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RAPID PUBLICATION

Existence in the Nucleus Incertus of the Cat of Horizontal-Eye-Movement-Related Neurons Projecting to the Cerebellar Flocculus

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SUMMARY AND CONCLUSIONS

1. Properties of nucleus incertus (NIC) neurons projecting to the cerebellar flocculus were studied in alert cats by using chronic unit and eye movement recording and antidromic activation. Projection of these neurons onto the flocculus was verified with retrograde transport of horseradish peroxidase after injections in the flocculus.

2. Bipolar stimulation electrodes were implanted into the "middle" zone of each flocculus because this zone is known to be involved in the control of horizontal eye movements. The dorsomedial aspect of the pontine tegmentum was explored with microelectrodes during stimulation of both flocculi. The majority of neurons antidromically activated from the flocculus were found in the caudal part of the NIC.

3. Of the 69 neurons activated from the flocculus, 44 were classified as burst-tonic (BT) neurons; 34 discharged in relation with horizontal movements of the eye, 10 in relation with vertical movements. Of the 14 remaining neurons, 6 were not related to eye movements and 8 were classified as burst neurons. The BT neurons of the NIC displayed a great sensitivity to both horizontal eye position and horizontal eye velocity.

4. This study demonstrates the presence of a new group of horizontal eye movement related BT neurons situated in the NIC. The fact that they project to the horizontal floccular zone emphasizes the importance of the functional specialization of the different Purkinje cell zones.

INTRODUCTION

Although the flocculus has been extensively studied in different species (Graf et al. 1988; Ito et al. 1977; Noda and Suzuki 1979; Waespe et al. 1981), its mode of information processing remains unknown. In contrast with this unsatisfying observation, the neuroanatomic organization of the flocculus is well known (Gerrits and Voogd 1982; Gerrits et al. 1984; Sato et al. 1983a,b; Tan et al. 1995) and offers a great opportunity to undertake an electrophysiological input-output approach toward the different floccular Purkinje cell zones.

In the flocculus, three longitudinal Purkinje cell zones have been described that respond selectively to large-field visual pattern movement. Thus Purkinje cells in a centrally located zone control horizontal eye movements, whereas those in the flanking zones control vertical eye movements (Sato and Kawasaki 1990). The central position of the "horizontal" zone facilitates its electrophysiological localization. Stimulation of this zone was recently performed to enable antidromic identification of its mossy fiber inputs with an origin in the medial vestibular nucleus (MVN) (Cheron et al. 1995) and the nucleus prepositus hypoglossi (NPH) (Escudero et al. 1995). Although these two nuclei are two important sources of floccular mossy fibers (Epema et al. 1990; Tan and Gerrits 1992), several studies have shown that the pontine reticular formation (PRF), including the raphe nuclei, provides another mossy fiber input (Alley 1977; Sato et al. 1983b).

The first electrophysiological analysis of these floccular projections from the PRF, performed in the anesthetized cat (Nakao et al. 1980), showed that electrical stimulation of the ipsilateral flocculus elicited antidromic activation of neurons located in and around the medial longitudinal bundle (mlb), in the nucleus raphe pontis (NRaP), and in the nucleus reticularis tegmenti pontis. Furthermore it was demonstrated that these PRF neurons provide the flocculus with eye-movement-related information.

The explored brain stem region contains a variety of neurons, located in and around the mlb and the adjacent periaqueductal gray. In the latter, a prominent cluster of neurons is present as the nucleus incertus (NIC) (Papez 1929). Until now, this nucleus has only been implicated is ascending connections to the medial preoptic area (Vertes 1988) and the interpeduncular nucleus (Hemmendinger and Moore 1984; Shibata et al. 1986).

In the present study we describe the properties of horizontal-eye-movement-related neurons in the NIC and the adjacent dorsomedial PRF with a projection to the flocculus in the alert cat.

METHODS

Four adult cats were prepared for chronic recording of neuronal activity in the brain stem and the cerebellum and for eye movement recording (magnetic search coil technique). Under general anesthesia and aseptic conditions, scleral search coils were implanted subconjunctivally on both eyes, bipolar silver stimulation electrodes were implanted intracranially on each sixth nerve at its exit from the brain stem, and three bolts were cemented to the skull to immobilize the head during the experimental sessions. Three holes, a central one and two lateral ones, were made in the skull. The dura mater was removed and a dental cement chamber was constructed around the holes. Bipolar tungsten microelectrodes were implanted in the middle zone of each flocculus (Fig. 1*A*). Further details of this chronic preparation have been described by Cheron et al. (1995).

Eight days after surgery, each animal was trained to accept restraining conditions without stress. Special care was taken to prevent any discomfort in the animals. Antidromic field potentials evoked by stimulation of the abducens nerve were used to map the

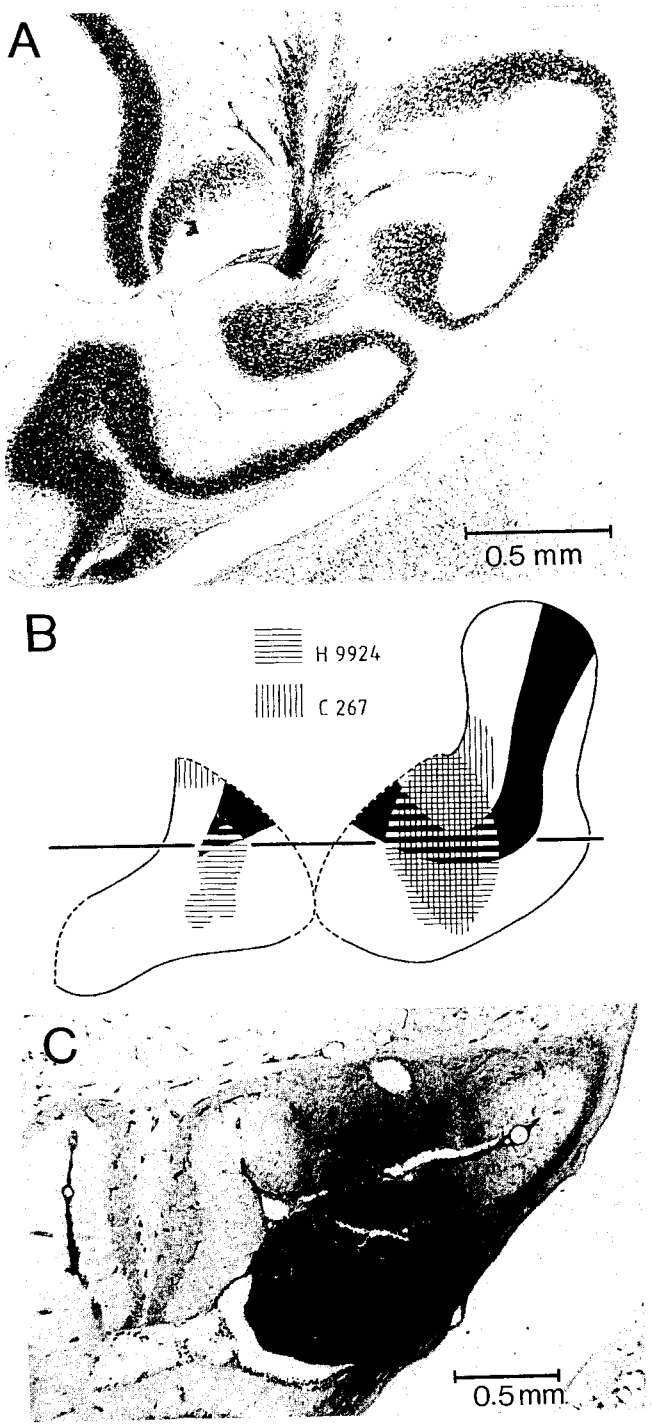

FIG. 1. *A*: transverse section showing the track of the stimulation electrode, which ends in the middle zone of the flocculus. *B*: diagram of the floccular complex (after Gerrits and Voogd 1982) illustrating the extent of the horseradish peroxidase (HRP) injection sites (H9924 and C276; hatching) and the clear overlap with the horizontal zone (black), here represented as the climbing fiber zone originating in the caudal dorsal cap of the inferior olive (Gerrits and Voogd 1982). Horizontal line: position of the sections from *A* and *C*. *C*: transverse section through the HRP injection site of H9924 at its largest diameter.

location and extent of the abducens nucleus, which was used as a landmark for further recording. The neuronal activity in the PRF was explored with glass micropipettes (1–2 MΩ impedance) during bipolar stimulation of the middle zone of both flocculi. We focused our attention only on antidromically activated neurons for which the latency was relatively short and constant and for which collision tests were succesful. The activity of each identified PRF neuron was recorded *1*) during spontaneous eye movements in the light and *2*) during horizontal sinusoidal stimulation of the vestibuloocular reflex (VOR) in complete darkness (amplitudes of 10, 20, 30, or 40 deg at 0.1 Hz). Identification of the VOR slow phases was performed automatically, using an algorithm developed by Baland et al. (1987). The saccadic velocity sensitivity (R_s) of the neurons was obtained by the method of Berthoz et al. (1989). The sensitivity to eye position during intersaccadic fixation (K_f), and the sensitivities to eye position during the VOR (K_v) and to eye velocity during the VOR (R_v) were obtained by a method described previously (Godaux and Cheron 1993). Recording and stimulation sites were verified and reconstructed from histological sections according to micrometer readings and electrolytic lesion marks. Two additional cats were available in which horseradish peroxidase (0.4 μl, 30% in saline) was injected into the caudal part of the flocculus in the same position where the stimulation electrodes were placed in the other animals (Fig. 1).

RESULTS

A total of 69 neurons were analyzed, antidromically activated from either flocculus (38 from the contralateral and 19 from the ipsilateral side; 12 neurons were situated in the midline). The majority (57 neurons) were located in the NIC (Fig. 2, *C* and *D*). In the cat, the NIC is a collection of small to medium-sized neurons with an oval-to-fusiform shape (Fig. 2, *E* and *F*). They are located in the periaquaductal gray directly dorsal to the mlb between the stereotactic planes P 5.5 and P 1.5 (Papez 1929). Caudally, the NIC abuts on the supragenual nucleus capping the facial genu, but its lateral and rostral borders are indistinct. The remaining PRF neurons (12 units) were located close to the midline in the NRaP and the central superior nucleus of the raphe (CS) (Fig. 2, *C* and *D*).

Figure 2*A* shows the antidromic activation of a NIC neuron from the contralateral flocculus (*left*) and the lack of activation of the same unit from the ipsilateral flocculus (*right*). The response latency of this neuron was constant, as was the case for all the other neurons. The variation in the peak latency was <30 μs, as would be expected for antidromic activation. Figure 2*B* illustrates the collision testing between spontaneous spikes and antidromic responses.

The 38 NIC neurons activated from the contralateral flocculus had an antidromic response latency of 1.32 ± 0.41 (SD) ms. For the 19 NIC neurons activated from the ipsilateral side, the antidromic latency was 1.16 ± 0.34 ms.

Of the 69 PRF neurons activated from either flocculus, 42 neurons modulated their firing rate during spontaneous horizontal saccades and could be divided into two categories. One group of 34 neurons changed their firing rate in a burst-tonic fashion (BT neurons). Another group of eight neurons modulated their burst activity only during saccades and not during subsequent gaze holding (B neurons). The NIC contained 31 BT neurons; 3 BT neurons were located in the NRaP and the CS (Fig. 2, *C* and *D*, ●). The BT neurons of the NIC were further classified as type I or type II, depending on whether their firing rate increased during horizontal head rotation toward or away from the recording side, respectively. The majority of the B neurons were located in the CS, the NRaP, or the mlb (Fig. 2, *C* and *D*, ▲). On the

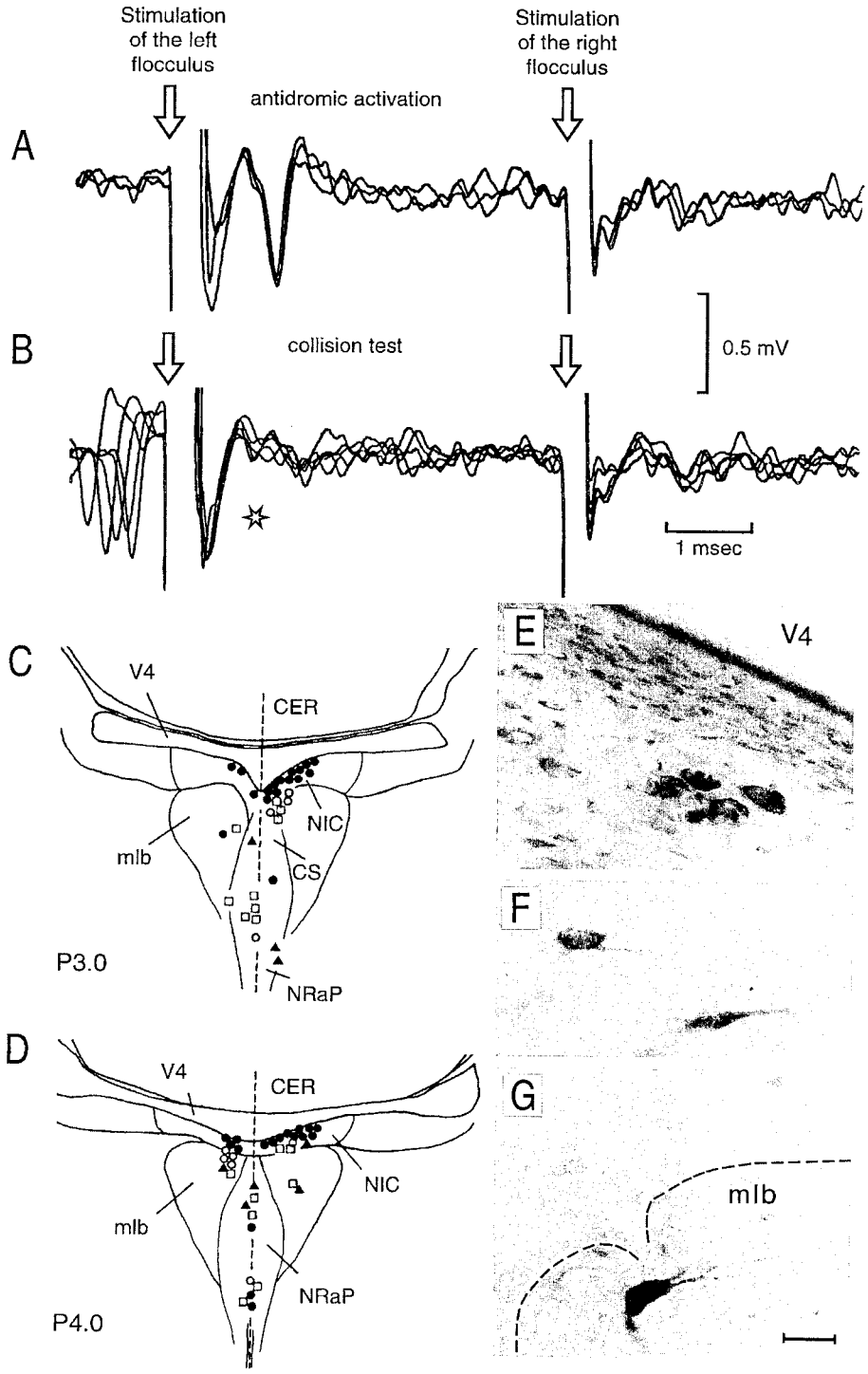

FIG. 2. *A*: superposition of 3 recording traces showing the antidromic activation of a burst-tonic (BT) neuron of the nucleus incertus (NIC) following stimulation of the contralateral flocculus and the lack of activation of the same unit from the ipsilateral flocculus. *B*: collision testing. Star: absence of the antidromic spike when it collided with the spontaneous spike. *C* and *D*: localization of the floccular projecting neurons in the pontine reticular formation (PRF) (see RESULTS for explanation of symbols). *E*: cluster of retrogradely labeled small neurons in the contralateral NIC (P4, Nissl counterstain). *F*: retrogradely labeled small oval and fusiform neurons in the contralateral NIC (P3). *G*: retrogradely labeled medium-sized polygonal neuron at the dorsomedial corner of the medial longitudinal bundle (mlb) (P3.5). Bar in *E–G*: 25 μm. CS, central superior nucleus of raphe; NRaP, nucleus raphe pontis; CER, cerebellum; V4, fourth ventricle.

border between the NIC and the mlb, 10 other BT neurons were found that did not respond to horizontal head rotation but modulated their activity exclusively during spontaneous vertical saccades (Fig. 2, *C* and *D*, ○). Retrogradely labeled neurons in this location were slightly bigger than the labeled NIC neurons (Fig. 2*G*). The 17 remaining neurons were not related to eye movements (Fig. 2, *C* and *D*, □).

All 34 horizontal-eye-movement-related BT neurons (17 activated from the contralateral and 14 from the ipsilateral flocculus, and 3 neurons located in the midline) responded to head rotation (27 as type I, 4 as type II). Figure 3 illustrates the spiking behavior of a representative BT neuron of the NIC activated antidromically from the contralateral flocculus. Before and during rapid eye movements, this neuron paused when the eyes moved toward the recording side and burst when the eyes moved in the opposite direction (Fig. 3, *A* and *B*). During intersaccadic fixation, the tonic discharge rate of this neuron increased with more eccentric

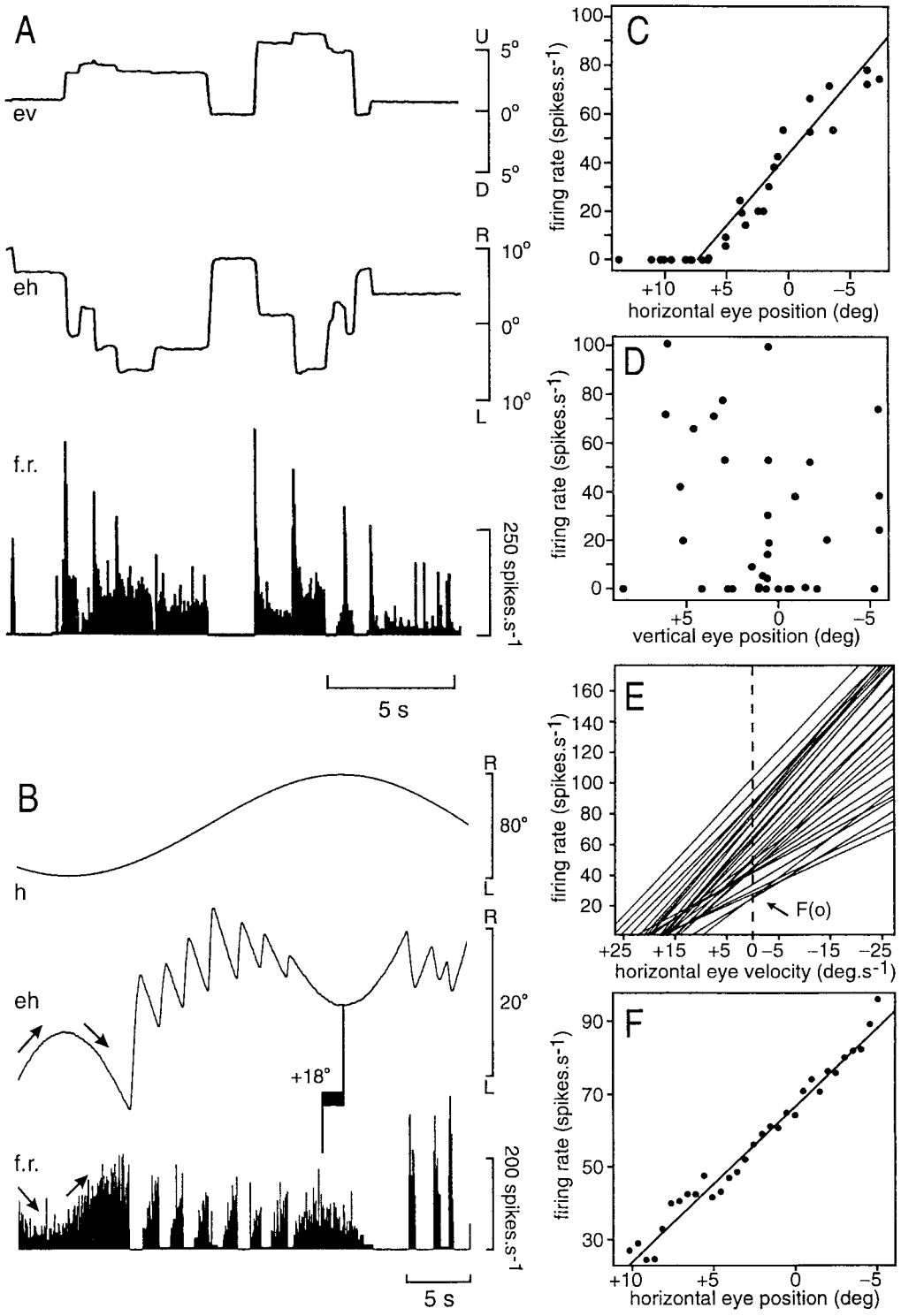

FIG. 3. *A* and *B*: behavior of a representative BT neuron of the NIC during spontaneous eye movements (*A*) and during the horizontal vestibuloocular reflex (VOR) (*B*). ev, vertical eye position; eh, horizontal eye position; f.r., firing rate; h, head position. Note that in this case the phase lead of the firing rate modulation is 18° with respect to eye position. *C*: scatter plot of mean instantaneous firing rate of the same BT neuron over horizontal eye position during intersaccadic fixation periods. The slope (K_f) of the linear regression between the horizontal eye position and the firing rate ($r = 0.85$) corresponds to the sensitivity to horizontal eye position ($K_f = 6.05$ spikes $\cdot s^{-1} \cdot deg^{-1}$). *D*: scatter plot of mean instantaneous firing rate of the neuron over vertical eye position. Note the absence of any significant relation. *E*: analysis of the eye velocity sensitivity (R_v) of this BT neuron. The slope of these different rate-velocity regressions (R_v) varied with eye position from 1.51 to 4.02 spikes $\cdot s^{-1} \cdot deg^{-1} \cdot sec^{-1}$. For each of these lines, the firing rate at 0 velocity, $F(0)$, was calculated by interpolation. *F*: relationship between $F(0)$ and horizontal eye position. The data points are well fitted by a linear regression line. The slope (K_v) of this line corresponds to the sensitivity of this neuron to eye position during the VOR ($K_v = 4.52$ spikes $\cdot s^{-1} \cdot deg^{-1}$).

(contralateral side) gaze position (Fig. 3*C*), but it did not change as a function of vertical eye position (Fig. 3*D*). During sinusoidal vestibular stimulation, the firing rate of this BT neuron showed a type I sinusoidal modulation interrupted by burstlike increases and by pauses corresponding to the quick phases, directed away or toward the recording side, respectively (Fig. 3*B*). For BT neurons in the NIC, the value of K_f ($n = 24$) ranged from 1.70 to 17.40 spikes $\cdot s^{-1} \cdot deg^{-1}$ with a mean ± SD of 8.68 ± 4.55 spikes $\cdot s^{-1} \cdot deg^{-1}$. The value of R_s ($n = 11$) ranged from 2.38 to 12.43 spikes $\cdot s^{-1} \cdot deg^{-1} \cdot s^{-1}$ with a mean of 6.76 ± 3.49 spikes $\cdot s^{-1} \cdot deg^{-1} \cdot s^{-1}$. The value of K_v ($n = 24$) ranged from 2.63 to 16.6 spikes $\cdot s^{-1} \cdot deg^{-1}$ with a mean of 7.63 ± 3.57 spikes $\cdot s^{-1} \cdot deg^{-1}$. The R_v during the slow phases of the BT neurons ($n = 24$) ranged from 0.94 to 17.03 spikes $\cdot s^{-1} \cdot deg^{-1} \cdot s^{-1}$ with a mean of 3.75 ± 1.98 spikes $\cdot s^{-1} \cdot deg^{-1} \cdot s^{-1}$.

DISCUSSION

In the explored region of the PRF, the majority of BT neurons that project to the horizontal zone of either the ipsilateral or contralateral flocculus were located in the caudal aspect of the NIC. Most of them (87%) responded to horizontal head rotation in a type I fashion. These neurons provide the flocculus with a signal related to both the velocity and the position of the eye. The BT neurons in the NIC that project to the flocculus showed a spiking behavior very similar to the BT neurons observed in the NPH (Escudero et al. 1995) and the MVN (Cheron et al. 1995), which were also antidromically activated from the horizontal zone of the flocculus.

The present study confirms the results of an earlier electrophysiological analysis of the PRF floccular projection in the anesthetized cat (Nakao et al. 1980). However, the BT neurons described in this study were located in the mlb, the CS, and the NRaP, but not in the NIC. Curthoys et al. (1981) demonstrated that BT neurons in the PRF could not be antidromically activated from the abducens nucleus, which indicates that they cannot be classified as premotor neurons.

The data presented in this study show that the majority of the horizontal BT neurons with a projection to the flocculus, recorded in the dorsolateral pontine tegmentum, are localized in a region hitherto not associated with the oculomotor system: the caudal part of the NIC.

Available data on this nucleus have shown that the NIC participates extensively in projection to the medial septum (Vertes 1988) and to the interpeduncular nucleus (Hemmendinger and Moore 1984; Shibata et al. 1986). Moreover, immunocytochemical studies have demonstrated that neurons in the NIC contain different peptides like somatostatin (De Leon et al. 1992a), neuropeptide Y (Covenas et al. 1990), neurokinin A (Marcos et al. 1993a), alpha-neo-endorphine (Marcos et al. 1993b), gastrin-releasing peptides (Marcos et al. 1994), substance P (Triepel et al. 1985), and cholecystokinin (De Leon et al. 1992b).

It remains to be determined whether the caudal NIC neurons are a subset of the paramedian tract cell groups associated with specific aspects of oculomotor behavior (Büttner-Ennever 1992). The absence of data on the precise connections of these neurons preclude conclusions concerning their functional role related to eye movements. Nevertheless, their high sensitivity for both eye position and eye velocity might indicate that they provide an efference copy of eye movement commands to the flocculus. The anatomic and physiological characteristics of the NIC neurons show a strong correspondence with the NPH floccular projecting neurons (Escudero et al. 1995), i.e., the superficial localization, the specific projection to the horizontal floccular zone and the spiking behavior. The convergence onto a single floccular zone of very similar eye-movement-related signals from different brain stem nuclei could represent an important element in the signal processing of the flocculus.

Furthermore, it remains to be determined whether single NIC axons collateralize to both cerebellum and basal forebrain. Apart from this question, it is important to determine which type of the efferent NIC neurons contains peptides and which of the peptidergic neurons are involved in oculomotor and cerebellar function.

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