Input of Anterior and Posterior Semicircular Canal Interneurons Encoding Head-Velocity to the Dorsal Y Group of the Vestibular Nuclei

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Blazquez, Pablo, Agis Partsalis, Nicolaas M. Gerrits, and Stephen M. Highstein. Input of anterior and posterior semicircular canal interneurons encoding head-velocity to the dorsal Y group of the vestibular nuclei. J. Neurophysiol. 83: 2891–2904, 2000. Neurons in the Y group of the vestibular nuclei are activated disynaptically from the ipsilateral VIIIth nerve and polysynaptically from the contralateral nerve. The ipsilateral anterior and posterior semicircular canals project to the Y group via interneurons in the vestibular nuclei. Candidate interneurons located in the rostrolateral corner of the superior (SVN) and in the caudal medial (MVN) vestibular nuclei were retrogradely labeled by the iontophoretic injection of biocytin into the Y group. The physiology of these interneurons named Y-group projecting neurons (YPNs) was studied in the SVN. SVN-YPNs were activated antidromically by electric pulse stimulation in the Y group. The properties of SVN-YPNs are distinct from those of SVN flocculus projecting neurons (FPNs). Namely, YPNs have a lower resting rate than FPNs, have more irregular interspike intervals, show a different phase and gain during the vestibuloocular reflex, and are located differentially within the SVN. After the injection of biocytin into the Y group, the locations of Purkinje cells that project to the Y group were confined to the vertical zones of the flocculus and ventral paraflocculus. However, mossy fibers originating in the Y group terminate in both the vertical and horizontal zones of the flocculus and ventral paraflocculus as well as in the ipsilateral nodulus.

INTRODUCTION

The vestibulococular reflex (VOR) is a spatially and temporally compensatory reflex producing eye movements of equal velocity but opposite direction to head movement. The neural circuit controlling a vertical (V) VOR contains multiple pathways from the vestibular nuclei to the vertical extra ocular muscles. For example, considering the superior rectus and inferior oblique there are two excitatory pathways, one via the superior vestibular nucleus (SVN) and another by way of the medial vestibular nucleus (MVN), and one inhibitory pathway via the SVN. An additional input is from the Y group of the vestibular nuclei whose role in vertical eye movements and in the VVOR and its plasticity has been firmly established (Chubb and Robinson 1984; Zhang et al. 1993). Pure vertical head-velocity neurons have been located mainly in the anterior half of the SVN sending direct projections to the ipsilateral and probably also to the contralateral flocculus and ventral paraflocculus (Zhang et al. 1993, 1995a). However, not all head-velocity neurons could be antidromically activated by stimulating electrodes placed within the cerebellum, suggesting that perhaps these nonactivatable neurons might have axonal projections to other sites.

Because the Y group is a significant link in VVOR processing, the signals carried by this putative interneuron(s) and its location and connectivity might assume some importance. The origin and synaptic linkage of this head-velocity information that Y group might receive. Namely, Y neurons express a head-velocity signal with characteristics of the vertical semicircular canals. However, the putative interneuron(s) in the pathway from the canal nerves have not been identified.

Zhang et al. (1993, 1995a), recording in the SVN, showed that pure head-velocity neurons, vestibular pause, and position vestibular units receive direct excitation from VIII nerve canal afferents. Some of these neurons increase their firing rate during up head movement, (posterior canal input), and others during down head movement, (anterior canal input) (Tomlinson and Robinson 1984; Zhang et al. 1993). Pure vertical head-velocity neurons have been located mainly in the anterior half of the SVN sending direct projections to the ipsilateral and probably also to the contralateral flocculus and ventral paraflocculus (Zhang et al. 1993, 1995a). However, not all head-velocity neurons could be antidromically activated by stimulating electrodes placed within the cerebellum, suggesting that perhaps these nonactivatable neurons might have axonal projections to other sites.

METHODS

Surgical procedures

Four female and two male squirrel monkeys (Saimiri sciureus) weighing between 600 and 800 g were prepared for chronic single-unit recording and electrical stimulation of the VIIIth nerve and brain stem. Multistage aseptic surgical procedures were carried out under in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
inhalation anesthesia with veterinary supervision in a sterile operating suite. A stainless steel head-fixation device was attached to the occiput. For the measurement of eye movements with the magnetic search coil technique (Fuchs and Robinson 1966), four turns of stranded, insulated stainless steel wire (Cooner) were sutured to the sclera at the limbus, the conjunctiva repaired, and the wire led subcutaneously to an occipital connector. Subsequently two recording/stimulating chambers, one aimed at the Y group and the second at the SVN were attached to the skull with dental cement. The bone beneath the chambers was removed, but the dura left intact.

Behavioral tasks and paradigms

Alert animals were seated in a primate chair placed atop a rate table (Contraves) in the center of an optokinetic drum 51 cm from their eyes. Alertness was maintained by oral β-amphetamine (0.1–0.5 mg/kg). Animals were placed with either their right or left sides down to stimulate the pitch axis and to eliminate utricular influences on the VVOR. Servo-controlled motors drove the chair and drum. Paradigms employed were the recording of spontaneous eye movements in the light and dark, VVOR in the light and dark, optokinetic following (OKN), and visual vestibular interaction such as suppression or enhancement of the VVOR. The gain of eye velocity with respect to head velocity was assumed to be 1 during VOR in the light at 0.5 Hz (Bello et al. 1991; Paige 1983).

Data collection and analysis

Vertical and horizontal eye position, chair and optokinetic drum velocity, and single-unit activity were recorded on videotape employing an eight-channel digitizing unit (Neurorecorder model DR-890). The sampling frequency for eye position, chair and optokinetic velocity was 11 kHz, and was 44 kHz for neuron activity. For studies of unit and field potential latency after electric pulse stimulation (cf. following text), only the unit activity or field potential was recorded at an 88-kHz sampling rate. Data was transferred to a PC using a Cambridge Electronic Design (CED) 1401 plus interface. The CED Spike 2 package was used for recording and signal analysis.

Eye-position sensitivity for each neuron was obtained by linear regression, and the mean firing rate with respect to eye position during steady fixation plotted. For analysis of the eye-velocity sensitivity, averages of at least five cycles of sinusoidal chair or OKN stimulation, or both, were performed. Horizontal and vertical eye velocity were extracted off-line from the eye position records using a low-pass filter differentiator. Saccadic eye movements including the postsaccadic slip. The locations of labeled cell bodies, fibers, and cerebellar glo- 

Histology

Biocytin in saline was iontophoretically injected into the dorsal Y group in three animals using glass microelectrodes (tip diam, ca. 15–20 μm) with positive currents (ca. 11 μA for 15 min, 70% duty cycle). Animals survived for 24 h were anesthetized deeply with pentobarbitol, heparinized (1,000 U iv) and then perfused transcardially with saline and fixative. Composition of the fixative was 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer. Frozen sections were cut at 80 μ and collected in phosphate buffer. Sections were treated with 1% Triton-X overnight, and the reaction product was developed using Vector ABC kits. Sections were rinsed and preincubated for 20 min with Ni-CO-acetate and diamino benzidine. Subsequently 0.03% hydrogen peroxide was added to the solution and tissue reacted until labeling was maximally developed, usually 10 min. Sections then were rinsed, dehydrated, and fitted with a cover slip. The locations of labeled cell bodies, fibers, and cerebellar glo- 

Results

Orthodromic activation and identification of Y neurons

A total of 49 neurons in three squirrel monkeys were classified as Y-group neurons. Cells were identified using stereotaxic coordinates and by their characteristic neuronal responses during visual following (VF), VOR in the dark (VORd), VOR in the light (VORl), enhancement of the
VOR (VORe), VOR reversal (VORr), and cancellation of the VOR (VORc) (Partsalis et al. 1995a). Recording sites also were identified as being located in the Y group by the locations of electrolytic lesions and by the location of iontophoretically injected biocytin (Fig. 5). Neuronal response during VORd, VORl, VORc, VORe, and VORr are shown in Table 1 for animals B and C and are consistent with previous reports on the firing properties of Y neurons (Chubb and Fuchs 1982; Partsalis et al. 1995a). (Animal A did not perform all paradigms adequately and thus Y neuron locations were verified by the sites of lesions and by stereotaxic coordinates.)

TABLE 1. Response of the population of Y cells recorded in monkey B and C during VOR dark, VOR light, VOR enhancement, VOR suppression and VOR reversal

<table>
<thead>
<tr>
<th>Subject</th>
<th>Stimulation</th>
<th>Neuronal Gain, spikes/s/deg/s</th>
<th>Neuronal Phase, deg</th>
<th>Eye Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey B</td>
<td>VORd</td>
<td>0.25 ± 0.12 (15)</td>
<td>−141 ± 79.3 (15)</td>
<td>0.81 ± 0.11 (15)</td>
</tr>
<tr>
<td></td>
<td>VORl</td>
<td>0.39 ± 0.02 (2)</td>
<td>−210 ± 4.65 (2)</td>
<td>1.13 ± 0.03 (2)</td>
</tr>
<tr>
<td></td>
<td>VORc</td>
<td>1.12 ± 0.54 (5)</td>
<td>−198 ± 7.04 (5)</td>
<td>1.91 ± 0.1 (5)</td>
</tr>
<tr>
<td></td>
<td>VORr</td>
<td>0.50 ± 0.30 (16)</td>
<td>4.22 ± 35.3 (16)</td>
<td>0.19 ± 0.17 (16)</td>
</tr>
<tr>
<td></td>
<td>VORc</td>
<td>0.90 ± 0.20 (4)</td>
<td>−8.7 ± 8.06 (4)</td>
<td>0.83 ± 0.06 (4)</td>
</tr>
<tr>
<td>Monkey C</td>
<td>VORd</td>
<td>0.25 ± 0.12 (15)</td>
<td>−202 ± 32.9 (15)</td>
<td>0.9 ± 0.13 (15)</td>
</tr>
<tr>
<td></td>
<td>VORl</td>
<td>0.25 ± 0.12 (6)</td>
<td>−185 ± 25.4 (6)</td>
<td>0.98 ± 0.09 (6)</td>
</tr>
<tr>
<td></td>
<td>VORc</td>
<td>0.25 ± 0.12 (17)</td>
<td>−201 ± 16.4 (17)</td>
<td>1.82 ± 0.25 (17)</td>
</tr>
<tr>
<td></td>
<td>VORr</td>
<td>0.8 ± 0.4 (16)</td>
<td>−14 ± 13.5 (16)</td>
<td>0.08 ± 0.03 (16)</td>
</tr>
</tbody>
</table>

Values are means ± SD with n in parentheses. VORd, VORl, VORc, VORe, and VORr, vestibuloocular reflex dark, light, enhancement, cancellation, and reversal, respectively.
Response of Y neurons after stimulation of the ipsilateral VIIIth nerve

The neuronal responses of 47 Y neurons were recorded after single-pulse electrical stimulation of the ipsilateral VIIIth nerve. Neurons were activated with variable latencies of 1.15–2.9 ms [Fig. 2, A and B (hollow arrows), and Table 2]. The latency of activation of individual Y neurons was also variable (Fig. 2, A and B). The current necessary to consistently activate Y neurons (>25% of the trials) varied between different cells and was also dependent on the latency of activation. Neurons with latencies <1.3 ms were driven using currents <175 μA, whereas neurons with latencies >1.5 ms were only driven using currents >175 μA. Table 2 documents the values obtained in three monkeys using pulses of 250 μA and 100 μs. The average latency in all cases was 1.2 and 2 ms indicating at least one interneuron interposed between the VIIIth nerve and the Y group.

No systematic comparison of the response latency of Y neurons to ipsilateral VIIIth nerve stimulation with their response to eye and head movement was made, but in the cases studied there was no obvious correlation between Y neurons with different latencies of activation and these parameters.

In Fig. 3, the response of three cells with different latencies of activation (1.2 ms, 1.5 ms, and no activation using currents ≤300 μA) to aspects of visual-vestibular stimulation are illustrated. Responses of the three cells are coupled tightly to the eye movement responses evoked by VORe (triangles), VORl (squares), VORs (diamonds), and VORr (inverted triangles). The three cells fell within the main sequence of response of Y neurons previously reported (Partsalis et al. 1995a).

Response of Y neurons to stimulation of the contralateral VIIIth nerve

In most cases, it was necessary to use 100 trials to observe a peak in the histogram after stimulation of the contralateral VIIIth nerve. The latencies of activation obtained were always >2 ms (Fig. 4A). After the first peak of activation, neurons exhibited a period of inhibition of discharge during the subsequent 4.5–9 ms. Many cells showed highly variable activation from the contralateral nerve, thus it was not possible to measure their latency accurately. The cell in Fig. 4A was considered activated, whereas the cell in B was not included in our sample (not activated) because the latency to activation was so variable. Table 2 documents the population study of the latency of activation from the ipsilateral and contralateral VIIIth nerves. The latency of activation from the ipsilateral nerve is ~1 ms shorter than from the contralateral. Also the number of cells activated from the ipsilateral nerve was greater than those activated from the contralateral. Currents used to activate Y cells from the ipsilateral nerve were always smaller than the currents needed to activate the same cell from the contralateral nerve. The latencies of activation of the population of Y group cells from the ipsilateral and contralateral VIIIth nerves was evaluated statistically and found to be significantly different for both monkeys tested (P < 0.00001).

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**Table 2. Activation latency of the population of cells in monkeys A–C**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Ear</th>
<th>Average</th>
<th>No. of Cells Activated</th>
<th>Total No. of Cells Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey A</td>
<td>Ipsilateral</td>
<td>1.93 ± 0.75</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Contralateral</td>
<td>2.82 ± 0.96</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Monkey B</td>
<td>Ipsilateral</td>
<td>1.58 ± 0.45</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Contralateral</td>
<td>1.31 ± 0.27</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Monkey C</td>
<td>Ipsilateral</td>
<td>2.75 ± 0.368</td>
<td>5</td>
<td>14</td>
</tr>
</tbody>
</table>

Values are means ± SD.
Location of neurons retrogradely labeled by biocytin injected into the Y group

Figure 5 illustrates an example of the reaction product produced after iontophoretic injection of biocytin into the Y group seen as a dense, dark area below the brachium conjunctivum (BC) and above the restiform body (CR). This injection was relatively well localized to the Y group and filled the entire nucleus, and anatomic results are plotted from sections taken from this animal. Results from injections in the other two animals confirm the illustrated results. As expected, retrogradely labeled cells were found in the ipsilateral flocculus and ventral parafoveolus of the cerebellum (cf. Figs. 14 and 15).

Figure 6, A and B, illustrates anterograde as well as retrograde label within the SVN and MVN. Nineteen SVN and 8 MVN neurons were labeled by this injection. SVN retrogradely labeled cells were concentrated in the most lateral and anterior corner of the ipsilateral nucleus (Figs. 6 and 7). The MVN cells were located caudal to the abducens nucleus and caudal to the acoustic stria. Thus they were caudal to the magnocellular MVN, lateral vestibular nucleus (LVN) group of position vestibular pause (PVP) neurons that project to the IIIrd and IVth nuclei. Labeled cells had a common morphology, with sparse radiating dendritic trees emerging from the cell body in a stellate pattern (Fig. 6, C and D). The dendritic trees were only visualized to the second or third ramification. Axons usually originated directly from the somata. Figure 7 illustrates the locations of the labeled cell bodies (*) within a series of coronal sections through the SVN and MVN. In Fig. 7, AP 0.0 mm corresponds to the −2.5 stereotaxic plane in the atlas of Emmers and Akert. Note that all labeled SVN neurons are located within the most rostral and lateral corner of the nucleus. No labeled cell bodies were identified within the contralateral vestibular nuclei, but axons could be followed from the injection site to the contralateral SVN. It is possible that these axons form boutons at anterior levels of the contralateral SVN or within the contralateral Y group; however, none were visualized in this study. Fibers from the injection site projected anterior crossing the middle line and ending with dense boutons at the level of the contralateral oculomotor nucleus. We conclude that there are two groups of interneurons that project monosynaptically to the Y group. One group in the ipsilateral rostral SVN and another in the ipsilateral MVN. The physiological responses of the SVN interneurons are documented in the next section.

SVN neurons antidromically activated from the ipsilateral Y group

To study the physiological properties of SVN neurons projecting to the Y group, the ipsilateral SVN was tracked with a recording electrode during electric pulse stimulation of Y. It initially was found that neurons without head-velocity sensitivity, e.g., cells demonstrating a relationship to eye position or eye velocity only were not antidromically activated from Y. Therefore these are not included in the present database. Indeed almost all the neurons in the rostrolateral part of the SVN with a head-velocity signal had little or no eye-position or -velocity sensitivity. The response of 52 head-velocity neurons was studied after electrical stimulation of the ipsilateral Y. Twenty could be activated antidromically from the Y group, whereas 32 were not activated.

The average latency of antidromic activation was 0.6 ms (range 0.5–0.8 ms). In all cases, antidromic activation was confirmed by the use of the collision test. A spontaneous action potential recorded from the SVN was used as the trigger for the collision test and collision occurred between 0.4 and 0.7 ms. Figure 8 illustrates a typical example. This particular neuron was activated at a latency of 0.5 ms, and the collision occurred at 0.5 ms. In Fig. 8 the hollow arrows on the left indicate spontaneous SVN action potentials and the solid arrows the stimulus artifact. The arrow on the right side of the top points to the antidromically activated cell. Note that there is no collision in the top record but in the lower traces, when the
triggering action potential was within 0.5 ms of the antidromically evoked spike, the evoked spike collided and the neuron was not invaded antidromically.

The field potential evoked in SVN by Y stimulation was studied in proximity to the sites where antidromically activated cells were found. The latency of the field potential was <0.65 ms in all cases. In Fig. 9 the closed field configuration of the field potential is illustrated. This closed field configuration suggests that the dendritic trees of the antidromically activated neurons are confined within the SVN nuclear borders. The collision test showed that for interstimulus intervals <1 ms there was a decrease in the amplitude of the second field potential (Fig. 9B, □), whereas full collision happened for interstimulus intervals <0.4 ms. Fig. 9B plots the amplitude of the test potential (□) as a function of interspike interval. It is apparent that the second potential decreased in amplitude smoothly between 0.5 and 1 ms.

Antidromically activated neurons were insensitive to eye position and/or velocity as neither slow horizontal or vertical or fast saccadic eye movements were accompanied by any change in discharge modulation (Fig. 10). Neuronal response of these neurons during head rotation in the light at 0.5–0.7 Hz (40–60° peak amplitude) was variable. Gain was always <0.5 spikes/s per °/s (Fig. 11). Figure 12 plots the phase and gain of these neurons in a polar plot. In sequential recordings in one animal, four neurons increased their firing rate for upward head movements, and three neurons increased their firing rate for downward head movements. Two neurons responded with a phase close to 90 or 270° so they could not be classified as upward or downward head-velocity cells. In another animal, two neurons increased their firing rate for upward head movements, and 10 neurons increased their firing rate for downward head movements.

Comparison of activated versus nonactivated SVN neurons

There are potentially two groups of head velocity only neurons located within the rostral SVN. Is there a distinct, separate class of YPNs or is there only one type of neuron, the flocculus projecting neuron (FPN) (Zhang et al. 1995a) that sends an axon collateral to the Y group? To evaluate these possibilities, the physiology of antidromically activated neurons (putative YPNs) and nonantidromically activated SVN head-velocity-only neurons (putative FPNs) were studied. Four issues were addressed.
RESTING RATE. The resting rate of YPNs was significantly lower than that of nonactivated vertical head-velocity SVN neurons (39.5 ± 14.7 vs. 62.4 ± 20.6 spikes/s respectively, \( P = 0.003 \)).

REGULARITY OF DISCHARGE OR COEFFICIENT OF VARIATION (CV) OF THE INTERSPIKE INTERVAL. The firing rate of YPNs was generally more irregular than putative SVN-FPNs (Fig. 11). The neuron in Fig. 11, A and C, was not antidromically activated, whereas the cell in B and D was activated. C and D are the interspike interval histograms (ISI) for cells A and B, respectively, during spontaneous eye movements. Cells not antidromically activated showed a sharp peak in the ISI, whereas activated cells had a much broader distribution of intervals. Additionally the mean firing rate is lower in C than in D (point 1, preceding section).

PHASE AND GAIN OF RESPONSE DURING VOR L. The population of YPNs has a very heterogeneous phase during head rotation (\( \Diamond \), \( \Box \), \( \bigcirc \), and \( \bullet \), Fig. 12). Most SVN-YPN neurons show either an in phase (\(-0.83 \pm 19.4^\circ, n = 12\)) or out of phase (\(181.4 \pm 83.85^\circ, n = 11\)) modulation during upward head motion. The sensitivity of upward neurons was 0.242 ± 0.14 spikes/s per °/s, and the sensitivity of downward neurons was 0.12 ± 0.031 spikes/s per °/s. Eighteen nonactivated neurons (58%) showed an increase of firing rate during upward head movement and 13
(42%) showed an increase during downward movement (▲, Fig. 12). The sensitivity of the upward neurons was 0.5 ± 0.315 spikes/s per °/s and of the downward neurons was 0.504 ± 0.294 spikes/s per °/s. The phase, for the upward neurons was 2.29.67 ± 9.7° and 2.192 ± 10.7 for downward neurons. An up on neuron is illustrated in Fig. 11. Statistical comparison using an ANOVA single-factor test, show significant differences between YPN and putative FPN neurons in their neuronal gain (down head-velocity populations P = 0.0088, and up head-velocity populations P = 0.0024) and no differences in their phases (P > 0.05).

LOCATION IN THE SVN. Figure 13 plots a reconstruction of the electrode penetration sites through the ipsilateral SVN. The plot was normalized across two monkeys using the position of the abducens nucleus (Fig. 13, ▫). YPNs (◼) were generally located more laterally than head-velocity neurons not antidromically activated (○). The location of these neurons compares favorably with the locations of labeled cells demonstrated by the retrograde transport of biocytin from the Y group (Fig. 7).

Considering the preceding four points, it may be concluded that YPNs and nonactivated vertical head-velocity SVN neurons (putative FPNs) are distinct, separate classes of head velocity only neurons located within the SVN and that axon collaterals of putative FPNs (if any) do not project to the Y group and vice versa.

Location of floccular and ventral parafloccular Purkinje cells and mossy fiber terminals after biocytin injection into the Y group

Figure 14 is a series of photomicrographs taken from the cerebellum. Figure 15 illustrates the distribution of retrogradely labeled Purkinje cells and anterogradely labeled mossy fibers and glomerular terminals after injection of biocytin in group Y. In Fig. 15, small dots are labeled mossy fiber glomeruli, large dots are labeled Purkinje cells, and thick lines in the molecular layer are labeled Purkinje cell dendrites. Retrogradely labeled Purkinje cells (Figs. 14A and 15) were present in both vertical cerebellar modules (V1 and V2), which are contiguous throughout the flocculus and ventral paraflocculus (Fig. 15). The majority of labeled cells (90%) were located in the medial vertical module (V1) (Voogd et al. 1996). The distribution is consistent with the location of labeled axons in the white matter compartments of these modules. Anterogradely labeled mossy fibers and glomeruli (Fig. 14, A, B, and D) were found in the flocculus and ventral paraflocculus and in lesser quantity in the uvula and nodulus of the posterior vermis (Fig. 14C). The labeled mossy fibers and glomeruli were most abundantly present in the medial half of the floccular/ventral parafloccular cortex but did not show a clear preference for any of the modules. They were present in the vertical, horizontal, and most lateral (C2) modules. In some instances there was a colocalization of labeled Purkinje cells and mossy fibers (Figs. 14A and 15).

The majority of mossy fiber glomeruli were 5–7 μm, and their parent axons, which could be frequently traced from the white matter, were either very thin (Fig. 14B) or very thick (Fig. 14, A and D). A third type of mossy fiber with very large (>10 μm) glomeruli was observed in small number in the lateral part of the ipsilateral uvula (Fig. 14C).
DISCUSSION

Sato and Kawasaki (1987) have shown that the dorsal Y group can be activated indirectly by both the ipsilateral and contralateral VIIIth nerve stimulation. Presently the pathways conveying vertical canal signals to the Y group have been delineated employing both electrophysiological and anatomic techniques. Further, the anatomic organization of the Purkinje cell input to Y, and the mossy fiber output from Y have been documented. Figure 16 is a schematic of the ipsilateral pathways to and from the dorsal Y group based on the results obtained in this study.

Activation of Y cells from the ipsilateral vestibular nerve

Individual Y neurons were activated from the ipsilateral vestibular nerve at variable latencies, and poststimulus time

FIG. 11. Response of 2 pure head-velocity cells in the SVN during sinusoidal rotation in the vertical plane are shown in A and B. Cell in A was not activated after stimulation of the dorsal Y group (currents ≥150 μA and 40 μs), whereas the cell in B was activated. C and D: interspike-interval histograms (ISIs) obtained for cells A and B, respectively, during no vestibular or optokinetic stimulation.
that the vertical VOR has a very extensive commissural component. Although Pompeiano et al. (1978) identified a Y-group commissural pathway, injection of the Y group with tracer in the present study failed to label contralateral cell bodies in squirrel monkeys. Thus the synaptic linkage of the contralateral polysynaptic pathway to the dorsal Y group remains to be elucidated.

Because Y neurons are excited not inhibited by contralateral VIIIth nerve stimulation, it may be suggested that this excitatory input arises from the same canal on the contralateral side that provides the dysynaptic input on the ipsilateral side. Thus either both anterior or both posterior canals would excite individual Y neurons. This type of connectivity stands in distinction to the push-pull arrangement that usually is assumed for commissurally connected central vestibular neurons (Shimazu and Precht 1966). However, Chen-Huang et al., (1997) have shown that individual central vestibular neurons may be either excited or inhibited by commissural inputs either increasing or decreasing their vestibular rotational gains. In keeping with that study, it may be suggested that the commissural excitation demonstrated here might increase the gain of Y neurons to rotary stimuli in the planes of the stimulated canals. Finally, the dysynaptic, rather than monosynaptic canal input to the Y group and the fact that this input is mediated via interneurons may be more permissive in allowing plasticity in this system in response to head velocity. In this view, the hard-wired three-neuron arc may be relatively unchangeable whereas the Y group, being more loosely connected to the canals, might serve to calibrate or adjust the brain’s assessment of head velocity during VOR adaptation.

**Responses of Y cells after stimulation of the contralateral VIII nerve**

In most cases, it was necessary to apply currents of 400 μA and 100-μs duration to activate contralateral Y neurons and produce a peak in the histogram. The latency of activation was always longer than that from the ipsilateral nerve ranging between 2.5 and 3.0 ms. The number of neurons activated was much smaller than those from the ipsilateral nerve. Thus in agreement with Sato and Kawasaki (1987) one, or probably more, interneurons are interposed in the pathway from the contralateral VIII nerve to the dorsal Y group. The contralateral polysynaptic activation observed in Y cells might be via an excitatory Y commissural pathway connecting the bilateral Y groups. This might be a plausible explanation, as it is known that the vertical VOR has a very extensive commissural component. Although Pompeiano et al. (1978) identified a Y-group commissural pathway, injection of the Y group with tracer in the present study failed to label contralateral cell bodies in squirrel monkeys. Thus the synaptic linkage of the contralateral polysynaptic pathway to the dorsal Y group remains to be elucidated.

Because Y neurons are excited not inhibited by contralateral VIIIth nerve stimulation, it may be suggested that this excitatory input arises from the same canal on the contralateral side that provides the dysynaptic input on the ipsilateral side. Thus either both anterior or both posterior canals would excite individual Y neurons. This type of connectivity stands in distinction to the push-pull arrangement that usually is assumed for commissurally connected central vestibular neurons (Shimazu and Precht 1966). However, Chen-Huang et al., (1997) have shown that individual central vestibular neurons may be either excited or inhibited by commissural inputs either increasing or decreasing their vestibular rotational gains. In keeping with that study, it may be suggested that the commissural excitation demonstrated here might increase the gain of Y neurons to rotary stimuli in the planes of the stimulated canals. Finally, the dysynaptic, rather than monosynaptic canal input to the Y group and the fact that this input is mediated via interneurons may be more permissive in allowing plasticity in this system in response to head velocity. In this view, the hard-wired three-neuron arc may be relatively unchangeable whereas the Y group, being more loosely connected to the canals, might serve to calibrate or adjust the brain’s assessment of head velocity during VOR adaptation.
Localization of the vestibular interneurons in the pathway to the ipsilateral dorsal Y group

Results obtained in this and other laboratories over the years indicate that the SVN is likely parcellated into functional cell groups based on axonal trajectories and terminations. SVN cells retrogradely labeled from the Y group are found mainly in the most anterior-lateral corner of the ipsilateral nucleus occupying a region of 0.7 mm in the anterior-posterior dimension. Vestibuloocular neurons are located centrally and medially in SVN (Mitsacos et al. 1983) and can be distinguished by their larger number of primary dendrites (between 4 and 9) than YPNs. Flocculus projecting neurons or FPN are located mainly in the central part of the SVN (Langer et al. 1985b). Flocculus terminals are concentrated in central SVN overlying VOR neurons but posterior to FPNs (Carpenter and Cowie 1985; Langer et al. 1985a). Peripheral portions of the nucleus receive commissural projections (Carpenter and Cowie 1985). Zhang et al. (1995a) confirmed these anatomic studies finding that most FPNs are located anteriorly in SVN, whereas FTNs are further posterior. We conclude that SVN-YPNs sending vestibular information to the dorsal Y group are located in the anterior-lateral corner of the SVN. Finally, we have no evidence that these neurons have an axon collateral going to the oculomotor complex nor to the flocculus of the cerebellum. The source of the anterograde label in SVN after labeling of the Y group is conjectural. It may represent the inadvertently labeled terminals of the VIIIth nerve or terminals of axon collaterals of rostrally projecting neurons.

A minority of labeled neurons also was found in the caudal MVN. The morphology of the dendritic trees and somata of these MVN interneurons are similar to their SVN counterparts. Although their physiological responses were not evaluated, it can be speculated that these MVN interneurons might convey posterior canal signals to the Y group as posterior canal primary afferent terminations have been shown to predominate in this portion of MVN (Gacek 1969).

Effect of stimulation in the Y on the SVN

To find and identify the putative SVN interneuron in the pathway between the VIIIth nerve and the Y group, we recorded within the SVN. Rotary stimulation in alert animals was employed to identify pure head-velocity neurons within the SVN the antidromic activation of which from Y then was tested. Using stimulus current intensities of \( \leq 100 \mu A \) and 40-\( \mu s \) duration latencies between 0.5 and 0.8 ms were documented.
Electrical stimulation in the Y group evokes an antidromic field potential in the SVN. Neurons in the SVN receive inputs from the anterior and posterior semicircular canals, responding mainly during vestibular stimulation in the vertical plane (Chubb et al. 1984; Zhang et al. 1993). Chubb et al. (1984) classified four types of neurons within the vestibular nuclei that respond during vertical visual-vestibular stimulation, namely, vestibular units, vestibular plus eye-position units, pursuit units, and miscellaneous units. We aimed our recordings to the most anterior half of the SVN. We mainly found neurons with head-velocity sensitivity and some neurons with eye position sensitivity. We failed to find neurons with only eye velocity or with gaze-velocity signals.

FIG. 15. Series of transverse sections through the flocculus and ventral paraflocculus illustrating the distribution of retrogradely labeled Purkinje cells and anterogradely labeled mossy fibers after injection of biocytin in group Y. Small dots, labeled mossy fiber glomeruli; large dots, labeled Purkinje cells; thick lines in the molecular layer, labeled Purkinje cell dendrites; thin lines, border between the central white matter and the granular layer; broken line, Purkinje cell layer; dark shading, laterally located labeled fibers; light shading, medially located labeled fibers; asterisk, location of the posterolateral fissure, separating the folia of the flocculus (ventral to the fissure) and the ventral paraflocculus (dorsal to the fissure). DPFL, dorsal paraflocculus.
Firing properties of YPNs

SVN neurons antidromically activated from the Y nucleus have a DC firing rate of ~40 spikes/s, lower than that observed in FPNs (~66 spikes/s), and much lower than that for FTNs (~124 spikes/s) (Partsalis et al. 1995a). Many vestibular neurons are reported to receive commissural inhibition (Carpenter and Cowie 1985; Shimazu and Precht 1966), but this was not evident in the present study as contralateral VIIIth nerve stimulation excited Y neurons.

YPNs have a more irregular-firing rate than FPNs. Partsalis et al. (1995b) injected muscimol into the ipsilateral flocculus depriving Y-group cells of their inhibitory floccular input. This resulted in a higher, more irregular firing rate at rest. The irregularity of YPNs might contribute to that observed in Y neurons after floccular inactivation.

Response of YPNs during visual stimulation

A common characteristic of the SVN-YPN cells is the lack of modulation during visually evoked eye movement. YPNs do not respond during saccades or during eye movement but only during head motion. Lisberger et al. (1994b) described a group of FTNs in the MVN that responded during head and eye movement.

Zhang et al. (1995a,b) described a group of FTNs in the SVN that showed a gaze-velocity signal. Until now, all FTNs described are related to eye velocity or eye position or both. These previous results are not surprising knowing that Purkinje cells in flocculus and ventral paraflocculus respond to eye velocity and eye position (Buttner and Waespe 1984; Graf et al. 1988; Stone and Lisberger 1990; Waespe and Henn 1981). We suggest that YPNs are not a part of the floccular output to the brain stem based on their response during visual stimulation and eye movement.

Response of Y cells during vestibular stimulation

YPNs respond to head movement modulating in phase or out of phase with respect to upward head velocity. There were more cells with an out of phase modulation than cells with an in phase modulation. That both anterior and posterior canal activated interneurons project to the dorsal Y group and the lack of Y cell modulation during VOR in the dark when the VOR gain is normal suggests that inputs from these two groups of interneurons might be balanced normally. When the VVOR gain is adapted to either high or low values, Y group neurons modulate either out of phase or in phase with upward head velocity respectively, suggesting that the ratio of anterior to posterior canal input strength has changed or been adapted.
Role of YPN in adaptation of the vertical VOR

Brain stem structures that adapt in parallel with changes in VOR gain generally have a direct inhibitory connection from the ipsilateral flocculus or paraflocculus (Lisberger et al. 1994a,b; Partsalis et al. 1995b). Therefore we suggest that no change should be expected in those neurons that do not get an inhibitory input from the cerebellum, e.g., the FPNs in SVN. As we described in the preceding text, YPNs do not behave as FTNs so we do not expect to find any change in their response after adaptation of the VOR.

The anatomic studies herein presented document that the majority (90%) of Purkinje cells that project to the Y group are located in the V1 zone of the flocculus and ventral paraflocculus with a minority (10%) in the V2 zone. On the other hand, the mossy fibers that originate from the Y group appear evenly distributed between the H and V zones although they are more prominent in the medial half of the cerebellar cortical complex. The exact localization of the Y group FPNs within the Y group and the physiology of these neurons await further anatomic and physiological study. However, to date our results indicate a physiologically uniform population in the Y group, thus we must assume that Y-group FPNs convey a true “efference copy” of the ascending motor command of the Y cell output to the extra ocular motor nuclei. A function for efference copy information in adaptive control of the VOR has been proposed (Hirata et al. 1999; Lisberger et al. 1994a,b; Miles and Lisberger 1981). It will be potentially revealing to compare the cerebellar projections of the paramedian tract cells (Buttner-Ennever et al. 1989) commonly assumed to convey efference copy to the flocculus and ventral paraflocculus with those of the FPNs in the Y group.


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