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Interconnections between the dorsal column nucleus and the cerebellum in a reptile

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ABSTRACT

Interconnections between the dorsal column nucleus and the cerebellum were examined in one group of reptiles, *Caiman crocodilus*. After anterograde tracer injections into the dorsal column nucleus, efferents terminated nearly exclusively in the white matter and ventral portion of the granule cell layer of the ipsilateral cerebellum. Subsequent to deposition of a retrograde tracer into the cerebellum, neurons in the central and ventral half of the dorsal column nucleus were labeled. When compared with the origin of midbrain and spinal cord projecting cells in *Caiman*, cerebellar projecting neurons arose from a more rostral location in the dorsal column nucleus than did neurons that terminated in either of these two other targets. The results of the present and previous experiments suggest that the dorsal column nucleus in this reptilian group is organized into sectors based on efferent target in a fashion similar to what has been described in certain mammals. Furthermore, the presence of this circuit in crocodilians and turtles suggests that his pathway from the dorsal column nucleus to the cerebellum arose early in amniote evolution.

Non-facial, somatosensory information from the periphery reaches the dorsal column nuclei in all tetrapods [8]. In mammals, the dorsal column nucleus is not a homogeneous structure but is organized into sectors based on several features. Efferent target is one character that distinguishes subdivisions of the dorsal column nucleus in mammals [4]. In crocodilians, which have the most well developed dorsal column system among reptiles [6], differential sectors have also been recognized based on efferent target location [10,11]. The identification of the cells of origin of a third projection area of the dorsal column nucleus, the cerebellum, was the focus of the present experiments. These results were then compared with the location of cells in the dorsal column nucleus which projected to the midbrain [11] or to the spinal cord [10] in this species as well as with similar studies in other amniotes.

Two types of experiments determined interconnections between the dorsal column nucleus and cerebellum. One was injection of an anterograde tracer into the dorsal column nucleus and determination of efferent projections to the cerebellum. Details for the methodology of these experiments are identical to a prior description [10]. The other approach was injection of a retrograde tracer into the cerebellum and identification of the location and distribution of labeled neurons in the dorsal column nucleus.

Cerebellar injections of a retrograde tracer were performed on 12 juvenile *Caiman* housed in aquaria in which the water temperature varied from 21.5 to 32 °C. Snout-vent length ranged from 10.3 to 17.4 cm. Animals weighed between 26.5 and 96 g. Details of anesthesia and skull fixation in a stereotactic apparatus modified for use in *Caiman* [9] have been described previously [11]. The cerebellum was exposed by craniectomy. After durotomy and removal of the overlying pia/arachnoid, retrograde tracer injections were made with horseradish peroxidase (HRP, Sigma type VI) in 11 cases and by HRP conjugated to wheat germ agglutinin (WGA) in one case. Injections were of two types: placement of a sephadex bead coated with concentrated HRP and attached to an insect pin [5] in one case or insertion of an insect pin whose tip was coated with concentrated HRP (10 cases) or HRP-WGA (1 case). After survival of 3–6 days, animals were euthanized with a lethal overdose of sodium pentobarbital and perfused according to methodology described previously [10]. Brains were removed, blocked in a standard plane [9], post-fixed, embedded in albumin–gelatin, and processed for HRP methodology as described previously [11]. Brains were cut at either 30 or 40 μm in either the sagittal (7 cases) or transverse (5 cases) plane. All procedures conformed to NIH and institutional guidelines.

The location of each injection site was drawn using a camera lucida. Surface reconstructions of injection sites were performed using a modified microprojector utilizing techniques described by others [3] except that the lateral borders of the cerebellar corti-

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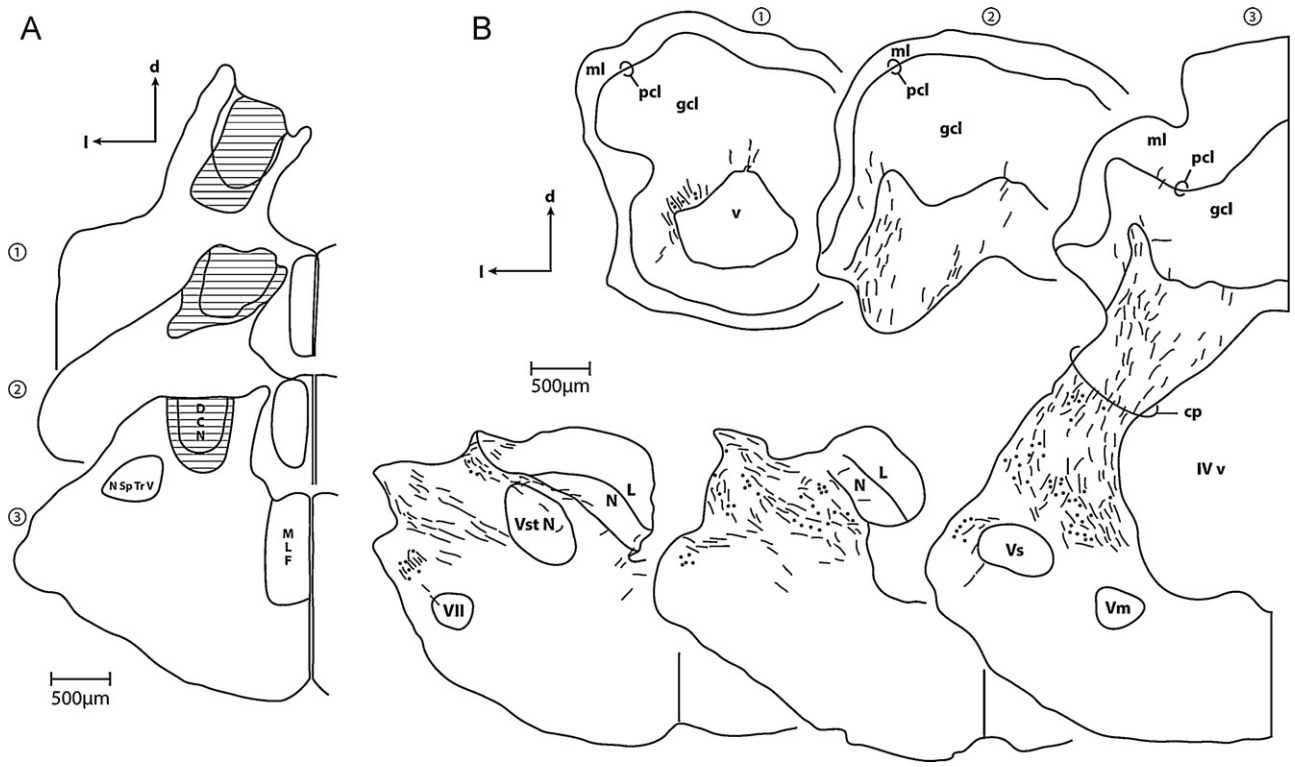

Fig. 1. Dorsal column nucleus projections to the cerebellum. Anterograde tracer injection (horizontal shading) into the dorsal column nucleus is shown in overlapping transverse sections (A). The caudal (A1), central (A2), and rostral (A3) extent of this injection is illustrated. Projections of the dorsal column nucleus to the cerebellum are depicted in overlapping transverse sections (B) arranged from caudal (B1) to rostral (B3). Dots represent axon terminals while short line segments indicate fibers. cp, cerebellar peduncle; DCN, dorsal column nucleus; d, dorsal; gcl, granule cell layer; IV v, fourth ventricle; l, lateral; MLF, medial longitudinal fasciculus; ml, molecular layer; NL, nucleus laminaris; N Sp Tr V, nucleus of the spinal trigeminal tract; pcl, Purkinje cell layer; v, ventricle; Vst N, vestibular nucleus; Vm, trigeminal motor nucleus; Vs, trigeminal sensory nucleus; VII, facial nucleus.

cal surface were not extended. A composite of multiple injection sites was mapped onto a single section using the center of the surface of the cerebellum as a reference point and only drawing the outermost borders of the cerebellum. The location of retrogradely labeled dorsal column nucleus neurons was drawn using a camera lucida. A composite of sections through the dorsal column nucleus in one case of retrogradely labeled neurons was mapped onto a single section using the center of each section as the reference point. Only the external borders of the overlapped sections were drawn. Similar methodology was used for the composite view that mapped the results of several cases onto a single section.

After injection of an anterograde tracer into the dorsal column nucleus (Fig. 1A), labeled axons leave the nucleus ventrolaterally and ascend in the dorsal half of the brainstem (Fig. 1B1–3) to reach the cerebellar peduncle (Fig. 1B3). Here, fibers ascend dorsally to enter the white matter of the cerebellum where they turn caudally. Some fibers reach the ventral part of the granular cell layer (Fig. 1B1–3). A rare fiber crossed the midline to end in the contralateral cerebellum (Fig. 1B1 and 2).

The locus of an injection of a retrograde tracer into the central portion of the right cerebellum is depicted on a surface view (Fig. 2A) and its maximal extent is illustrated in a sagittal section (Fig. 2B). The distribution of retrogradely labeled dorsal column nucleus neurons is shown is equally spaced sagittal sections (Fig. 2C). Cerebellar projecting neurons were mainly located in the ventral and caudal half of the dorsal column nucleus sparing its most rostral pole. Oftentimes, retrogradely labeled cells were clustered together (Fig. 2C1, 3, 4, and 6). Only a rare retrogradely labeled cell was seen contralaterally. Each such cell was located rostro-ventrally (Fig. 2C7 and D).

To provide an overview, several cases (example shown in Fig. 2 and 5 others) were mapped onto a single composite drawing showing all the injection sites grouped as a single injection on a surface view of the right cerebellum (Fig. 3A) as well as the distribution of retrogradely labeled neurons mapped onto a single, composite dorsal column nucleus section (Fig. 3B). Injections primarily involved the central portion of the cerebellum (Fig. 3A) as this was the most easily accessed. Because of the natural curvature of the cerebellum, the rostral and caudal poles of the cerebellum were more difficult to reach. The vast majority of retrogradely labeled neurons were located in the central and ventral half of the ipsilateral dorsal column nucleus sparing its most rostral part (Fig. 3B). Contralaterally labeled cells were rare and were mainly located in a central and ventral part of the dorsal column nucleus (Fig. 3B). No obvious relationship between cerebellar injections and locus and distribution of retrogradely labeled cells in the dorsal column nucleus could be determined from these cases.

Transversely sectioned material (data not shown) demonstrated retrogradely labeled neurons primarily in a central portion of the dorsal column nucleus. Clustering of retrogradely labeled cells in the dorsal column nucleus neurons was also seen.

The trajectory of neurons that project to the cerebellum and their location in the dorsal column nucleus are described in this analysis in *Caiman*. The contralaterally labeled neurons in the dorsal column nucleus (Figs. 2C7, D and 3B) seen after cerebellar injections with a retrograde tracer which did not cross the midline are most likely explained by the occasional dorsal column nucleus axon that reached the contralateral cerebellum (Fig. 1B1 and 2).

In *Caiman*, spinal cord projecting neurons originate mainly from the caudal–ventral portion of the dorsal column nucleus [10]. While midbrain projecting dorsal column nucleus cells are also located