Neuronal Organization of the Cerebellar Projection to the Pretectum in the Cat

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The projections from the cerebellar nuclei to the pretectum of the cat were studied by injecting a retrograde tracer, biotinylated dextran amine, into the pretectum. Labeled neurons were distributed bilaterally in the lateral and medial cerebellar nuclei, and contralaterally in the anterior and posterior interpositus cerebellar nuclei. We analyzed the size and shape of the labeled neurons in the cerebellar nuclei and identified three different shapes in the pretectal projecting neurons: multipolar, trigonal, and bipolar. Based on the cell size, these neurons were divided into three subclasses: small ($50–200\mu m^2$), medium-sized ($200–450\mu m^2$), and large ($450–1500\mu m^2$) cells. Multipolar neurons were mostly large, and bipolar neurons were mostly small in size. These results suggest that several different types of cerebellar neurons project to the pretectum.

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Key words：cerebellar nuclei, pretectum, projecting neuron, cat

INTRODUCTION

Based on Golgi studies, neurons of the cerebellar nuclei have been classified into three groups of different size: large ($40–50\mu m$), medium-sized ($25–35\mu m$) and small ($12.5–20\mu m$) cells[1)2)]. These cells of different sizes have different dendritic patterns. Large neurons were multipolar, polygonal or radiate in shape, whereas medium-sized neurons were polygonal, fusiform or spindle in shape. These large and medium-sized neurons were considered to be projecting neurons. On the other hand, small neurons were oval or spindle in shape and belong to a category of short-axoned cells that were considered to be interneurons. Recent studies evaluated, however, that the small neurons of the cerebellar nuclei also project to the brainstem structures[3)4)] and the cerebellar cortex[5)] in the rat.

The projections from the cerebellar nuclei to the pretectum have been studied by using silver impregnation, autoradiographic tracing, retrograde horseradish peroxidase tracing, and retrograde wheat germ agglutinin conjugated to horseradish peroxidase tracing methods in the cat[6)–12)]. After injection of retrograde tracers into the pretectum, labeled neurons were reported to distribute bilaterally in the medial cerebellar nuclei, and contralaterally in the interpositus and lateral cerebellar nuclei[8)12)]. Types of pretectal projecting neurons, however, are not yet studied in the cerebellar nuclei.

The aim of this study was to know which type of cerebellar neurons project to the pretectum. We quantitatively analyzed the size and shape of cerebellar nuclear neurons in the cerebellar nuclei projecting to the pretectum by retrograde labeling technique using biotinylated dextran amine (BDA).

METHODS

Experimental Animals

A total of six adult cats of both sexes, weighting 3.0–4.2 kg, were used in this study. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and with the guidelines for care and use of animals established by the Animal Care and Use Committee of Gifu University.

Injection of BDA

The animals were sedated with an intramuscular injection of ketamine hydrochloride (50mg/kg), followed with an injection of sodium pentobarbital (20mg/kg). The animals were placed in a stereotaxic apparatus and a small craniotomy was done over the appropriate stereotaxic position of the pretectum. The injection glass micropipettes (tip diameter 20–30 μm) were located in the pretectum and $0.1–0.3\mu\ell$ solution of 20% biotinylated dextran amine (BDA) (BDA-10,000 : Molecular Probes, Eugene, OR, USA) was injected under intermittent gas pressure (0.7–1.0kPa, 20–30msec, 5–20times)

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using a Picospritzer 2 (General Value, Fairfield, NJ, USA). The amount of the tracer injected was determined by movement of the level of the tracers observed under an operation microscope.

Perfusion

After a survival period of two weeks, the animals were anesthetized with intraperitoneal administration of a lethal dose of sodium pentobarbital (80mg/kg) and perfused transcardially with 1 liter of saline followed by 2 liter of 4% paraformaldehyde in 0.1M phosphate buffer (pH7.3). After two days immersion in 20% sucrose solution in the buffer, 50–μm thick sections through the pretectal region and cerebellum were cut frozen in the frontal plane.

Immunohistochemistry

One-in-four serial sections were reacted with the Elite ABC kit (Vector, Burlingame, CA, USA) and then were incubated with diaminobenzidine tetrahydrochloride (DAB; Wako) with glucose oxidase (Sigma) for BDA histochemisty[13]. Another one-in-four serial sections were processed for Nissl-staining with Cresyl violet. The contours of the sections and the locations of the labeled neurons were observed under a brightfield microscope (Nikon, Tokyo, Japan), and the contour of the labeled neurons was drawn using a camera lucida. Quantitative analysis of the labeled neurons was performed with NIH Image software.

RESULTS

A total of 454 cerebellar neurons were retrogradely labeled after the pretectal injections. Fig. 1 shows one case, which has the most extensive injection sites among six brains. In this case, the tracer covers the most part of the pretectum and encroaches upon the dorsolateral part of the central gray and the lateral posterior nucleus of the thalamus, but does not encroach upon the superior collicolus, red nucleus and the oculomotor complex. In other cases, the injections cover most of the pretectum but slightly involve the lateral posterior nucleus of the thalamus. The tracer diffuses into the lateral posterior nucleus along the tract of the injection micropipette.

Fig. 2 shows the distribution of labeled neurons of the case CAT 11. We analyzed this case and other cases. Most of the labeled cells were situated in the contralateral lateral cerebellar nucleus (76%). As was already reported[10], most of these neurons were seen in the ventral part of the contralateral lateral cerebellar nucleus, with some neurons scattered in the dorsocaudal part. In addition, we found a few neurons in the ipsilataral lateral cerebellar nucleus. A few labeled neurons were also seen bilaterally in the caudal half of the medial cerebellar nucleus (15%) and contralaterally in the anterior and posterior interpositus cerebellar nuclei (9%) (Table 1).

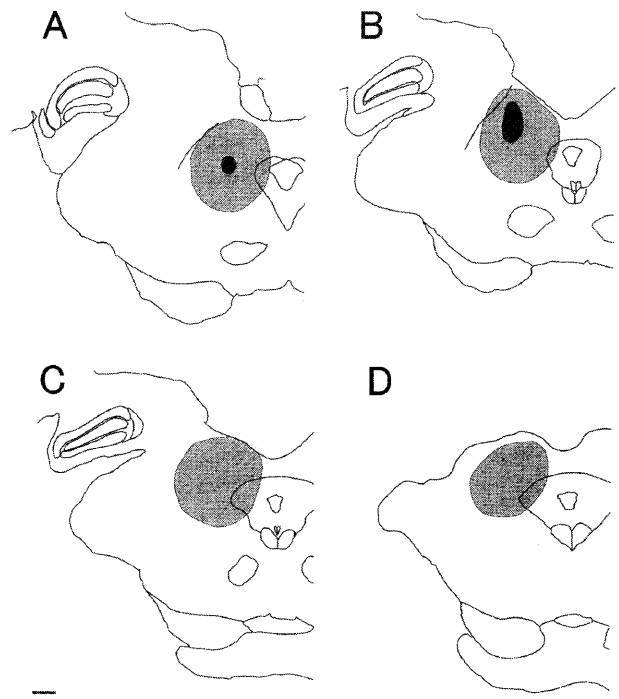

Fig. 1 Outline drawings of frontal sections through the pretectal region to show the extent of the injection site in the case CAT11. In this fig. the drawings are arranged from rostral (A) to caudal (D). Scale bar=1mm

We analyzed the shape of the labeled pretectal projecting neurons and divided them into three types: multipolar (Fig. 3 A, D, G), trigonal (Fig. 3B, E, H), and bipolar neurons (Fig. 3C, F, I). The size of these neurons ranged from 50μm^2 to 1500μm^2. We arbitrarily divided these neurons into three subclasses: small (50–200μm^2, Fig. 3G, H, I), medium-sized (200–450μm^2, Fig. 3D, E, F), and large (450–1500μm^2, Fig. 3A, B, C) cells.

Analysis of a total of labeled cells (454) showed that most of the large cells (65%) were multipolar in shape, whereas most of the small cells (59%) were bipolar in shape. In the lateral cerebellar nucleus, 41% of the cells (143) were large, 47% of the cells (162) were medium-sized, and 12% (42) were small cells. Most of the large (68%) and medium-sized (51%) cells were multipolar in shape, whereas, most of the cells (60%) were bipolar in shape. In the interpositus cerebellar nucleus, 40% of the cells (16) were large, 47% of the cells (19) were medium-sized, and 13% (5) were small cells. Most of the labeled cells in the interpositus nucleus were multipolar in shape (53%). In the medial cerebellar nucleus, 34% of the cells (23) were large, 52% of the cells (35) were medium-sized, and 14% (9) were small cells. Most of the labeled cells in the medial nucleus were multipolar in shape (57%) (Table 1).

Fig. 4 shows the result of a quantitative histological analysis of the numerical and metrical parameters of the projection neurons to the pretectum. To compare our data to those shown by Palkovits *et al.*[14], the longest (L) and the perpen-

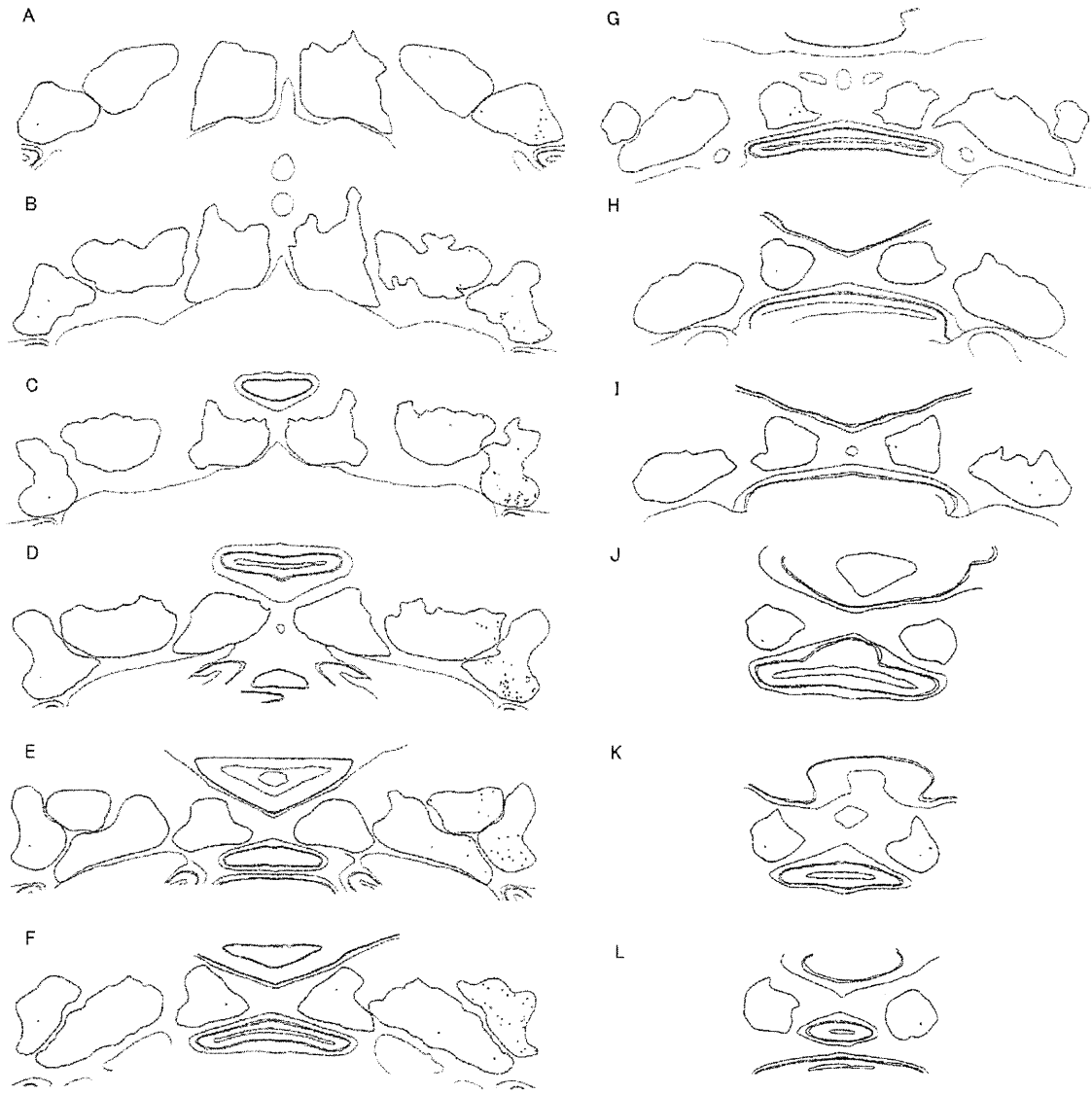

Fig. 2 Outline drawing to show the distribution of retrogradely labeled neurons (dots) in the cerebellar nuclei of one case. In this fig. sections arranged from rostral (A) to caudal (L). Scale bar=1mm

Table 1 Summary of the number of retrogradely labeled neurons in all cases in which BDA was injected into the pretectum. In all cases, retrogradely labeled neurons were observed in the lateral nucleus (LCN), the interpositus nucleus (IN) and the medial nucleus (MCN).

	Large			Medium-sized			Small				
	Multi	Tri	Bi	Multi	Tri	Bi	Multi	Tri	Bi	SUM	%
LCN	97	33	13	82	36	44	13	4	25	347	76%
IN	8	6	2	12	2	5	1	1	3	40	9%
MCN	14	7	2	21	4	10	3	1	5	67	15%
	119	46	17	115	42	59	17	6	33		
SUM	182			216			56			454	
%	40%			48%			12%				100%

dicular bisecting diameter (B) of the labeled neurons were measured in accordance with general principles of karyometry[14]. The areas of labeled neurons were quantitatively evaluated using the NIH Image software. From each of cerebellar nuclei, 40–347cells were measured. We calculated the excentricity of these cells (Ex= L/B) and evaluated the square area (S) of these cells. Log S of each cell was then plotted against the excentricity of the same cell, so that in the diagram of Fig. 4 each dot corresponds to one cell and each diagram is given for each cerebellar nucleus. By this procedure cell populations of different size and shape should appear as concentrations of dots. The dots appeared over large, medium-sized, and small cell regions without clear gap of distribution.

DISCUSSION

We analyzed the shape of the labeled pretectal-projecting neurons and divided them into three types: multipolar, trigonal, and bipolar neurons. These neurons were further divided into three subclasses: small, medium-sized, and large cells. In the lateral cerebellar nucleus, most of the large and medium-sized labeled cells were multipolar in shape and small labeled cells were mostly bipolar in shape. In the interpositus and me-

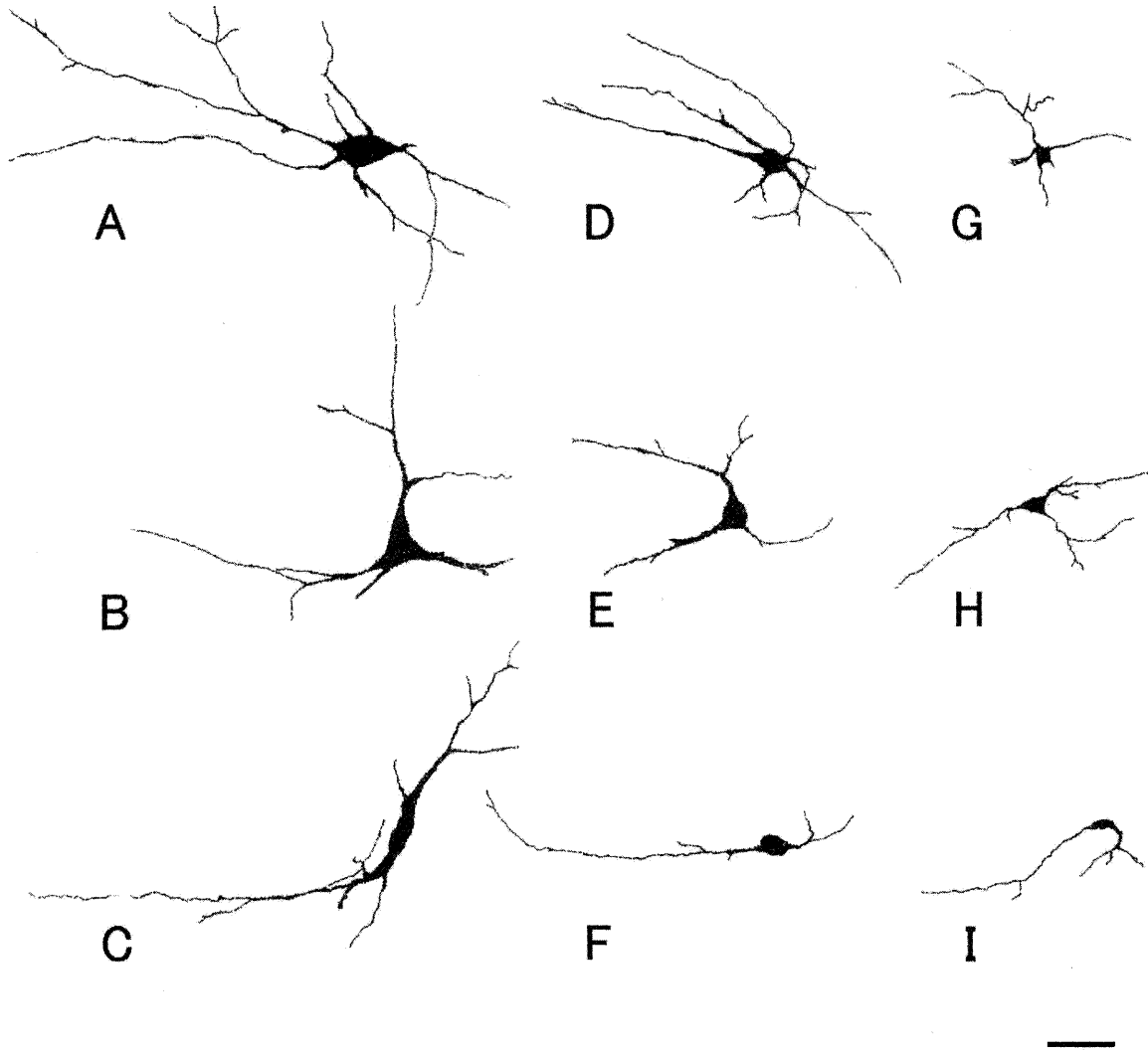

Fig. 3 Drawing of different types of celebellar nuclei neurons labeled retrogradely with BDA injected into the pretectum. (A, B, C). Large neurons. (D, E, F). Medium-sized neurons. (G, H, I). Small neurons. (A, D, G). Multipolar neurons. (B, E, H). Trigonal neurons. (C, F, I). Bipolar neurons. Scale bar=50μm

dial cerebellar nucleus, most of the labeled cells were multipolar in shape.

By using Golgi method, Matsushita and Iwahori reported that neurons of the cerebellar nuclei have three different sizes: large (40–50μm), medium-sized (25–35μm) and small (12.5–20μm) cells[1)2)]. They reported that large neurons were multipolar, polygonal or radiate in shape. Medium-sized neurons were polygonal, fusiform or spindle in shape, while small neurons were oval or spindle in shape. Their polygonal and radiate neurons are considered to be multipolar neurons in the present study. On the other hand, fusiform and spindle-shaped neurons described by them are bipolar neurons. Because of the shrinkage (10.2%–64.8%)[15)] in fixation and embedding processes of the Golgi preparation, we are not able to compare the present measurement directly with those of Matsushita and Iwahori, but the features of these classes with different sizes are basically the same as those shown in the present study. Matsushita and Iwahori considered that large and medium-sized neurons were projecting neurons and small neurons were interneurons[1)], however, the present results provide the evidence that not only the large and medium-sized cells but also small cells project to the pretectum.

Gamma aminobutyric acid (GABA) and glutamate (Glu) are shown to be immunoreactive in the cerebellar nuclei[16)17)]. Batini *et al*. showed that small neurons of cerebellar nuclei are GABA immunoreactive and large and medium-sized neurons are Glu immunoreactive[5)]. Because GABA and Glu are thought to be the principal substances in the central nervous system responsible for neuronal inhibition and excitation, we suggest that the large and medium-sized projecting neurons to the pretectum are excitatory and the small projecting neurons to the pretectum are inhibitory.

In the present study, several different types of neurons in the cerebellar nuclei are shown to project to the pretectum. This is in accordance with previous studies that showed the existence of several different projections from the cerebellar nuclei to the pretectum. For example, neurons of the ventrolateral part of the contralateral lateral nucleus project to the

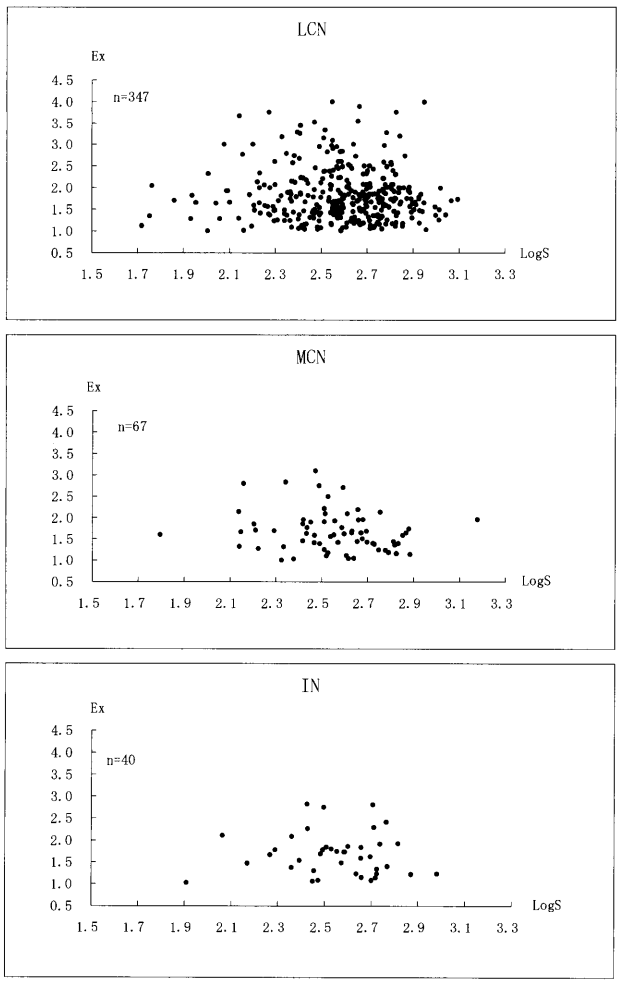

Fig. 4 Dot-diagrams for the separation of different cell populations in the various cerebellar nuclei on the basis of cell size and shape characteristics. Each dot represents one cell by plotting excentricity quotient Ex (=longest: bisecting perpendicular diameter) against log area (Log S). The large cells are considered that have an area larger than450μm^2 (Log450=2.7). The medium-sized cells have an area ranging from 200μm^2 to 450μm^2. The small cells have an area smaller than200μm^2 (Log200=2.3). The dots appeared over large, medium-sized, and small cell regions without clear gap of distribution.

rostrodorsal portions of the nucleus of the posterior commissure, the ventral regions of the posterior pretectal nucleus, and the reticular part of the anterior pretectal nucleus[9)12)]. The posterior half of the medial cerebellar nucleus projects bilaterally to the nucleus of the posterior commissure and the reticular part of the anterior pretectal nucleus of the pretectum[7)10)11)]. The lateral part of the anterior cerebellar interpositus nucleus projects contralaterally to the ventral half part of the posterior pretectal nucleus and the compact part of the anterior pretectal nucleus[8)9)]. The lateral part of the posterior cerebellar interpositus nucleus projects contralaterally to the nucleus of the posterior commissure[9)]. We thus suggest that different types of neurons may project to different pretectal nuclei.

Most of the pretectal projection neurons were distributed in the lateral nucleus. The lateral cerebellar nucleus receives cerebellar cortical afferents from the most lateral part of the anterior lobe, the paramedial lobe, crus I and crus II, and the paraflocculus and flocculus in the cat[18)~23)]. The paraflocculus and flocculus receive afferents from the reticular pontine tegmental nucleus[24)25)] and inferior olive[26)]. These brainstem structures are the link in the pathway mediating visual input from the tectum[27)28)] and the nucleus of the optic tract[26)] to the ventrolateral region of the lateral cerebellar nucleus. The lateral cerebellar nucleus, in turn, projects to the pretectum. This projection may mediate orientation movement of the eye and other parts of the body. The pretectum also receives projections from the caudal half of the medial cerebellar nuclei bilaterally. The caudal half of the medial cerebellar nuclei receives afferents from Larsell's VI and VII lobes, a vermal portion which is known to receive visual impulses and is connected to the rhombencephalic structures participating in the regulation of eye movements[29)~33)]. This projection may mediate the regulation of eye movements.

Several studies have demonstrated that the pretectum, which receives inputs from all the cerebellar nuclei, also receives inputs from the visual cortex, somatosentory and auditory structures[34)35)]. The pretectum plays a role not only in eye movement[36)] but also in the body orientation movements[12)], it is possible that the projection from the different cerebellar nuclei to the pretectum may mediate different functions. Additionally, different cell classes may mediate different role in each functions, so it is interest to investigate the projections from different types of cerebellar neurons to the subnuclei of the pretectum in the future.

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ネコ・小脳核の視蓋前域投射ニューロンの構成

逆行性標識物質のビオチン化デキストランアミンをネコの視蓋前域に注入して，小脳核の視蓋前域投射ニューロンの構成を調べた。逆行性に標識されたニューロンは両側の内側核と外側核および反対側の中位核に認められた。これらの標識ニューロンの大きさと形を解析した。視蓋前域への投射ニューロンは形から，multipolar（多極性），trigonal（三極性），bipolar（双極性）の三つのタイプに分類された。さらにこれらのニューロンは大きさからそれぞれ三つのグループ（小細胞，中等大細胞，大細胞）に分類された。多極性ニューロンの大部分は大型で，双極性細胞の大部分は小型であった。これらの結果から，小脳—視蓋前域投射は異なる種類のニューロンから構成されていることが明らかとなった。

キーワード：小脳核，視蓋前域，投射ニューロン，ネコ