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Research report

Retinal projection to the dorsal raphe nucleus in the Chilean degus (*Octodon degus*)

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Abstract

A substantial projection from the retina to the dorsal raphe nucleus (DRN) has been demonstrated in the Chilean degus, a diurnal/crepuscular hystricomorph rodent. Following intraocular injection of cholera toxin subunit B (CTB), immunocytochemically labeled CTB-positive axons and terminals were observed in all major retinorecipient nuclei as well as in the DRN and periaqueductal gray (PAG) of the mesencephalon. Two streams of optic axons to the DRN were observed: one descending from the optic tract at the level of the pretectum and anterior superior colliculus, the other emerging as a small fascicle at the anterior pole of the inferior colliculus and descending bilaterally through the PAG. Contralateral retinal afferents in the DRN appeared to terminate primarily in the dorsomedial and lateral subdivisions of the DRN, and a less extensive ipsilateral component also was observed. Axonal arborizations were characterized by short branches and multiple varicosities, both in the DRN and in the PAG. The extent and density of DRN retinal afferents were not as extensive as previously observed in Mongolian gerbils using identical techniques, but the retinal-DRN projection is considerably larger in degus than in rats. The functional significance of the retinal-DRN pathway remains to be determined, although a variety of evidence indicates that light may directly affect the activity of neurons and serotonin levels in the DRN.

Theme: Sensory systems

Topic: Subcortical visual pathways

Keywords: Subcortical visual system; Non-imaging forming visual system; Environmental light stimulation; Periaqueductal gray; Serotonin

1. Introduction

The vast majority of serotonergic (5-HT) neurons that project to the forebrain are located in the dorsal raphe nucleus (DRN) of the ventral mesencephalon [15,37]. Despite the wide range of targets in the neocortex, striatum, limbic system, and diencephalon innervated by 5-HT projections from the DRN, little information is currently available with regard to the localization and density of afferent terminals in the DRN. DRN efferents also innervate a number of structures in the central visual system, including the lateral geniculate nuclear complex, superior colliculus, and visual cortex. Some evidence indicates that light stimulation can directly affect neuronal activity in the DRN [11,16], and also that DRN serotonin levels can be altered by light independently of circadian factors [2].

At present, little is known about the neural circuitry whereby photic stimulation may directly influence the serotonergic nuclei that are involved in arousal, affective and/or emotional states via their ascending projection systems. One possible route is a direct optic pathway to the DRN that has been described in several species: cat [10], rat [9,18,34], and Mongolian gerbil [9]; this projection remains a relatively unexplored component of mammalian central visual pathways. The distribution of retinal afferents to the DRN and the retinal ganglion cells from which this pathway originates have been described recently in Mongolian gerbils and laboratory rats [9]. The retinal-DRN projection is more extensive in gerbils than in rats,

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which may be related to the relative importance of vision in these two species; gerbils are active primarily during daylight hours, while rats are predominantly nocturnal. Also, the gerbil retina contains a substantially larger population of cone photoreceptors [14] than rats, and gerbils have a well-defined visual streak in the superior retina [25].

In the present study, the retinal-DRN projection was investigated in the Chilean degus, a highly visual South American hystricomorph rodent that is diurnal in its natural environment and shows 'robust' responses to both photic and non-photic circadian zeitgebers [13]. Results indicate that the projection pathway is quite well-developed in degus, but neither the density nor distribution of retinal terminals in the degus DRN is as extensive as found previously in Mongolian gerbils [9].

2. Materials and methods

Adult female *Octodon degus* (170–220 g) were anesthetized with an intraperitoneal injection (mixture of ketamine (55 mg/kg), xylazine (0.5 mg/kg) and acepromazine (1 mg/kg)). All procedures were approved by the University of Massachusetts Institutional Animal Care and Utilization Committee. Following a topical application of corneal anesthetic (0.5% proparacaine hydrochloride), the needle of a 10-μl Hamilton microsyringe was inserted into the posterior chamber just behind the corneal margin, and 6–8 μl of 2% (w/v) solution of cholera toxin subunit B (CTB, low salt, List Biological Labs) dissolved in 2% dimethyl sulfoxide (to facilitate CTB uptake) was slowly injected into the eye, and the needle left in place for 10 min to minimize leakage of tracer from the eye. Subsequently, the needle was withdrawn, the injection site washed immediately with saline, and antibiotic ointment (Bacitracin) applied topically to the site. All intraocular injections were unilateral.

Animals were allowed to survive for 6–7 days post-injection, and were anesthetized for transcardial perfusion. The descending aorta was clamped, the heart injected with 1 ml of heparin (5000 USP U/ml), and perfusion with saline was followed with 400 ml of chilled 4% paraformaldehyde in phosphate buffer (PB, pH 7.2). The brain was subsequently removed, post-fixed in the same fixative overnight at 4°C, and immersed in 30% sucrose in PB overnight at 4°C. Serial, coronal sections throughout the thalamus and mesencephalon were cut on a freezing microtome at 40 μm thickness.

The CTB immunocytochemistry (ICC) protocol was based on that described previously by Angelucci et al. [1] (see also Ref. [9]). Sections were rinsed four times (5 min each) in 0.1 M phosphate-buffered saline (PBS, pH 7.4); incubated in 0.3% H_2O_2 in PBS for 20 min; rinsed in PBS three times (5 min each); incubated in 0.1 M glycine in PBS for 30 min; rinsed three times (5 min each) in PBS; and incubated in 4.5% normal rabbit serum (NRS, Vector), 2.5% bovine serum albumin (BSA, Sigma) and 0.4% Triton X-100 (TX) in PBS overnight at 4°C. Sections were then rinsed two times (5 min each) in PBS and incubated in a goat anti-CTB IgG (1:2700, List Biological Labs) in PBS containing 2% NRS, 2.5% BSA and 2% TX in PBS for 4 days at 4°C. Sections were rinsed four times (15 min each) in PBS; incubated in 2% NRS and 2.5% BSA in PBS for 10 min; then incubated in biotinylated rabbit anti-goat IgG antibody (Vector, diluted 1:200) with 2% NRS, 2.5% BSA and 1% TX in PBS for 1.5 h. Sections were rinsed four times (15 min each) in PBS and again incubated in 2% NRS, 2.5% BSA in PBS for 10 min. Sections were incubated in a 1:100 ABC (ABC Elite; Vector) solution in PBS for 1 h, rinsed four times in PBS (15 min each), then rinsed twice (5 min each) in 0.05 M Tris buffer (TB; pH 7.4), incubated in 0.5% $CoCl_2$ in TB for 10 min, then rinsed in TB for 2 min followed by two rinses in PBS (5 min each). Sections were then preincubated in 3,3′-diaminobenzidine (DAB; 0.05%) in PBS for 5 min and reacted for 3 min by adding 0.01% H_2O_2 to the DAB solution. Sections were then rinsed five times (1 min each) in PBS, mounted on chromium-subbed slides, allowed to air dry, cleared with Hemo-De, and coverslipped with Permount.

Sections containing CTB-labeled axons and terminals were charted serially using both bright- and darkfield microscopy with reference to a standard rat atlas [29]. The borders of the DRN were determined using alternate brain sections from one of the CTB-injected degus processed immunocytochemically for serotonin. The CTB-immunocytochemical protocol (described above) was modified to demonstrate DRN serotonergic neurons as follows: the goat anti-CTB antibody was replaced with a rabbit anti-5-HT antibody (Protos Biotech Corp., New York) at a dilution of 1:1500, the rabbit anti-goat IgG antibody was replaced with goat anti-rabbit IgG antibody (Vector) diluted 1:200, and the normal rabbit serum was replaced with normal goat serum (Vector).

3. Results

All major retinorecipient nuclei contained densely labeled, CTB-positive axons and terminals. In addition, sparse retinal afferents were observed in a variety of other sites, including the lateral posterior nucleus, periventricular gray, periaqueductal gray (PAG), and parabrachial nucleus. Retinal axons appeared to innervate the DRN via at least two descending streams. At the level of the pretectum and the anterior pole of the contralateral superior colliculus, a stream of CTB-positive axons emerged from the optic tract near the olivary pretectal nucleus and descended medially through the commissure of the superior colliculus to enter the PAG (Fig. 1A). Some CTB-positive axons in the PAG contained short branches with conspicuous varicosities,

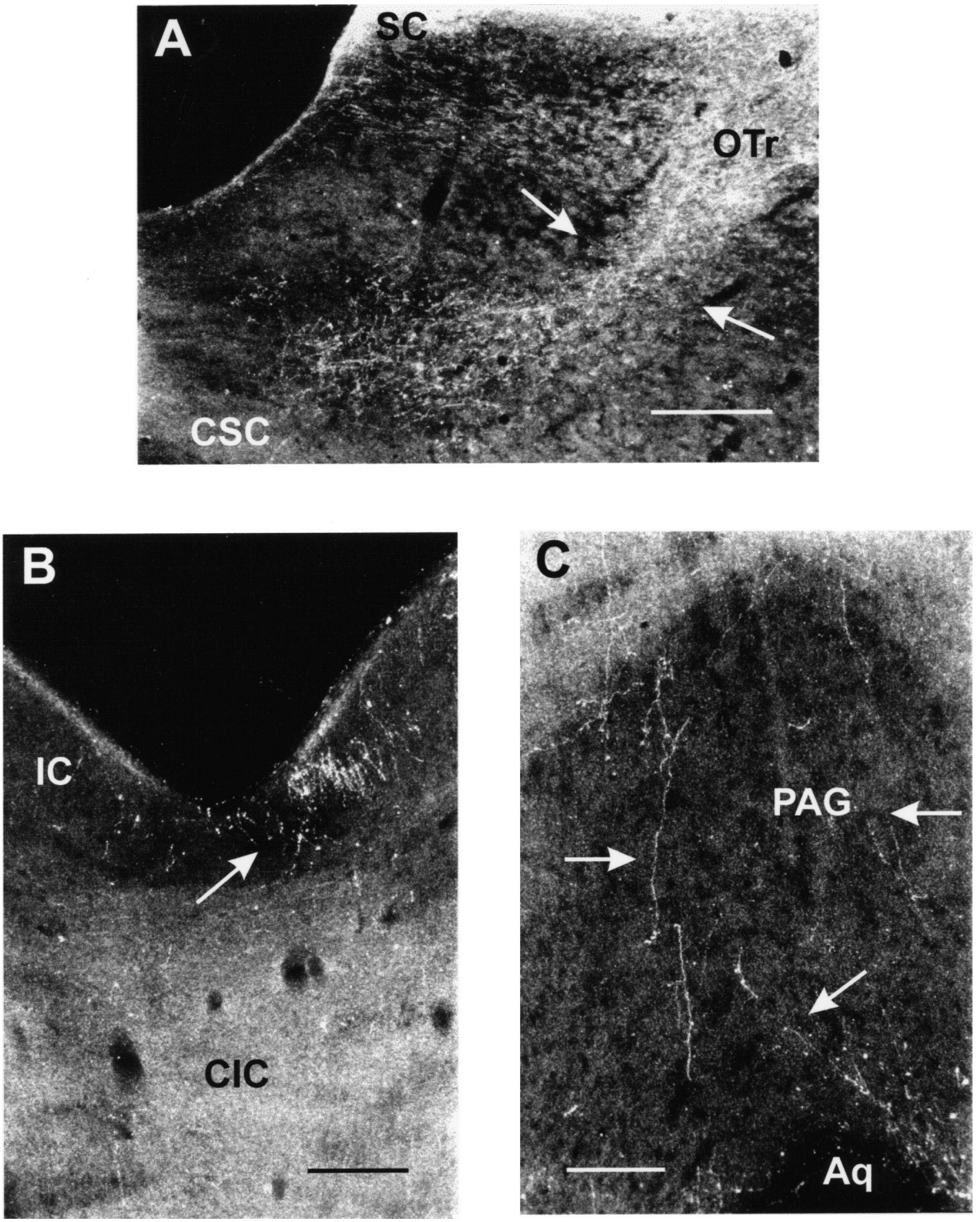

Fig. 1. Darkfield photomicrographs of CTB-positive optic axons in the anterior mesencephalon of the Chilean degus following an intraocular injection of CTB. (A) Optic axons (arrows) emerge from the optic tract (OTr) at the level of the rostral superior colliculus (SC) and pretectal region and descend towards the commissure of the superior colliculus (CSC). (B,C) Optic axons at the anterior pole of the inferior colliculus (IC) descending through the commissure of the inferior colliculus (CIC) into the periaqueductal gray (PAG) (arrows). Aq, cerebral aqueduct. Scale bars: A=250 μm; B,C=50 μm.

some of which entered the ependymal layer lining the aqueduct, particularly in the region immediately dorsal to the rostral pole of the DRN. In addition, a second stream of CTB-labeled axons was observed at the anterior pole of the inferior colliculus; these axons descended bilaterally through the lateral PAG into the caudal regions of the DRN (Fig. 1B,C).

CTB-positive axonal arborizations in the contralateral DRN were observed primarily in the dorsomedial and dorsolateral portions of the nucleus (Figs. 2 and 3), with a smaller number of ipsilateral, retinal afferents in the dorsolateral DRN near the aqueduct. CTB-positive axons and terminals also occurred in the lateral and ventrolateral PAG, extending from the anterior pole of the superior colliculus to the posterior pole of the DRN. In both the DRN and PAG, CTB-positive optic axons were characterized by short branches with multiple varicosities, often occurring in clusters (Fig. 4).

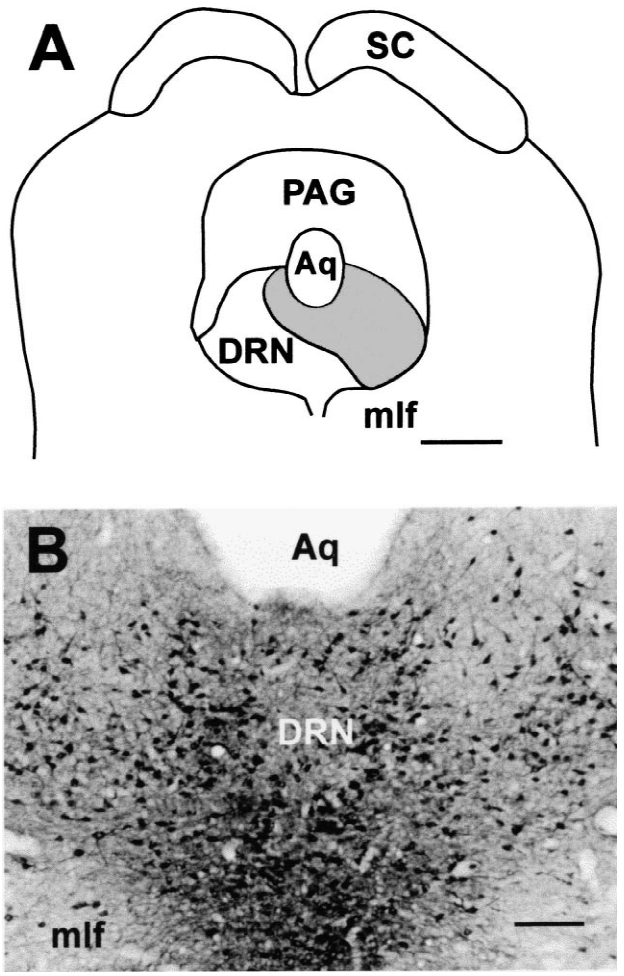

Fig. 2. (A) Schematic coronal section showing the location and extent of CTB-positive optic axons (shaded area) in the dorsal raphe nucleus (DRN) following an intraocular injection of CTB into the left eye. (B) Photomicrograph of serotonin-positive neurons used to determine the boundaries and extent of the dorsal raphe nucleus. Aq, cerebral aqueduct; mlf, medial longitudinal fasciculus; PAG, periaqueductal gray; SC, superior colliculus. Scale bars: A=500 μm; B=250 μm.

4. Discussion

The direct retinal pathway observed in the Chilean degus appears similar to that reported in several other rodents, including gerbils and rats [9,18,34]. However, both the density of optic terminals and the size of the DRN retinorecipient zone were less extensive in degus than previously observed in gerbils using the same techniques [9]. In degus, the retinal-DRN afferent projection is primarily contralateral, with a smaller ipsilateral component; whereas in gerbils, the retinal-DRN projection is more bilaterally symmetrical. The location and greatest density of retinal terminals in the DRN of all three species suggest that this optic pathway is well-placed to influence serotonergic neurons, particularly in the lateral cell groups, many of which project to the superior colliculus and/or LGN [17,26,38] and/or to the visual cortex [39].

Previous studies have shown that serotonergic afferents from the raphe nuclei can modulate neuronal activity at the initial stages of visual processing and transmission in the brain. The effects of the serotonergic projection to the dorsal LGN are primarily inhibitory and tonic in nature [19,23,28]. Serotonin also can inhibit retinotectal transmission in the superior colliculus by acting on presynaptic 5-HT_{1B} receptors that are located on retinal terminals [27,28]. In the visual cortex, serotonin also inhibits the induction of long-term potentiation, presumably by acting through 5-HT_{1A} and 5-HT_2 receptors [5,6,8,12].

The retinal-DRN pathway may be associated with what has been described as a ‘non-image-forming’ subsystem of retinal afferents that includes the retino-hypothalamic projection to the suprachiasmatic nucleus, as well as sparse retinal afferents to the lateral habenular nucleus [31], lateral hypothalamic area [4,22,40], paraventricular nuclei [40], medial amygdala and peri-amygdaloid area [4,7], piriform cortex, and the olfactory tubercle [4,24,40]. All of these retinal afferents are characterized by fine-caliber axons with multiple varicosities that arborize in regions that lie more medially than those typically associated with the classically defined ‘image-forming’ and visuomotor pathways. This non-image-forming subset of retinal afferents may be involved in the photic regulation of arousal, neuroendocrine and circadian functions, and they appear to encode the temporal rather than the spatial characteristics of light stimulation [3,4].

Although the functional significance of the retinal-DRN pathway remains unknown at present, previous studies have shown that DRN neurons may respond to visual stimulation [11,16,32,35]. Also, the concentration of extracellular DRN serotonin appears to parallel neuronal activity in the DRN [30], and oscillations of serotonin in the pineal gland and in serum show high levels of

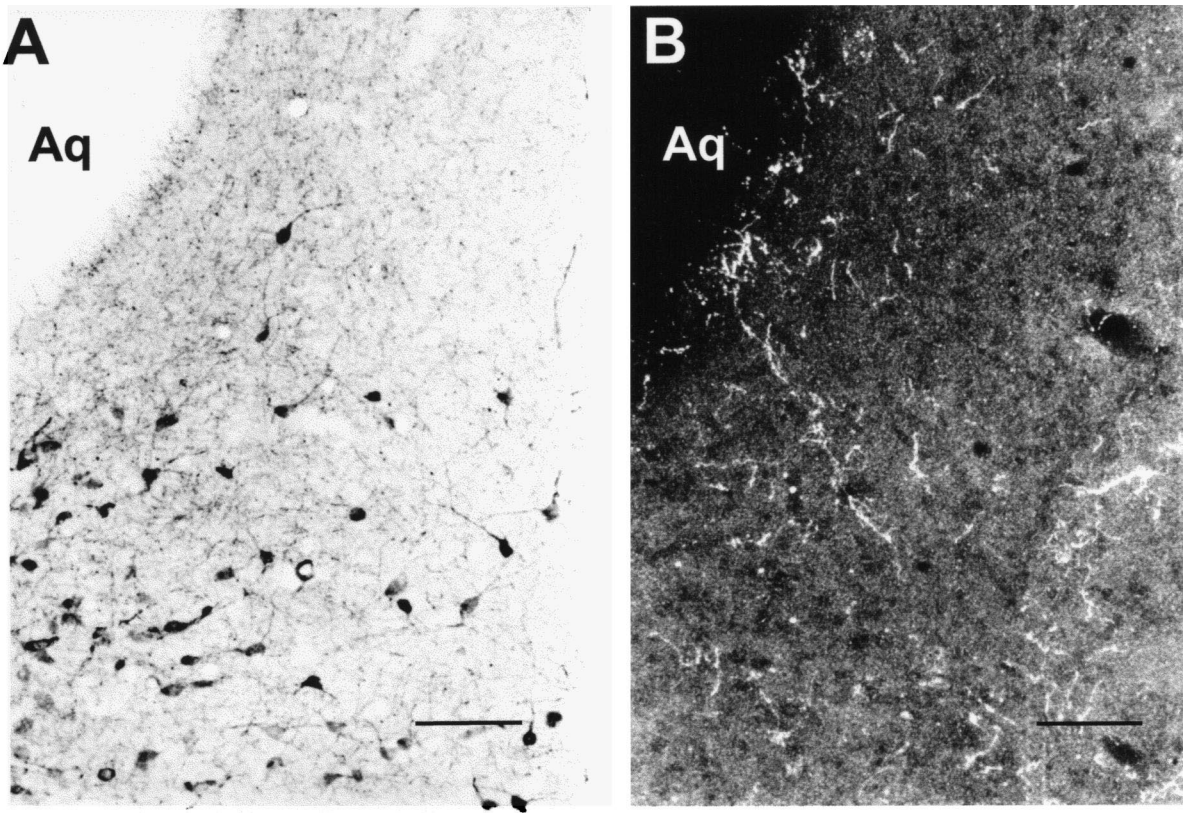

Fig. 3. Dorsomedial region of the contralateral dorsal raphe nucleus. (A) Serotonin-immunoreactive neurons. (B) CTB-positive optic axons. Aq, cerebral aqueduct. Scale bars=100 μm.

serotonin during the day and low levels at night. Seasonal variations in serotonin metabolism have been reported as well [20,21], and the effectiveness of light therapy for seasonal depression is thought to involve central serotonergic mechanisms and metabolism [33,36]. Ultimately, the retinal-DRN pathway may prove to be an important

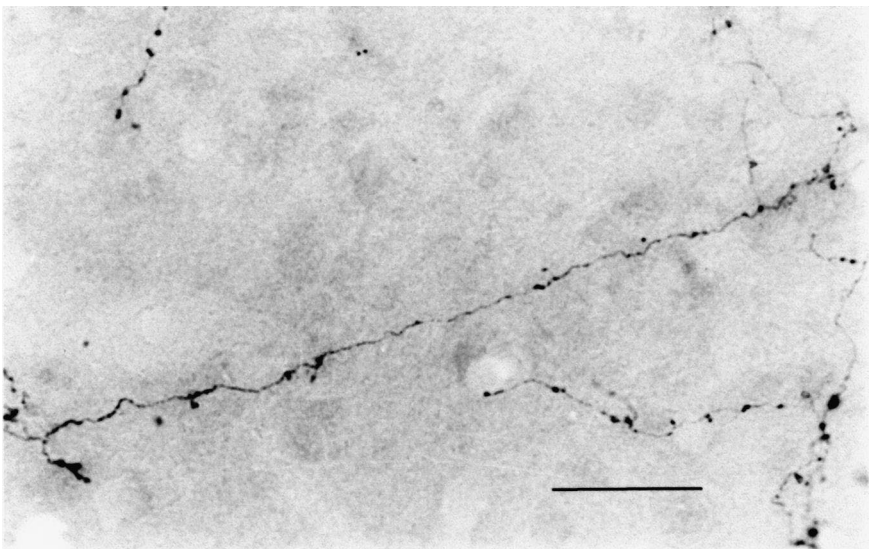

Fig. 4. CTB-positive optic axons in the lateral DRN showing multiple branches and varicosities. Scale bar=50 μm.

afferent channel whereby the intensity, total flux, and/or phase characteristics of environmental light stimulation may influence serotonergic activity in the brain, particularly in more diurnal species that rely extensively on vision for adaptation and survival.

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