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Brain Research 973 (2003) 146–150
BRAIN RESEARCH
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Short communication

Retinal ganglion cells projecting to the dorsal raphe and lateral geniculate complex in Mongolian gerbils

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Accepted 28 February 2003

Abstract

Injections of rhodamine-B into the dorsal raphe nucleus (DRN) and Fluoro-Gold into the lateral geniculate nucleus (LGN) revealed double-labeled retinal ganglion cells (DL RGCs) projecting to both nuclei. The soma-size distribution of DL RGCs was compared with three other distributions: DRN-projecting RGCs, LGN-projecting RGCs, and a large sample of RGCs labeled via the optic nerve with DiI. DL RGC soma diameters fell primarily within the mid-to-upper size range of all three distributions. DL RGCs may provide information to both nuclei concerning comparable aspects of light and visual stimulation via collateralized axons.

Theme: Sensory systems

Topic: Subcortical visual pathways

Keywords: Optic axon collateral; Double-labeled retinal ganglion cell; Serotonin system; Rodent visual system

The large, serotonergic dorsal raphe nucleus (DRN) receives direct retinal afferents in a number of species, including cats [10], rats [9,32], Chilean degus [7], tree shrews [31] and Mongolian gerbils [9]. Gerbils are highly visual animals with a diurnal/crepuscular activity rhythm [12,15,30], and in this species, retinal-DRN terminals are well placed to influence serotonergic neurons, particularly those in the large, lateral subdivision which project to the superior colliculus and lateral geniculate complex [9,17]. The retinal-DRN pathway in gerbils originates from RGCs distributed in nonrandom fashion over the entire retina and comprise about 1% of the total RGC population [9]. The soma-diameter size range of DRN-RGCs is comparable to that of the overall RGC population, suggesting that this pathway may include collaterals of optic afferents that innervate other retinorecipient nuclei. Extensive evidence for collateralized optic axons has been described in rodent visual systems [3–6,20,27]. The retinal-DRN pathway shows several morphological features [7,9] comparable to those of the 'nonimage-forming' subsystem of retinal afferents, which encodes primarily the photic and temporal characteristics of visual stimulation [2,8,22,23,26].

In order to determine whether the retinal-DRN pathway in gerbils may contain collaterals of optic axons that innervate the large LGN complex, rhodamine-B was injected into the DRN and Fluoro-Gold was injected into the LGN complex. RGCs positive for both tracers were identified and analyzed in whole-mount retinas with regard to soma sizes and retinal location. In addition, the soma-size distribution of double-labeled (DL) RGCs was compared with a previously described DRN–RGC distribution [9], with a population of LGN-projecting RGCs, and with a large, random sample of RGCs labeled via the transected optic nerve.

Adult, male, Mongolian gerbils weighing 50–70 g (*Meriones unguiculatus*) were used as subjects. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts in accordance with NIH and USDA guidelines. Animals were anesthetized with an intraperitoneal injection (mixture of ketamine, 100 mg/kg and xylazine, 10 mg/kg). A total of nine animals each received injections of two different

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0006-8993/03/$ – see front matter
doi:10.1016/S0006-8993(03)02549-6

fluorescent tracers. Using previously determined stereotaxic coordinates [17], rhodamine-B-isothiocyanate (RITC, Sigma) was injected into the DRN using a 10-μl Hamilton syringe, needle and microdrive assembly. Approximately 0.8 μl of 3% RITC dissolved in 2% DMSO was injected and the needle left in place for 10 min. A 5% solution of Fluoro-Gold (FG, Fluorochrome) dissolved in distilled water was injected into the LGN complex of the same animal via a micropipette (inner diameter, 80–100 μm). After 7–12 days, the animal was anesthetized and perfused transcardially with 150 ml of 0.9% saline, followed by 400 ml of cold 4 °C paraformaldehyde in phosphate buffer (PBS; pH 7.4). The eyes and brain were removed and the eyes injected with 0.25 ml of fixative; subsequently, the retinas were removed and were mounted, ganglion-cell layer upward onto subbed slides, air-dried for 30–40 min, and coverslipped. In a second group of three animals, the eyes were removed and small crystals of the fluorescent tracer DiI (1,1-dioctadecyl-3-3,-3′,tetramethylindocarbocyanine percholate) were applied to the transected optic nerve at the retrobulbar exit point. Eyes were immersed in 4% paraformaldehyde and incubated in a 40 °C oven for 7–10 weeks. Subsequently, the retinas were removed intact and flat-mounted, ganglion-cell side upward.

For analysis of injection sites, brains were postfixed in 4% paraformaldehyde overnight at 4 °C, then transferred to a solution of 30% sucrose in PBS, and were cut on a freezing microtome in serial, 40-μm thick sections from rostral thalamus to the caudal pole of the superior colliculus. Alternate sections were mounted on gelatin-coated glass slides and coverslipped with Krystalon. Whole-mount retinas and brain sections containing injection sites were analyzed with a Leica DRM fluorescent microscope using ultraviolet (emission λ_{max}=365) and rhodamine filters (emission λ_{max}=547), respectively. Fluorescent images were obtained using a Kodak DC290 digital camera system and were analyzed using PHOTOSHOP software and a computer-assisted morphometry system (SIGMA SCAN 3). A minimum of eight, nonoverlapping sample areas (0.45 μm×0.30 μm) were selected from all four retinal quadrants (superior-temporal, inferior-temporal, superior nasal, inferior-nasal) and were analyzed with regard to the number and soma sizes of DL RGCs (both FG and RITC positive). DL RGCs were identified in 145 sample areas taken from the contralateral retinas of four animals with the most well-localized injection sites in both target nuclei, and soma-size measurements were combined into a single distribution (n=289). Soma-size measurements also were obtained from a large, sample (n=1950) of LGN-projecting FG-labeled RGCs, and from DiI-positive RGCs (n=4927) in five retinas. Eight sample areas were selected from the central and peripheral regions of each retinal quadrant, and from four sample areas in the visual streak. Measurements were corrected for 11–12% tissue expansion that occurred during the long incubation period required for DiI labeling.

Four of nine animals injected showed well-localized tracer injections in both target structures, and no leakage of either tracer into any other retinorecipient nuclei was observed. DL RGCs (Fig. 1C and D) had elongated or triangular soma with 1–3 primary dendrites and were observed primarily in contralateral retinas, since the LGN receives a predominantly crossed projection this species. In each of these four cases, the LGN complex was completely filled with FG label (Fig. 1A). DRN injections were localized primarily in the central and lateral regions of the nucleus (Fig. 1B) where optic axons are relatively dense [9]. Numbers of DL RGCs observed in these four cases were 84, 81, 72 and 52, respectively. Soma diameters ranged from 7 to 28 μm (mean 17.4±0.2 μm), and 95% were 10–25 μm in diameter (Fig. 2). A somewhat larger percentage (59%) of DL RGCs occurred in peripheral vs. central retinal sampling areas, but no major differences were observed among the four retinal quadrants with regard to DL-RGC numbers or soma diameters. Comparison of the soma-size distribution of DL RGCs was made with that of a larger population of DRN-projecting RGCs reported previously (cholera toxin B was injected into the DRN using comparable injection procedures as in the present study; see [9] for methodological details). The DL RGC population clearly falls within the mid-upper range of the larger DRN-RGC soma-size distribution. A similar result was obtained when comparing the DL RGC distribution with LGN-projecting RGCs (n=1950, mean=11.7±0.06 μm; Fig. 3). Finally, comparing the DL-RGC population with a very large number of DiI-positive RGCs sampled over the entire retina (n=4927, mean=13.4±0.04 μm) showed that DL RGCs lie in the mid-upper size range of RGCs repesenting the overall retinal population (Fig. 4).

These results are consistent with prior studies showing that RGCs projecting to multiple target nuclei are among the largest RGCs in the retina. In rats, Dreher et al. [4] showed that the large, contralaterally projecting Class I RGCs send axonal branches to both the LGN and superior colliculus, results also confirmed by Kondo et al. [19]. Farid Ahmed and co-workers [5,6] reported that ipsilateral DL RGCs projecting to both the dorsal LGN and superior colliculus were larger than those that innervate only the superior colliculus. Large, alpha ganglion cells have been demonstrated previously in the gerbil retina [28], and in mammals, α-RGCs generally account for less than 10% of all RGCs. Functionally, they appear to serve the more global aspects of image processing as described extensively for Y-type ganglion cells [29].

Some evidence indicates that light stimulation can affect the firing rate of DRN serotonergic neurons [11,13,27,33]. Also, DRN serotonin levels vary over the light–dark cycle, with a peak occurring early in the light period, and this pattern is substantially altered in continuous darkness [1]. Expression of c-Fos in the DRN also varies over the diurnal cycle [16], and an intense flashing light (2–3 Hz) reduces DRN c-Fos expression, particularly during the

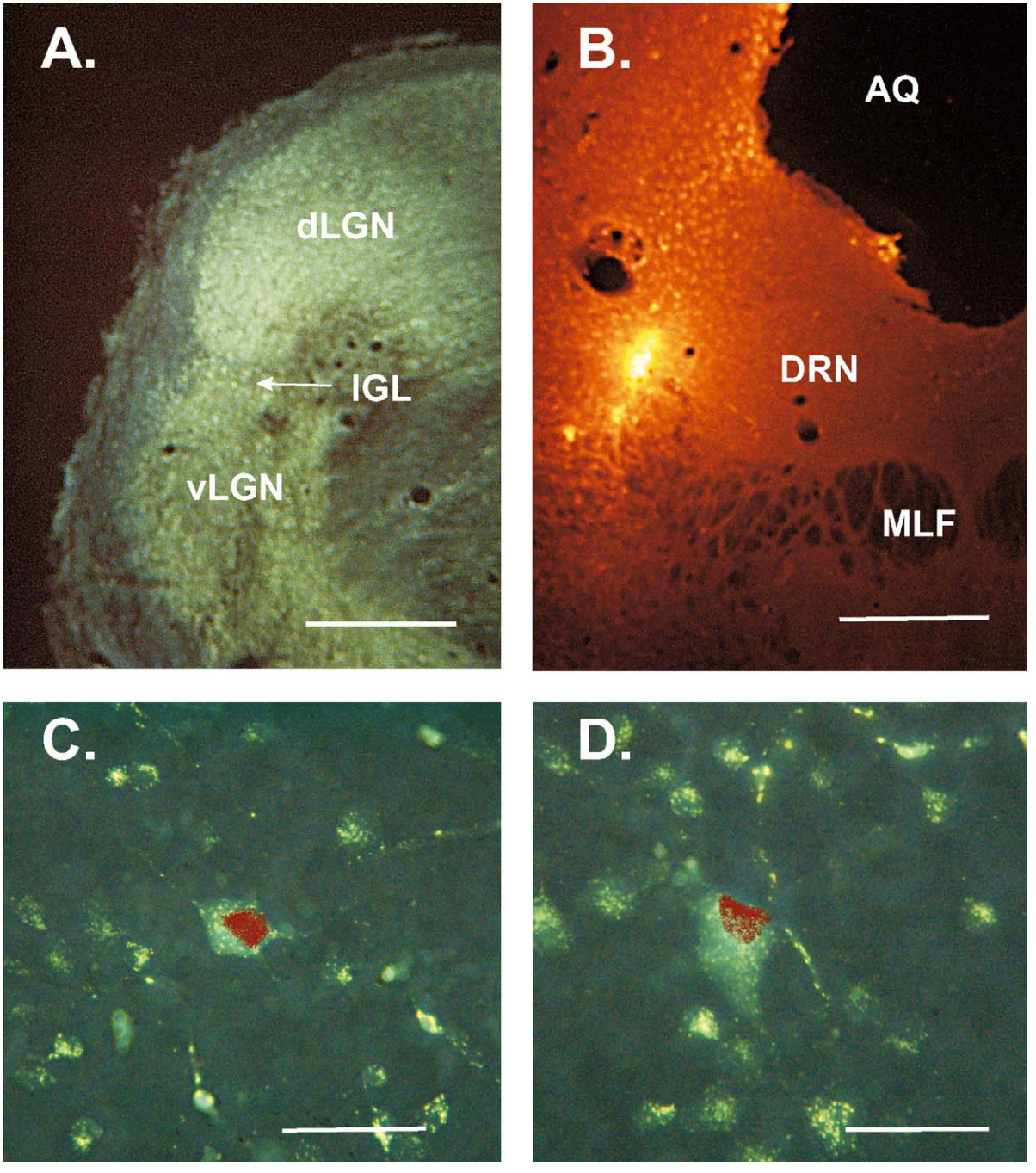

Fig. 1. Fluorescent microscopy images obtained from one brain with well-localized tracer injections: (A) Fluoro-Gold (FG) injected into the lateral geniculate nuclear complex (LGN) included the dorsal (dLGN), ventral (vLGN) and intergeniculate leaflet (IGL); (B) rhodamine injected into the lateral region of the dorsal raphe nucleus (DRN). (C) and (D) show large retinal ganglion cells (RGCs) double-labeled with FG (yellow) and rhodamine (yellow/red). Scale bars: 200 μm in (A,B); 25 μm in (C,D); Aq, aqueduct; MLF, medial longitudinal fasciculus.

normal light period [Wu and Fite, unpublished data]. The lateral DRN subdivisions that receive the greatest density of optic afferents in gerbils send serotonergic efferents to the LGN and superior colliculus (SC) [9], and some DRN serotonergic neurons may send axonal branches to both structures [34]. Serotonergic efferents terminate densely in the retinorecipient superficial layer of the SC [24] and exert an inhibitory, modulatory effect on SC neurons as well as in the LGN [14,18,21,25].

Functionally, retinal afferents to the DRN may play an important role in the actions of serotonin both in the SC and LGN complex. Conceivably, the excitatory effects of retinal afferents on LGN neurons may be modulated, presynaptically, by serotonergic neurons that are influ-

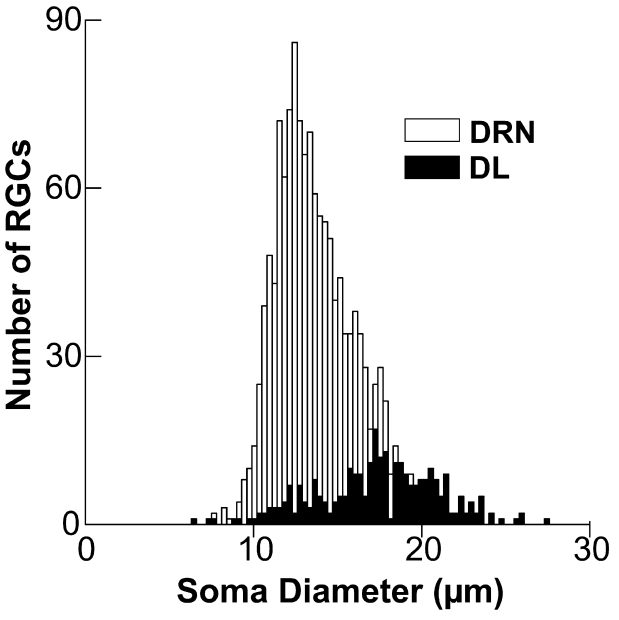

Fig. 2. Soma-size frequency distribution of double-labeled (DL) retinal ganglion cells (RGCs) from four retinas (n=289, mean=17.4±0.4 μm, range 7–27 μm) compared with that of RGCs projecting to the DRN (n=1330, mean=13±0.6 μm, see [9]).

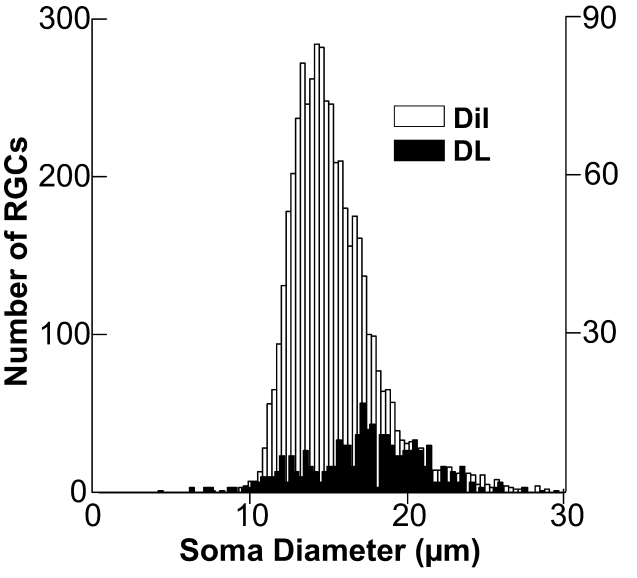

Fig. 4. Soma-size frequency distribution of double-labeled (DL) retinal ganglion cells (RGCs) (left y axis) compared with a large random sample of DiI-labeled RGCs (right y axis) (n=4927, mean 13.4±0.4 μm, range 6–29 μm).

enced by direct retinal input to the DRN. Determining the mode of action of these retinal afferents represents a crucial next step in understanding how light stimulation can affect the DRN serotonin system and its widespread efferent projections to the forebrain.

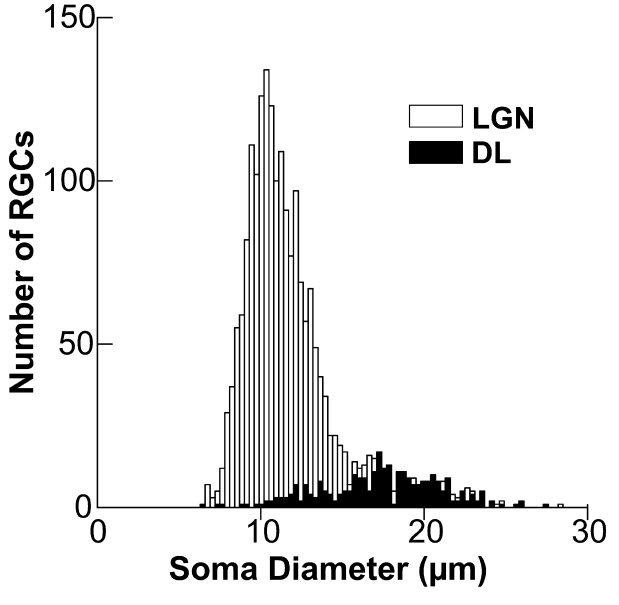

Fig. 3. Soma-size frequency distribution of double-labeled (DL) retinal ganglion cells (RGCs) compared with a large, random sample of Fluoro-Gold labeled LGN-projecting RGCs (n=1950, mean 11.7±0.06 μm, range 7–22 μm).

Acknowledgements

This research was supported by the University of Massachusetts-Baystate Collaborative Biomedical Research Program and by NSF IBN-0091660. We thank Lynn Bengston for her excellent technical assistance.

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