TOPOGRAPHICAL ORGANIZATION OF INFERIOR OLIVE CELLS PROJECTING TO TRANSLATION AND ROTATION ZONES IN THE VESTIBULOCEREBELLUM OF PIGEONS

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Abstract—Previous electrophysiological studies in pigeons have shown that the vestibulocerebellum can be divided into two parasagittal zones based on responses to optic flow stimuli. The medial zone responds best to optic flow resulting from self-translation, whereas the lateral zone responds best to optic flow resulting from self-rotation. This information arrives from the retina via a projection from the accessory optic system to the medial column of the inferior olive. In this study we investigated inferior olive projections to translational and rotational zones of the vestibulocerebellum using the retrograde tracer cholera toxin subunit B. Extracellular recordings of Purkinje cell activity (complex spikes) in response to large-field visual stimuli were used to identify the injection sites. We found a distinct segregation of inferior olive cells projecting to translational and rotational zones of the vestibulocerebellum. Translation zone injections resulted in retrogradely labeled cells in the ventrolateral area of the medial column, whereas rotation zone injections resulted in retrogradely labeled cells in the dorsomedial region of the medial column.

Motion of any object through space, including self-motion of organisms, can be described with respect to translation and rotation in three-dimensional space. Our results show that, in pigeons, the brainstem visual systems responsible for detecting optic flow are segregated into channels responsible for the analysis of translational and rotational optic flow in the inferior olive, which is only two synapses from the retina.

Key words: optokinetic, optic flow, accessory optic system, visual–vestibular integration, self-motion, cholera toxin subunit B.

The motion of any object through space can be described with respect to its translation between two points in space, and its rotation relative to some frame of reference. Any organism moving through space has a nervous system designed to provide information about self-motion. The vestibular apparatus contains the semicircular canals and the otolith organs, which are sensitive to head rotation and translation, respectively. Numerous other sensory systems contribute to the analysis of self-motion, including the visual system. That vision can serve as a proprioceptive sense was emphasized by Gibson, who noted that, because the environment contains numerous stationary visual stimuli, self-motion induces "flow fields" or "optic flow" across the entire retina. Self-rotation results in a rotational flow field that is opposite to the direction of one's head rotation. The flow field resulting from self-translation consists of a "focus of expansion" (fe), which is a point in the direction of translation from which all visual images radiate outward. Along the axis of translation, but in the direction opposite to the translation vector, is a "focus of contraction", a point to which all visual images converge. The accessory optic system is a component of the visual system dedicated to the analysis of optic flow. Nuclei of the accessory optic system project to areas of the inferior olive (IO) that provide climbing fiber input to the contralateral vestibulocerebellum (VbC). In pigeons, the VbC consists of folium X (nodulus), the ventral lamella of folium IXc,d (ventral uvula) and the auricle (flocculus), which is the lateral extension of these folia. Previous electrophysiological studies of the pigeon VbC have shown that complex-spike (CS) activity of Purkinje cells (which reflects climbing fiber activity) responds best to specific patterns of optic flow resulting from either self-translation or self-rotation. Translation- and rotation-sensitive neurons are organized into two parasagittal zones. Cells in the medial VbC (nodulus and ventral uvula) and lateral VbC (flocculus) respond best to translational and rotational optic flow, respectively. The boundary between the two zones was located approximately 1.8-2 mm from the midline.

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Abbreviations: BDA, biotinylated dextran amine; CS, complex spike; CTB, cholera toxin subunit B; DAB, diaminobenzidine; dl, dorsal lamella; fe, focus of expansion; IO, inferior olive; mc, medial column; PB, phosphate buffer; PBS, phosphate-buffered saline; VA, vertical axis; VbC, vestibulocerebellum; vl, ventral lamella; XII, cranial nerve XII (hypoglossal nerve).
Table 1. A summary of the locations of injection sites and retrogradely labeled cells in the inferior olive

<table>
<thead>
<tr>
<th>Injection site</th>
<th>Distance from midline (range in µm, medial-lateral)</th>
<th>Rostral</th>
<th>Caudal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preferred visual stimulus</td>
<td>Folium</td>
<td>Distance from midline (mm)</td>
<td></td>
</tr>
<tr>
<td>Translation zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>feVAd</td>
<td>Ventrail IXc, d</td>
<td>2.3</td>
</tr>
<tr>
<td>10</td>
<td>feVAv</td>
<td>Ventrail X</td>
<td>1.6</td>
</tr>
<tr>
<td>12 (left side*)</td>
<td>fe45i</td>
<td>Ventrail X</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>feVAd</td>
<td>Dorsal X</td>
<td>2.2</td>
</tr>
</tbody>
</table>

The side of the injection site.

The visual response types, the folia injected and the distance from the midline of each injection site are indicated. The mediolateral range of retrogradely labeled cells in the IO at four rostrocaudal levels (200–250 µm apart) is also indicated for each case.

The pigeon IO consists of a dorsal (dl) and ventral (vl) lamella, which are joined by the medial column (mc; see Fig. 4B). Arends and Vosog1 reported that the VbC receives climbing fiber input largely from the contralateral mc, and to lesser extent the vl and dl. It was the purpose of this study to investigate the organization of the IO projections to the physiologically identified translational and rotational zones of the VbC using the retrograde tracer cholera toxin subunit B (CTB).

EXPERIMENTAL PROCEDURES

Surgery

The methods reported herein conformed to the guidelines established by the Canadian Council on Animal Care and were approved by the Biociences Animal Care and Policy Committee at the University of Alberta. These guidelines required that efforts were made to ensure that animal discomfort was minimized and that as few animals as possible were used. Experiments were performed on Silver King and Racing Homer pigeons (Palmetto Pigeon Plant, Sumpter, N.C., U.S.A.) anesthetized with a ketamine (90 mg/kg)/xylazine (15 mg/kg) mixture (i.m.); supplemental doses were administered as necessary. The animals were placed in a stereotaxic device with pigeon ear bars and beak adapter so that the orientation of the skull conformed with the atlas of Karten and Hodos.13 Sections of bone and dura were removed to expose the dorsal surface of the flocculus (auricle) of the cerebellum in the area contained by the anterior canal of the vestibular apparatus. On initial penetrations, extracellular recordings were made with glass micropipettes (4 µm tip diameter) filled with 2 M NaCl which were oriented 45° to the sagittal plane.

Once CSs of Purkinje cells were isolated, their optic flow field preferences were determined using stimuli described in detail elsewhere.29 As a large (about 90° × 90°) stimulus, consisting of a pattern of dark lines and dots on a light background, moving in the central region of each visual field (i.e., along the inter-aural axis), was initially used to identify the CS responses. With this stimulus, translation and rotation cells are easily distinguishable. The former prefer the same direction of motion in this region of both visual fields, whereas the latter prefer the opposite directions in the two visual fields.29,31 In some cases, we also used a full-field planetarium projector to present rotational flow fields,30 and a full-field “translator” projector to present translational flow fields.30 As in other species,2,7 rotation cells in the pigeon VbC can be functionally classified into two types: rVA cells prefer rotation about the vertical axis (VA), whereas r45 cells prefer rotation about a horizontal axis that is oriented at 45° contralateral azimuth.30 Translational cells can be classified into four functional groups,29,31,32 that are most easily described with reference to the location of the fe in the flow field that results in maximal excitation. We have dubbed these four groups feVAd, feVAv, fe45i, and fe135i. The feVAd neurons are excited in response to translational optic flow along the VA with the fe dorsal to the animal’s head. The feVAv neurons have the opposite direction preference. They prefer translation along the VA but with the fe ventral to the animal’s head. The flow fields that maximally excite feVAd and feVAv cells would result from the pigeon ascending and
descending, respectively. The two other types of cells prefer translational optic flow along horizontal axes. The \( \text{fe}45^i \) cells respond best to a translational flow field with an \( \text{fe} \) at 45° ipsilateral azimuth. The \( \text{fe}135^i \) cells respond best to a translational flow field with an \( \text{fe} \) at 135° ipsilateral azimuth.

Once the flow field preference was identified, the recording electrode was removed and replaced with a pipette (tip diameter 16–20 µm) containing CTB (Sigma, St Louis, MO, U.S.A.; 1% in phosphate-buffered saline, PBS). The solution was pressure injected at the recorded location with a PicoSpritzer II (General Valve Corp.). Subsequent to the CTB injection, biotinylated dextran amine (BDA; Molecular Probes; 10% in 0.1 M PBS) was iontophoretically injected (+3 µA, 1 s on, 1 s off) for 2–5 min using micro-pipettes with tip diameters of 8–12 µm. Recordings were first made with the BDA injection electrode to ensure that the tip was within the identified zone. Following the BDA injection, the electrode was left undisturbed for an additional 5 min. Injections of BDA were used to help visualize the injection site because the CTB injection sites were very diffuse. Thus, the BDA injection represented the approximate center of the CTB injection site.

Processing for biotinylated dextran amine and cholera toxin subunit B

After a survival time of three to six days, the animals were given an overdose of sodium pentobarbital (100 mg/kg) and immediately perfused with saline (0.9%) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The brains were extracted, postfixied for 2 h (4% paraformaldehyde, 20% sucrose in 0.1 M PB), cryoprotected in sucrose overnight (20% in 0.1 M PB) and frozen sectioned in the coronal plane at 40 µm thickness. Alternate sections were collected for CTB and BDA processing. The BDA protocol we used was based on the procedure of Wild and Veenman. Sections were washed in PBS (at 10-min intervals), incubated in 1% \( \text{H}_2\text{O}_2 \) with 25% methanol for 20 min, washed in 0.1 M PBS, incubated in ExtrAvidin peroxidase (Sigma; 1:1000) with Triton X-100 (0.4%) for 1.5 h at room temperature, washed in PBS, then visualized with diaminobenzidine (DAB). After 10 min in 0.025% DAB in 0.1 M PBS, 0.005% \( \text{H}_2\text{O}_2 \) was added and reacted up to 2 min. The tissue was subsequently washed several times with PBS.

The CTB protocol we used was based on Wild. The tissue was incubated for 30 min in 4% rabbit serum (Sigma; in PBS) with 0.4% Triton X-100, followed by goat anti-CTB (List Biological Laboratories; 1:20,000 in PBS) with 0.4% Triton X-100 for 20–24 h at 4°C. The tissue was washed with 0.1 M PBS, and then placed in biotinylated rabbit anti-goat (Vector Laboratories; 1:600 in PBS) with 0.4% Triton X-100 for 1 h, washed with PBS, followed by 1.5 h in ExtrAvidin with 0.4% Triton X-100, rinsed with PBS and then visualized with DAB, as with the BDA procedure. In some cases the reaction product was intensified with 0.002% CoCl\(_2\). The tissue was subsequently washed several times with PBS. Sections were then mounted on to gelatin-coated
RESULTS

Injections of CTB were made in 10 pigeons. Two pigeons received bilateral injections, providing 12 cases. In four instances (nos 5, 10, 12 left side, 15) injections were made into the translation zone. Likewise, in four cases (nos 6, 8, 13 right side, 16) injections were made into the rotation zone. In two cases (no. 12 right side, no. 13 left side) injections included both rotation and translation zones (on the same side of the brain). In two other cases (nos 17 and 18), injections were made in the vermis to determine if other areas of the cerebellum receive projections from the mc. BDA injections were performed on all pigeons except for case nos 5, 6 and 8. Table 1 shows a summary of each individual case, listing the cell response types that were isolated, the folia that were injected and the approximate distance of injection sites from the midline. The table also shows the location (distance from midline) of retrogradely labeled cell bodies in the IO from four rostrocaudal levels (200–250 µm apart).

In all VbC cases, retrogradely labeled cells were found throughout the rostrocaudal extent of the mc; however, there was a distinct medial–lateral segregation between cells projecting to translation and rotation zones. The cells projecting to translation zones were found in the ventrolateral regions of the mc, whereas cells projecting to rotation zones were concentrated in the dorsomedial regions of the mc. From Table 1, the average range of the distance from the midline of retrograde labeled cells, collapsed across all four rostrocaudal levels, was 291 ± 14.8 to 504 ± 16 µm for rotation zone injections, and 476 ± 25 to 676 ± 31 µm for translation zone injections.

Translation zone injections

In these four cases, the injection sites were centered 0.4–0.75 mm from the midline and found to be located in the ventral uvula or nodulus. In all cases the retrogradely labeled cell bodies were located in the ventrolateral regions of the mc and were bordered laterally by cranial nerve XII (XII). A few cells were found lateral to XII in the medial areas of the dl and vl. Figure 1 shows a series of coronal section drawings from case no. 10, illustrating the injection site.
Inferior olive projections to the vestibulocerebellum in pigeon

(Fig. 1A) and distribution of retrogradely labeled cells in the IO (Fig. 1B–J). Photomicrographs of the retrogradely labeled cells in the ventrolateral margin of the mc resulting from translation zone injections are shown in Fig. 4A and C.

**Rotation zone injections**

These injection sites were centered 2.2–3.1 mm from the midline. In all cases the retrogradely labeled cells were concentrated in the dorsomedial regions of the mc, including the area described as the dorsal cap by Arends and Voogd1 (see Fig. 2D–G). Figure 2 shows a series of coronal section drawings illustrating the characteristic distribution of retrogradely labeled cells in the dorsomedial mc (see IO on the left side of the brain in Fig. 3). Photomicrographs of the retrogradely labeled cells resulting from rotation zone injections are shown in Fig. 4B and D. Note the distinction between the locations of cells labeled from translation zone injections (Fig. 4A, C) and rotation zone injections (Fig. 4B, D).

**Injections including both translation and rotation zones**

In case no. 12 the injection into the translation zone of the left VbC extended across the midline to include the translation zone on the right side. A subsequent injection was made laterally in the rotation zone of the right VbC. In case no. 13 (left side), the injection site was centered 1.6 mm from the midline but spread laterally to include the rotation zone. At this injection site, r45°c cells and feVAv responses were recorded 150 µm apart. Thus, in these two cases, the retrogradely labeled cells in the IO represent projections to both translation and rotation zones. In both cases retrogradely labeled cells were found distributed throughout the mediolateral extent of the mc, and a few cells were found lateral to XII, in the medial areas of the dl and vl. Figure 3 shows a series of coronal section drawings illustrating the distribution of retrogradely labeled cell bodies throughout the mc from case no. 13 (left side; see IO on the right side of the brain in Fig. 3). The average range of the distance from midline of the retrogradely labeled cells from both cases was 210 ± 25 to 714 ± 52 µm. This range encompassed the measurements derived from cases where the injection was confined to either the translational or rotational zone.

**Vermal injections**

Vermal injections were conducted to determine if other areas of the cerebellum received projections from the mc. The vermal Purkinje cells at the
injection sites exhibited no modulation in response to visual stimuli. In case no. 17 the injection was centered 1.6 mm from the midline in the lateral-most aspect of the vermis (see Fig. 5A). Retrogradely labeled cells were found in a small strip in the ventromedial mc and some were found in the vl. Figure 5 is a series of coronal section drawings showing the distribution of retrogradely labeled cells in the ventromedial mc and vl. A photomicrograph of retrogradely labeled cells in the ventromedial mc from this case is shown in Fig. 4F.

In case no. 18, the injection was centered near the midline (see Fig. 6A) and resulted in bilateral retrograde labeling in the IO. The medial aspect of the dl,
lateral to XII, was extensively labeled on both sides, and a few cells were found in the medial vl. No retrogradely labeled cells were found in the mc. Figure 6 shows the distribution of retrogradely labeled cells in the dl. A photomicrograph of the retrogradely labeled cells in the dl, found lateral to XII, is shown in Fig. 4E.

DISCUSSION

In this report we have shown that zones in the pigeon VbC, containing Purkinje cells responsive to either translational or rotational optic flow, receive differential climbing fiber inputs from the IO. Injections of CTB in the translation zone retrogradely labeled cells in the ventrolateral mc. In contrast, injections of CTB in the rotation zone retrogradely labeled cells in the dorsomedial mc. Thus, just two synapses from the retina, this specific visual pathway is already segregated into channels specifying translational and rotational optic flow.

It is very likely that the rotation and translation areas of the IO are segregated further. In mammals, the flocculus and the nodulus contain cells sensitive to rotational optic flow, but the different subtypes are organized in parasagittal zones that receive differential climbing fiber input. Cerebellar zones containing rVA cells receive input from the caudal dorsal cap, whereas r45°c cells receive input from the rostral dorsal cap and ventrolateral outgrowth. Nonetheless, in the present study, we found no appreciable differences in the location of retrograde labeling in the IO following injections at locations containing rVA vs r45°c cells. Likewise, we found no obvious differences in the locations of retrogradely labeled cells following injections in areas of the translation zone at locations containing different response types. However, our injections were not small and likely included any subzones contained within the translation and rotation zones.

Comparison with previous studies

Arends and Voogd divided the pigeon cerebellum into five parasagittal zones (zones A–C, E and F) based on climbing fiber projections. The most medial zone, zone A, received input from the dl, which was confirmed in the present study (case no. 18). The anterior and posterior aspects of zone B received
input from the caudal vl and ventromedial mc, respectively. Zone C received input from the vl. Zone E received input from the ventral mc, which was confirmed in the present study (case no. 17). Finally, the most lateral zone, zone F, consisted only of the lateral half of the VbC (flocculus) and received input from the dorsal mc. These five zones transcended all folia, including folia IXc,d and X of the VbC, with the exception that zone A in the ventral uvula and nodulus received input from the mc. However, Arends and Voogd did state that the zonal organization of climbing fiber inputs to the VbC was not completely determined in their study.

Our data from the present study suggest that the VbC does not contain the same zonal organization as the vermis (this is also the case in mammals, where the zones of the flocculus and nodulus do not correspond to those of other parts of the cerebellum). Like Arends and Voogd, we found that both medial and lateral aspects of the VbC receive input from the mc, but we have also shown that this projection is subdivided further. We could not confirm the presence of a zone C (which is rather wide in the drawings of Arends and Voogd) in the nodulus or ventral uvula, as retrogradely labeled cells in the vl were rarely observed.

Comparative considerations

The fundamental difference between the VbC in pigeons and mammals is very clear from electrophysiological studies. In pigeons, the flocculus contains cells responsive to rotational optic flow, whereas in the nodulus and ventral uvula cells are responsive to translational optic flow. In rabbits, there are zones in the ventral uvula, nodulus and flocculus that contain cells responsive to rotational optic flow but, as yet, Purkinje cells responsive to translational optic flow have not been identified in the cerebellum of species other than the pigeon (it is possible that cells responsive to translational optic flow are peculiar to flying animals, but there are no data available from flying mammals. Likewise, it is possible that cells responsive to translational optic flow are peculiar to head-bobbing birds, but there are no data available from other bird species). Given these findings, it is not surprising that, in rabbits, the ventral uvula, nodulus and flocculus...
receive input from the same areas of the IO, namely the dorsal cap and ventrolateral outgrowth.\textsuperscript{7,16,23} In fact, Takeda and M. aekawa\textsuperscript{3,4,5} have shown that individual IO neurons in rabbits project to the uvula/nodulus and the flocculus. From the present study we conclude that the dorsomedial mc of pigeons is functionally equivalent to the dorsal cap and ventrolateral outgrowth of mammals, because these structures provide input to rotation cells.

The nodulus/uvula of rabbits also receives a large climbing fiber input from the beta subnucleus of the IO to the most medial zone.\textsuperscript{2,16,23} Barmack et al.\textsuperscript{3,4,5} have shown that many neurons in this zone and the beta subnucleus respond to static head tilt, thus demonstrating a direct input originating in the otolithic organs. Although not self-translation per se, the medial zone of the nodulus/uvula and the beta subnucleus in rabbits receive inputs related to linear acceleration during self-motion, as does the medial VbC in pigeons. For these reasons, we suggest that the beta subnucleus in mammals might be functionally similar to the ventrolateral mc in pigeons.

**Conclusions**

The vestibulocerebellum in pigeons can be divided into two parasagittal zones that contain Purkinje cells whose CS activity is maximally modulated by either translational or rotational optic flow stimuli. The lateral zone of the VbC contains rotation cells and receives climbing fiber input from the dorsomedial region of the mc of the IO. The medial zone of the VbC contains translation cells and receives climbing fiber input from the ventrolateral region of the mc of the IO.

**References**


(Accepted 7 November 1997)