

Research report

Optic afferents to the parabrachial nucleus

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Abstract

Following intraocular injection of cholera toxin subunit B (CTB), optic afferents to the dorsal pontine region were observed in Mongolian gerbils, Chilean degus, and laboratory rats. CTB-positive optic axons emerge at the caudal pole of the superior colliculus, descend through the periaqueductal gray, and innervate the lateral parabrachial nucleus. This projection appears to be a continuation of the retinal pathway that innervates the dorsal raphe nucleus in these same species. © 2002 Elsevier Science B.V. All rights reserved.

Theme: Sensory systems

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1. Introduction

Retinal afferents innervate a number of brain regions that lie outside the classically defined set of central visual nuclei that are essential for perceptual and visuomotor functions. Described as a ‘non-image-forming’ subsystem [6], these include diencephalic retinal afferents terminating in the suprachiasmatic nucleus, in the preoptic and anterior hypothalamic nuclei [28,35], lateral hypothalamic area [24], paraventricular and periventricular regions [26,36], supraoptic nuclei [32,36], medial and lateral preoptic areas, retrochiasmatic area and subparaventricular zone [7,24], lateral habenular nucleus [35], anterodorsal thalamus, and lateroposterior nucleus [13,21,28]. Retinal afferents also innervate the olfactory tubercle, piriform cortex, and amygdaloid complex in the basal forebrain [5,6,10,18,26,28,31,35]. In the mesencephalon, optic afferents also have been observed in the periaqueductal gray and dorsal raphe nucleus in a variety of species [12–14,22,36].

Several characteristic features are associated with this subset of retinal afferents, the majority of which terminate more medially than do retinal afferents to the lateral

geniculate complex, superior colliculus, pretectal, and accessory optic nuclei. Also, their fine-caliber axons are varicose and branch extensively as they arborize, suggesting a non-topographic mode of termination [6,12,26]. Depending upon the specific site of termination, these non-image-forming retinal afferents are thought to be involved in photic regulation and/or modulation of neuroendocrine, seasonal reproductive, or circadian functions and appear to encode the duration, intensity, photoperiodic, and/or phase-related characteristics of environmental light.

The present study describes optic afferents that project into the dorsal pontine region and terminate in the lateral component of the parabrachial (PB) nucleus in three species: laboratory rats, Mongolian gerbils and Chilean degus, following intraocular injection of an anterograde tracer, cholera toxin subunit B. These optic axons show morphological characteristics similar to those previously described for the retinofugal projection to the dorsal raphe nucleus (DRN) [12,13]. Furthermore, this optic afferent pathway to the PB appears to be a caudal extension of the retinal pathway that innervates the DRN in each of these same species.

2. Materials and methods

Adult animals of three rodent species, laboratory rats

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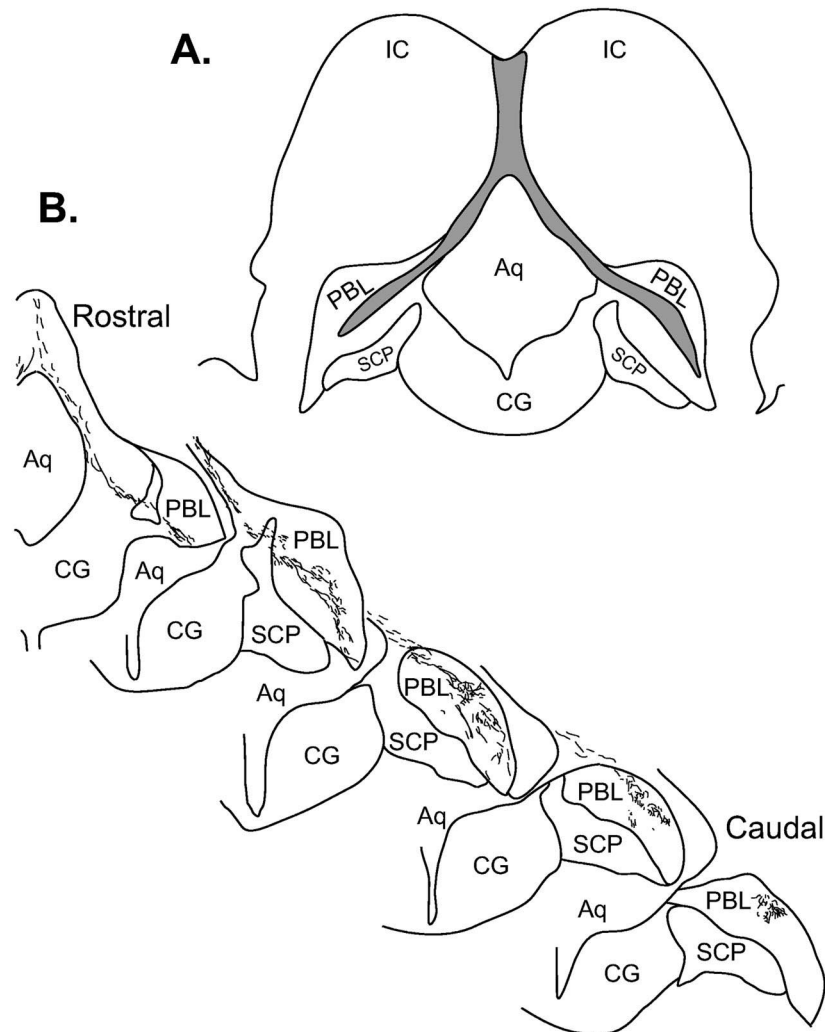
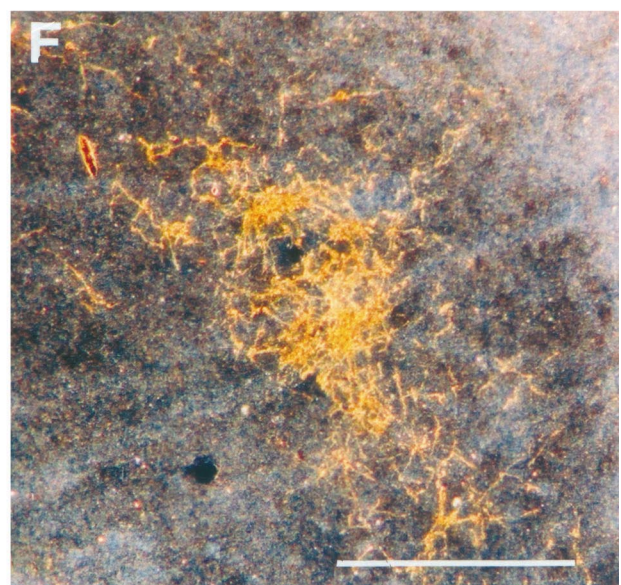
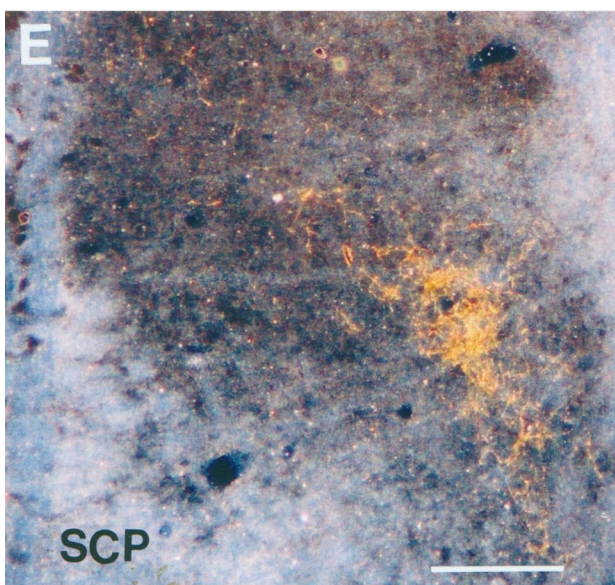
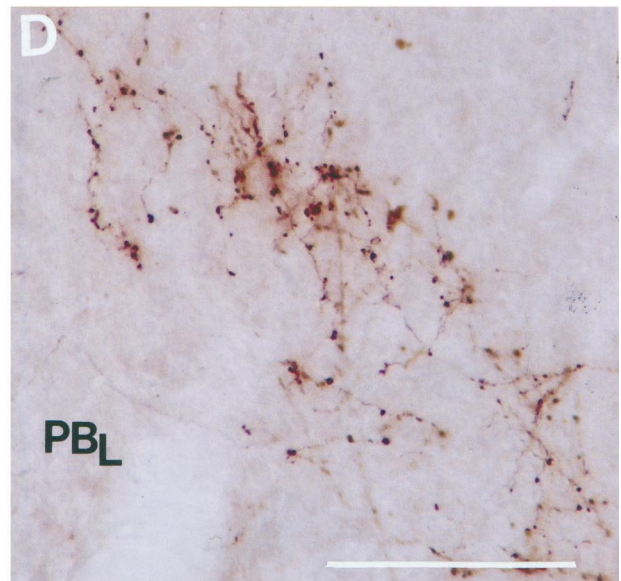
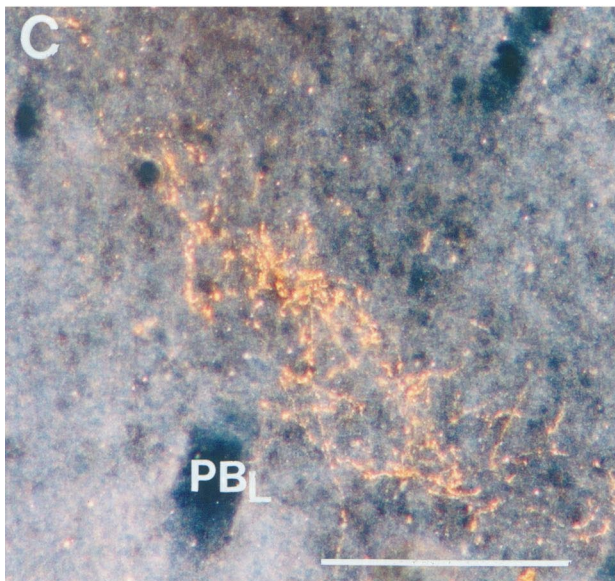
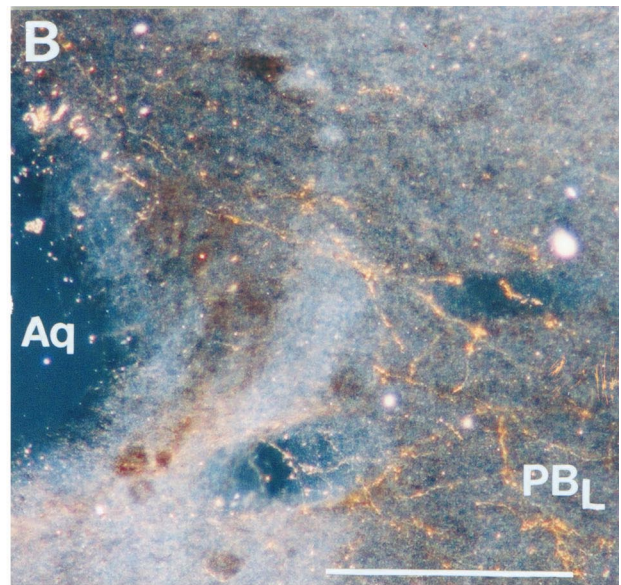
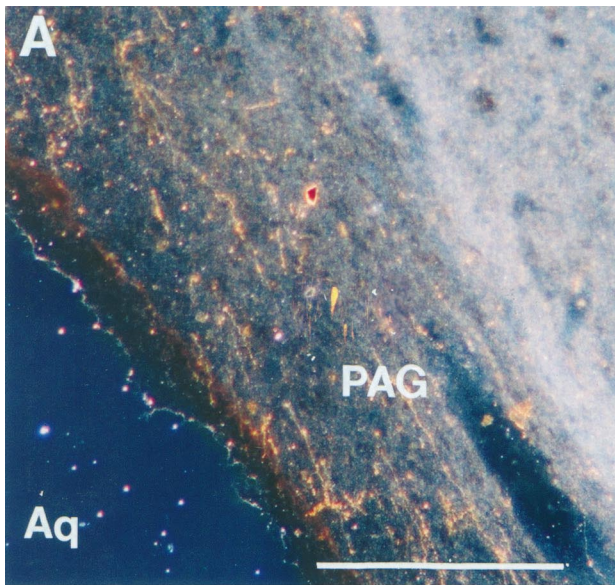


Fig. 1. (A) Camera lucida drawing of a coronal section at the anterior pole of inferior colliculus (IC) showing the course (shaded zone) of CTB-positive optic axons that project to the lateral parabrachial nucleus (PBL) in Chilean degus. (B) CTB-positive optic axons charted as they descend through the periaqueductal gray (PAG) and enter the PBL; (a–e) coronal sections at intervals of approximately 140 μm . Aq, aqueduct; CG, central gray; IC, superior colliculus; SCP, superior cerebellar peduncle.

(*Rattus norvegicus*, $n = 4$), Mongolian gerbils (*Meriones unguiculatus*, $n = 4$) and Chilean degus (*Octodon degus*, $n = 3$), received unilateral intraocular injections of cholera toxin subunit B (CTB, low-salt; List Biological). All surgical procedures were performed in accordance with NIH and USDA guidelines and approved by the University of Massachusetts Institutional Animal Care and Use Committee. Animals were anesthetized with an intraperitoneal injection [rats: ketamine (90 mg/kg) and xylazine (4 mg/kg); gerbils: ketamine (100 mg/kg) and xylazine (10 mg/kg); degus: ketamine (55 mg/kg) and xylazine (0.5

mg/kg) and acepromazine (1 mg/kg)]. Local anesthetic (0.5% proparacaine hydrochloride) was applied to the cornea prior to intraocular injection. The needle of a 10 μl Hamilton microsyringe was inserted into the posterior chamber at the conjunctival margin, and 5–7 μl of 2% CTB in 2% dimethyl sulfoxide were slowly injected. The needle was held in place for an additional 10–12 min to allow diffusion of CTB away from the tip of the needle in the posterior chamber. After the needle was withdrawn, the injection site was rinsed with saline and Bacitracine antibiotic ointment applied topically to the site.

Fig. 2. (A) Dark-field image of CTB-positive optic axons (orange) in Chilean degus descending through the periaqueductal gray (PAG); (B, C) optic axons leaving the PAG and entering the rostral pole of the lateral parabrachial nucleus (PBL); (D) bright-field image of CTB-positive optic axons in the central portion of the PBL showing fine-caliber, varicose arborizations; (E, F) dark-field images of a dense cluster of optic axons in the caudal region of the PBL. Scale bars 100 μm .



Animals survived for 7–8 days post-injection and were perfused transcardially with 4% paraformaldehyde in phosphate buffer (pH 7.2). The brain was removed, post-fixed at 4 °C, and immersed in 30% sucrose in phosphate buffer overnight. Brains were serially sectioned in the coronal plane on a freezing microtome (section thickness 40 µm) from the level of the anterior thalamus to the rostral pole of the cerebellum. The immunocytochemical procedures used to demonstrate anterograde transport of CTB in optic axons have been described previously [1,12,13]. In at least three animals of each species, sections containing CTB-labelled axons and terminals were charted from the posterior pole of the superior colliculus to the anterior pole of the cerebellum using both bright- and dark-field light microscopy.

3. Results

Anterograde, CTB-positive optic afferents were observed in all thalamic, pretectal, and accessory optic retinorecipient nuclei, in the superior colliculus, and in the DRN. In addition, a diffuse stream of CTB-positive optic axons was observed that emerged from the optic tract at the caudal pole of the superior colliculus/anterior pole of the inferior colliculus. These CTB-positive axons descended bilaterally through the central gray adjacent to the cerebral aqueduct (Figs. 1 and 2A), with en passant short branches and varicosities as was also observed with retinal axons that descend through the periaqueductal gray and terminate in the DRN of these three species [12,13]. Caudally, at the level of the trochlear nerve (nIV) and ventrolateral edge of the cerebral aqueduct, CTB-positive axons changed course to enter the lateral subdivision of the PB nuclear complex. (Figs. 1B and 2B). The course of these optic afferents was similar in all three species; however, the extent of the terminal field in the lateral PB was most extensive in Chilean degus, where retinal afferents arborized in the central and dorsal portions of the lateral PB. In rats, a relatively small number of CTB-positive axons were observed, primarily in the central portion of the lateral PB. Both axons and terminal arborizations were of finer caliber in gerbils than in degus or rats, as previously observed for retinal afferents to the DRN in these same species [12,13].

4. Discussion

Functionally, the PB nuclear complex has been described as a major integrative and relay station for ascending gustatory and visceral, sensory information from the brainstem to the forebrain [15,33]. The PB is physically subdivided into medial and lateral regions by the brachium conjunctivum. The medial PB receives the major output of

the gustatory solitary nucleus, while the lateral PB receives and relays general visceral sensory information associated with cardiovascular, respiratory and nociceptive afferents from the dorsal horn of the spinal cord [9,25,37]. In rats, the PB has been further subdivided into 10–12 subnuclei based upon cytoarchitecture, afferent and efferent connections, and different neurotransmitters [8,15,17,33]. Efferent targets of the lateral PB include the lateral hypothalamus, the ventrobasal and visceral relay nuclei of the thalamus (median preoptic nucleus, ventroposterior parvocellular nucleus) [8,33], which relay nociceptive and visceral information to the limbic forebrain, to the insular cortex and to the central nucleus of the amygdala. The dorsolateral subnucleus is a major target for ascending nociceptive somatosensory information from lamina I of the dorsal horn of the spinal cord and for ascending visceral sensory information associated with autonomic functions and reflexes [20]. Hypothalamic afferents innervate the central and dorsal subnuclei of the lateral PB [33], suggesting a strong association with the autonomic functions and reflexes mediated by these subnuclei.

In relation to the visual system, cholinergic afferents from the rostral PB region including the laterodorsal and pedunculopontine tegmental nuclei terminate in, and affect neuronal activity in, the LGN [2–4,11,19,23,27,29,30,38,39]. Also, PB efferent terminals appear to contact the same dendritic region of LGN neurons as do retinogeniculate terminals [11]. Recently, Billett and co-workers [4] reported that some PB neurons project both to the dorsal LGN and to the intermediate gray of the superior colliculus from which premotor command signals originate that control orienting movements of the head and eyes. The PB also receives afferents from the ventral geniculate nucleus [34], which is closely associated with the intergeniculate leaflet. Both are involved in mediating phase shifts in circadian rhythms and photoperiodic responses [16].

5. Conclusions

In conclusion, in each of the three rodent species investigated in the present study, the lateral subdivision of the PB complex receives bilateral optic afferents via what appears to be a caudal extension of a previously documented, direct retinal projection to the DRN [12,13]. Functionally, this afferent pathway may provide a route whereby environmental light stimulation could modulate PB functions and potentially influence a variety of behavioral and visceral states, arousal, and attention-gating mechanisms at higher CNS levels. Further investigations are needed to determine the physiological responses, properties and functional role of this unusual optic pathway and whether it may exist in other mammalian species as well.

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