Projections and Firing Properties of Down Eye-Movement Neurons in the Interstitial Nucleus of Cajal in the Cat

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INTRODUCTION

Many studies have shown that the interstitial nucleus of Cajal (INC) plays an important role in the control of vertical eye and head movements (for review see Fukushima 1987; Fukushima et al. 1992). The INC projects to the oculomotor nuclei related to vertical eye movements (Carpenter et al. 1970; Graybiel and Hartwieg 1974; Kokkoroyannis et al. 1996; Steiger and Büttner-Ennever 1979). There are neurons in and around the INC that exhibit a burst-tonic firing rate for vertical eye movements (Büttner et al. 1977; Fukushima et al. 1990a; King and Fuchs 1977; King et al. 1981; Shiraishi and Nakao 1995). Lesions in the INC produce impairment of vertical gaze-holding (Anderson et al. 1979; Crawford et al. 1991; Fukushima and Fukushima 1992; King and Leigh 1982). On the basis of these studies, it has been suggested that the INC is a brain stem region that is essential in the control of vertical eye movement, particularly in velocity-to-position integration (Robinson 1975; see Fukushima et al. 1992 for review).

There are efferent projections from the INC to at least two supranuclear regions related to vertical eye movements: the contralateral INC (Carpenter et al. 1970; Kokkoroyannis et al. 1996) and the ipsilateral vestibular nuclei (VN) (Carpenter and Cowie 1985; Fukushima et al. 1982; Kokkoroyannis et al. 1996; Pompeoia and Wallberg 1957). Moschovakis and his colleagues (see Moschovakis 1995), using a morphophysiological technique, showed that a functionally identified burst-tonic neuron in the primate INC sent its axon to the contralateral INC through the posterior commissure (PC). Lesions in the PC impair vertical gaze holding in the monkey (Partalis et al. 1994), suggesting an important role of this fiber bundle in the control of vertical eye position. Regarding the descending projection, some vertical eye position-related neurons in the INC were found to be activated antidromically from the ipsilateral VN (Chimoto et al. 1992) and from the pontine reticular formation or the medial longitudinal fasciculus (MLF) (Fukushima et al. 1990a; Shiraishi and Nakao 1994). The INC, in turn, receives afferent inputs from the VN (Fukushima et al. 1982; King et al. 1980). In particular, VN neurons that carry vertical eye-position signals have been shown to project to the INC through the MLF in both the cat and monkey (Iwamoto et al. 1990b; McCrea et al. 1987). Some vertical eye-position-related INC neurons receive disynaptic excitatory input from the contralateral vestibular nerve (Chimoto et al. 1992; Fukushima et al. 1991). Thus there appears to be a close functional coupling between the INC and the VN to generate vertical eye-position signals. In agreement with this assumption, vertical eye-position-holding ability is impaired after MLF cuts in monkeys (Evinger et al. 1977).

In the present study, our aim was elucidating the afferent and efferent connectivity of vertical eye-position-related INC neurons with respect to the contralateral INC and the VN. This paper describes neurons with downward on directions (here called d-INC neurons). First, we examined whether efferent projections of d-INC neurons to the contralateral INC via the

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posterior commissure and to the ipsilateral VN via descending paths originate from the same or two separate populations of neurons. In the present report, two separate INC populations are found to convey vertical eye-position and saccadic signals to either the contralateral INC or the ipsilateral VN. Quantitative analysis of discharge properties of d-INC neurons was made in relation to eye movements after identifying their projections. The axonal arborization of these neurons in the contralateral INC or the ipsilateral VN also was corroborated electrophysiologically. Second, we investigated responses of d-INC neurons with commissural or descending projections to electrical and natural vestibular stimulation. By applying rotational stimuli in two mutually orthogonal vertical planes, the preferred direction was estimated. Comparison of dynamic characteristics between d-INC neurons and previously studied eye-position-related secondary vestibular neurons with downward on directions (DPV neurons) (Iwamoto et al. 1990a,b) was made to evaluate the significance of the INC and the VN in the integration of velocity signals for saccades and the vestibuloocular reflex.

Preliminary reports of a part of this study appeared previously (Chimoto et al. 1993; Iwamoto et al. 1993, 1994).

METH ODS

Animal preparation

Experiments were performed using nine adult cats. Under pentobarbital sodium anesthesia (initial dose, 40 mg/kg ip, supplemented by 2–5 mg/kg h−1 iv) and aseptic conditions, each animal underwent the following surgical procedures. No adverse reactions were observed during anesthesia. A coil of Teflon-coated stainless steel wire was implanted beneath the insertions of the four recti of the right eye to measure eye movements using the magnetic search coil technique. The tympanic bulla on each side was opened, and silver ball electrodes were placed on the round window to stimulate the vestibular nerve. Wires from the eye coil and labyrinth electrodes were led subcutaneously to the surface of the skull and soldered to small connectors. As part of the head-restraint system for painless head fixation, two metal tubes were attached securely to the skull with dental acrylic and small screws. A craniotomy was made in the posterior part of the parietal bone to gain access to the rostral mesencephalon. Another craniotomy was made in the posterior part of the parietal bone to reach the VN and/or the medial part of the pons. The cerebrum and cerebellum were kept intact. A cylindrical chamber with a diameter of 5–7 mm was placed over each craniotomy and fixed to the skull with dental acrylic. All surgical and experimental procedures, including the anesthetic regime, were approved by the Animal Experiment Committee at the University of Tsukuba, which is operated in accordance with Japanese Governmental Law (No. 105).

Recording

During recording sessions, the animal was restrained gently in a cloth bag and mounted on a turntable. The animal’s head was fixed to a stereotaxic frame and pitched nose down 26.5° from earth horizontal. Four stainless steel rods that could be attached to the frame were inserted into the metal tubes in the implant to have the animal’s head fixed. Eye movements were measured by the use of a magnetic search-coil system, and the fixation to the point 10° down from the earth-horizontal plane (or 16.5° up from the cat’s Horsley-Clarke horizontal plane) was taken as the standard vertical position. Glass-coated tungsten electrodes (shaft diameter of 250 μm) were used for extracellular single-unit recordings in the midbrain. They were inserted through the cerebrum in a track that was tilted 26.5° caudally to the stereotaxic frontal plane and 8–10° laterally to the sagittal plane. In this orientation of electrodes, all the tracks that reached the INC passed through the superior colliculus. To insert an electrode in the medial midbrain and pons accurately and reproducibly across recording sessions, a 21-gauge needle permanently implanted into the dental acrylic served as the zero reference point for all three dimensions. Before each penetration the recording electrode was aligned (with the use of a ×40 surgical microscope) with this point, and all tracts were converted to depth, mediolateral and anteroposterior dimensions. Ten to 50 penetrations were made per cat. The entrance to the superior colliculus was recognized readily by clear visual responses of neurons. The depth of the collicular surface proved to be a reliable reference for locating the INC. The visual receptive field of collicular neurons gave us a good estimate of the mediolateral and rostrocaudal location of an electrode. The INC was identified physiologically by the presence of vertical eye-movement-related unit activities and the disynaptic negative field potentials evoked by stimulation of the contralateral vestibular nerve.

Raw and filtered unit activity, eye-position signals, and turntable-position signals and velocity signals were displayed on a thermosensitive chart recorder. These signals were stored on magnetic tape with a data recorder (Sony PC-108 M) for later quantitative analyses.

Stimulation

The vestibular nerves were stimulated monopolarly with chronically implanted silver-ball electrodes. To identify axonal projection by antidromic activation, glass-coated Elgiloy or resin-coated acupuncture needle electrodes (shaft diameters, 300 and 220 μm, respectively) were implanted chronically in the INC, PC, or the medial part of the pons. In one animal, four Elgiloy electrodes were implanted in the medial pontine reticular formation and another one in the PC. Electrodes were inserted stereotaxically and fixed to the skull with dental acrylic.

For tracking with antidromic microstimulation, a movable tungsten electrode was inserted through the cerebrum to the contralateral INC or through the cerebellum to the ipsilateral VN. The INC was identified physiologically as described in the preceding section. To locate the VN, N1 field potentials induced by stimulation of the vestibular nerve (Shimazu and Precht 1965) were monitored as the electrode was advanced. Unit activities related to eye movements or induced by head rotation were observed and used to orient ourselves in the VN. The INC, PC, VN, and medial part of the pons were stimulated monopolarly with single cathodal current pulses of 100- or 200-μs duration. The stimulus currents were increased to 240 μA when there was no response with a lower stimulus intensity. However, stimulus currents for evoking antidromic response were in most cases much lower, and currents > 240 μA were not used. Single-pulse stimulation of the regions previously described did not produce any sign of pain or discomfort in the animal.

For rotational vestibular stimulation, the table was rotated sinusoidally in the light in two vertical planes that were orthogonal to each other and oriented 45° away from the pitch and roll planes. These rotation planes were approximately coplanar with the contralateral posterior and ipsilateral anterior canal (c-pci/ac) pair and the ipsilateral posterior and contralateral anterior canal (i-pci/ac) pair and are called c-pci/ac or i-pci/ac planes in this paper. We used a fixed frequency of 0.5 Hz and a peak table velocity of 3.8 or 7.6°/s.

Histology

Stimulation sites and some recording sites were marked by making electrolytic lesions at the termination of experiments. The animals were killed by an overdose of pentobarbital sodium and then perfused through the ascending aorta with 1–2 L of saline followed by 4% paraformaldehyde or 20% formalin. Transverse sections (100-μm thick) were cut parallel to the electrode tracks. Electrolytic lesions...
were identified histologically and recording sites of neurons were reconstructed with reference to the marked spots.

Data analysis

For the analysis of responses to electrical stimulation, neuronal activity was amplified (bandwidth 0.01–8 kHz) and sampled on-line at 100 kHz for display and storage. Latencies of antidromic and orthodromic excitatory responses were measured from 10 to 30 superimposed traces. For the responses to ipsilateral vestibular nerve stimulation, 100–200 traces were superimposed to detect inhibitory effects.

The relationships between neuronal discharges and eye movements were analyzed off-line. Filtered neuronal activity (bandwidth 0.3–8 kHz) was sampled at 25 or 33.3 kHz and stored in a computer using an interface and software (CED1401 plus, Spike2). This allowed us to inspect the entire waveform of each spike on a monitor screen to ensure reliable spike detection using an amplitude criterion. Horizontal and vertical eye position were sampled at 500 Hz and instantaneous component velocity was computed as the slope of the line fitted to position samples contained in a 10-ms moving window. The position sensitivity was defined as the slope of the regression line fitted to scatter plots of the firing rate versus vertical eye position during intersaccadic intervals. The firing rate was calculated for each intersaccadic interval except for the initial and last 50-ms portions to exclude activity changes associated with saccades. As a measure of burst activity associated with saccades, the saccadic sensitivity was computed as follows. We defined the burst component as firing activity in excess of the component proportional to instantaneous eye position during saccade. We first calculated the latter component using the rate-position relationship and vertical position trace and expressed it in number of spikes. We then subtracted it from the total number of spikes in the burst to obtain the burst component. The burst component was plotted against the amplitude of vertical saccades to obtain the saccadic sensitivity (spikes/degree) as the slope of the regression line fit through the data. To analyze responses of neurons for vertical sinusoidal head rotations, eye position, table-position, and table-velocity signals were sampled every 10 ms (200 bins/cycle). Discriminated spikes were sampled at 10 kHz and converted to a spike density function. The spike density function was calculated by substituting a raised cosine bell, which has unit area below it, with a fixed width (half-width 10 or 20 ms, depending on the firing rate) for each spike. The spike density was averaged over 5–30 cycles of record that contained no saccade, and the response fundamental was calculated by a least-squares procedure. Because neurons exhibited eye-position-related discharges, cycles during which the animal made a saccade were not used for analysis. The gain of response was defined as the ratio of the amplitude of the response fundamental to the stimulus velocity amplitude. The phase was measured as the difference between the peak of the response fundamental to the stimulus velocity amplitude. The orientation of the vertical plane at which rotation would evoke a maximum response (optimal plane) was estimated from the responses in c-p/c/-ac and c-ac/c-p planes, assuming a linear vectorial summation of the two responses.

All the data were expressed as means ± SD. Statistical comparisons of response parameters between groups of neurons with different projections were conducted with nonparametric Mann-Whitney U tests.

RESULTS

Extracellular recordings were made from neurons with activity related to eye movements in and near the INC. The explored region extended 0–2 mm rostral to the rostral end of the oculomotor nucleus, 0–3 mm lateral to the midline, and 4–8 mm ventral to the surface of the superior colliculus. More than 400 neurons were studied in nine alert animals. Three hundred and fifty-one of them showed spike activity related to vertical eye position during intersaccadic intervals. Of these, 237 (68%) had tonic firing rates that increased with downward eye positions and 114 (32%) with upward eye positions. This paper describes eye-position-related neurons with downward on directions, called d-INC neurons. Most d-INC neurons exhibited a burst of spikes for downward saccades followed by a position-related tonic activity (Fig. 1A). The intensity of the burst varied widely from neuron to neuron. Some d-INC neurons showed almost no saccade-related burst activity (Fig. 1B). Activity changes of d-INC neurons associated with upward saccades were less conspicuous but many neurons showed a decrease or a pause in activity.

Quantitative analysis of discharge pattern

The relationship between the firing rate during intersaccadic intervals and vertical eye position was examined in 94 d-INC neurons for which we could collect sufficient data for quantitative analysis. As exemplified in Fig. 2A, linear regression analysis indicated that the correlation was highly significant for all the neurons examined. Correlation coefficients ranged from 0.77 to 0.98 with a mean of 0.91 ± 0.04. The slope of regression lines representing the eye-position sensitivity ranged from 1.3 to 7.2 (spikes/s)/° with a mean of 3.7 ± 1.3 (spikes/s)/° (Fig. 2B). The y-axis intercept representing the firing rate at the standard vertical eye position (15.0° upward from the cat’s Horsley-Clark horizontal plane) (cf. Iwamoto et al. 1990a) ranged from −26 to 114 spikes/s with a mean of 61 ± 25 spikes/s. Only four neurons had a negative value and were silent at the standard position. The threshold eye position at which the firing rate became zero was estimated by extrapolation and ranged from 4.3° down to 63.9° up with a mean of 18.8° in the upward direction. Thus most neurons were active for the entire range of eye positions.
The burst activity associated with downward saccades was examined quantitatively in 72 d-INC neurons. Figure 2C exemplifies the relationship between the burst component expressed in spike count (see METHODS) and the amplitude of downward saccades. For most neurons (63/72), the correlation between the two parameters was statistically significant ($P < 0.01$, t-test for linear regression). For the population of neurons examined, the correlation coefficients for the linear regressions ranged from 0.10 to 0.98 with a mean of 0.85 ± 0.14. The saccadic sensitivity, defined as the slope of the regression line, ranged from 0.02 to 1.82 spikes/° with a mean of 0.96 ± 0.42 spikes/° (Fig. 2D). The distribution of saccadic sensitivity was unimodal, indicating that d-INC population ranges from neurons with intense bursts to those with little or no burst activity.

Identification of efferent projections of d-INC neurons

Efferent projections of d-INC neurons were identified by antidromic stimulation at the contralateral INC or the PC and the ipsilateral VN or the medial part of the pons. Figure 3A shows an example of antidromic excitation of a d-INC neuron. The antidromic nature of evoked spikes was indicated by a clearly defined threshold, a fixed latency at a threshold-straddling stimulus intensity, the ability to follow high-frequency repetitive stimuli, and collision with spontaneous spikes (Fig. 3A).

Ninety-four neurons were tested for antidromic activation after stimulation of the contralateral INC. Forty-five of them (48%) were activated antidromically with latencies ranging from 0.44 to 1.28 ms. Antidromic activation after stimulation of the PC was found in 9 of 43 d-INC neurons with latencies ranging from 0.35 to 0.60 ms. Thus in total, 54/137 d-INC neurons (39%) were found to be antidromically excited by stimulation of the contralateral INC or the PC.

Antidromic responses to stimulation of the ipsilateral VN were examined in 71 d-INC neurons. Twenty-two of them (31%) were activated with latencies ranging from 0.55 to 1.60 ms. The effective stimulation sites were found in the superior vestibular nucleus and the ventral part of the medial and lateral vestibular nuclei close to the border between the two nuclei. In four animals, the ipsilateral MLF at the level of the trochlear nucleus was stimulated (Fig. 3B). Antidromic activation was found in 35 of 99 neurons tested with latencies ranging from 0.40 to 0.90 ms. All the d-INC neurons that were activated from the VN were activated antidromically from the MLF as well, indicating that axons of these neurons descend in or near the MLF on their way to the VN. In total, 54/159 d-INC neurons (34%) were found to be activated antidromically from the ipsilateral VN or the MLF at the level of the trochlear nucleus. To estimate the location of descending axons at more caudal levels, four stimulation electrodes were implanted in the pontine tegmentum between the trochlear and abducens nuclei in one animal. The mediolateral positions of the stimulation sites were from the midline to 3 mm lateral, with each separated by ∼1 mm (Fig. 3C, a–d). Sixteen of 35 d-INC neurons tested were activated antidromically from at least one of these sites with latencies ranging from 0.45 to 0.95 ms. For these 16
neurons, the frequency of occurrence of antidromic responses from sites c, b, and a was 94% (13/16), 80% (12/15), and 54% (6/13), respectively. Stimulation of the most lateral site (d) did not evoke antidromic spikes in any of the tested neurons (0/15). These results indicate that descending fibers of d-INC neurons were located in a region adjacent to the MLF. To confirm this, systematic tracking of stimulation sites was made in a transverse plane 5.0 mm caudal to the trochlear nucleus to find distribution of thresholds for antidromic activation of a single d-INC neuron (Fig. 3D). The most effective site (threshold of 10 μA) gave an accurate estimate of the location of its descending axon. It was outside the MLF, 1.0 mm lateral to the midline and 1.2 mm ventral to the floor of the fourth ventricle.

Comparison of d-INC neurons contributing to commissural and descending pathways

The results described above showed that d-INC neurons contribute to the commissural and descending pathways from theINC. Table 1 demonstrates that these two pathways arise from two separate populations. A total of 113 d-INC neurons was tested for the responses to stimulation of both the commissural and descending pathways. Of these, 44 (39%) were activated antidromically from the commissural pathway (the PC or the contralateral INC) but not from the descending pathway (the ipsilateral pons or VN). Forty (35%) were activated from the descending pathway but not from the commissural pathway. No neurons were activated from both pathways.

Thus the vast majority (74%) of the neurons were output neurons that contributed to only one of the two pathways.

To further characterize these two subgroups of d-INC neurons, the locations, discharge properties, and responses to electrical and natural vestibular stimulation of the two populations were compared.

LOCATION OF NEURONS. Figure 4 shows locations of 33 d-INC neurons collected in one animal that were activated antidromically from either the ipsilateral VN (○, n = 16) or the contralateral INC (○, n = 17). Neurons with descending projections were located mainly within the boundary of the INC. Neurons with commissural projections tended to be located more dorsally, some being outside the INC. The tendency for the two populations to be located in dorsoventrally different regions was further suggested by the following observations. In five animals, spikes of both commissural and descending types of neurons were isolated along a single electrode track. In 11/12 penetrations, the neuron we encountered first was of the commissural type.

DISCHARGE PROPERTIES. To determine if any functional differences existed between commissural and descending types of neurons, their firing behavior during saccades and intersaccadic intervals was compared. Position sensitivity and saccadic sensitivity were determined for 51 neurons of which 24 and 27 were of the commissural and descending types, respectively. The mean position sensitivity was 4.6 ± 1.2 (spikes/s)/° for the descending group and 2.9 ± 0.8 (spikes/s)/° for the commissural group. The mean saccadic sensitivity tended to be higher for the commissural group (1.1 ± 0.3 spikes/°) than for the descending group (0.7 ± 0.3 spikes/°). These differences in both position sensitivity (Mann-Whitney U test, P < 0.001) and saccadic sensitivity (Mann-Whitney U test, P < 0.002) were statistically highly significant. In Fig. 5, saccadic sensitivity is plotted against position sensitivity for individual neurons in each group. Although there is some overlap, the data points for the commissural group (○) and the descending group (●) largely are segregated. To evaluate the significance of this separation, Mahalanobis’ squared distance between the two groups was calculated from a linear discriminant function using Fisher’s approach. The diagonal line in Fig. 5 represents a line of optimal discrimination estimated from the linear discriminant function. A statistical test using the distribution of Mahalanobis’ squared distance (cf. Lachenbruch 1975) indicated that the difference between the two groups was highly significant (F(2,48) = 26.93, P < 0.001).

<table>
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<th>TABLE 1.</th>
<th>Summary for the occurrence of antidromic activation from the commissural (PC or c-INC) and descending (i-Pons or i-VN) pathways</th>
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Numeral indicates number of neurons. PC, posterior commissure; c-INC, contralateral interstitial nucleus of Cajal; i-VN, ipsilateral vestibular nucleus; i-Pons, medial part of the ipsilateral pons.
EFFECTS OF CONTRALATERAL AND IPSILATERAL VESTIBULAR NERVE STIMULATION. Figure 6 compares responses to vestibular nerve stimulation in two groups of d-INC neurons. As exemplified in Fig. 6A, neurons with descending projections were consistently excited with short latencies after stimulation of the contralateral vestibular nerve. Figure 6E, ■, shows the distribution of the shortest latency in 57 neurons with descending projections. Fifty-seven of 59 (97%) neurons exhibited excitatory responses with latencies of 1.4 to 2.3 ms. Because the latency of the monosynaptic volley of secondary vestibular axons recorded in the INC was ~1.0 ms, the excitation was mostly disynaptic from the contralateral vestibular nerve. Stimulation of the ipsilateral vestibular nerve suppressed spikes of neurons with descending projections (Fig. 6B). The suppression of spikes was induced in 11 of 12 neurons examined. Latency of the suppression was measured from the stimulus to the last spike before the suppression in 100–200 superimposed traces. The latencies ranged from 1.5 to 2.2 ms, suggesting disynaptic inhibition from the ipsilateral vestibular nerve.

In contrast, the majority of neurons with commissural projections (33/43) exhibited no short-latency excitation after stimulation of the contralateral vestibular nerve as shown in Fig. 6C. The remaining 10 neurons exhibited excitatory responses to contralateral vestibular nerve stimulation with longer latencies (1.8–3.2 ms; Fig. 6E, □). Stimulation of the ipsilateral vestibular nerve did not suppress spikes of 18 neurons with commissural projections (Fig. 6D).

RESPONSE TO VERTICAL HEAD ROTATION. Responses to whole-body rotations were examined in 29 d-INC neurons of which 6 were antidromically identified as the commissural type (Fig. 7A) and 10 were identified as the descending type (Fig. 7B). All of these neurons had a similar spatial preference: they exhibited clear modulation of the firing rate during rotation in the c-pc/i-ac plane (Fig. 7, A and B, left). Rotation in the i-pc/c-ac plane (Fig. 7, A and B, right) induced a much weaker response. The mean gain of the responses in the c-pc/i-ac plane was $2.25^{+0.91}_{-0.91}$ (spikes/s)/°(s) (range 0.86–4.64). The mean gain of response in the i-pc/c-ac plane was $0.45^{+0.31}_{-0.31}$ (spikes/s)/°(s) (range 0.05–1.22), which was about one-fifth of that in the c-pc/i-ac plane (gain ratio: $0.21^{+0.12}_{-0.12}$). The orientation of the optimal plane estimated from responses in the two orthogonal planes (see METHODS) was shifted toward roll from the c-pc/i-ac plane by 4.3° on the average, ranging from 18° toward pitch to 24° toward roll. The estimated gain in the optimal plane ranged from 0.90 to 4.68 (spikes/s)/°(s) with a mean of $2.30^{+0.92}_{-0.92}$ (spikes/s)/°(s).

For the preceding 29 neurons, the phase lag of responses with respect to head velocity for rotations in the c-pc/i-ac plane was distributed continuously from 34.1 to 71.2° with a mean of $51.6^{+9.6}_{-9.6}$°. However, there was a difference in this response parameter between the two neuronal groups with different projections. The response of d-INC neurons with commissural projections ($n = 6$) lagged nose-up head velocity by $54.6^{+7.6}_{-7.6}$° (range: 47.6–66.9). Neurons with descending projections ($n = 10$) exhibited a phase lag of $45.0^{+5.5}_{-5.5}$° (range: 36.1–53.3). This difference was significant (Mann-Whitney U test, $P < 0.02$). There was no significant difference in response gain for the c-pc/i-ac plane between the two types of neurons ($P > 0.2$), the mean gain
being 2.07 ± 0.71 (spikes/s)/° (range: 1.34–3.19) for the commissural and 2.63 ± 1.05 (spikes/s)/° (range: 1.46–4.64) for the descending type. The response gain in the i-pc/c-ac plane ranged from 0.15 to 0.96 (spikes/s)/° with a mean of 0.52 ± 0.32 (spikes/s)/° for the commissural type and from 0.06 to 0.92 (spikes/s)/° with a mean of 0.44 ± 0.31 (spikes/s)/° for the descending type, the difference not being statistically significant (P > 0.5). The shift of optimal orientations from the c-pc/i-ac plane was also not significantly different (P > 0.5), 6.7 ± 13.7°.

**FIG. 6.** Effects of vestibular nerve stimulation on d-INC neurons. A: short-latency excitation after stimulation of the contralateral vestibular nerve in a neuron with a descending projection. B: spike suppression of the same neuron as in A, induced by ipsilateral vestibular nerve stimulation. C: effect of stimulation of the contralateral vestibular nerve on a neuron with a commissural projection. D: effect of stimulation of ipsilateral vestibular nerve on the same neuron as in C. E: latency histogram for orthodromic response to contralateral vestibular nerve stimulation in neurons with commissural (□, n = 10) and descending (■, n = 57) projections.

**FIG. 7.** Responses of d-INC neurons to vertical head rotation. A: averaged response of a neuron with a commissural projection during sinusoidal rotation at 0.5 Hz in the light. Left: response to rotation in the plane of the contralateral posterior-ipsilateral anterior canal pair. Traces are, from top, firing rate, vertical and horizontal eye position, and table velocity. Right: response to rotation in the plane of the ipsilateral posterior-contralateral anterior canal pair. B: response of a neuron with a descending projection. Note that vertical and horizontal eye-position traces do not represent actual eye movement amplitudes in the pitch or yaw planes because the eyes are rotating in a vertical canal plane.
toward roll for the commissural and $2.6 \pm 9.1^\circ$ toward roll for the descending type.

Arborization of efferent fibers of d-INC neurons in the contralateral INC and the ipsilateral VN

CONTRALATERAL INC. To investigate possible axonal arborization of electrophysiologically identified c-INC-projecting d-INC neurons, the depth profile of threshold and latency for antidromic activation was constructed by moving a stimulation electrode through the contralateral INC and its surroundings. We interpreted stimulation results as indicating axonal branching when at least one of the following two criteria was met: the depth profile of threshold in a single track exhibited multiple low-threshold peaks and the antidromic latency varied within a narrow range of depth. The second criterion included cases in which the latency changed discontinuously to a shorter value as the stimulus intensity was gradually increased at a fixed depth (cf. Jankowska and Roberts 1972) (see DISCUSSION).

Figure 8 shows a d-INC neuron that appeared to arborize in the contralateral INC. This cell exhibited a burst-tonic firing pattern with a vertical eye-position sensitivity of 2.3 (spikes/s)/° and a saccadic sensitivity of 1.2 spikes/°. Stimulation applied at the site, indicated by arrow $a$ in Fig. 8C, in the dorsal part of the INC induced spikes of this d-INC neuron with a fixed latency of 0.76 ms with a threshold of 11 µA (Fig. 8A, top). Collision testing confirmed the antidromic nature of the evoked spikes (Fig. 8A, middle). The recording site for this neuron was in the dorsal part of the INC (Fig. 8B). Tracking for antidromic stimulation showed that the threshold dropped markedly as the stimulation electrode moved into the INC and that effective sites for antidromic activation were distributed throughout the INC along this track (Fig. 8C). The depth profile of threshold for antidromic activation (Fig. 8D) showed multiple low-threshold peaks at several depths in the INC. The latencies of antidromic activation within the nucleus were longer than those obtained in more dorsal regions and varied from site to site by as much as 0.70 ms (0.76–1.46 ms). Moreover, even at a single stimulation site, this neuron showed two or more discrete latencies when stimulus currents gradually were changed. For example, at the depth indicated by arrow $b$ in Fig. 8C, antidromic spikes were evoked with a latency of 1.24 ms and a threshold of 20 µA. As the stimulus intensity increased, the latency remained constant until the stimulus current reached 34 µA, at which point the latency jumped from 1.24 to 0.94 ms (Fig. 8A, bottom). These findings were interpreted as the existence of fine axonal branches in the vicinity of the stimulation site. Similar jumping of latencies was found at several other depths in the INC (Fig. 8D).

Figure 9 shows distribution of effective sites for antidromic activation of another d-INC neuron, which had a vertical eye-position sensitivity of 3.3 (spikes/s)/° and a saccadic sen-
sensitivity of 1.6 spikes/°. Stimulation tracks were made at four different rostrocaudal levels (Fig. 9A, a–d). In b, multiple low-threshold peaks and varied latencies (0.65–1.4 ms) were found at the depths corresponding to the INC (Fig. 9B, left). At more dorsal and rostral stimulation sites (Fig. 9A, c), latency was fixed at 0.6 ms for a wide range of stimulus intensities and stimulation sites. The depth-threshold curve along c had a parabolic shape with a single low-threshold peak (Fig. 9B, right). The latency after stimulation of the dorsal sites gradually increased as the stimulation site moved caudally from d (0.6 ms) to a (0.7 ms). These results led to an interpretation that the stem axon coursed ventrocaudally from the lateral edge of the PC and gave off axonal branches in the INC.

Of 10 d-INC neurons with commissural projections that were examined with tracking in the INC and surrounding structures, 9 showed antidromic activation with discrete latencies, and 5 of those showed multiple low-threshold sites in the INC. One neuron had a depth-threshold curve with a single peak and little variation in latency. Thus the vast majority of neurons with commissural projections appeared to issue axonal arborization in the INC.

Of 10 neurons with commissural projections, 5 were activated antidromically from the contralateral MLF at the level of the trochlear nucleus as well as from the contralateral INC, suggesting that axons of some neurons descendent in or near the MLF after giving off axonal branches in the INC.

**IPSI LATERAL VN.** The depth profile of threshold and latency for antidromic activation was examined by moving a stimulation electrode in the ipsilateral VN. Figure 10 shows a VN-projecting d-INC neuron that exhibited a vertical eye-position sensitivity of 3.1 (spikes/s)° and a saccadic sensitivity of 0.5 spikes/°. The distribution of effective stimulation sites for antidromic activation was obtained from six tracks at two rostrocaudal levels (300 μm apart) in the superior vestibular nucleus (Fig. 10, A and B, left). Depth-threshold curves for two tracks (a and b) also are shown (right). In each track, the threshold curve had two low-threshold peaks with varied latencies. At the lowest threshold site in b, the latency changed abruptly from 1.20 to 0.90 ms as the stimulus intensity gradually increased, suggesting that the axon of this neuron probably gave off branches in the superior vestibular nucleus. Of 18 VN-projecting d-INC neurons that were examined with tracking, 12 neurons appeared to arborize in the ipsilateral VN. Eight of them showed both marked changes in latency and multiple low-threshold peaks, three showed marked latency changes only, and one showed multiple low-threshold peaks only. The remaining six neurons had a depth-threshold curve with a single peak and little variation in latency.

**DISCUSSION**

**Efferent organization of d-INC neurons**

The present study revealed that many d-INC neurons were activated antidromically from the contralateral INC or the ipsilateral VN. This finding is consistent with several previous studies. Moschovakis (1995) showed, using horseradish peroxidase (HRP) staining, that one stained vertical burst-tonic INC neuron sent its axon to the contralateral INC. A population of vertical burst- and tonic neurons in and near the INC was found to be activated antidromically from the medial part of the pons (Fukushima et al. 1990a; Shiraishi and Nakao 1994) and from the VN (Chimoto et al. 1992). Our data extend these findings by showing that the vast majority of d-INC neurons are output neurons contributing to the commissural or descending pathways. As many as 74% (84/113) of d-INC neurons were activated antidromically from the commissural pathway (contralateral INC and the PC) or the descending pathway (ipsilateral VN and the ipsilateral medial pons). In the remaining 26% of neurons, no antidromic response was observed. This implies that there may be some d-INC neurons the axons of which are localized within the nucleus. However, the actual ratio of output neurons is probably higher than 74%, because the intensity of stimulus currents (≥240 μA) was not sufficient to cover all parts of the VN or INC, resulting in false negative response in some cases.

An important finding in the present study was a lack of d-INC neurons that projected to both the contralateral INC and the ipsilateral VN. None of 113 neurons were activated antidromically from both the commissural and descending pathways. This suggests that the two pathways arise from different groups of d-INC neurons. Considering the possibility of false negative findings, the existence of d-INC neurons with bifurcating axons projecting to both the contralateral INC and the ipsilateral VN could not absolutely be excluded. However, a large number of positive findings (40 commissural, 44 descending) make it unlikely that such neurons, if any, are a major subpopulation of d-INC neurons.

To confirm axonal arborization in the INC and the VN, we tracked thresholds of antidromic microstimulation originally developed by Jankowska and Roberts (1972). Multiple peaks...
of the depth-threshold curve and latency variation (as large as 1.0 ms) of antidromic activation within a narrow range of stimulation sites (as small as 1.0 mm) were interpreted as indicating that there was fine axonal branching in that area. This interpretation was justified by several previous studies: there was a good agreement between findings obtained by the tracking of antidromic microstimulation and intraaxonal HRP staining with respect to branching of axons of secondary vestibular neurons (Ishizuka et al. 1980; McCrea et al. 1981; Nakao et al. 1982) and of inhibitory burst neurons (Hikosaka and Kawakami 1977; Yoshida et al. 1982) in the cat abducens nucleus. Although the morphological study with HRP staining of single axons is of great advantage to finding the extent and features of their terminal arborization, antidromic microstimulation was sufficient for the present purpose to provide evidence that the antidromically stimulated axons were of those giving off branches in the nucleus.

Comparisons between d-INC neurons with commissural projections and those with descending projections

The two groups of d-INC neurons differed not only in their axonal projection but also in afferent inputs from the vestibular labyrinth. It was reported previously that downward burst-tonic neurons in the INC best respond to head rotation in the plane close to the c-pc/i-ac plane (Fukushima et al. 1990b). Our data on the commissural and descending types of d-INC neurons were in good agreement with this observation; both types of neurons exhibited responses consistent with inputs predominantly from the c-pc/i-ac pair; excitatory input from the c-pc and/or inhibitory input from the i-ac. However, the responses to electrical stimulation of the vestibular nerve were quite different in the two groups. Neurons with descending projections received disynaptic excitatory input from the contralateral vestibular nerve and disynaptic inhibitory input from the ipsilateral vestibular nerve, whereas most neurons with commissural projections lacked such disynaptic inputs. Taken together, results of natural and electrical stimulation strongly suggest that neurons with descending projections receive canal signals directly from secondary vestibular neurons, whereas neurons with commissural projections receive signals from the same canal pair through polysynaptic pathways.

The second major difference between the two groups of d-INC neurons was the relative strength of the two basic components of their firing activity: the eye-position-related tonic component and the saccade-related burst component. Both groups of neurons exhibited a wide spectrum of firing behavior, ranging from burst-tonic to pure tonic discharge patterns. For the whole population, the histogram of position sensitivity showed unimodal distribution, and there appeared to be no dichotomy. The mean position sensitivity was 3.8 (spikes/s)/°, which agrees well with the value of 3.9 (spikes/s)/° obtained in a previous study (Fukushima et al. 1990a). However, when projection pattern of each neuron was taken into account, clear differences were found between the two groups. Position sensitivity of neurons with descending projections (mean 4.6 (spikes/s)/°) was significantly higher than that of neurons with commissural projections (mean 2.9 (spikes/s)/°). Likewise, the distribution of saccadic sensitivity was unimodal for the whole population of neurons, but when they were divided into two groups according to their axonal projections, saccadic sensitivity was significantly higher for neurons with commissural projections than for neurons with descending projections. Thus the relative strength of eye-position component was distinctly higher in neurons with descending projections. This might reflect a greater degree of processing of the velocity-to-position integration of saccadic signals in the descending type than in the commissural type (see following text).

Possible connections among eye-position-related neurons in the INC and VN

The present study has provided data that lead us to suggest several possible synaptic connections. First, the great majority of d-INC neurons that project to the vestibular nucleus have been shown to receive a direct excitatory connection from secondary vestibular neurons on the contralateral side. Our previous studies have shown that one of the excitatory sources of vertical eye-position signals to the INC is a group of secondary vestibular neurons that are located in the contralateral VN (Iwamoto et al. 1990a,b). These neurons, called downward-position vestibular (DPV) neurons, are excited monosynaptically by electrical stimulation of the vestibular nerve and exhibit responses consistent with excitatory input from the posterior canal. During saccades, they exhibit a pure tonic discharge pattern with downward on directions. Using an intraaxonal HRP injection technique, it has been demonstrated that axons of contraterally projecting DPV neurons terminate in the INC as well as in the trochlear and oculomotor nuclei. Moreover, spike-triggered averaging experiments have shown that these projections are excitatory in nature. These findings strongly support the idea that d-INC neurons with descending projections receive a direct excitatory connection from DPV neurons (Fig. 11).

Second, the descending projection from d-INC neurons to the ipsilateral VN suggests, in turn, the existence of a population of neurons in this area that carries vertical eye-movement signals. As a target of VN-projecting d-INC neurons, DPV neurons are an unlikely candidate. In terms of spatial response properties, d-INC neurons and contralateral posterior canal afferents respond similarly to rotations. However, DPV neurons have a preferred direction similar to that of the ipsilateral posterior canal. The d-INC neurons and DPV neurons on the same side thus have preferred directions that are orthogonal to each other. It is therefore extremely unlikely that d-INC neurons make connections with DPV neurons in the ipsilateral VN. Considering the preferred plane of rotation, neurons that receive efferent projection from d-INC neurons should be those responding to rotation in c-pc/i-ac plane. Kasahara and Uchino (1974) have shown that there is commissural inhibitory interaction between the bilateral VN in the vertical canal system: secondary vestibular neurons that are excited from the ipsilateral anterior canal are inhibited from the contralateral posterior canal via inhibitory type II neurons (Fig. 11). Fukushima et al. (1983) suggested that vestibular neurons with excitation from the contralateral vestibular nerve received direct excitation from the ipsilateral interstitio-vestibular neurons. Using the spike-triggered average technique, we have shown in the alert cat that functionally identified VN-projecting d-INC neurons exert excitatory synaptic actions in the ipsilateral VN (Iwamoto
 Movements, a neural signal proportional to eye velocity must be integrated mathematically to produce a signal proportional to eye position. In saccadic eye movements, a burst of spikes that encodes eye velocity is integrated to produce sustained discharges proportional to eye position to fixate on a target. During head rotation, eye movements are produced in the opposite direction by the vestibuloocular reflex (VOR) that subserves stabilization of the visual scene. The discharges of the vestibular nerve proportional to head velocity are integrated to produce a signal that is closer to eye position. Robinson suggested that the velocity-to-position transformation of signals is performed by a common integrator for all types of conjugate eye movements.

It has been hypothesized that integration is accomplished by positive feedback (Arnold and Robinson 1997; Cannon et al. 1983; Galiana and Outerbridge 1984; Kamath and Keller 1976). Galiana and Outerbridge (1984) proposed a bilateral model in which mutual inhibition of secondary vestibular neurons through the commissural pathway interconnecting the bilateral VN (Shimazu and Precht 1966) contributes to the realization of the brain stem integrator and suggested that it is shared by the VOR and visual reflexes. In the horizontal canal system, there appears to be a closed-loop pathway between the bilateral VN, and the feedback loop can be positive. The same argument may be applied to the vertical canal system between neurons that belong to the anterior canal-posterior canal pair (Fig. 11). The positive feedback loop in the vertical canal system may subserve position-related discharges of DPV neurons in the VN.

In addition, as shown in Fig. 11, the position signals arising from, say, the left DPV neurons are sent to the right d-INC neurons via the MLF and then via a descending pathway to the right VN. We assume here that d-INC neurons with descending axons make an excitatory connection with ipsilateral inhibitory type II neurons. The left DPV-right d-INC-right VN pathway then can extend back to the original left DPV neurons via a vestibular commissural pathway, thus forming a positive feedback loop that also could contribute to the integrator circuit in the vertical eye movement system. Evinger et al. (1977) reported that, after bilateral MLF cuts, the monkeys could not maintain eccentric vertical eye positions of fixation. This at least in part may be explained by the interruption of both the ascending pathway from DPV neurons and the descending pathway from d-INC neurons, which would open the positive feedback loop assumed above.

The present and previous studies are compatible with the notion that there is a third positive feedback loop in the posterior commissure system between bilateral INCs. As discussed in a previous section, d-INC neurons that contribute to commissural pathways may make inhibitory connections with contralateral INC neurons having upward ON directions. Moreover, it has been shown that there is a population of upward burst-tonic INC neurons that projects to the INC on the other side of the brain stem (Iwamoto et al. 1993). Partsalis et al. (1994) showed that after injecting lidocaine in the posterior commissure in the monkey, vertical gaze holding was severely affected and vertical VOR phase was advanced, suggesting that this fiber bundle linking bilateral INCs is important in the velocity-to-position integration. The three loops, i.e., vestibular commissure, VN-INC-VN, and posterior commissure loops, may be only a part of the vertical integrator circuitry. There are probably several other loops, unidentified to date, involving the

![Figure 11](image-url)
VN, INC, and other parts of the brain stem and possibly the cerebellum. The whole integrator may consist of multiple, positive feedback loops that are overlapped and connected with one another.

Finally, comparison of quantitative data in the present and our previous studies may shed some light on how velocity signals are processed within the integrator circuitry. Three groups of cells, i.e., d-INC neurons with commissural projections, those with descending projections and DPV neurons, have been found to exhibit different combinations of velocity and position signals. Furthermore the relative strength of the position component was shown to differ for two different types of eye movements, namely saccades and the vestibuloocular reflex. In the case of saccades, the relative strength of the position component with respect to velocity component was distinctly higher in d-INC neurons with descending projections than that of d-INC neurons with commissural projections. DPV neurons were shown to have a pure tonic discharge pattern, with no burst or pause (Iwamoto et al. 1990a). These differences among the three neuronal populations might reflect different degrees of processing of the velocity to position integration. Saccadic velocity signals probably originate from vertical burst neurons in the rostral midbrain (Büttner-Ennever and Büttner 1978; Büttner et al. 1977; King and Fuchs 1979; Moschovakis et al. 1991a,b; Nakao et al. 1988). In the monkey, it was shown that vertical burst neurons in the rostral interstitial nucleus of MLF project to and terminate in the INC (Moschovakis et al. 1991a,b). Saccadic velocity signals thus enter the integrator at the INC, generating a burst component of discharges of d-INC neurons. At the level of DPV neurons, located far from saccadic velocity input, velocity signals already have been converted completely to position signals, probably through the vestibular commissure loop. Saccadic velocity signals thus seem to be processed sequentially from d-INC neurons with commissural projections to those with descending projections and then to DPV neurons. In the case of the vestibuloocular reflex, there were differences in response phase among the three neuronal populations: 54.6° for d-INC neurons with commissural neurons, 45.0° for d-INC neurons with descending neurons, and 36.6° for DPV neurons (Iwamoto et al. 1990a). DPV neurons, which receive head velocity input directly from canal afferents, thus carry the least integrated signals. The d-INC neurons with descending projection, which probably receive input directly from DPV neurons, carry a more integrated signal, and d-INC neurons with commissural projections, farthest from canal afferents, have the most integrated signals. The order in processing of vestibular signals is reversed compared with that of saccadic signals. Thus for both saccadic and vestibular eye movements, it appears that discharges of neurons that directly receive velocity input still reflect this input and that the relative strength of the position component increases as the signals are passed on to succeeding populations of neurons.

Contribution of the INC to the formation of motoneuron discharge

Both DPV neurons and INC neurons are immediate premotor neurons (Iwamoto et al. 1990b; Partsalis et al. 1994). Motoneuron discharges can be regarded as the sum of eye velocity- and eye position-related components (Robinson 1975). To explain motoneuronal discharge for saccades, the two input signals from burst neurons and DPV neurons appear to be sufficient. Actually, however, d-INC neurons, by their direct projection to motoneurons, also contribute to both the burst and tonic components of motoneuron discharges. The signals conveyed by d-INC neurons might seem redundant if merely saccades are taken into consideration. The need for premotor signals from the INC is clearer in the vestibuloocular reflex. For sinusoidal vertical head rotation, mean phase lag of DPV neurons relative to head velocity (Iwamoto et al. 1990a) was smaller than that of motoneurons (60–70° at 0.5 Hz for medial rectus and abducens motoneurons) (de la Cruz et al. 1989; Delgado-Garcia et al. 1986; Fukushima et al. 1992). This suggests the existence of premotor neurons, other than DPV neurons, introducing an additional phase lag in motoneurons. The present study showed that the phase lag of d-INC neurons was larger than that of DPV neurons, probably comparable with that of motoneurons. INC neurons thus might contribute to the formation of motoneuronal responses with an appropriate phase during the vestibuloocular reflex.

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