Subdivisions of the dorsal raphe nucleus projecting to the lateral geniculate nucleus and primary visual cortex in the Mongolian gerbil

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The mammalian dorsal raphe nucleus (DRN) is composed of subdivisions with different anatomical and functional properties. Using cholera toxin subunit B as a retrograde tracer, DRN subdivisions projecting to the lateral geniculate nucleus and to the primary visual cortex were examined in the Mongolian gerbil. DRN neurons projecting to the lateral geniculate nucleus were observed in the lateral DRN (rostrally) and in the ventromedial DRN (caudally), while DRN cells projecting to the primary visual cortex were observed at all rostral–caudal levels in the ventromedial DRN. These results demonstrate a significant overlap between the DRN projections to the lateral geniculate and superior colliculus, and show that only the caudal ventromedial DRN projects to all three major visual targets: the lateral geniculate nucleus, primary visual cortex, and superior colliculus. Since the DRN is involved in depression and other neuropsychiatric disorders, as well as is affected by many psychotropic substances, these data may help to develop new treatments and therapies targeting specific DRN subdivisions. *NeuroReport* 14:459–462 © 2003 Lippincott Williams & Wilkins.

Key words: Dorsal raphe nucleus; DRN; Lateral geniculate nucleus; Primary visual cortex; Rodent visual system; Serotonin (5-HT); Topography

INTRODUCTION

The dorsal raphe nucleus (DRN) of mammals contains the majority of serotonergic neurons that innervate forebrain structures. Recent studies have demonstrated that the DRN is composed of several subdivisions, each of which may have specific neuroanatomical, neurochemical, and functional characteristics [1–4]. Understanding how DRN subdivisions interact with different structures in the visual system is relevant to both fundamental and clinical neuroscience. A number of studies have shown that DRN neurons modulate visual processing at both subcortical and cortical levels [5–8]. Light therapy is a highly effective treatment in some forms of clinical depression, such as seasonal affective disorder, which may be associated with abnormal changes in the DRN serotonin system [9,10]. However, the neural circuitry that mediates the therapeutic effects of light in humans remains unknown.

Functional interactions between the DRN and visual system structures cannot be fully understood without a more complete knowledge of the internal organization of the DRN itself. In rats, the superior colliculus and the lateral geniculate nucleus receive projections from neurons located in the lateral wings of the DRN, whereas neurons projecting to the primary visual cortex are primarily located in the ventromedial portions of the DRN [1,2,11]. In addition, a direct retinal projection to the DRN has been demonstrated recently in several mammalian species, with retinal axonal terminals distributed heterogeneously in different DRN subdivisions [12,13]. The functional significance of this direct retinal pathway is not known, although it appears to be well placed to mediate the effects of visual and environmental light stimulation on a broad range of behavioral, arousal, and affective states, some of which are affected in depressed patients with abnormalities in specific DRN subdivisions [14].

In the present study, DRN neurons that project to the lateral geniculate nucleus and to the primary visual cortex were investigated in Mongolian gerbils (*Meriones unguiculatus*) using retrograde labeling techniques. This species offers a good model for investigating the relationship between different DRN subdivisions and major visual system structures. Unlike most rodents, gerbils show a crepuscular activity pattern [15], and have a well-developed visual system [16] with a direct retinal projection to the DRN [12]. In addition, specific subdivisions of the gerbil DRN have been described with respect to their projections to the superior colliculus [3] and with regard to variations in the expression of c-Fos over the light–dark cycle [4].

MATERIALS AND METHODS

All experiments were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts in accordance with the NIH and USDA guidelines. Adult male gerbils were anesthetized with an i.p. injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), and 2% cholera toxin B (CTB low salt, List Biological Laboratories, Inc., Cat. no. 104) dissolved in 2% dimethyl sulfoxide was pressure-injected into each target structure bilaterally. (Bilateral injections provided better visualization of clusters of retrogradely labeled cells in some DRN subdivisions.) Animals were placed in a stereotaxic frame, and the incisor bar was adjusted such that the surface of the skull was level. In eight animals, CTB was injected into the lateral geniculate nucleus (LGN), using lambda as the reference point and the following coordinates: anterior (AP) 3.5–3.8 mm, lateral (ML) 3.0–3.5 mm, ventral (DV) 3.8–4.1 mm. The most accurate injection sites were obtained with AP 3.5 mm, ML 3.0 mm and DV 3.8 mm. In six cases, a pulled glass micropipette (i.d. 80–100 μm) attached to a 10 μl Hamilton syringe was used to inject ~0.07 μl CTB. In the other two cases, large (0.6–1.0 μl) injections were delivered with a 10 μl Hamilton syringe. The needle was left in place for 5–15 min after each injection was completed. In four animals, CTB was injected into the primary visual cortex (V1). The stereotaxic coordinates were based on the delineation of area 17 by Ellard and Chapman [17] and were AP 0.0 mm, ML 1.5–3.5 mm, DV 1.0 mm. A 10 μl Hamilton syringe was used, and the injected volume of CTB ranged from 0.4 μl to 2.2 μl. The needle was left in place for 10–15 min after each injection. The skull opening was then sealed with bone wax, and the incision was closed with stainless steel wound clips.

After a survival period of 6–9 days (LGN injections) or 8–12 days (V1 injections), animals were deeply anesthetized, and the exposed heart was immediately injected with 0.3 ml heparin (5000 USP units/ml). Animals were perfused with saline followed by 400 ml chilled 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.2). The brains were removed, postfixed in the same fixative overnight at 4°C, and transferred to 30% sucrose in PB. Serial, coronal sections were cut on a freezing microtome at 40 μm and were stored in cryoprotectant (30% sucrose with 1% polyvinylpyrrolidone and 30% ethylene glycol). CTB immunocytochemistry was performed using a modification [3,12] of the highly sensitive protocol developed by Angelucci *et al.* [18]. Brain sections were mounted on chromium-subbed slides, air-dried, cleared in Hemo-De, and coverslipped. The distribution of CTB-labeled cells was charted using a camera lucida system.

RESULTS

In animals with well-localized CTB injections in the LGN, dense CTB deposits were observed either in the dorsal LGN or in both dorsal and ventral LGN. In these cases, the vast majority of retrogradely labeled cells were observed in the lateral DRN, at rostral to mid-levels (Fig. 1a, Fig. 2a). At more caudal levels, a small number of retrogradely labeled cells were also observed in the ventromedial and interfascicular subdivisions. The mean (± s.e.m.) numbers of labeled cells per section were 41.0 ± 3.7 in the rostral lateral DRN (one side); 37.4 ± 3.5 in the mid-level lateral DRN (one side); and 8.6 ± 1.6 in the caudal ventromedial and interfascicular DRN. In several other cases, the injection site involved the most lateral portion of the LGN, with some tracer leakage into lateral portions of the hippocampus. In these instances, clusters of retrogradely labeled cells were observed in the dorsomedial subdivisions at mid- to caudal coronal levels of the DRN.

In animals that received CTB injections into the primary visual cortex (V1), the amount of injected CTB varied from one case to another (see Materials and Methods). In several of these cases, some leakage of CTB into the extrastriate visual cortex may have occurred, as indicated by the presence of retrogradely labeled cells in the lateral posterior thalamic nucleus. However, the distribution of retrogradely labeled cells in the DRN showed little variation across different animals, and was virtually identical in all these cases. Retrogradely labeled cells occurred in the ventromedial and interfascicular subdivisions at all coronal levels (Fig. 1b, Fig. 2b), and some CTB-labeled cells were observed in the most ventral portion of the ventromedial DRN, just dorsal to the medial longitudinal fasciculi (Fig. 1b). The mean numbers of labeled cells per section in the ventromedial–interfascicular DRN at rostral, mid- and

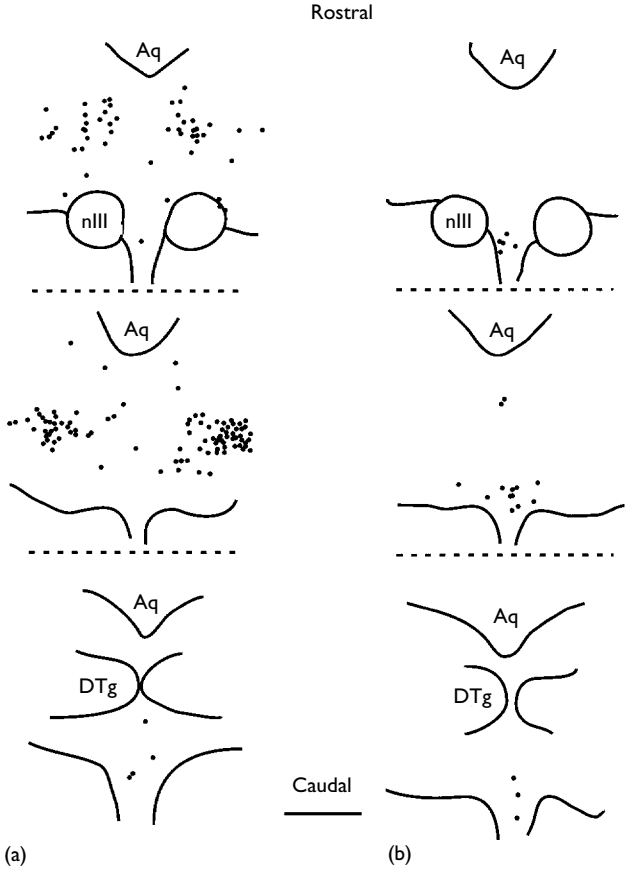

Fig. 1. Camera lucida drawings of the distribution of CTB-labeled cells in representative sections at rostral, middle and caudal levels of the DRN following a bilateral CTB injection into the LGN (**a**) and into VI (**b**). Each dot represents a CTB-labeled cell. Aq, aqueduct; DTg, dorsal tegmental nucleus; nIII, oculomotor nucleus. Bar = 400 μm.

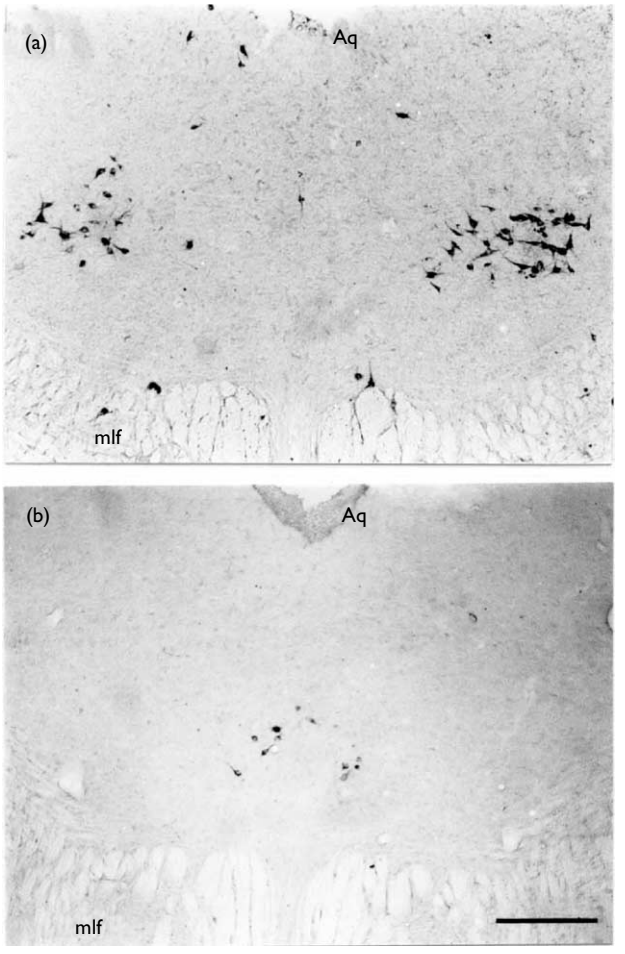

Fig. 2. (**a**) CTB-labeled cells in the DRN (middle coronal level) following a bilateral CTB injection into the lateral geniculate nucleus. (**b**) CTB-labeled cells in the DRN (middle coronal level) following a bilateral CTB injection into the primary visual cortex (VI). Aq, aqueduct; mlf, medial longitudinal fasciculus. Bar = 200 μm.

caudal levels were 1.6 ± 0.8, 4.4 ± 1.9, and 3.4 ± 1.0, respectively.

DISCUSSION

The distribution of retrogradely labeled cells in the DRN following CTB injections into the LGN was virtually the same as that observed with CTB or Fluoro-Gold injections into the superior colliculus (SC) reported previously [3]. In both LGN- and SC-injected animals, virtually all retrogradely labeled cells were observed in the lateral wings at rostral to mid-DRN coronal levels, which is also consistent with the distribution of LGN- and SC-projecting cells described in rats [2]. In the gerbil, CTB-positive cells in the caudal DRN were restricted to the ventromedial and interfascicular subdivisions. In addition, Waterhouse *et al.* [2] reported a substantial number of labeled cells in the rat dorsomedial DRN. In the present study, several cases with CTB injections targeted to the LGN also showed retrogradely labeled cells in the dorsomedial subdivision of the DRN. However, in these cases, CTB leakage into the adjacent hippocampus was apparent, suggesting that, at least in gerbils, the dorsomedial DRN may project not to the LGN, but to the forebrain structures. Alternatively, the DRN projection to the LGN may have a finer topographic organization, such that only the lateral, but not medial, portions of the LGN receive projections from the dorsomedial DRN. Further studies using very small and localized tracer injections will be needed to resolve this question.

Interestingly, in humans, the ventrolateral subdivision contains the highest density of 5-HT_{1A} receptors in the DRN [19]. Since 5-HT_{1A} receptors act as autoreceptors with inhibitory effects on serotonergic neurons, the activity of neurons in the ventrolateral subdivision may be highly dependent on intrinsic 5-HT levels. Since a large number of neurons in the lateral DRN project both to the LGN and to the SC [11], fluctuations of 5-HT levels in the DRN could affect 5-HT release simultaneously in both the LGN and SC. Electrical stimulation of the DRN has been shown to inhibit neurons in the LGN and SC [6–8], suggesting that neurons in the lateral DRN may gate sensory input to both of these major visual structures in relation to an animal's behavioral state (sleep–wake cycle) and/or attention/arousal level.

Following CTB injections into the primary visual cortex, retrogradely labeled neurons were observed in the ventromedial subdivisions throughout the DRN, and some of these neurons were located near or between the fascicles of the medial longitudinal fasciculi. A similar distribution of visual-cortex-projecting cells also has been reported in rats [1] and humans [20].

In gerbils, the caudal ventromedial DRN appears to be the only DRN subdivision that projects to all three major visual structures: the SC, the LGN, and V1. This caudal ventromedial region also appears to be functionally distinct, because it shows a prominent diurnal variation in c-Fos expression [4]. It is yet to be determined whether all these cells that project to the visual cortex also project to the SC and LGN, and whether these cells are the same cells that show diurnal variation in c-Fos immunoreactivity.

Mapping the specific efferent and afferent connections of specific subdivisions of the DRN, as well as their internal relationships, is important for understanding the functional relationships that exist between the DRN and its many target structures. This information also should be of considerable value in aiding the future development of new and innovative treatments for a variety of neuropsychiatric disorders that involve the serotonin system.

CONCLUSION

The lateral geniculate nucleus and the primary visual cortex are innervated by specific subdivisions of the gerbil DRN. The only DRN subdivision that projects to all three major visual structures (the LGN, SC and V1) is the caudal ventromedial DRN. A similar organization of the DRN may exist in primates, understanding of which will aid in developing new treatments targeting specific DRN subdivisions.

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