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Difference of climbing fiber input sources between the primate oculomotor-related cerebellar vermis and hemisphere revealed by a retrograde tracing study

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ABSTRACT

The cerebellar flocculus–paraflocculus complex, vermal lobule VII (V-7) and hemispheric lobule VII (H-7) are involved in learning-dependent smooth pursuit eye movement control. To locate the sources of climbing fiber inputs to the H-7 and V-7, we injected retrograde tracers and examined the locations of retrogradely labeled neurons in the inferior olive in 4 monkeys. After the injection of cholera toxin B (CTB) into the H-7, retrogradely labeled neurons were observed abundantly in cell group *d*, i.e., dorsal cap, of the caudal medial accessory olive (MAO) and ventral lamella of principal olive (PO). After injections of fast blue (FB) into the V-7, retrogradely labeled neurons were observed mainly in cell group *b* of MAO, but rarely in cell group *d* or PO. Cell group *d* is known to receive inputs from the nucleus optic tract (NOT) and project climbing fibers to the flocculus and ventral paraflocculus, and cell group *b* is known to receive inputs from the superior colliculus. These results suggest that the three oculomotor cerebellar areas may use different visual signals for the control of smooth pursuit: the flocculus–paraflocculus complex and H-7 receive visual climbing fiber inputs derived mainly from the NOT via cell group *d*, while the V-7 receive visual climbing fiber inputs derived mainly from the superior colliculus via cell group *b*.

The cerebellum plays an important role for eye movement control. Monkey lesion studies suggest that the flocculus–paraflocculus complex [8,22,28], vermal lobule VII (V-7) [24], and hemispheric lobule VII (H-7) [19] are involved in smooth pursuit control. After lesions of either one of these cerebellar areas, velocities of smooth pursuit were decreased appreciably [8,19,22,24,28], and particularly the retinal slip-induced adaptations in velocities of smooth pursuit [19,24] or ocular following response [20] were impaired. Climbing fiber inputs are considered to play a very important role in the cerebellum-dependent motor learning (e.g., [9]). Although the sources of climbing fiber inputs to the flocculus and paraflocculus have been already clarified in the monkey [7,18,27], those of the H-7 are not well known. Here, we compared the sources of climbing fibers between the hemisphere and vermis using retrograde axonal transport techniques.

The experimental protocol is approved by the management committees of RIKEN and Jichi Medical University. One cynamolgus (*Macacus fascicularis*) and three rhesus (*Macaca mulatta*) monkeys were used. They were anesthetized with intramuscular administration of 0.2 ml ketamine (50 mg/ml) and intraperitoneal administration of 1.5 ml sodium pentobarbital (50 mg/ml). After the removal of the cranial bone covering the right vermis or hemisphere, the dura mater was cut to directly expose the cerebellum under an operating microscope. One cynamolgus monkey (IW) was injected with 15 μl of 1% CTB (low salt; Research Biochemical International, Natick, MA) dissolved in saline into the right hemisphere. In three rhesus monkeys (LE, JA, YU), 5 μl of 3% FB (Sigma, St. Louis, MO) in distilled water was injected into the vermis. We did not inject CTB and FB in the same monkey, because the transverse sinus which covers the dorsal cerebellum prevented us from exposing the surface of the vermis and hemisphere for more than 5 mm. The tracers were injected directly into the cerebellar areas (0.5 μl at each site) by air pressure through a glass micropipette (tip diameter, 20–30 μm) attached to an IM-300 microinjector (Narishige, Tokyo, Japan), or by the 10 μl microsyringe (701N, Hamilton, NV) attached to a standard micromanipulator. Seven days after injections, the animals were deeply anesthetized with ketamine and sodium

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pentobarbital. They were perfused with 1000–3000 ml of saline, followed by 3000 ml of a fixative composed of 4% paraformaldehyde, 0.05% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4). Frontal sections were cut at 40 or 60-μm thick on a freezing microtome. To visualize CTB injection sites and CTB-labeled structures in monkey IW, the sections were incubated with a goat antiserum to CTB (diluted 1:10,000 in Tris-buffer solution; Vector Laboratories, Burlingame, CA) for 1 h at room temperature and overnight at 4 °C. They were then incubated with biotinylated rabbit anti-goat IgG (diluted 1:200; Vector) for 1 h before ABC (Elite kit, diluted 1:100; Vector) treatment for 1 h. Finally, they were treated with 0.05% DAB in TBS containing 0.003% hydrogen peroxidase and cobalt chloride. The sections were mounted on slide glasses, naturally dried, dehydrated and coverslipped. To visualize FB injection sites and FB-labeled structures in monkeys LE, JA, YU, the sections were just mounted and coverslipped with an aqueous mounting medium. We followed the nomenclature of Paxinos et al. [21] and Larsell and Jansen [14] in general cerebellar structure, and Bowman and Sladek [3] in medial accessory olive (MAO). Sources of mossy fiber projections in monkey IW were already reported [25].

In monkey IW, CTB was injected into the right crura I and II of H-7 (12–14 mm from the midline and 16–19 mm posterior to the inter-auricular line), and a small portion of the adjacent paramedian lobule (Fig. 1A). CTB-labeled neurons were seen abundantly in the bilateral pontine nuclei with the predominance in the contralateral side [25]. In the inferior olive, labeled neurons were seen exclusively in the contralateral side. Clusters of labeled neurons were seen in the most caudal part of MAO (Fig. 1B and C), cell group *d*, and throughout the principal olive (PO), predominantly in the ventral lamella (Fig. 1E–I and Table 1). However, labeled neurons were seen only rarely in cell group *a–c*, *f* or dorsal accessory olive (DAO, Fig. 1D–I and Table 1).

Table 1
Density of labeled neurons within the subnuclei.

Monkey	MAO					DAO	PO
	a	*b*	*c*	*d* and *e*	*f*		
IW	–	–	–	+	–	–	+
LE	+	++	++	–	++	+	–
JA	+	+	+	–	+	–	–
YU	+	+	+	–	–	+	–

Labeled density was measured by counting the number of labeled neurons using the open-source software *Image*, scaled as ++, highly labeled. +, labeled: –, very weakly or not labeled. *a–f*, cell group *a–f*. DAO, dorsal accessory olive. PO, principal olive.

In monkeys LE, JA and YU, FB was injected into the right vermis. The injection covered most wide areas in monkey LE, including the

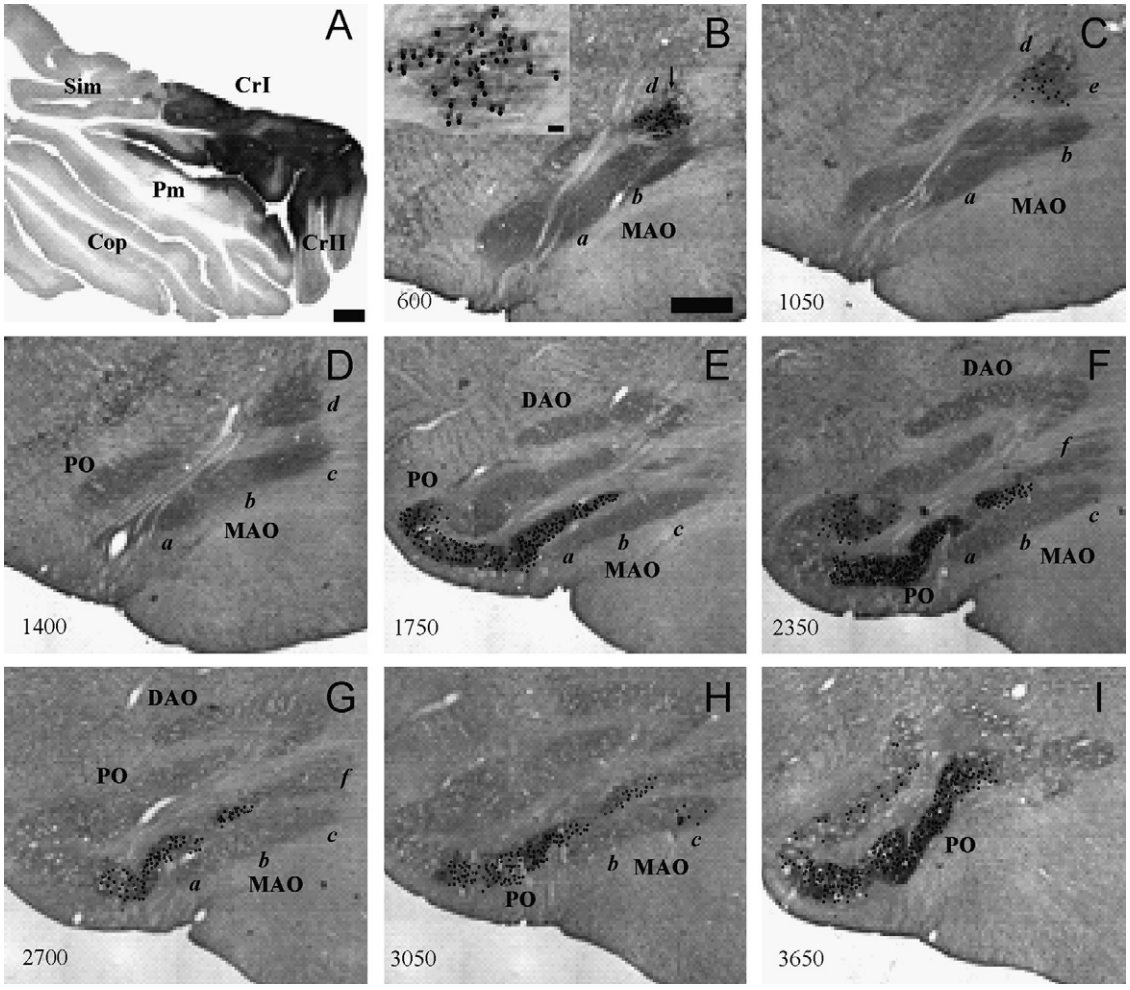

Fig. 1. Distribution of retrogradely labeled neurons in the inferior olive after injection of CTB into the H-7 in monkey IW. (A) A photograph for injection site in the right hemisphere. (B–I) Photographs for labeled neurons in the left inferior olive. Black dots plotted on the photographs show labeled neurons. Inset in (B) show labeled neurons indicated by an arrow. Note that labeled neurons are seen in cell group *d*, but very rarely in cell group *a–c* in MAO. *a–f*, cell group *a–f*. Cop, copula of pyramis. CrI, crus I of H-7. CrII, crus II of H-7. DAO, dorsal accessory olive. Pm, paramedian lobule. PO, principal olive. Sim, simple lobule. Numbers in the left lower quadrant indicate the distance from the caudal end of inferior olive. Scale bar: 1 mm (A), 0.5 mm (B–I) and 0.05 mm (inset for B).