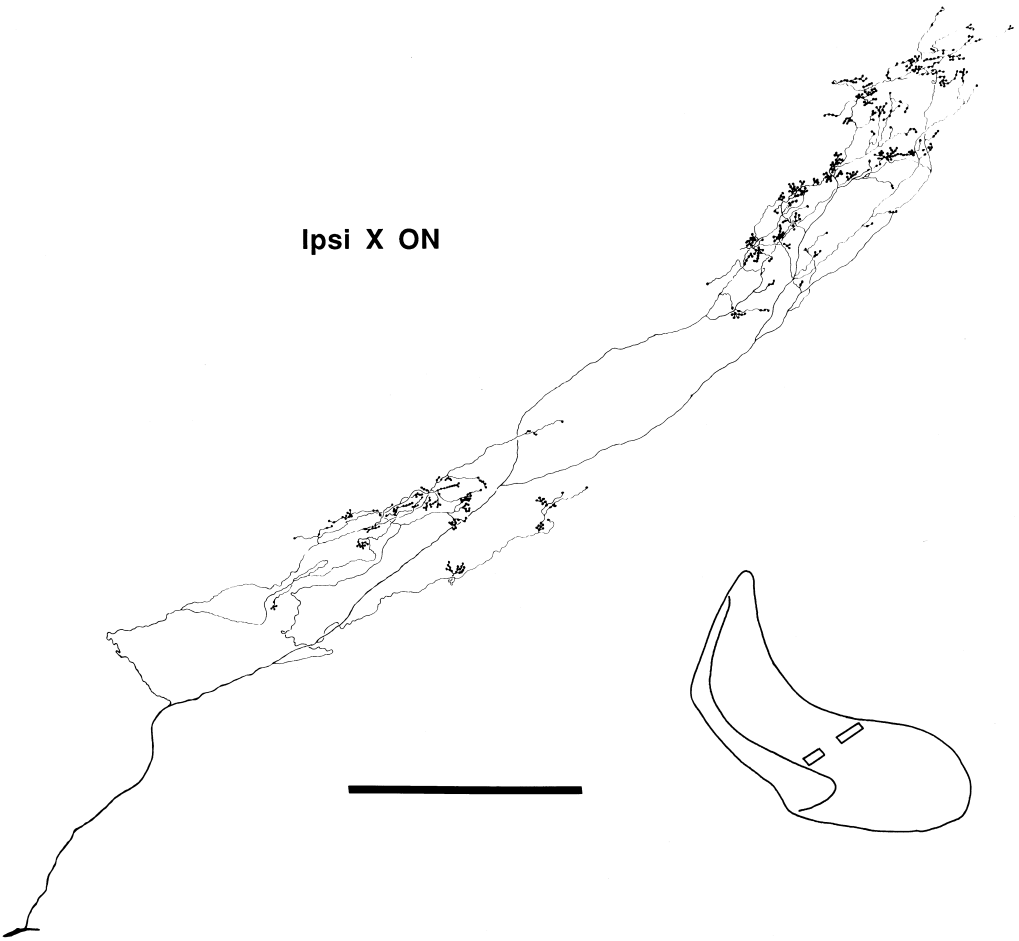

Fig. 2. An ipsilaterally projecting ON-center X axon. This axon had an OX latency of 0.8 ms and a receptive field center diameter of 1.2° centered at an eccentricity of 25°. With respect to the normal termination patterns of ipsilaterally projecting X axons (e.g., Ref. [14]), this axon also has two distinct arbors located in inappropriate regions of the LGN. Scale bar: 250 μm.

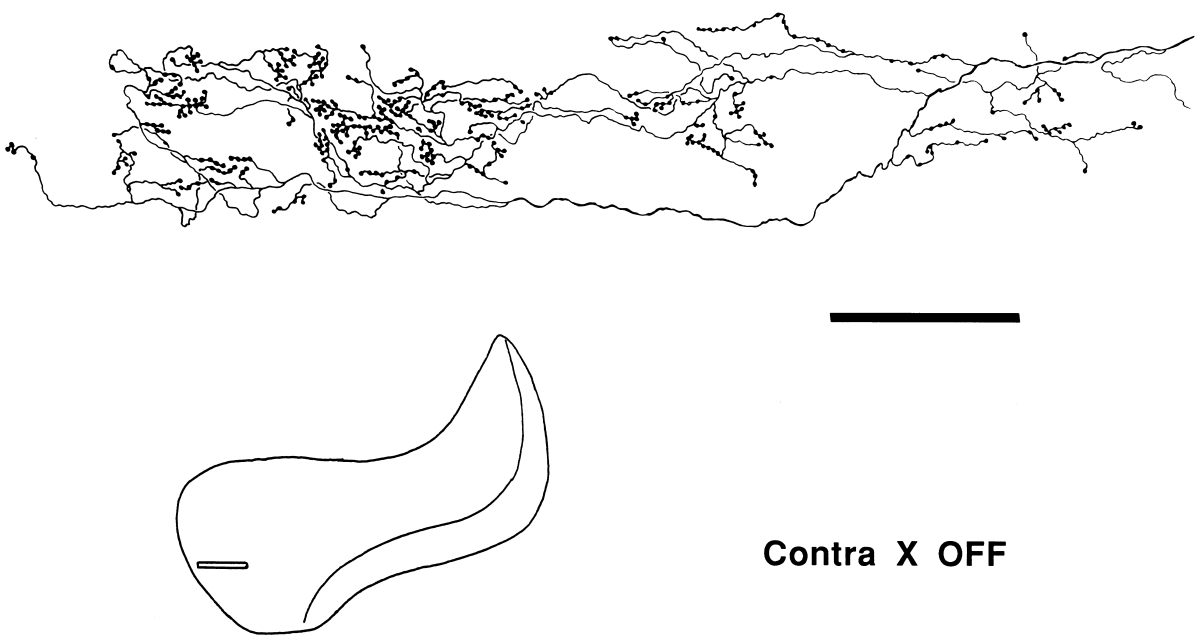

Fig. 3. A contralaterally projecting OFF-center X axon. This axon had an OX latency of 0.9 ms and a receptive field center diameter of 4.5° centered at an eccentricity of 57°. With respect to contralaterally projecting X axons in normal adult cats, this axon's arbor is appropriately located in the LGN. Scale bar: 100 μm.

2.2° in diameter centered at an eccentricity of 15°. In normal adult cats, Y axons project ipsilaterally to lamina A1 [2,14]. In contrast, the axon illustrated in Fig. 1 terminates dorsally and ventrally in the magnocellular layer in zones we interpret to be cryptic laminae A and C, and avoids the middle region that we interpret to be cryptic lamina A1 [5]. In normal adult cats, spatially separated dorsal and ventral arborizations are typically characteristic of contralaterally projecting Y axons terminating in laminae A and C [2,14].

Fig. 2 illustrates an ipsilaterally projecting X axon with a similar termination pattern. This axon had an OX latency of 0.8 ms, with a receptive field measuring 1.2° in diameter located at an eccentricity of 25°. As for the Y axon depicted in Fig. 1, this X axon has two distinct arborizations in regions we interpret to be laminae A and C. In normal cats, ipsilaterally projecting X axons terminate only in lamina A1 (e.g., Ref. [14]), an area that is apparently avoided by these axonal arbors. However, a small proportion of contralaterally projecting X axons in normal cats display a comparable pattern of termination [14].

Fig. 3 illustrates the termination of a contralaterally projecting X axon that terminates with a single arbor in the dorsal region of the magnocellular layer. This axon had an OX latency of 0.9 ms, and a receptive field center diameter of 4.5° at an eccentricity of 57°. This pattern of termination is typical of most contralaterally projecting X axons in normal cats [14].

All of the Y axons were abnormal in one of two ways. They were either abnormally tall, spanning the entire magnocellular division of the LGN [5], or they were normal in size but located in inappropriate parts of the nucleus (see below). All of the X axons were normal in size, but several were inappropriately located. We should note that identifying axonal arbors as inappropriately located is based on the assumption that cryptic lamination exists within the LGN (cf., Ref. [5]). After E36 monocular enucleation, as in cats monocularly enucleated on E44, only two layers are evident in the LGN, a dorsal magnocellular layer and a ventral parvocellular layer (cf. Refs. [3,5]). For the E44 enucleated cats, we have suggested that the magnocellular layer is a composite of the A-laminae and the magnocellular division of layer C. The pattern of terminations of the axons recovered from the E36 enucleated cat are consistent with a similar interpretation, but we *emphasize* the tenuous nature of this assumption due to our inability to partition the magnocellular layer with any other objective index.

4. Discussion

The afferent projection to the A-laminae of the cat lateral geniculate nucleus (LGN) arises from X and Y retinal ganglion cells (see Ref. [6] for review). As schematically illustrated in Fig. 4, Y axons in normal adult cats project contralaterally to laminae A and C and ipsilaterally to lamina A1 (and, infrequently, to lamina C1 [e.g., Ref. [2]]). X axons project contralaterally to lamina A and ipsilaterally to lamina A1. In addition to these differences, X and Y retinogeniculate arbors differ in their propensity to form or elaborate terminations in geniculate territory denervated by monocular enucleation. Thus, if one eye is removed in newborn kittens (P0), at which time the ocular segregation of retinogeniculate axons is virtually complete [9,10], and the animals are studied as adults, Y axons are found to have expanded into geniculate territory formerly innervated by the enucleated eye, but X axons are appropriately restricted (see Fig. 4). Because the waves of neurogenesis of X and Y retinal ganglion cells are temporally displaced, with X cell genesis being initiated first [15,16], it remained possible that X axon arbors were appropriately restricted because they were relatively more mature at the time of the enucleation.

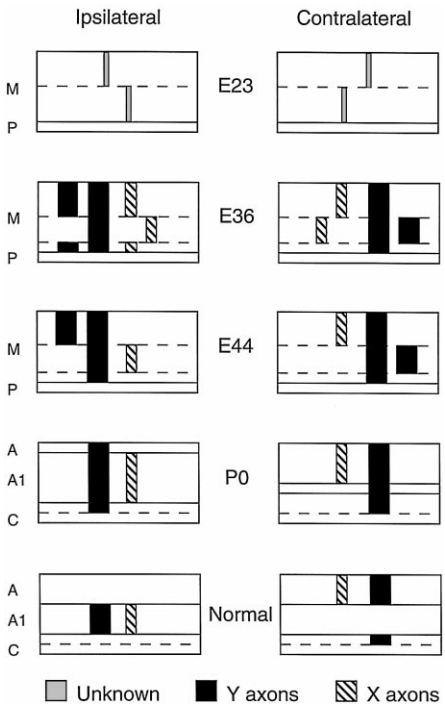

Fig. 4. A schematic summary of the projection patterns of ipsilaterally and contralaterally projecting X and Y axons in normal adult cats, and in cats that underwent monocular enucleation at various times during development. M: magnocellular layer; P, parvocellular layer; P0, day of birth; E44, E36, E23: embryonic days 44, 36, and 23.

To examine this possibility, we studied adult cats that had undergone monocular enucleation on embryonic day 44 (E44; birth = E65 = P0), a time when axonal arbors from the two eyes are maximally overlapped in the LGN [9]. Similar to the cats enucleated on P0, all Y axons have terminations in regions of the LGN that would have normally represented the removed eye (see Fig. 4). On the other hand, all X arbors are normally proportioned and appropriately located [4]. Thus, even when ongoing binoc-

ular interactions are eliminated 3 weeks earlier (E44 vs. E65 or P0), X and Y axons still differ with regard to whether or not they can expand into (or elaborate normally transient side-branches in) geniculate territory that would have normally represented the removed eye.

4.1. Comparison of the effects of monocular enucleation on E36 vs. E44

In most respects, monocular enucleations on either E36 or E44 have comparable effects on X and Y axons. All the Y axons from the E36 enucleated cat are abnormal in one of two ways. They are either abnormally tall, spanning the dorsal magnocellular layer of the LGN, or they are of normal height, but located in what we interpret to be inappropriate zones of the LGN. In contrast, all X axons from the E36 enucleated cat are of normal height, as was the case after E44 (and P0) enucleation. However, several of the X axons from the E36 enucleated cat are inappropriately located, something not seen after E44 enucleation. Finally, in fetuses studied on E59 after E23 monocular enucleation, a time when retinal axons have not yet reached the optic chiasm [9], bulk-filled retinogeniculate axons are found to terminate in two tiers roughly corresponding to laminae A and A1 [12]. Clearly, physiological characterization of the E59 axons is not possible but just as clearly, X axons are not abnormally expanded, though many may be abnormally located. Why some (presumably Y) axons do not span both tiers after E23 monocular enucleation is obviously not known. Perhaps the propensity of Y axons to elaborate arbors in inappropriate regions of the LGN is enabled by interactions between Y axons from the two eyes as they first encounter one another at the optic chiasm. Clearly, such interactions were not possible with E23 enucleation, but they certainly were with E44 or P0 enucleation. An interesting possibility is that the two-tiered structure of the LGN after E23 enucleation reflects a functional segregation, perhaps of X and Y or ON and OFF inputs.

The fact that X axons do not seem to require ongoing binocular interactions to restrict the height of their arbors should not be taken to imply that they never transiently involve areas ultimately taken over by axons from the opposite eye. Sretavan and Shatz [13] noted that, at E44, most retinogeniculate axons have side branches in areas destined to become dominated by axons from the opposite eye. Since X axons are probably the first to reach the geniculate anlage and outnumber Y axons by about 10 to 1, it seems more than likely that many of the fibers with mislocated side branches at E44 are X axons. Therefore, the difference in the capacities of X and Y axons to develop expanded arbors after monocular enucleation on E36, E44, or P0 cannot be due simply to whether or not side branches exist in inappropriate areas. Rather, these two classes of axons differ in their capacity to stabilize and maintain the normally transient expanded innervation [10] of inappropriate geniculate regions. Therefore, developing X and Y axons must differ in terms of intrinsically directed maturational programs, sensitivity to modifications of extrinsic milieu, or both (see Ref. [17]).

Acknowledgements

We thank Carla Shatz and David Sretavan for their surgical proficiency, and for kindly providing us with the cat. Supported by EY07023 (M.S.).

References

[1] J.C. Adams, Technical considerations on the use of horseradish peroxidase as a neuronal marker, Neuroscience 2 (1977) 141–145.
[2] D.B. Bowling, C.R. Michael, Terminal patterns of single, physiologically characterized optic tract fibers in the cat's lateral geniculate nucleus, J. Neurosci. 4 (1984) 198–216.
[3] L.M. Chalupa, R.W. Williams, Organization of the cat's lateral geniculate nucleus following interruption of prenatal binocular competition, Hum. Neurobiol. 3 (1984) 103–107.
[4] P.E. Garraghty, C.J. Shatz, D.W. Sretavan, M. Sur, Axon arbors of X and Y retinal ganglion cells are differentially affected by prenatal disruption of binocular inputs, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 7361–7365.
[5] P.E. Garraghty, C.J. Shatz, M. Sur, Prenatal disruption of binocular interactions creates novel lamination in the cat's lateral geniculate nucleus, Vis. Neurosci. 1 (1988) 93–102.
[6] P.E. Garraghty, M. Sur, Competitive interactions influencing the development of retinal axonal arbors in cat lateral geniculate nucleus, Physiol. Rev. 73 (1993) 529–545.
[7] P.E. Garraghty, M. Sur, S.M. Sherman, Role of competitive interactions in the postnatal development of X and Y retinogeniculate axons, J. Comp. Neurol. 251 (1986) 216–239.
[8] P.E. Garraghty, M. Sur, R.E. Weller, S.M. Sherman, Morphology of retinogeniculate X and Y axon arbors in monocularly enucleated cats, J. Comp. Neurol. 251 (1986) 198–215.
[9] C.J. Shatz, The prenatal development of the cat's retinogeniculate pathway, J. Neurosci. 3 (1983) 482–499.
[10] C.J. Shatz, P.A. Kirkwood, Prenatal development of functional connections in the cat's retinogeniculate pathway, J. Neurosci. 4 (1984) 1378–1397.
[11] C.J. Shatz, D.W. Sretavan, Interactions between retinal ganglion cells during the development of the mammalian visual system, Annu. Rev. Neurosci. 9 (1986) 171–207.
[12] D.W. Sretavan, C.J. Shatz, Prenatal development of cat retinogeniculate axon arbors in the absence of binocular interactions, J. Neurosci. 6 (1986) 990–1003.
[13] D.W. Sretavan, C.J. Shatz, Prenatal development of retinal ganglion cell axons: segregation into eye-specific layers within the cat's lateral geniculate nucleus, J. Neurosci. 16 (1986) 234–251.
[14] M. Sur, M. Esguerra, P.E. Garraghty, M.F. Kritzer, S.M. Sherman, Morphology of physiologically identified retinogeniculate X- and Y-axons in the cat, J. Neurophysiol. 58 (1987) 1–32.
[15] C. Walsh, E.H. Polley, The topography of ganglion cell production in the cat's retina, J. Neurosci. 5 (1985) 741–750.
[16] C. Walsh, E.H. Polley, T.L. Hickey, R.W. Guillery, Generation of cat retinal ganglion cells in relation to central pathways, Nature 302 (1983) 611–614.
[17] R.W. Williams, K. Herrup, The control of neuron number, Annu. Rev. Neurosci. 11 (1988) 423–453.