

## Projections from the medial column of the inferior olive to different classes of rotation-sensitive Purkinje cells in the flocculus of pigeons

Douglas R.W. Wylie<sup>\*</sup>, Ian R. Winship, R.G. Glover

*Department of Psychology, University of Alberta, Edmonton, AB, Canada T6G 2E1*

Received 15 February 1999; received in revised form 26 April 1999; accepted 26 April 1999

### Abstract

In the flocculus of pigeons, as in other species, there are two major types of Purkinje cell responses to rotational optokinetic stimuli. One type prefers rotation about the vertical axis (VA neurons) whereas the other prefers rotation about an horizontal axis oriented at 135° ipsilateral azimuth (H-135 neurons). In this study, we injected the retrograde tracer cholera toxin subunit B into the VA and H-135 zones in attempt to determine the origin of inferior olive inputs. We found that VA and H-135 zones received input from the caudal and rostral margins of the medial column of the inferior olive, respectively. There is a similar pattern of connectivity in mammalian species. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Climbing fiber; Optokinetic; Vestibulocerebellum; Flocculus; Inferior olive; Retrograde tracer; Pigeon

Previous electrophysiological studies in mammals, have shown that the complex spike activity of Purkinje cells in the vestibulocerebellum (VbC; flocculus, nodulus and ventral uvula) is modulated by rotational optic flow stimuli [3,4,6,9]. In this regard, there are two major types of neurons. One type responds best to rotational optic flow about the vertical axis, whereas the other type responds best to rotation about an horizontal axis that passes through 135° ipsilateral azimuth (and 45° contralateral azimuth) [3,9]. We refer to these as VA and H-135 neurons. In pigeons VA and H-135 neurons are also found in the flocculus, but Purkinje cells in the ventral uvula and nodulus respond best to translational optic flow stimuli [13–16].

In mammals, the climbing fiber (CF) input to the VA zones arises from the caudal dorsal cap (dc) of the inferior olive (IO), whereas the CF input to the H-135 zones is from the rostral DC and the ventrolateral outgrowth (vlo) [8,11]. In pigeons, the CF inputs arise from the medial column (mc) of the IO [1], and recently we have shown that the rotation and translation areas receive CF inputs from the medial and lateral margins of the mc, respec-

tively [7]. In this study we attempted to determine different areas of the mc project to the VA and H-135 zones in the pigeon flocculus.

Methods for anesthesia, surgery and extracellular recording can be found elsewhere [7,14,15]. Anaesthetized animals were placed in a stereotaxic device and the dorsal surface of the flocculus was exposed. Extracellular recordings were made with glass micropipettes (5 µm tip diameter) containing 2 M NaCl and Purkinje cell CS activity was recorded in response to rotational optic flow stimuli [14]. In mammals, studies have shown that the VA and H135 cells are found localized to specific parasagittal zones. For example, in the rabbit flocculus there are two VA zones and two H135 zones [11]. We have yet to identify the precise location and number of zones in the pigeon flocculus, although the VA and H135 cells were found in clusters, and not intermingled. After identification of the cell as either a VA or H-135 neuron, the recording electrode was removed and replaced with a micropipette (12–18 µm tip diameter) containing low-salt cholera toxin subunit B (CTB; Sigma, St. Louis, MO; 1% in phosphate-buffered saline). CS activity was also recorded with the injection electrode to confirm the cell type, and the solution was iontophoretically injected (+3 µA, 7 s on, 7 s off) for 3–5 min. After a survival time of 3–5 days, the animals were given an overdose of sodium pentobarbitol (100 mg/kg) and

<sup>\*</sup> Corresponding author. Tel.: +1-780-492-5274; fax: +1-780-492-1768.

E-mail address: dwylie@ualberta.ca (D.R.W. Wylie)

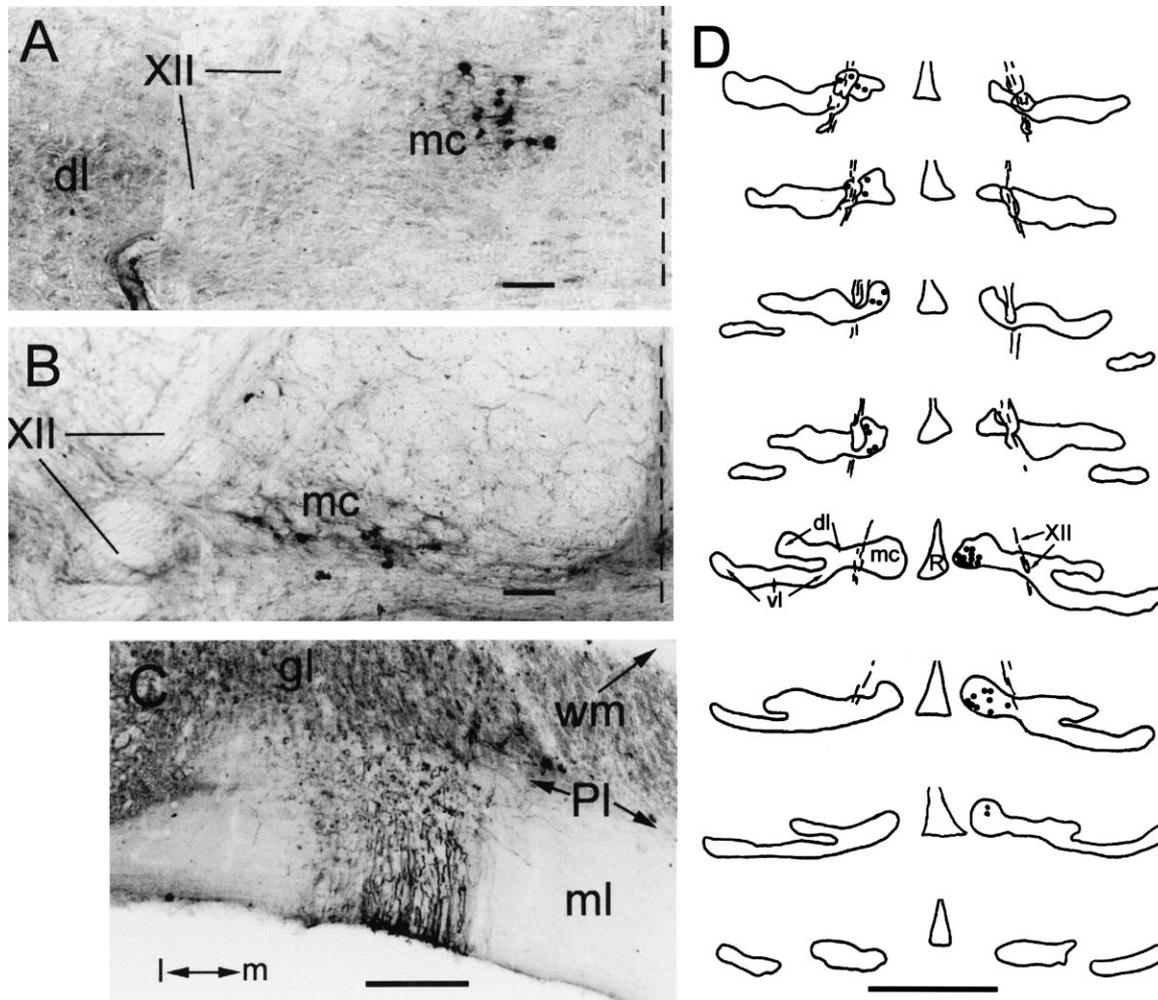


Fig. 1. Location of retrograde-labeled neurons in the inferior olive (IO) after injections into VA and H-135 zones of the pigeon flocculus. (A,B) Respectively, show representative photomicrographs of retrogradely labeled cells in the medial column (mc) of the inferior olive resulting from injections into VA (from case No. 3) and H-135 (from case No. 5) zones. In these sections the broken lines indicate the midline. The section shown in (B) was located in the extreme caudal margin of the IO, about 1mm rostral to the section shown in (A). (C) Shows the injection in the ventral lamella of folium IXc,d from the left side of case No. 1. Most apparent from the injection sites was the labeled climbing fibres (and Purkinje cell dendrites) extending into the molecular layer (ml). (D) Shows a series of sections, (caudal to rostral; about 170  $\mu\text{m}$  apart), through the inferior olive of case No. 1. The retrogradely labeled cells in the left and right IO resulted from injections in the VA and H-135 zones of the flocculus, respectively. dl, dorsal lamella of the inferior olive; gl, granule layer; PI, Purkinje cell layer; wm, cerebellar white matter; XII, cranial nerve XII. Scale bars, 80  $\mu\text{m}$ , (A,B); 160  $\mu\text{m}$  (C); 1 mm (D).

immediately perfused with saline (0.9%) followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were extracted and 44- $\mu\text{m}$  thick coronal sections through the IO and cerebellum were processed for CTB using the protocol described by Wild [12] (for additional details see Ref. [7]). The tissue was mounted on gelatin-coated slides, lightly counterstained with Neutral Red, and examined using light microscopy.

In total, there were seven injections in the five pigeons: in two cases the flocculus was injected bilaterally, and in three cases the injection was unilateral. In pigeons, the flocculus consists of the lateral extension of folia IXc,d and X. In case No. 1, a bilateral injection, VA cells were recorded at the injection site in the right flocculus and H-135 cells were

recorded at the injection site in the left flocculus. Both of these injection sites were located in the ventral lamella of folium IXc,d. Case No. 2 was a unilateral injection in ventral IXc,d of the left flocculus at a site where VA cells were recorded. Cases Nos. 3 and 4 were unilateral injections at sites containing H-135 cells. In case No. 3 the injection was restricted to dorsal IXc,d, whereas in case No. 4, the injection was concentrated in ventral IXc,d, but there was evidence of spread into dorsal IXc,d and folium X. In case No. 5, a bilateral injection, VA cells were recorded at the injection site in the right flocculus (ventral X) and H-135 cells were recorded at the injection site in the right flocculus (ventral IXc,d). Fig. 1C shows the injection site from case No. 3. The injection site can easily be distinguished by the

Table 1

Distribution of retrogradely labeled cells in the medial column (mc) after injections of cholera toxin subunit B into the VA and H-135 zones of the pigeon flocculus<sup>a</sup>

Sections	Case No. 1 VA (%)	Case No. 2 VA (%)	Case No. 5 VA (%)	Case No. 1 H-135 (%)	Case No. 3 H-135 (%)	Case No. 4 H-135 (%)	Case No. 5 H-135 (%)
–30 to –25	0	1 (1)	22 (22)	0	8 (9)	0	0
–24 to –20	11 (29)	14 (14)	48 (49)	1 (1)	15 (17)	2 (2)	0
–19 to –15	13 (34)	45 (45)	22 (22)	2 (3)	10 (11)	17 (13)	4 (13)
–14 to –10	12 (32)	28 (28)	5 (5)	6 (8)	0	19 (15)	9 (29)
–9 to –5	2 (5)	11 (11)	1 (1)	35 (48)	13 (14)	64 (50)	14 (45)
–4 to 0	0	0	0	27 (37)	40 (44)	26 (20)	3 (10)
+1 to 5	0	0	0	2 (3)	4 (4)	0	1 (3)

<sup>a</sup> The total number of cells observed in five successive coronal sections is tabulated for the rostro-caudal extent of the mc for each of seven cases. The sections were numbered relative to the rostral tip of the nucleus of cranial nerve XII (–ve, caudal; +ve, rostral). The percentage of the total number of cells for each case is indicated in parentheses.

abundance of CTB-labeled CFs and/or Purkinje cell dendrites extending into the molecular layer (ml) of the cerebellar cortex.

In all cases, retrogradely labeled cells were found in the medial margin of the contralateral mc. The difference in the distribution of retrogradely labeled cells resulting from the VA and H-135 injections was consistent and unambiguous. From the VA injections, retrogradely labeled cells were found in the caudal mc, whereas from the H-135 injections, retrogradely labeled cells were found in the rostral mc. Photomicrographs of coronal sections through the IO showing retrogradely labeled cells from injections into the H-135 and VA zones are shown in Fig. 1A,B, respectively. The section in Fig. 1B shows the extreme caudal margin of the mc, and was located about 1 mm caudal to the section shown in Fig. 1A. Fig. 1D shows camera lucida drawings of a caudal-to-rostral series of coronal sections through the IO from case No. 5. Retrogradely labeled cells in the left and right IO resulted from injections into VA cells in the right flocculus and H-135 cells in the left flocculus, respectively. Note the clear rostral-caudal difference in the distribution of cells in the mc. Table 1 summarizes the distribution of retrogradely labeled cells from all cases. For this table, the coronal sections were numbered relative to the rostral tip of the nucleus of cranial nerve XII (caudal, –ve; rostral +ve). Table 1 clearly shows that in all cases of injections in VA and H-135 zones, labeling was found in the caudal and rostral mc, respectively. The only exception was case No. 3, an injection in a H-135 zone. The majority of the labeling was found rostrally, but there was a second smaller group of cells found more caudally. We suggest that the injection may have encroached upon a neighboring VA zone in this case.

The results of the present study bear a striking similarity to the connectivity in mammalian species. In the mammal flocculus, the VA zones receive input from the caudal dc, whereas the H-135 zones receive input from the rostral dc and vlo [8,11]. Similarly, we have found that the VA and H-135 zones in the pigeon flocculus receive CF input from the caudal and rostral mc, respectively. Although the mc in

birds is not morphologically identical to the dc in mammals, our results suggest that the general features of the olivary projection to the rotation zones of the VbC are highly conserved, and may exist in stem reptiles.

Despite this striking similarity in the projection pattern of the rotation system in birds and mammals, there are also striking differences. In mammals, those areas of the IO projecting to the VA and H-135 zones in the flocculus, also project to VA and H-135 zones in the ventral uvula and nodulus [2,5,8,11]. In fact, some individual neurons in the dc project to both the flocculus and the nodulus [10]. In the pigeon, the nodulus and ventral uvula do not contain VA and H-135 zones, but rather respond best to translational optic flow along one of three orthogonal axes [13,15]. These translation areas of the pigeon VbC receive CF input from the ventro-lateral margin of the mc [7].

This research was supported by grant funding from the Natural Sciences and Engineering Research Council of Canada and the Alberta Heritage Foundation for Medical Research to D.R.W.W.

- [1] Arends, J.J.A. and Voogd, J., Topographic aspects of the olivocerebellar system in the pigeon. *Exp. Brain Res.*, 17 (1989) 52–57.
- [2] Balaban, C.D. and Henry, R.T., Zonal organization of olivonodulus projections in albino rabbits. *Neurosci. Res.*, 5 (1989) 409–423.
- [3] Graf, W., Simpson, J.I. and Leonard, C.S., Spatial organization of visual messages of the rabbit's cerebellar flocculus. II. Complex and simple spike responses of Purkinje cells. *J. Neurophysiol.*, 60 (1988) 2091–2121.
- [4] Kano, M., Kano, M.S., Kusunoki, M. and Maekawa, K., Nature of optokinetic response and zonal organization of climbing fibre afferents in the vestibulocerebellum of the pigmented rabbit. II. The nodulus. *Exp. Brain Res.*, 80 (1990) 238–251.
- [5] Katayama, S. and Nisimaru, N., Parasagittal zonal pattern of olivonodular projections in rabbit cerebellum. *Neurosci. Res.*, 5 (1988) 424–438.
- [6] Kusunoki, M., Kano, M., Kano, M.S. and Maekawa, K., Nature of optokinetic response and zonal organization of climbing fibre afferents in the vestibulocerebellum of the

- pigmented rabbit. I. The flocculus, *Exp. Brain Res.*, 80 (1990) 225–237.
- [7] Lau, K.L., Glover, R.G., Linkenhoker, B. and Wylie, D.R.W., Topographical organization of inferior olive cells projecting to translation and rotation zones in the vestibulocerebellum of pigeons. *Neuroscience*, 85 (1998) 605–614.
- [8] Ruigrok, T.J.H., Osse, R.J. and Voogd, J., Organization of inferior olivary projections to the flocculus and ventral paraflocculus of the rat cerebellum. *J. Comp. Neurol.*, 316 (1992) 129–150.
- [9] Simpson, J.I., Graf, W. and Leonard, C., The coordinate system of visual climbing fibres to the flocculus. In A.F. Fuchs and W. Becker (Eds.), *Progress in Oculomotor Research*, Elsevier, Amsterdam, 1981, p. 475.
- [10] Takeda, T. and Maekawa, K., Olivary branching projections to the flocculus, nodulus and uvula in the rabbit. II. Retrograde double labelling study with fluorescent dyes. *Exp. Brain Res.*, 76 (1989) 323–332.
- [11] Tan, J., Gerrits, N.M., Nanhoe, R.S., Simpson, J.I. and Voogd, J., Zonal organization of the climbing fibre projection to the flocculus and nodulus of the rabbit. A combined axonal tracing and acetylcholinesterase histochemical study. *J. Comp. Neurol.*, 356 (1995) 23–50.
- [12] Wild, J.M., Descending projections of the songbird nucleus robustus archistrialis. *J. Comp. Neurol.*, 338 (1993) 225–241.
- [13] Wylie, D.R.W., Bischof, W.F. and Frost, B.J., Common reference frame for the coding translational and rotational optic flow. *Nature*, 392 (1998) 278–282.
- [14] Wylie, D.R. and Frost, B.J., Responses of pigeon vestibulocerebellar neurons to optokinetic stimulation: II. The 3-dimensional reference frame of rotation neurons in the flocculus. *J. Neurophysiol.*, 70 (1993) 2647–2659.
- [15] Wylie, D.R.W. and Frost, B.J., Complex spike activity of Purkinje cells in the ventral uvula and nodulus of pigeons in response to translational optic flowfields. *J. Neurophysiol.*, (1999) in press.
- [16] Wylie, D.R., Kripalani, T.K. and Frost, B.J., Responses of pigeon vestibulocerebellar neurons to optokinetic stimulation: I. Functional organization of neurons discriminating between translational and rotational visual flow. *J. Neurophysiol.*, 70 (1993) 2632–2646.