A Study on the Projection Patterns of Aberrant Retinal Ganglion Cells in the Albino Rat

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Summary: Injecting the retrograde fluorescent neuronal tracer fluoro-gold into the dorsal nucleus of the lateral geniculate body (dLGN), the olivary pretectal nucleus (OPN), and the superior colliculus (SC) revealed the existence of some ganglion cells (RGCs) scattered outside the temporoventral crescent of the ipsilateral retina in the adult albino rats. We studied the projection patterns, topographic distribution, number, and the soma size of these aberrant RGCs in 12 adult albino rats. We estimated a mean of 50 aberrant cells projecting to the dLGN. Their soma size ranged from 40.6 to 211.0 μm², with an average of 108.6 μm². The soma size of the 45.5 aberrant cells projecting to the OPN ranged from 41.5 to 312.5 μm², with an average of 147.2 μm². The SC received projection from 38.3 aberrant cells whose soma size ranged from 42.0 to 315.1 μm², with an average of 120.7 μm². These cells were almost equally distributed between the central and peripheral portions of the ipsilateral nasal retina. The mean cell count of the SC-projecting population was significantly lower than those of the other 2 groups. The mean soma size of the OPN-projecting aberrant cells was larger, and their soma size histogram was significantly different from those of the other 2 groups, whose histograms were almost alike. Though their physiological role in processing visual information is not fully understood, the aberrant RGCs might project their axons to — in addition to the dLGN, OPN, and SC — other visual centers.

The mammalian retinal ganglion cells (RGCs) project their axons to many visual centers in the brain ipsilaterally (Yamadori 1977; Stone et al. 1978; Cooper and Pettigrew 1979; Yamadori 1981; Yamadori and Yamauchi 1981; Yamauchi and Yamadori 1982; Sefton and Dreher 1985; Lund et al. 1987; Yamadori et al. 1989; Dong et al. 1992; Rahman et al. 1992; Moriya and Yamadori 1993), contralaterally (Yamadori 1977; Stone et al. 1978; Cooper and Pettigrew 1979; Yamadori 1981; Yamauchi and Yamadori 1982; Sefton and Dreher 1985; Yamadori et al. 1989; Dong et al. 1992; Moriya and Yamadori 1993), or bilaterally (Cunningham and Freeman 1977; Cowey and Perry 1979; Jeffery et al. 1981). In adult albino rats, the contralaterally projecting cells spread all over the retina in contrast to the ipsilaterally projecting population that is usually confined to the temporoventral crescent of the retina (Cowey and Perry 1979; Dräger and Olsen 1980) and rats (Dreher et al. 1985). These aberrant cells were seen in all stages of postnatal development in the nasal retina of normal rats and rats with neonatal lesions (Chan et al. 1989). Stone et al. (1978) observed similar cells scattered throughout the nasal retina of Siamese cats after sectioning the contralateral optic tract.

Injecting a neuronal tracer into the optic tract or cutting it did not characterize the central connections of this minor population in correlation with their morphology or distribution pattern. Earlier, Lund et al. (1974) observed that retinal lesions outside of the normally ipsilaterally projecting locations produced faint degeneration in the dorsal lateral geniculate nucleus (dLGN) of pigmented and albino rats. Consequently, they concluded indirectly that the dLGN receives some ipsilateral afferents from the nasal retina. Similar to the aforementioned methods, this anterograde degeneration technique did not answer the following questions: how many aberrant cells project to the dLGN, what are their morphological and distribution patterns, do these aberrant cells project to — beside the dLGN — other terminal specifications? These issues were investigated in the present study by using a retrograde labeling technique. We specified the efferent projec-
tions, and described the topographic distribution, morphology, count and soma size of these aberrant cells.

Materials and Methods

Animals

Experiments were carried out on 12 adult male Wistar albino rats (Japan Clea Co., Japan), whose body weight ranged from 250 to 400 gm. We divided them into 3 groups (4 rats per group).

Operation

The animals were anaesthetized with 10% chloral hydrate (with a dosage of 300 mg/kg b.wt., i.p.) before mounting the head in a stereotaxic frame. We drilled a small hole in the skull and inserted a glass micropipette (tip diameter about 25 μm) vertically into the target, i.e. the center of the dLGN in the first group, the OPN in the second group, and the centromedial portion of the SC in the third group. The injection sites (the locations of the holes and the depth of penetration of the micropipette) were decided according to the coordinates of Paxion's and Watson's atlas (1986). The retrograde fluorescent neuronal tracer fluoro-gold (FG, Fluorochrome, Englewood, CO) was dissolved in distilled water (4% w/v) and injected by pressure using a 5 μl Hamilton syringe. The total amount of the tracer injected in each animal was 0.03 μl (less amount into the OPN due to its smaller area).

Preparation of the retinae

The survival time ranged from 48–72 hours before perfusion. The animals were re-anaesthetized with 10% chloral hydrate (with a dosage of 300 mg/kg b.wt., i.p.) before mounting the head in a stereotaxic frame. We drilled a small hole in the skull and inserted a glass micropipette (tip diameter about 25 μm) vertically into the target, i.e. the center of the dLGN in the first group, the OPN in the second group, and the centromedial portion of the SC in the third group. The injection sites (the locations of the holes and the depth of penetration of the micropipette) were decided according to the coordinates of Paxion's and Watson's atlas (1986). The retrograde fluorescent neuronal tracer fluoro-gold (FG, Fluorochrome, Englewood, CO) was dissolved in distilled water (4% w/v) and injected by pressure using a 5 μl Hamilton syringe. The total amount of the tracer injected in each animal was 0.03 μl (less amount into the OPN due to its smaller area).

Results

Localization of the injected tracer

The serial brain sections showed that labeling with the retrograde fluorescent tracer FG was almost confined to the dLGN (in group 1), the OPN (in group 2), and the SC (in group 3). The other cases, in which the labeling was insufficient or was found to diffuse outside these visual centers, were discarded and not included in this study. However, the injection track had to pass through the superior brachium in order to reach the underlying small OPN.

Morphology and distribution of the aberrant cells

Fluoro-gold labeled distinctly the RGCs in the experimental animals. The labeled cells were characterized by conspicuous fluorescent gold colored granules within the perikaryon and neurites (Fig. 1). Some cells looked whitish. The majority of the ipsilaterally projecting labeled cells (i.e. the ordinary RGCs) were seen concentrated in the temporal crescent. On the other hand, the aberrant cells were seen sparsely scattered all over the nasal retina. Though they had no specific pattern of spatial distribution, those aberrant cells were equally distributed in the central and peripheral portions of each nasal
Fig. 1. Photomicrographs showing fluoro-gold labeled aberrant RGCs. A-C, low power; E-H, high power. D, high power photomicrograph showing number of labeled ordinary RGCs of various classes (differing in somal and dendritic size and dendritic morphology) for comparison. The finer dendrites could be seen by changing the focus of the microscope. Bar = 10 μm.
Fig. 2. Camera-lucida drawings of flat-mounted ipsilateral retinae from rats 3(A), 5(B), and 11(C). The dots indicate the locations of the aberrant RGCs projecting to the dLGN(A), OPN (B), and SC (C). Note that their distribution appears fairly uniform. The solid area represents the temporoventral crescent where the ordinary RGCs were located. The central circle represents the optic nerve head. D, dorsal; N, nasal; T, temporal; V, ventral.
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Consequently, there was no significant difference in the cell density between the centrally located and the eccentric aberrant RGCs. The topographic distribution of the ordinary and aberrant RGCs in 3 representative cases is shown in Fig. 2.

The dLGN-projecting aberrant cells

Their count ranged from 45 to 54 cells with an average of 50 cells. They were fairly distributed throughout the nasal retina (Fig. 2A). Their soma size ranged from 40.6 to 211.0 \( \mu \text{m}^2 \), with a mean of 112.3 \( \mu \text{m}^2 \) (Table 1). The soma size increased with eccentricity of the cells. Fig. 3A shows the soma size distribution in rat 3.

The OPN-projecting aberrant cells

Their count ranged from 38 to 52 cells with an average of 45.5 cells. They were evenly distributed throughout the nasal retina (Fig. 2B). Their soma size ranged from 41.5 to 312.5 \( \mu \text{m}^2 \), with a mean of 147.2 \( \mu \text{m}^2 \) (Table 1). The smaller cells were centrally located while the larger cells were seen more often at the periphery. Fig. 3B shows the soma size distribution in rat 5.

The SC-projecting aberrant cells

Their count ranged from 32 to 43 cells with an average of 38.3 cells. They were equally distributed throughout the nasal retina (Fig. 2C). Their soma size ranged from 42.0 to 315.1 \( \mu \text{m}^2 \), with a mean of 120.7 \( \mu \text{m}^2 \) (Table 1). The eccentric cells were comparatively larger than the central ones. Fig. 3C shows the soma size distribution in rat 11.

Comparison between the different groups

The mean soma size of the OPN-projecting aberrant RGCs was larger, and their soma size histogram was significantly different in comparison with those of the other 2 groups (\( p < 0.05 \)). The difference between the soma size histogram of the dLGN-projecting cells and that of the SC-projecting group was not statistically significant.

The mean cell count of the SC-projecting population was significantly lower than those of the other 2 groups (\( p < 0.05 \)). There was no significant difference in the mean cell count between the dLGN-projecting cells and the OPN-projecting group.

Discussion

Choice of the animal and tracer

By backfilling the optic nerve cells with FG, we could identify the projection patterns, topographic distribution, cell count and soma size of aberrant cells in the adult albino rats (Rahman et al. 1992). We used albino rats in this study mainly because of their small uncrossed retinofugal projection (Lund 1965; Guillery et al. 1973; Lund et al. 1974; Creel and Giolli 1976; La Vail et al. 1978; Dräger and Olsen 1980; Dreher et al. 1985). The fluorescent retrograde axonal tracer FG was chosen to label the RGCs because of its unique properties (Schmued and Fallon 1986). These include bright fluorescence and extensive filling of the ganglion cells' perikarya and processes, high resistance to fading, and no diffusion outside the labeled cells.

Efferent projections of the aberrant RGCs

Previous investigations — by injecting HRP into the optic tract of rats (Dreher et al. 1985) or mice (Dräger and Olsen 1980), or sectioning the optic tract of Siamese cats (Stone et al. 1978) — could not specify the target of axonal projections of the aberrant cells. After massive HRP injections into the dLGN, no labeled ganglion cells were seen in the nasal retina of Siamese cats (Cooper and Pettigrew 1979) denoting that the aberrant cells observed by Stone et al. (1978) do not project their axons to this structure. However, their projection to the dLGN was indirectly proved by anterograde degeneration technique in pigmented and albino rats (Lund et al. 1974). In the present study, by retrograde fluorescent labeling, we directly confirmed this phenomenon, and analyzed this aberrant projection quantitatively.

By means of anterograde tracing techniques, the
Fig. 3. Histograms showing the soma size distribution of the aberrant RGCs shown in figure 3. (A) The dLGN-projecting group; (B) the OPN-projecting population; (C) the SC-projecting cells. N, total number of aberrant cells; mean, mean soma size.
pars oralis of the OPN was found to be the main component of the pretectal complex that receives uncrossed retinofugal input (Scalia 1972; Scalia and Arango 1979). We confirmed this finding in our previous investigation (Yamadori 1977) by silver impregnation technique; the degenerating nerve terminals were seen conspicuously in the medial nucleus of the optic tract (according to Bucher and Nauta’s nomenclature, 1954). In our present study, we proved that the origin of this ipsilateral retinopretectal pathway is mainly located in the temporal crescent, as in the case of the uncrossed retinotegmental and retinotectal pathways (Cowey and Perry 1979; Moriya and Yamadori 1993; Yamadori et al. 1989). In addition, we gave evidence that the OPN receives some afferent projections from some RGCs in the nasal retina.

The uncrossed retinotectal pathway was reported to originate from the temporal retinal crescent and terminate in the anteromedial to posterior (Lund et al. 1987), or postero medial to posterior part (Yamadori and Yamauchi 1981) of the superior colliculus. Our present findings proved that the origin of this projection is not confined to the temporal retina only, but it extends sporadically into the nasal retina as well.

Although we investigated the projection patterns of the aberrant cells to the dLGN, OPN and SC, the possible central connections of these cells with other visual terminals should not be overlooked.

Aberrant RGCs’ count

Chan et al. (1989) found more than 1000 RGCs in the nasal retina of normal rats on day 0. A process of cell death took place and the figure continuously declined until reaching a steady level of less than 200 cells on day 21. In other words, more than 80% loss of the aberrant population occurs during the postnatal development of the uncrossed pathway (Jeffery 1984; Jeffery and Perry 1982; Laemle and Labriola 1982; Linden and Serfaty 1985; Perry et al. 1983). Interestingly, a significant surge in their number was recorded in rats with neonatal lesions on day 2 before the onset of the progressive cell death (Chan et al. 1989).

The aberrant cells’ count in our study, collectively, agrees with Chan et al.’s finding (1989), but it appears bigger than the average count of 43 cells in mice reported by Dräger and Olsen (1980) using HRP. This discrepancy may be attributed to the following: (1) the bigger RGCs population in the rat (about 110,000 cells, Perry et al. 1983) than in the mouse (about 65,000, Dräger and Olsen 1980), and consequently the ipsilaterally projecting population is bigger, and (2) the incorporation of the FG molecules into the axon terminals is much easier than the macromolecules of HRP; therefore, more cells — especially the smaller ones with fine axons — could transport the tracer. However, it should be noted that there is a possibility that some of the aberrant cells project to more than one terminal specification like some of the ordinary cells, and then the total number of the aberrant cells would be less than the sum of the aberrant RGCs counts of the various groups investigated in the present study. The significant difference between the dLGN-projecting aberrant cell count and that of the SC-projecting group is similar to our previous finding regarding the ordinary uncrossed retinofugal pathways (Moriya and Yamadori 1993).

Soma size of the aberrant RGCs

We found that the aberrant RGCs in adult albino rats are of the small and medium types only. This agrees with previous observations in the mice (Dräger and Olsen 1980) and Siamese cats (Stone et al. 1978). However, the OPN-projecting aberrant cells had significantly larger soma size than the other groups. We do not know whether this finding has some functional implication or not. As the larger RGCs tend to send branching axons to more than one central terminal (Sefton and Dreher 1985), we think that relatively more aberrant OPN-projecting cells are centrally connected with other targets, i.e. multiprojecting.

Our finding, that the soma size of the aberrant cells increases with their eccentricity, corresponds to the data about the soma size distribution pattern of the ordinary RGCs obtained from studies of the retiniae of the mouse (Dräger and Olsen 1981), rat (Dreher et al. 1985), rabbit (Provis 1979), cat (Stone 1978), and monkey (Bunt et al. 1975). In all these animals, this may reflect the influence of the density of all classes of ganglion cells; both somata and dendritic trees of RGCs in the region of peak density (as area centralis) are smaller than those located in medium- and low-density ganglion cell regions (Dreher et al. 1985). An interesting contradiction is with the tree shrew (Drenhaus et al. 1994) where RGCs become smaller the further they are peripheral (the smaller cells lie in the ventro nasal periphery, and the larger cells in the temporocentral retina).

There was no significant difference between the soma size histogram of the dLGN-projecting group and that of the SC-projecting cells. This means that the arbor-volume-effect on soma size (Dräger and Olsen 1980) was not evident in case of the aberrant cells. This might be due to the smaller number of the aberrant RGCs.

Concluding Remarks

Outside the area subserving the binocular vision...
of the albino rat, exist some ipsilaterally projecting RGCs of small and medium size. These aberrant cells are evenly distributed in the nasal retina and send efferent projections to the dLGN, OPN, and SC. Further investigations are needed to clarify their other projection characterizations and the implication of their existence for the functional architecture of the visual system of the albino rat.

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References

