VESTIBULAR RESPONSES OF NEURONS IN THE NUCLEUS RETICULARIS GIGANTOCELLULARIS OF RABBIT: AN ELECTROPHYSIOLOGICAL AND ANATOMICAL STUDY

by

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To my children, Beth, Sally and Rob

for their encouragement and support

and for the role models they provided

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ABSTRACT

Neurons in the reticular formation respond to vestibular stimulation. Using extracellular recording, we characterized the responses of neurons in a restricted region of the reticular formation, the medial aspect of the caudal nucleus reticularis gigantocellularis (NRGc). Most neurons in the medial aspect of the caudal NRGc have increased activity with static roll tilt to the contralateral side. This response polarity is opposite to that of vestibular nucleus responses. Over half of these also responded to transient vestibular stimulation. At least one quarter of vestibularly responsive neurons have a response phase that varies with the orientation of the stimulus plane ("variable phase" neurons) suggesting convergence of spatially and temporally differing inputs. This proportion of "variable phase" neurons is higher than that observed in the vestibular nuclei indicating that some convergence of vestibular inputs occurs in the medial NRGc.

Since input from the two vertical semicircular canals is topographically organized in both the β-nucleus of the inferior olive and the uvula/nodulus of the cerebellum, we were interested in testing whether vestibular information is topographically organized in the NRGc. We compared the location of each neuron to its response characteristics, i.e., response to static tilt and optimal response plane. We were not able to identify a topographical organization of vestibular information in this nucleus.

To examine the pathways that transmit vestibular information to the NRGc, we used injections of phaseolus leucoagglutinin into the medial and descending vestibular

nuclei (MVN and DVN). We identified fibers extending from the MVN and DVN and filled terminals bilaterally in the medial NRGc. Projections extend from the MVN and DVN in two distinct fiber tracts. Fibers in these tracts appear to terminate among three groups of neurons adjacent to these tracts. Using horseradish peroxidase (HRP) injections into the medial NRGc, we demonstrated that the projection from the DVN is primarily ipsilateral while that from the MVN is primarily contralateral. We also identified glutamic acid decarboxylase-positive varicosities in the region of the fiber tracts suggesting that the projection from the MVN and DVN is GABAergic. Using HRP injections into the vestibulocerebellum, the medial vestibular nucleus and nucleus prepositus hypoglossi, we identified the locations of neurons in the medial NRGc that project to these nuclei. These anatomical findings may be useful in further exploration of the organization of vestibular information in the nucleus on a more localized level.

NRGc neurons are known to project to the spinal cord and to have strong effects on motoneurons. Our results together with these previous findings suggest that the medial NRGc may play an important role in molding postural.

ABBREVIATIONS

CFR	climbing fiber response
CRF	corticotropin releasing factor
dmcc	dorsomedial cell column
DVN	descending vestibular nucleus
EMG	electromyographic
GABA	γ-aminobutyric acid
HRP	horseradish peroxidase
MVN	medial vestibular nucleus
NRGc	nucleus reticularis gigantocellularis
NRL	nucleus reticularis lateralis
NRPO	nucleus reticularis pontis oralis
NRPC	nucleus reticularis pontis caudalis
NRPc	nucleus reticularis parvicellularis
NRV	nucleus reticularis ventralis
PHA-L	phaseolus leucoagglutinin
PRN	paramedian reticular nucleus
STC	spatiotemporal convergence
VCR	vestibulocollic reflex

INTRODUCTION

Sensory systems of vertebrates convey a wide range of specific information about the environment and forces of the environment acting on the animal. However, the specific information encoded by a sensory system or set of systems in response to a particular set of stimuli must first be segregated and combined for the control of the appropriate muscles.

This research is concerned with the processing of vestibular information for the control of posture. One aspect of postural control that has received attention is the stabilization of the head. For the stabilization of the head, the vestibular end organs provide sensory information about the spatial and temporal aspects of the position and movements of the head (Lowenstein and Sand, 1940b; Lowenstein and Sand, 1940a; Lowenstein and Roberts, 1949). This information is used by various postural reflexes controlling neck, back and limb muscles (Schor and Miller, 1995; Wilson and Maeda, 1974; Bolton et al. 1992a; Schor and Miller, 1981; Wilson et al. 1979; Bilotto et al. 1982; Wilson et al. 1990a). For example, during the vestibulocollic reflex, the appropriate neck muscles must be selected to counteract the forces of gravity or the movement of the head and to control the timing of each muscle to initiate correction of head movement, to control for overshoot of this head movement. Neck and other postural muscles are controlled by vestibulospinal and reticulospinal input to motor neurons (Wilson and Yoshida, 1969; Roucoux et al. 1989; Hayes and Rustoni, 1981;

Peterson et al. 1978; Peterson et al. 1980; Peterson et al. 1979; Wilson et al. 1979; Bilotto et al. 1982).

We have examined the responses of neurons in the reticular formation to natural vestibular stimulation. We focused our attention on a limited region of the reticular formation, the medial aspect of the caudal nucleus reticularis gigantocellularis (NRGc), addressing the following questions. From which peripheral vestibular organs do the vestibular responses of NRGc neurons originate? Is vestibular information topographically organized in this nucleus? What pathways convey vestibular information to the NRGc? What kind of processing of vestibular information in preparation for motor control might take place in the NRGc?

This introduction provides an overview of current ideas about the pathways that convey vestibular information, segregation and topography of vestibular information in the central nervous system, the vestibulocollic reflex, the transformation of vestibular information by convergence of inputs, and the role of the reticular formation in vestibular function.

Pathways and transmitters of the vestibular system

Vestibular information for the control of motor functions is conveyed in several pathways (Fig. 1). Primary vestibular afferents terminate in the vestibular nuclei (Stein and Carpenter, 1967; Walberg and Bowsher, 1958; Gacek, 1969). The transmitter for primary vestibular afferents has not been identified conclusively but is probably glutamate (Takeda and Maekawa, 1980; Raymond et al. 1984). Neurons in the lateral

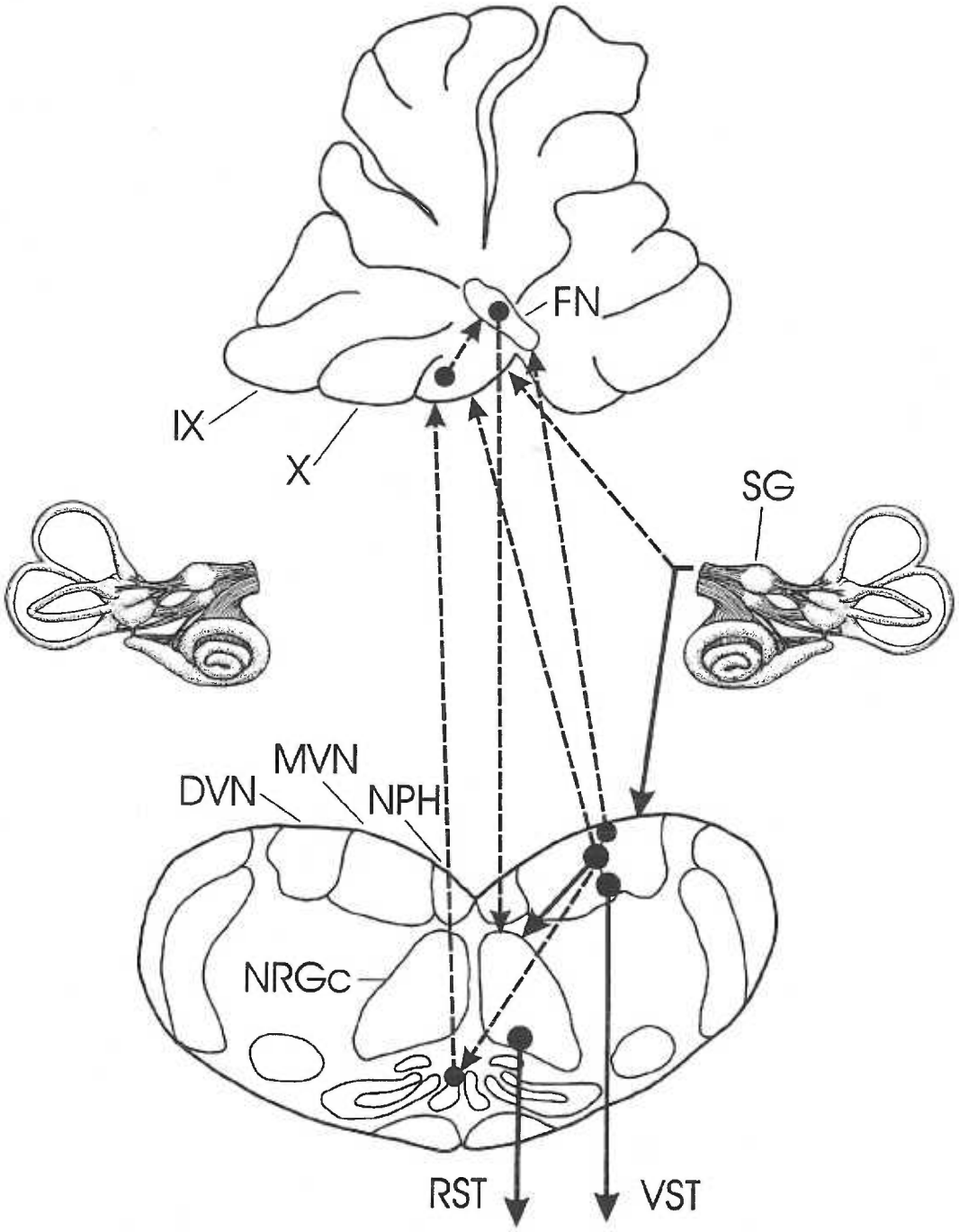

FIG. 1. Transmitter-specific pathways of the vestibular system. Direct vestibulomotor pathways are indicated by solid lines. Pathways through the cerebellum are indicated by dashed lines. Abbreviations: DVN, descending vestibular nucleus; FN, fastigial nucleus; MVN, medial vestibular nucleus; NPH, nucleus prepositus hypoglossi; NRGc, nucleus reticularis gigantocellularis; RST, reticulospinal tract; SG, scarpa's ganglion; VST, vestibulospinal tract; IX, cerebellar lobule IX or uvula; X, cerebellar lobule X or nodulus.

and descending vestibular nuclei project in the vestibulospinal tract to neurons in the spinal cord motor nuclei (Minor et al. 1990; Rose et al. 1992; Coulter et al. 1979; Petras, 1967; Wilson and Peterson, 1981; Manzoni, 1988).

Neurons in all four of the vestibular project to the reticular formation (Ferraro et al. 1940; Ladpli and Brodal, 1968; Brodal, 1972; Brodal, 1974). Fibers from the vestibular nuclei have been traced to nuclei in the reticular formation including: the nuclei reticularis pontis oralis (NRPO) and caudalis (NRPC), gigantocellularis (NRGc), and parvicellularis (NRPc) (Ferraro et al. 1940; Ladpli and Brodal, 1968). The transmitter for the projection from the vestibular nuclei to the reticular formation has not been identified.

Neurons in the reticular formation convey information to motor nuclei in the spinal cord. Reticulospinal neurons are located predominantly in the NRGc and NRPC but are also found in the nuclei reticularis ventralis (NRV) and lateralis (NRL) (Valverde, 1961). Axons of neurons in the dorsal NRGc of rat project to the spinal cord in the ventral reticulospinal tract and those in the ventral NRGc project in the ventrolateral reticulospinal tract (Zemlan et al. 1984). Axons of neurons in the nucleus reticularis ventralis and the nucleus reticularis magnocellularis project in the lateral reticulospinal tract.

Vestibular information is also conveyed to the cerebellum. More than 70% of primary vestibular afferents project as mossy fibers to the cerebellar nodulus terminating in the granule cell layer (Barmack et al. 1993a). Ionotropic glutamate receptors are expressed by vestibular nuclear neurons as well as in cerebellar granule

cells and Purkinje cells (Smith and Darlington, 1988; De Waele et al. 1990; Doi et al. 1990; Smith and Darlington, 1991; Petralia and Wenthold, 1992; Gallo et al. 1992; Danbolt et al. 1992; Martin et al. 1993). Secondary vestibular afferents from the medial and descending vestibular nuclei also project to the uvula/nodulus and the flocculus of the cerebellum as well as to both the ipsilateral β-nucleus and bilaterally to the dorsomedial cell column (dmcc) of the inferior olive (Olson et al. 1987; Walberg, 1974; Saint-Cyr and Courville, 1979; Barmack et al. 1992a; Barmack et al. 1992b; Barmack and Fagerson, 1994). The transmitter for the projection from the MVN and DVN to the inferior olive is γ-aminobutyric acid (GABA) while one of the transmitters to the cerebellum is acetylcholine (Nelson et al. 1986; Nelson et al. 1989; Barmack et al. 1992a; Barmack et al. 1992b).

Neurons in both the β-nucleus and the dmcc project as climbing fibers to the cerebellum terminating on Purkinje cells in the contralateral uvula/nodulus (Maekawa and Simpson, 1973). The transmitter for these pathways is probably glutamate (Matute et al. 1987; Wiklund et al. 1982). Corticotropin releasing factor (CRF) is also released by these projections to the uvula/nodulus (Wynn et al. 1984; DeSouza et al. 1985; DeSouza, 1987; Mugnaini and Nelson, 1989; Barmack and Young, III, 1990).

The fastigial nucleus receives input from neurons in the vestibular nuclei as well as GABAergic input from Purkinje cells in the uvula/nodulus (Najlerahim et al. 1990; Schiffmann and Vanderhaeghen, 1990). The transmitter for the projection from the vestibular nuclei has not been identified.

Neurons in the vestibular nuclei, the cerebellar uvula/nodulus and the fastigial nucleus as well as in the β-nucleus and dmcc of the inferior olive respond to vestibular stimulation (Barmack et al. 1993b; Barmack, 1987; Barmack and Fagerson, 1994). The predominant response to stimulation in vertical planes of neurons in the vestibular nuclei, the uvula/nodulus and the fastigial nucleus is increased activity during tilt to the **ipsi**lateral side while that of neurons in the β-nucleus and dmcc of the inferior olive is increased activity during tilt to the **contra**lateral side (Barmack et al. 1993b). The reversed polarity of the responses of neurons in the β-nucleus and dmcc is likely a result of the inhibition resulting from the release of GABA in this nucleus.

Segregation and topography of vestibular information

Sensory information must be retained in an organized fashion for it to be used effectively in motor control. Retention of vestibular sensory information in an organized fashion has been demonstrated in several nuclei. Following lesions of the vestibular ganglia of monkey, fiber degeneration in the medial and descending vestibular suggests an organization of information from individual vestibular organs (Stein and Carpenter, 1967; Gacek, 1969; Lowenstein and Roberts, 1949). Ipsilateral semicircular canal information is represented in the superior vestibular nucleus (SVN) and the rostral MVN and DVN. Otolith input is also represented caudal to that from the semicircular canals and extends caudally throughout most of the length of these nuclei. Input from the utriculus terminates in the medial DVN while input from the sacculus terminates in the lateral DVN.

Secondary vestibular input to the β-nucleus of the inferior olive retains the segregation of anterior and posterior canal information (Barmack et al. 1989; Barmack et al. 1993b). Extracellular recordings in rabbit β-nucleus during vertical vestibular stimulation demonstrated that the optimal response planes of neurons in the rostral β-nucleus are close to the plane of the ipsilateral posterior semicircular canal while the optimal response planes of neurons in the caudal β-nucleus are close to the plane of the ipsilateral anterior semicircular canal.

A similar organization of vertical canal information has also been identified in climbing fiber responses in the cerebellar uvula/nodulus of rabbit (Sato and Barmack, 1985; Barmack and Shojaku, 1992; Barmack and Shojaku, 1995). During natural vestibular stimulation, the optimal response planes of climbing fiber responses of Purkinje cells in a medial sagittal strip on the ventral surface of the uvula and in the nodulus were located in the plane of the ipsilateral posterior semicircular canal, whereas the optimal response planes of Purkinje cells in a more lateral sagittal strip were located in the plane of the anterior semicircular canal. Visual stimulation in vertical planes coinciding with the planes of the anterior or posterior canal also resulted in climbing fiber responses in the lateral or medial strip, respectively (Sato and Barmack, 1985; Barmack and Shojaku, 1992; Barmack and Shojaku, 1995). Horizontal visual stimulation in the posterior→anterior direction was represented in cells in the ventral nodulus between the medial and lateral strips containing vertical canal information. Thus, two modes of sensory input are combined in these cerebellar

sagittal strips but each mode is segregated based on the directional content of the information.

Vestibular contribution to head stabilization

Vestibular information contributes to the stabilization of the head through the vestibulocollic reflex. This reflex coordinates with the cervicocollic reflex that responds to proprioceptive input from neck muscles. The two reflexes stabilize the head by stimulating neck muscles to oppose movement of the head or the force of gravity (Wilson et al. 1979; Bilotto et al. 1982; Dutia and Price, 1987; Goldberg and Peterson, 1986; Peterson et al. 198; Wilson et al. 1990b; Wilson et al. 1990a). Voluntary control also contributes to the stabilization of head position (Keshner et al. 1992b; Keshner et al. 1992a).

Neck muscles respond to stimulation of vestibular afferents (Wilson and Maeda, 1974; Wilson et al. 1979; Baker et al. 1985; Bolton et al. 1992a). Experiments using stimulation of individual ampullar nerves, while recording intracellularly from neck extensor motoneurons in cat, indicate that neck muscles receive both excitatory and inhibitory input from several canals (Wilson and Maeda, 1974). Some neck muscles, biventer cervicus and complexus, are maximally excited by stimulation of both anterior semicircular canals. Similar experiments using stimulation of the utricular nerve, indicate that neck motoneurons receive disynaptic excitatory and tri- or polysynaptic inhibitory input from the utriculus (Bolton et al.

1992a). The motoneuron responses in these experiments were functionally appropriate to counteract a head movement suggested by the nerve stimulated.

The phase lag and the gain of neck muscle electromyographic (EMG) activity decreased as the frequency of sinusoidal polarizing current applied to individual ampullary nerves increased (Wilson et al. 1979). On the other hand, simultaneous extracellular recordings in the vestibular nuclei indicated that the response of vestibular nuclei neurons followed the stimulus as the frequency of stimulation was increased, with the phase of the response approximately in phase with the stimulus. The gain of the vestibular neuronal responses increased with increases in frequency rather than decreasing as was observed in the muscle EMG. This difference between the muscle responses and vestibular neuron responses and the similarity of vestibular responses to the stimulus profile indicates that a transformation of the timing of vestibular information must occur in pathways to neck muscles beyond the vestibular nucleus.

Other transformations of vestibular information

Another transformation of vestibular information occurs in the pathway from the vestibular organs to neck muscles. A combined spatial and temporal transformation of vestibular information was demonstrated in EMG activity in individual neck muscles during natural vestibular stimulation (Baker et al. 1985). The optimal response plane of stimulation was identified for each neck muscle at stimulus frequencies above 0.5 Hz. At low frequencies of stimulation in vertical planes, the optimal response plane was poorly defined because the response phase shifted with changes in the orientation

of the stimulus plane. These data were taken to indicate that neck muscle responses were generated by convergence of input from two sources with differing spatial and temporal characteristics. The phenomenon was termed spatiotemporal convergence (STC) and was assumed to be the result of convergence of vertical canal and otolith inputs.

A spatial transformation also occurs in the transmission of vestibular information to neck muscles. Some muscles are maximally activated in pitch planes and some neck motoneurons receive input from the two anterior canals (Wilson and Maeda, 1974; Baker et al. 1985).

Since the discovery of spatial and temporal transformations, experiments have focused on identifying the level at which these transformations take place (Baker et al. 1984b; Baker et al. 1984a; Wilson et al. 1990b; Wilson et al. 1992; Peterson et al. 1992; Bolton et al. 1992b; Graf et al. 1993; Endo et al. 1994). Explorations have taken place in the vestibular nuclei, the reticular formation, and in spinal cord commissural neurons with the goal of finding neurons with properties that indicate a spatial convergence such as that of two canals across the midline (e.g., right anterior + left anterior) or a spatial and temporal convergence such as convergence of canal and otolith input (Wilson et al. 1990b; Bolton et al. 1992b; Endo et al. 1994). There was no evidence of significant convergence between the two anterior or the two posterior canals at any of these levels. There was evidence for spatiotemporal convergence in small fraction of vestibular nucleus neurons (9%) and in medullary reticular formation neurons (10%) (Wilson et al. 1990b; Bolton et al. 1992b). However, since

spatiotemporal convergent behavior is so prominent in the vestibulocollic reflex, a larger fraction of spatiotemporal convergent neurons was expected. These results have led to the conclusion that significant convergence occurs at a point closer to the motor neuron.

Role of the reticular formation in vestibular functions

The reticular formation is appropriately positioned in the vestibulomotor pathways to provide the substrate for the convergence of vestibular information to control muscles (Fig. 1).

The reticular formation receives vestibular input. Evidence from both galvanic stimulation of vestibular afferents and from natural vestibular stimulation experiments indicate that the reticular formation receives vestibular information. First, the reticular formation receives both di- and polysynaptic input from vestibular afferents (Peterson et al. 1975a; Peterson and Abzug, 1975; Nauta and Kuypers, 1958; Peterson et al. 1980). With stimulation of the vestibular nerve, short latency IPSPs were concentrated in the dorsorostral NRGc while short latency EPSPs were distributed throughout this nucleus (Peterson et al. 1975a). Neurons identified as reticulospinal neurons received only short latency EPSPs but not IPSPs. When stimulation was applied to the vestibular nuclei on the same side similar responses were usually evoked (Peterson and Abzug, 1975). Vestibular neurons could also be activated antidromically by stimulation of the contralateral medial reticular formation. However, many reticular

neurons receiving input from the vestibular nuclei do not receive vestibular information since they could not be activated by stimulation of the vestibular nerve.

Second, neurons in the reticular formation respond to natural vestibular stimulation of both the semicircular canals and the otoliths (Duensing and Schaefer, 1960; Orlovsky and Pavlova, 1972; Fukushima et al. 1977; Manzoni et al. 1983; Spyer et al. 1974; Bolton et al. 1992b). Neurons that responded to horizontal rotation were located near the boundary of the nucleus prepositus hypoglossi in the dorsal brainstem and concentrated just caudal to the abducens nucleus (Fukushima et al. 1977). Neurons in the NRGc and NRV respond to low frequency (0.026 Hz) sinusoidal stimulation in vertical planes (Manzoni et al. 1983). The majority of these neurons especially in the NRGc and rostral NRV have increased activity during rotations onto the contralateral side and had a response phase that correlated to the displacement of the head during sinusoidal stimulation frequencies from 0.026 to 0.051. At higher frequency stimulation, the response phase of a minority (16%) of these neurons advanced so that the response was correlated with the angular velocity of displacement. These results indicate that the majority of reticulospinal neurons receive input mediated by the otoliths (Bolton et al. 1992b). This is in contrast to vestibulospinal neurons that receive input mediated predominantly by semicircular canal input (Kasper et al. 1988; Wilson et al. 1990b).

The planes of stimulation resulting in an optimal response for most reticulospinal neurons lie in roll quadrants (i.e., the maximal response occurs during rotation to the side rather than during nose down-nose up rotations). There was no

attempt to correlate the location of the neurons in these studies in the reticular formation with the optimal response plane.

Although previous anatomical studies have demonstrated connections of the vestibular nuclei with the pontomedullary reticular formation, they have not identified conclusively the laterality of the projections from the medial and descending vestibular nuclei (Ferraro et al. 1940; Ladpli and Brodal, 1968). The Marchi method does not allow identification of terminals (Ferraro et al. 1940) whereas with the Nauta technique fiber and terminal degeneration were identified in the dorsomedial contralateral NRGc following lesions in the DVN (Ladpli and Brodal, 1968). However, because of possible interruption by the lesion of fibers from other vestibular nuclei that also terminate in the NRGc, it was not possible to conclude that fibers from the MVN terminate in the ipsi- or contralateral NRGc. Thus, the laterality of the projections from the MVN and DVN to the NRGc have not been demonstrated.

The transmitter for the pathway from the vestibular nuclei to the reticular formation has not been identified. Physiological studies comparing the responses to vestibular stimulation of vestibular nuclei neurons to those of reticular formation neurons have demonstrated that the polarity of the majority of responses in the reticular formation is opposite to that of the majority of vestibular neurons (Manzoni et al. 1983). Consequently it was suggested that the vestibular input to the reticular formation may be inhibitory.

The peripheral origin of vestibular responses in the pontomedullary reticular formation has been inferred from the dynamic responses of reticular formation neurons

to vestibular stimulation by comparison to the responses of vestibular afferents from the semicircular canals and otoliths (Manzoni et al. 1983; Bolton et al. 1992b). Most semicircular canal afferent responses of squirrel monkey have an advancing phase lead and increasing gain as the frequency of stimulation increases (Fernandez and Goldberg, 1971). In the mid-frequency stimulus range, the phase lead of the response with respect to head position is 90 deg. Most otolith afferent responses have relatively constant gain with increasing stimulus frequency and only small increases in phase lead (Fernandez and Goldberg, 1976). Also, the response phase is near head position or 0 deg. However, some otolith afferent responses are similar to canal afferent responses in that they have large increases in gain and phase with increasing stimulus frequency. Thus, analysis of dynamics does not conclusively identify the peripheral origin of vestibular responses. Response to static tilt, however, has been taken as conclusive evidence of otolith input since semicircular canal afferents do not respond to static tilt (Vidal et al. 1971; Fernandez et al. 1972).

Reticulospinal connections with motor neurons. Reticulospinal neurons that project to neck motoneurons make monosynaptic connections with motor neurons allowing direct control of muscle action (Wilson and Yoshida, 1969; Roucoux et al. 1989). However, reticulospinal neurons that project to lumbar motoneurons may have only polysynaptic connections (Wilson and Yoshida, 1969). While many hind limb motor neurons in cat are activated by stimulation of the lateral vestibular nucleus, these neurons not activated by stimulation of the medial reticular formation. Neck motor neurons, on the other hand, are often activated by both vestibular nucleus and reticular

formation stimulation. Limb motor neurons may be activated by polysynaptic pathways involving cervical motor neurons. This suggestion is supported by evidence that retrograde tracer injected in the lumbar spinal cord labels neurons in cervical segments but not in the NRGc where stimulation activates lumbar back muscles (Roucoux et al. 1989). On the other hand, neurons in the ventral region of NRGc and NRV were retrogradely labeled but stimulation in these regions did not activate back muscles. However, some reticulospinal neurons in the pontomedullary reticular formation project to both the brachial and lumbar spinal enlargements (Hayes and Rustoni, 1981).

Although no topographical organization of specific vestibular inputs to the reticular formation has been demonstrated, topographical organization of some motor outputs has been demonstrated in the reticular formation. The lateral and medial reticulospinal tracts originate in different regions of the reticular formation (Nyberg-Hansen, 1966; Petras, 1967; Ito et al. 1970; Peterson et al. 1975b; Tohyama et al. 1979). The pontomedullary reticular formation can be divided into a number of zones with distinct patterns of connections (Peterson et al. 1979). Microstimulation in the reticular formation of chronically implanted unanesthetized cats indicates that there is an organization based on patterns of EPSPs and IPSPs in neck back and limb motor neurons and on patterns of muscle activation, i.e., neck muscle activation with various combinations of right and left, fore and hind limbs (Drew and Rossingnol, 1990a; Drew and Rossingnol, 1990b). The strongest responses in the ipsilateral fore limb were evoked by stimulation in rostrodorsal regions while those in the contralateral

forelimb were evoked by stimulation caudoventrally. Responses of neck and axial muscles were evoked by stimulation throughout the medullary reticular formation. However, stimulus loci evoking these muscles were concentrated in the most caudal regions.

The medial aspect of the caudal NRGc. In summary, the reticular formation lies midway along the pathways from the vestibular end organs to motor output. This position in vestibulomotor pathways allows convergence of other inputs. For example, some other inputs to the reticular formation include neck proprioceptive, tectal, cortical and cerebellar inputs (Brodal et al. 1962; Peterson et al. 1976). There may also be convergence of more than one vestibular input on neurons in the reticular formation.

One region of the caudal reticular formation is structurally different from the surrounding regions (Brodal, 1957). One of three cerebellar projecting nuclei in the reticular formation is located in the caudomedial reticular formation, the paramedian reticular nucleus (PRN). In the rabbit, this nucleus is included in the NRGc (Meessen and Olszewski, 1949). The PRN contains neurons that have a morphology similar to sensory neurons with short highly ramified dendrites while neurons in the surrounding regions of the reticular formation have few long sparsely ramified dendrites (Leontovich and Zhukova, 1963). The dendritic morphology of neurons in the PRN seems to make these neurons well suited to receiving convergent inputs from many sources. The PRN is located ventromedial to the fibers of the hypoglossal nerves and dorsal to the middle third of the inferior olive (Brodal, 1953; Brodal, 1957; Kotchabhakidi et al. 1980). In the rabbit, the PRN is included in the nucleus reticularis

gigantocellularis (NRGc) lying in the medial aspect of the caudal NRGc (Meessen and Olszewski, 1949).

Previous physiological studies of vestibular responses of neurons in the reticular formation have examined a broad region of the pontomedullary reticular formation including the nuclei reticularis pontis caudalis, magnocellularis, gigantocellularis and ventralis (Spyer et al. 1974; Peterson et al. 1976; Peterson et al. 1979; Peterson et al. 1980; Manzoni et al. 1983; Bolton et al. 1992b). This study has focused on a more limited region of the reticular formation, the medial 500 μm of the caudal NRGc of rabbit. This region has similar boundaries to the PRN of cat and rat. It lies dorsal to the rostral two thirds of the inferior olive and ventromedial to the hypoglossal nerve. In contrast to most previous physiological studies, we have attempted to correlate physiological findings with anatomical variations in the reticular formation addressing issues of topographical organization and convergence of inputs.

SUMMARY

The goal of this dissertation is to characterize several aspects of the role of the medial aspect of the NRGc in the segregation and processing of vestibular information for muscle control. Two sets of experiments were performed as presented in the following two manuscripts.

First, we examined the kinds of vestibular information conveyed by NRGc neurons and we tested the hypothesis that vestibular information is topographically organized within the NRGc. To examine the peripheral origin (semicircular canal or otolith) of the vestibular input, we examined the responses of NRGc neurons using sinusoidal, static and exponential "step" vestibular stimulation while recording extracellularly from single neurons. We determined the optimal response plane for each neuron. The location of each neuron was examined in relation to the optimal response plane or the response to exponential "step" stimulation for each neuron.

Second, we examined the anatomical connections of the medial aspect of the caudal NRGc. By injecting an orthogradely transported lectin, phaseolus-leucoagglutinin (PHA-L), into the medial and descending vestibular nuclei, we determined the likely pathways by which vestibular information reaches neurons in the NRGc. These pathways were also identified with injections of the retrograde tracer, horseradish peroxidase (HRP), into the NRGc. We tested the hypothesis that the transmitter for the pathway conveying vestibular information from the vestibular nucleus to the NRGc was GABA using glutamic acid decarboxylase immunohistochemistry. We also examined some of the projections from this region of

the NRGc using HRP injections into the MVN and DVN and into the uvula/nodulus and flocculus of the cerebellum.

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RESPONSES TO VERTICAL VESTIBULAR STIMULATION OF NEURONS IN THE NUCLEUS RETICULARIS GIGANTOCELLULARIS IN RABBITS

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SUMMARY AND CONCLUSIONS

1. Since the nucleus reticularis gigantocellularis (NRGc) receives a substantial descending projection from the caudal vestibular nuclei, we used extracellular single unit recording combined with natural vestibular stimulation to examine the possible peripheral origins of the vestibularly modulated activity of caudal NRGc neurons located within 500 μm of the midline. Chloralose-urethane anesthetized rabbits were stimulated with an exponential "step" and/or static head-tilt stimulus, as well as sinusoidal rotation about the longitudinal or interaural axes providing various combinations of roll or pitch, respectively. Recording sites were reconstructed from electrolytic lesions confirmed histologically.

2. More than 85% of the 151 neurons, in the medial aspect of the caudal NRGc, responded to vertical vestibular stimulation. Ninety-six percent of these responded to rotation onto the contralateral side (β responses). Only a few also responded to horizontal stimulation. Seventy-eight percent of the neurons that responded to vestibular stimulation, responded during static roll-tilt. Half of these neurons also responded transiently to the change in head position during exponential "step" stimulation, suggesting input mediated by otolith and semicircular canal receptors or tonic-phasic otolith neurons.

3. Seventy-five percent of the responsive neurons had a "null plane." The planes of stimulation resulting in maximal responses, for cells that responded to static stimulation, were distributed throughout 150 deg in both roll and pitch quadrants. Five

of these cells responded only transiently during exponential "step" stimulation and responded maximally when stimulated in the plane of one of the vertical semicircular canals.

4. The phase of the response of the 25% of medial NRGc neurons that lacked "null planes" gradually shifted approximately 180 deg during sinusoidal vestibular stimulation as the plane of stimulation was shifted about the vertical axis. These neurons likely received convergent input with differing spatial and temporal properties.

5. The activity of neurons in the medial aspect of the caudal NRGc of rabbits was modulated by both otolithic macular and vertical semicircular canal receptor stimulation. This vestibular information may be important for controlling the intensity of the muscle activity in muscles such as neck muscles where the load on the muscle is affected by the position of the head with respect to gravity. Some of these neurons may also shift muscle function from an agonist to an antagonist as the direction of head tilt changes.

INTRODUCTION

The nucleus reticularis gigantocellularis (NRGc) receives inputs from many areas of the nervous system including ascending inputs from the spinal cord, primary fibers from cranial nerves IX, X and V and secondary fibers from cranial nerve nuclei, spinal trigeminal and vestibular nuclei (Rossi and Brodal, 1957; Anderson and Berry, 1959; Mehler et al. 1960; Clarke and Bowsher, 1962; Kerr, 1962; Ladpli and Brodal, 1968; Brodal, 1972; Brodal, 1974; Kerr, 1975; Maisky et al. 1978; Peterson and Abzug, 1975). It also receives descending inputs from the cerebellum, lateral hypothalamus, pallidum, superior colliculus and cortex (Rossi and Brodal, 1957; Nauta, 1958; Johnson and Clemente, 1959; Walberg et al. 1962; Nauta and Mehler, 1966; Rafols and Matzke, 1970; Kawamura et al. 1974). The diversity of these inputs implies a heterogeneity of neuronal responses in the NRGc.

The NRGc is also implicated in postural responses to vestibular input. The medial pontomedullary reticular formation receives input from the vestibular nuclei (Ladpli and Brodal, 1968; Peterson and Abzug, 1975). Reticular neurons respond to vestibular stimulation of semicircular canal (Duensing and Schaefer, 1960; Orlovsky and Pavlova, 1972; Fukushima et al. 1977; Manzoni et al. 1983; Bolton et al. 1992) and otolith receptors (Duensing and Schaefer, 1960; Spyer et al. 1974; Peterson et al. 1980; Manzoni et al. 1983; Bolton et al. 1992). The descending reticulospinal system originates from these neurons (Torvik and Brodal, 1957; Kuypers et al. 1962; Nyberg-Hansen, 1965; Petras, 1967; Kuypers and Maisy, 1975; Coulter et al. 1979; Tohyama et al. 1979; Hayes and Rustoni, 1981). The reticular formation also projects

extensively to the cerebellum, especially to lobulus simplex and the nodulus (Kotchabhakidi et al. 1980).

We were interested in learning what kind of vestibular information modulates the activity of NRGc neurons. In previous experiments, we have used a vestibular "null technique" (Estes et al. 1975) to detect the peripheral origin of vestibularly modulated activity of central neurons (Sato and Barmack, 1985; Shojaku et al. 1991; Barmack and Shojaku, 1992; Barmack et al. 1993). Neurons in the β-nucleus of the inferior olive and in the cerebellar nodulus responded maximally when the plane of one of the vertical semicircular canal was coplanar with the plane of stimulation. These neurons were found in anatomically discrete groups according to the vertical canal to which they were most sensitive. Some of these neurons were also sensitive to static tilt stimulation.

In the present experiment, we have recorded from neurons in the medial aspect of the NRGc during natural vestibular stimulation in anesthetized rabbits. Each neuron was characterized by its sensitivity to dynamic (semicircular canal and/or tonic-phasic otolith afferents) and static (otolith afferents) vestibular stimulation using sinusoidal, static tilt, and exponential "step" vestibular stimulation.

We have found that 85% of the neurons in the medial aspect of the caudal NRGc respond to vertical vestibular stimulation with increased activity when the contralateral side is down. The activity of most of these NRGc neurons was modulated primarily by otolith and to some extent vertical semicircular canal mediated input.

Some of these results have been presented previously in an abstract (Fagerson and Barmack, 1993).

METHODS

Surgical methods for electrophysiology

Twelve adult pigmented rabbits weighing 1.0-1.7 kg were anesthetized intravenously with α-chloralose (50 mg/kg) and urethane (500 mg/kg) administered through a 22-gauge Teflon catheter inserted into the marginal ear vein. Rectal temperature was monitored and maintained at 37° C by a servo-control system.

A 2-cm midline incision was made starting below the occiput and reaching the first cervical vertebrae. The neck muscles were retracted exposing the *cisterna magna*. A small window was cut through the *dura mater*, draining some cerebral spinal fluid, and exposing a 3.2 mm region extending from the obex to the caudal aspect of cerebellar lobule 9c. Meningeal attachments between the cerebellum and the brainstem were severed with microscissors. This helped reduce brain movements associated with the heart beat, and gave a better exposure of the more rostral regions of the brainstem.

Vestibular stimulation

The head of the rabbit was attached by implanted head bolts to a restraining bar, maintaining the head in the center of rotation of a three-axis vestibular rate table. The plane of the horizontal semicircular canals corresponded to earth horizontal. The rate table was sinusoidally oscillated about the vertical axis (yaw), about the longitudinal

axis (roll) or about the interaural axis (pitch) ($\pm$10 deg). For most neurons, data was collected during sinusoidal stimulation at only one frequency usually 0.2 - 0.60 Hz. A systematic analysis of response dynamics was not carried out. During vestibular stimulation, the vision of the rabbit was occluded. The body of the rabbit was encased in foam rubber and fixed with elastic straps to a semicircular plastic tube aligned with the longitudinal axis.

A "null technique" was used to help determine the peripheral origin of vestibularly modulated activity in the NRGc. While the table rotated sinusoidally about the longitudinal axis, the orientation of the head of the rabbit was shifted systematically around the vertical axis, effectively changing the plane of vestibular stimulation (Fig. 1A-F). The body of the rabbit was held in line with the longitudinal axis of the rabbit's head during rotation around the vertical axis so that cervical proprioceptive units were not stimulated. By changing the plane of stimulation between the extremes of pure pitch and roll, we could detect a head angle at which stimulus-driven activity was minimum, a "null plane." On either side of this null plane, the phase of the response to vestibular stimulation shifted 180 degrees.

The angle that the longitudinal axis of the rabbit's head (dotted line in Fig. 1) made with the axis of rotation (solid line in Fig. 1) is called here the "head angle." At a head angle of 0 deg, the longitudinal axis of the head was colinear with the axis of rotation and perpendicular to the plane of stimulation, producing pure roll stimulation (Fig. 1C). During rotation about the longitudinal axis, when the head was shifted in a clockwise direction (viewed from above) from an initial head angle of 0 deg, the head

angle was designated as a negative angle (Fig. 1A and B). For example a head angle of -45 deg corresponded to the **optimal plane** of stimulation for the right anterior and left posterior semicircular canals (Fig. 1B). Conversely, a head angle of -45 deg corresponded to a **null** plane for the left anterior and right posterior semicircular canals. Deviations of the head in counterclockwise directions resulted in stimulation planes at positive head angles (Fig. 1 D and E). Planes of stimulation at head angles of $\pm$ 90 deg produced pitch stimulation (rotation about the interaural axis, Fig. 1A and E).

For the purposes of our analysis, we use 45 ± 10 deg as the angle that the anterior or posterior canal makes with the sagittal plane. Two laboratories have measured the angle that the anterior and posterior semicircular canals make with the sagittal plane using anatomical criteria (Simpson and Graf, 1981; Barmack and Pettorossi, 1988). There was considerable variation in the results. The measurements for the anterior canals were 37 deg and 50 deg and for the posterior canals were 57 deg and 42 deg, respectively. However, physiological measurements made from semicircular canal induced responses of inferior olivary neurons suggest optimal response planes closer to 45 deg from the sagittal plane (Barmack et al. 1993).

A "static tilt" test was used to find out whether the discharge of a neuron in the medial NRGc was related to otolithic stimulation. In this test, the rabbit was tilted 5-10 deg about the longitudinal axis. After an adaptation period of 30 s, the average discharge frequency was measured for the next 30 s. Then the rabbit was tilted in the opposite direction and the measurements were repeated. We used the arbitrarily

selected criterion, a difference in average discharge frequency for tilt stimuli of two different directions of at least 20%, as evidence that the response was mediated in part by an otolithic input (Barmack et al. 1993).

An exponential "step" stimulation was used further to characterize the discharge of NRGc neurons. This stimulus was a low frequency (0.02-0.05 Hz, $\pm$10 deg), exponential "step" about the longitudinal and/or interaural axis with a time constant of 1.5 s. A transient response during the change in head position, without a response during the static portions, was taken to indicate semicircular canal input to the neuron. A sustained response during the static portion of the stimulus cycle was taken to indicate otolithic influence on the evoked neuronal activity.

Microelectrode recording

A microdrive with an electrode holder was attached to the head restraining bar. Tungsten microelectrodes were used to obtain extracellular single neuronal recordings. After exposing the dorsal aspect of the brainstem, the electrode was advanced to the NRGc 2.0 - 3.2 mm rostral to the obex. The signal from the microelectrode was amplified (bandwidth 0.1-10.0 kHz), discriminated and fed to a computer for further data reduction.

Histological localization

At the completion of each electrode track, one or more electrolytic marking lesions (3.5 μA, 20 sec, electrode negative) were made at depths at which stimulus-

driven neuronal activity was found. At the completion of each experiment the animal was perfused with saline followed by 10% formalin. The brainstem was cryoprotected, sectioned and stained with Neutral Red to identify the location of the lesions. The location of each cell was either identified from a marking lesion, or inferred from known vertical distances from a marking lesion within the same electrode penetration.

Data analysis

The data were analyzed using peristimulus histograms of the spike frequency averaged over 3-20 cycles. The maximum and minimum discharge frequency as well as the phase were measured on histogram printouts. The gain and phase used in this study were referenced to position. The log gain was defined as the $\log_{10}$ (peak-to-peak amplitude of the response/peak-to-peak amplitude of stimulus function) (Anderson et al. 1977). In those cases in which the neuron was silent during part of the stimulus cycle, the gain was defined as the ratio of the maximum change in firing rate to the change in stimulus magnitude over that portion of the cycle during which the neuron was firing (Melvill Jones and Milsum, 1971). The gain of the response to each plane of stimulation was analyzed for 20 cells. The phase in relation to the sinusoidal forcing function was measured on each histogram for each plane of stimulation for each neuron. Since most neurons in this study responded in phase with contralateral side down, zero phase was taken to indicate a peak excitatory response in phase with contralateral side down. Most of the neurons tested were on the right side of the

brainstem. The data from the few cells in the left NRGc were translated to represent the response as if the cells were on the right.

RESULTS

Sensitivity to vestibular stimulation

We identified the responses to natural vestibular stimulation of 151 neurons in the caudal NRGc within 500 μm of the midline. Nearly all (85%) neurons in the medial aspect of the caudal NRGc responded to vestibular stimulation. Every electrode penetration in the medial aspect of the caudal NRGc isolated from one to 20 neurons. We encountered these neurons at depths of 700 - 3500 μm below the dorsal surface of the brainstem. Some cells were less than 100 μm apart. These neurons were responsive primarily to vertical vestibular stimulation (rotation about the longitudinal and interaural axes). Typical vestibular responses of a NRGc neuron were evoked by sinusoidal roll (Fig 2A) and exponential "step-roll" (Fig. 2B) stimulation about the longitudinal axis. This neuron discharged as the animal was tilted onto the contralateral side. According to the nomenclature developed by Duensing and Schaefer (1959) these responses in otolith afferents are designated as "β responses." Increased activity with ipsilateral side down is designated as "α responses." Of all the NRGc neurons responding to otolith afferent stimulation, 96% showed β responses and 4% showed α responses.

While 87% of the vestibularly responsive neurons responded only to vertical vestibular stimulation, 13% of the neurons responded to both horizontal and vertical vestibular stimulation. These neurons were found primarily in the dorsal half of the NRGc close to the hypoglossal nucleus or close to the nucleus raphe magnus. The horizontal responses could **not** be attributed to misalignments of the head of rabbits in the rate table. The responses were not modified significantly by ± 10 deg static head tilts about either the longitudinal or interaural axes while the rabbit was sinusoidally rotated about the vertical axis. Eighteen (90%) of these horizontally-sensitive neurons responded to rotation toward the contralateral side (Type II response according to the nomenclature of Duensing and Schaefer for horizontal canal responses, 1959). Only two neurons responded exclusively to horizontal vestibular stimulation.

Classification of neurons using static tilt and exponential "step" stimulation

We recorded the responses of 117 neurons to "static tilt" and/or exponential "step" vertical vestibular stimulation. Ten neurons were tested only with "static tilt" stimulation. The majority (87%) of those neurons that responded during the static part of the exponential "step" test were excited by tilt to the contralateral side. The remainder responded to tilt onto the ipsilateral side.

The 102 cells that responded to the "step" test were assigned to one of three categories based on analysis of peristimulus histograms of their responses (Fig. 3). The activity of the first group of neurons (30 cells) increased during static tilt of the rabbit onto the side contralateral to the recording site and decreased during static tilt

onto the ipsilateral side (Fig. 3A). These neurons received otolith-mediated input and the group is called here the "static" group. The activity of a second group of neurons (51 cells) increased transiently during the change in position onto the contralateral side and decreased during the change in position onto the ipsilateral side (Fig. 3B). In addition, they had β responses during the static tilt ("static + transient" group). These neurons received an otolith-mediated input and may have received an input mediated by one of the vertical semicircular canals or by tonic-phasic otolith neurons. The activity of a third group of neurons (21 cells) increased transiently during tilts onto the contralateral side and decreased transiently during tilts onto the ipsilateral side (Fig. 3C). The firing rate of these neurons was not influenced by static tilt stimulation ("transient" group). These neurons did not receive otolith-mediated input and received an exclusive vertical semicircular canal input.

Classification of neurons based on vestibular null plane

We used a "null technique" to analyze the modulated activity of 133 NRGc neurons during sinusoidal vestibular stimulation. As described in METHODS, the null plane was the plane of stimulation at a head angle that resulted in a minimum modulated activity, i.e., the gain was near zero. On either side of this null plane, there was a 180-deg shift in the phase of the response with respect to the sinusoidal stimulus.

For most, but not all, neurons (84 cells) a null plane could be determined. These neurons are called "fixed phase" neurons since the phase of the response is

constant except at the null plane. The responses of a "fixed phase" NRGc neuron to vestibular stimulation during null plane analysis are shown in Fig. 4. When the rabbit's head was at an angle of -44 deg with respect to the longitudinal axis of rotation, the left anterior-right posterior semicircular canals were approximately coplanar with the plane of stimulation. At this head angle, the neuron discharged as the animal was rotated onto the caudal, **contralateral** side (Fig. 4A). This was the optimal "response plane" for this neuron. At a head angle of +46 deg (i.e., the plane of stimulation was perpendicular to the left anterior-right posterior semicircular canals), the response of the neuron was not modulated (Fig. 4B). When the head was rotated further in the counterclockwise direction to +66 deg, the activity of the neuron was modulated, but the neuron discharged as the animal was rotated onto the caudal, **ipsilateral** side (Fig. 4C). This is a result of the reversal of the receptor orientation in relation to the shear force of the stimulus beyond the null plane. This neuron did not respond to the static portion of exponential "step" stimulation.

We verified the null plane obtained during stimulation by analysis of peristimulus histograms composed of 3 to 20 cycles of stimulation. Each histogram was obtained during stimulation at a different head angle. For example, the "fixed phase" neuron in Fig. 5 had two null planes: in two approximately coplanar planes of stimulation at head angles of - 60 deg (Fig. 5C) and +125 deg (Fig. 5G). At head angles of -125 and -90 deg, the neuron responded as the table was rotated toward the right or ipsilateral side (Fig. 5A and B). At head angles of 0 to +90 deg (Fig. 5D to F), the neuron responded as the table was rotated toward the left or contralateral side

(Fig. 5D-F). When the animal was rotated in the optimal response plane (Fig. 5E), the phase of the evoked discharge led head position by 10 deg.

To illustrate the relationship of the change in phase to the change in gain, we plotted the gain and phase against the head angle for each plane of stimulation for another "fixed phase" neuron (Fig. 6). For this neuron, the gain of the response was near zero at the null plane. The phase shifted approximately 180 deg on either side of the null plane. This phase shift occurred within a range of head positions of less than thirty degrees. These two characteristics: 1) A near zero gain, and 2) A 180-deg phase shift of the evoked activity with respect to the sinusoidal vestibular stimulus, characterized the null planes of "fixed phase" neurons.

We expected that neurons with transient responses during exponential "step" stimulation might be maximally excited by stimulation in the planes of the semicircular canals. The optimal "response planes" (90 deg from the null plane) were plotted for the three groups (Fig. 7). All five cells, in the transient group, had optimal "response planes" aligned with the anterior or posterior semicircular canals (at head angles near +45 and -45 deg) (Fig. 7C). The optimal "response planes" for the "static" and "static + transient " groups of neurons (Figs. 7A and B), were distributed over head angles from -70 to +80 degrees. The mean phase lead for the three groups was 3 ($\pm$ 25 s.d.) deg, 10 ($\pm$ 19 s.d.) deg and 32 ($\pm$ 46 s.d.) deg, for the "static," "static + transient" and "transient" groups, respectively.

Neurons lacking null planes

In some neurons, null planes could not be determined. The evoked activity of these neurons had no well defined minimum at any head position, and the phase of the response shifted gradually as the plane of stimulation was changed (Fig 8). This gradual phase shift characterized "variable phase" neurons. To illustrate the difference between the "fixed phase" and "variable phase" neurons, we plotted the gain and phase for a "variable phase" neuron against the head angle of the plane of stimulation (Fig.9). The activity of the neuron was modulated at all head angles. There was no null plane. However, the phase lead increased as the head angle was changed from -122 to +127 deg.

We characterized 33 cells as "variable phase" neurons. For 28 of these neurons, we plotted the phase of the responses to stimulation at a variety of head angles (Fig. 10). During stimulation at a head angle of 0 deg (pure roll), 94% of the "variable phase" neurons responded to rotation onto the contralateral side and the phase is near position. At head angles producing pitch stimulation ($\pm$90 deg), the phase is closer to maximum velocity. For clarity, the cells are divided into two groups: those for which the phase increased gradually as the plane of stimulation was changed from negative to positive head angles (upper plot) and those for which the phase decreased (lower plot). Most of these neurons fall into the first category.

Twenty-four of the neurons, with "variable phase" responses received exponential "step" stimulation. Twenty-one (88%) had transient responses to the dynamic portion of the stimulus. We classified 46% of the "variable phase" neurons as "transient," 42% as "static + transient," and only 8% as "static." (Four percent of

"variable phase" neurons did not respond to exponential "step" stimulation.) This is in contrast to the "fixed phase" neurons of which 7%, 61% and 28% were classified respectively as "transient," "static + transient" and "static." (Four percent of "fixed phase" neurons did not respond.)

Although these neurons did not have null planes, the size of the gain varied as the plane of stimulation changed. We estimated the optimal "response plane" for these neurons based on maximal gains, rather than the more sensitive method of phase reversal. The phase lead of these neurons during stimulation in the optimal response plane was similar for the three groups: means 36 (± 45) deg, 29 (±41) deg and 36 (±44) deg for the transient, static + transient and static groups, respectively.

Location of neurons within the NRGc

Of the 151 vestibularly-sensitive neurons from which we recorded, 142 were localized to the medial aspect of the caudal NRGc, 5 in cell group d (Meessen and Olszewski, 1949) and 4 in nucleus raphe magnus (Fig. 11). The responses of the non-NRGc neurons were similar to those of NRGc neurons and have been grouped together in the present analyses. All responsive neurons were located within 500 μm of the midline. The neurons were in the region where fiber tracks and terminal fields of projections from the caudal medial and descending vestibular nuclei were identified by orthograde transport of phaseolus leucoagglutinin (PHA-L) and retrograde transport of HRP (Fagerson and Barmack, unpublished data). The highest density of neurons

responding to vestibular stimulation was observed 100-200 μm from the midline. Fewer neurons were encountered as the electrode tracks were moved laterally.

TOPOGRAPHY. The location of "fixed phase" neurons within the medial aspect of the caudal NRGc was examined for topographical organization based on the orientation of the optimal plane of stimulation for each neuron. The neurons were classified into five groups: two groups contained neurons whose optimal "response plane" orientations were within 15-20 deg of the planes of the two vertical semicircular canals (31 to 65 deg from the sagittal plane, anterior semicircular canal, 125 to 149 deg from the sagittal plane, posterior semicircular canal) and the three remaining groups contained neurons with optimal "response plane" orientations greater than (0 to 30 deg), less than (150 to 180 deg) or between the two semicircular canal groups (66 to 124 deg, (Fig. 11). Using this classification, we were unable to distinguish a topographical pattern for cells responding to vertical vestibular stimulation. Nor did we find a topographical pattern for "variable phase" neurons that were found throughout the medial aspect of the NRGc. We also examined the possibility of topographical organization of neurons in the NRGc based on their responses to exponential "step" stimulation. We were unable to identify a topographical organization of "static," "static + transient," and "transient" neurons. The 20 neurons that responded to horizontal and vertical vestibular stimulations were in the dorsal half of the NRGc or, within or immediately lateral to, the nucleus raphe magnus.

DISCUSSION

Methodological considerations

ANESTHESIA. All experiments were carried out under anesthesia. However, a previous study (Barmack and Hess, 1980) compared the effects of chloralose urethan and sodium pentobarbital on visually evoked activity of the dorsal cap of the inferior olive. The response was virtually identical under the two anesthetics. Nevertheless it is possible that some subtleties of vestibularly-evoked activity were masked by chloralose-urethan anesthesia.

DETERMINATION OF PERIPHERAL ORIGINS OF VESTIBULAR RESPONSES. Ideally we could have tested neurons for the absence of otolith input by orienting the head of the rabbit such that the plane of a vertical semicircular canal was horizontal. Thus, the semicircular canal could be stimulated by rotation about the vertical axis without concomitantly modulating the gravitational vector acting on saccular and utricular hair cells (Estes et. al. 1975). However, maintaining isolation of single NRGc neurons under this stimulus condition was not possible.

Sensitivity to vertical vestibular stimulation

Although the reticular formation is anatomically diffuse and receives inputs from many areas of the brain, our recordings from the medial aspect of the caudal NRGc suggest that vestibular stimulation evokes a remarkably uniform behavior from cells in this region. These neurons responded primarily to tilt onto the contralateral side, β response, (Duensing and Schaefer, 1959). In other experiments, we have

injected phaseolus leucoagglutinin (PHA-L) into the medial and descending vestibular nuclei and injected horseradish peroxidase into the medial NRGc. These experiments showed that the projection from the vestibular nuclei to the NRGc is primarily ipsilateral (Fagerson and Barmack, unpublished observations). Using immunohistochemistry for glutamate decarboxylase (GAD), we have further shown that this projection is, at least in part, GABAergic. Since vestibular nucleus neurons in rabbit, cat and rat respond to ipsilateral tilt, an α response (Adrian, 1943; Duensing and Schaefer, 1959; Fujita et al. 1968; Hiebert and Fernandez, 1965; Peterson, 1970; Kubo et al. 1977), the presence of an inhibitory transmitter in neurons that project from the vestibular nuclei to the medial NRGc could explain the β responses of NRGc neurons. The few α responses might be attributed to a few projections from the vestibular nuclei that are contralateral (Fagerson and Barmack, unpublished observations). Alternatively, some ipsilaterally projecting vestibular neurons may receive inputs from hair cells peripheral to the striola on the macula. These hair cells have a functional polarity that is opposite to that of hair cells medial to the striola (Goldberg et al. 1990).

Possible origins of vestibular responses

For methodological reasons cited above, we cannot be certain of the source of the input to single neurons in the "static + transient group." The transient response to the change in position during the exponential "step" stimulation in these neurons could

be a result of either semicircular canal or tonic-phasic otolith neuron mediated input converging with tonic otolith neuron mediated input.

On the other hand, our data suggest that the medial aspect of the caudal NRGc receives input mediated by both the otoliths and the semicircular canals. We inferred the peripheral origin of NRGc responses by comparing the responses of these neurons to the responses of identified vestibular primary afferents. Several characteristics distinguish otolith afferents from semicircular canal afferents: 1) response to static tilt, 2) orientation of the optimal "response plane," 3) phase of the response and 4) response dynamics. These are reviewed below.

First, the firing rate of otolith afferents increases in response to static tilt onto the ipsilateral side (Adrian, 1943; Hiebert and Fernandez, 1965; Peterson, 1970; Curthoys and Markham, 1971; Goldberg et al. 1984). In this study, we assume that a lack of response to static tilt shows a lack of input from the otoliths. Second, in utricular afferents of the chinchilla, optimal "response plane" orientations are broadly distributed, corresponding to the location and orientation of the associated receptor(s) on the macula, (Goldberg et al. 1990; Goldberg et al. 1990). Third, primary afferents from the otoliths are more likely to have a response phase corresponding to head position, while those from the canals are more likely to have a phase corresponding to head velocity (Goldberg and Fernandez, 1971; Fernandez and Goldberg, 1976). In this study, these three characteristics were identified using static tilt or exponential "step" stimulation and the null technique applied during sinusoidal stimulation. Neurons that responded to static tilt were classified as receiving otolith mediated input. This

classification was either supported or opposed by the optimal "response plane" orientation and the response phase in the optimal "response plane" of that neuron.

The fourth characteristic is response dynamics (Spyer et al. 1974; Blanks et al. 1978; Manzoni et al. 1983; Bolton et al. 1992). Otolith afferents are more likely to have small increases in gain and small decreases in phase with respect to the force profile as the stimulus frequency is increased. Primary afferents of the semicircular canals are more likely to have large gain enhancements and large phase leads (Fernandez et al. 1972). However, while velocity dependent responses have been attributed to stimulation of semicircular canals (Spyer et al. 1974; Manzoni et al. 1983; Bolton et al. 1992), velocity dependent changes in response phase have also been observed in otolith afferents (Vidal et al. 1971; Fernandez and Goldberg, 1976; Anderson et al. 1977) and in vestibular neurons of animals after canal plugging (Schor and Miller, 1982). This implies an otolithic origin for these changes. We did not systematically evaluate the response dynamics of NRGc neurons.

Otolithic and semicircular canal mediated inputs to the NRGc

The three lines of evidence that suggest that the vestibular inputs to the medial aspect of the caudal NRGc originate at least in part from the otoliths are as follows: 1) 78% of NRGc neurons were responsive to static vestibular stimulation. 2) For the neurons that had defined null planes, "fixed phase" neurons, most of the optimal "response plane" orientations were not aligned with the vertical semicircular canals. 3) The two groups of NRGc neurons that responded to static vestibular stimulation

("static" and "static + transient" neurons) also had smaller phase leads with respect to head position than the "transient" group. The "static" group discharged almost in phase with position (Fig. 7).

It is also likely that some input to the medial NRGc is mediated by semicircular canal receptors. We assumed that neurons that did not respond to static tilt or the static portion of the exponential "step" stimulation did not receive input mediated by the otoliths. Of the neurons that responded only to the transient portion of the "step" stimulus, five were "fixed phase" neurons (8%). These neurons were likely to receive input mediated by the vertical canals for two reasons. The optimal "response planes" for these neurons were approximately aligned with the vertical semicircular canal planes and the phase leads for these neurons were larger than those of the "static" and "static + transient" groups. Also, 46% of the "variable phase" neurons responded only to the transient portion of exponential "step" stimulation. These "variable phase" neurons may have received convergent inputs from the semicircular canals.

Spatio-temporal convergence

Twenty-five percent of medial NRGc neurons ("variable phase" neurons) behaved as if they received spatially and temporally differing convergent (STC) inputs. The activity of these neurons was modulated in all planes of stimulation. The phase of the response for these neurons shifted continuously as the plane of stimulation was

changed. In contrast, the activity of "fixed phase" neurons is not modulated in the null plane and the response phase is constant with changes in the plane of stimulation.

Convergence of vestibular inputs (vertical canals, horizontal canals, otoliths) has been shown in vestibular nuclei (Duensing and Schaefer, 1959; Curthoys and Markham, 1971; Wilson and Felpel, 1972; Kubo et al. 1977; Baker et al. 1984). Responses that imply spatial and temporal convergence (STC) have been observed in otolith afferent fibers (Fernandez and Goldberg, 1976; Dickman et al. 1991), in neurons in the vestibular nuclei (Baker et al. 1984; Angelaki et al. 1992; Bush et al. 1992; Angelaki et al. 1993), and recently, in reticulospinal neurons (Bolton et al. 1992). These responses include excitatory responses to orthogonal shearing forces (Fernandez and Goldberg, 1976), undefined null plane accompanied by responses to sinusoidal stimulation in any vertical plane (Baker et al. 1984), response phase that varies with the plane of stimulation (Baker et al. 1984) and changes in optimal response planes with changes in frequency (Bolton et al. 1992).

In vestibular nucleus neurons, STC responses appear to result from convergence of information from the canals and otoliths with different spatial alignments (Baker et al. 1984). When a neuron receives input from two receptors with both differing optimal "response planes" and differing response phases, the sum of the responses to sinusoidal stimulation of those receptors by the neuron would result in a shift in the response phase as the plane of stimulation is changed. The reason for this is that the relative influence of the two receptors on central neurons depends on the plane of stimulation. Since the null planes of the two inputs lie in different planes, at

least one of the inputs will be modulated in all planes of stimulation. Theoretically, the gain resulting from the sum of the two inputs would depend on both the size of the difference between the optimal response planes of the two inputs and the relative magnitudes of the gains of the two inputs.

It is possible that the STC behavior of NRGc neurons may be a result of several types of convergence. Since 42% of the "variable phase" neurons responded to both the static and transient portions of exponential "step" stimulation, these may receive either canal and otolithic input or two otolith-mediated inputs at least one of which has tonic-phasic properties. Since 8% of our "variable phase" neurons responded exclusively to the static portion of exponential "step" stimulation, it is conceivable that these neurons receive two differently aligned tonic otolithic inputs. Alternatively, many "variable phase" neurons may receive input from two semicircular canals since 46% of the "variable phase" neurons were responsive primarily to the transient portion of exponential "step" stimulation.

Function of reticular neurons

The targets of efferent projections from the NRGc suggest a role for NRGc neurons in the control of neck and postural musculature. These projections include the descending reticulospinal system, an extensive projection to all parts of the cerebellum except the paraflocculus especially lobulus simplex and the nodulus, and projections to thalamic nuclei, the caudate and lentiform nuclei (Torvik and Brodal, 1957; Nauta and Kuypers, 1958; Kuypers et al. 1962; Nyberg-Hansen, 1965; Petras, 1967; Lynch et al.

1973; Bowsher, 1975; Kuypers and Maisy, 1975; Coulter et al. 1979; Tohyama et al. 1979; Kotchabhakidi et al. 1980; Hayes and Rustoni, 1981). There also may be a projection from the medial NRGc back to the vestibular nuclei (preliminary observations of this laboratory, (Grottel and Jakielska-Bukowska, 1993).

Neurons of the NRGc may relay information about head position and to some extent head velocity. This study has identified two types of responses of medial NRGc neurons that may be designed to meet the specific needs of various neck or postural muscles. For "fixed phase" neurons, the temporal relationship of this information to head position was relatively constant over a range of stimulus orientations. On the other hand, the timing of the responses of "variable phase" neurons varied with the plane of stimulation.

To illustrate the difference between the responses of the "fixed phase" and "variable phase" neurons in all orientations of the vertical stimulus plane, we have plotted the responses of two neurons, a "fixed phase" and a "variable phase" neuron, to stimulation in all vertical planes using polar coordinates to indicate the head angles for each direction of tilt (Figs. 12 and 13). See the appendix for a description of the construction of these figures.

The maximal response for both the “fixed phase” and the “variable phase” neuron occurred during downward tilt (Figs. 12A and 13A). The "fixed phase" neuron responded maximally to tilt backward to the right (21 impulses per sec at a head angle of +62 deg). For this neuron the phase lead of the response (filled circles on the horizontal plane) for all tilts between tilt to the right and backward tilt (head angles

between 0 and +90 deg) was constant (between 65 and 70 deg). The neuron was silenced during downward tilt in the inhibitory direction in the optimal plane (Fig. 12A, head angle -118 deg). As the animal was returned to the upright in the optimal plane, the inhibition was released and the neuron began firing again (peak in the responses in Fig. 12B, head angle -118 deg). Note that the maximal response for the neuron was attained during the next portion of the sinusoidal stimulus cycle plotted in Fig.12A.

The "variable phase" neuron responded maximally to tilt forward to the left (18 impulses per sec at a head angle of -120 deg) and the phase lead, in this plane of stimulation, was 75 deg (Fig. 13). However, as the plane of stimulation changed, the response phase gradually decreased to a 35-deg lag at a head angle of +120 deg as the rabbit's head returned toward upright from the tilt position (filled circle in Fig. 13B). This neuron was also silenced during downward tilt in the optimal plane in the inhibitory direction and began firing again as the head approached the upright position at a head angle of +60 deg (Fig. 13B).

The difference between the responses of the two types of neurons is subtle. Both neurons had a maximal response in an optimal plane (at head angles of +62 deg for the "fixed phase" and -120 deg for the "variable phase" neuron). They also had similar phase leads in that plane (65 deg for the "fixed phase" and 75 deg for the "variable phase" neuron). However, the "fixed phase" neuron had a null plane at head angles of -30 and +150 deg (most easily identified in Fig. 12B). If the "variable phase" neuron had a null plane, it would also have occurred at head angles of

approximately -30 and +150 deg. However, in this plane, the activity of the "variable phase" neuron was modulated but the phase had decreased to 0 deg (Fig. 13). A similar variation in response timing has been observed in muscle activation patterns associated with agonist and antagonist functions (Flanders et al. 1994). The timing of the responses of "variable phase" neurons may control a shift in muscle function from that of an agonist to that of an antagonist as the direction of head tilt changes.

Some neck muscles involved in the vestibulocollic reflex have a response phase that varies with the orientation of the stimulus (Baker et al. 1985). The function of these neck muscles may shift gradually from that of an agonist to that of an antagonist as the direction of head tilt changes. Since some NRGc neurons project to the cervical spinal cord (Torvik and Brodal, 1957; Kuypers and Maisy, 1975; Hayes and Rustoni, 1981), the similarity between this timing shift in muscle activation and the response phase shift of the "variable phase" neurons in this study implies that the "variable phase" neurons may be involved in the control of these neck muscles. To demonstrate this, the other known properties of neck muscle responses should be compared to those of the "variable phase" neuronal responses. Two properties of neck muscle responses that this study does not address are the response dynamics (an advancing phase with increasing stimulus frequency) (Baker et al. 1985) and a synergistic action between neck and vestibular inputs (Dutia and Hunter, 1985; Peterson et al. 1985). A more thorough sampling and analysis of response properties should be carried out in these neurons.

For a few of the "variable phase" neurons, the direction of the shift in the response phase was opposite to that of a majority of these neurons (lower part of Fig. 10). The neuron represented in Figure 13 belongs to the majority and the response phase of this neuron spirals outward in a counterclockwise direction while that of the minority of "variable phase" neurons spirals outward in clockwise direction. The neurons in the minority may control muscles that are antagonists to those controlled by the majority of "variable phase" neurons and that shift to an agonist function as the direction of tilt changes. Further studies of agonist and antagonist actions of neck muscles is necessary to understand the role of variable phase neurons in shifting agonist and antagonist muscle actions is possible.

To summarize, these data imply that neurons in the medial aspect of the caudal NRGc play a role in processing vertical vestibular information, especially otolithic input. The firing rate of the neurons may adjust postural muscle activity based on changes in head tilt. "Fixed phase" neurons may also excite or inhibit an agonist (or antagonist) muscle depending on the direction of head tilt. "Variable phase" neurons may adjust the timing of muscle activation and shift the function of the muscle from that of an agonist to that of an antagonist as the direction of head tilt changes.

APPENDIX: CONSTRUCTION OF POLAR PLOT

The data from the peristimulus histogram analysis of responses to sinusoidal stimulation were divided artificially into four segments. For example responses to sinusoidal roll stimulation were divided as follows: 1) responses to tilt from an upright position toward left side down, 2) responses to a return to upright position from the left side down position, 3) responses to tilt from upright position toward right side down, and 4) responses to return to upright from right side down. This was carried out for the data for responses to stimulation at each head angle. The data for each head angle from segments number 1 and 3 were combined along a diameter of the polar plot to create the downward tilt figure and that from segments number 2 and 4 were combined to create the upward tilt figure.

The radius of the polar plot was selected to indicate both degree of head tilt from 0 deg at the origin to 10 deg on the perimeter and the phase of the response with respect to head position from 90 deg at the origin to 0 deg on the perimeter of the figures. Since the plot is based on data from responses to sinusoidal stimulation, the radius also represents velocity and acceleration of stimulation. Maximum velocity occurs at the origin while maximum acceleration occurs at the perimeter. The angle of the radius represents the plane of stimulation. A head angle of 0 deg indicates right side down, -90 deg nose down, $\pm$180 deg left side down, and +90 deg tail down. The activity of each neuron (impulses/sec) during the selected segment of the sinusoidal stimulation in each plane of stimulation was plotted on the vertical axis. A surface was fit to the data using a distance-weighted least squares smoothing method. Responses at

the moment when the head was in the upright position (maximum velocity) were plotted at the origin of each figure while responses at the moment when the head was maximally tilted (maximum acceleration) were represented at the perimeter of the figure.

Since the firing frequency at the moment the head is in the upright position depends on the direction from which the head approaches the upright position (i.e., the stimulus plane), there are multiple values for this variable at the origin. There are two for each stimulus plane, one in the excitatory plane and one in the inhibitory plane. Rather than force the smoothing function to include all these points on the surface fit to the data, we allowed the smoothing function to interpolate the firing frequency at the origin from data for points near the origin on each stimulus plane.

These plots divide a continuous function into four discontinuous segments in order to approximate the discharge that might occur during normal head movements. This allows one to compare similar movements in all vertical planes.

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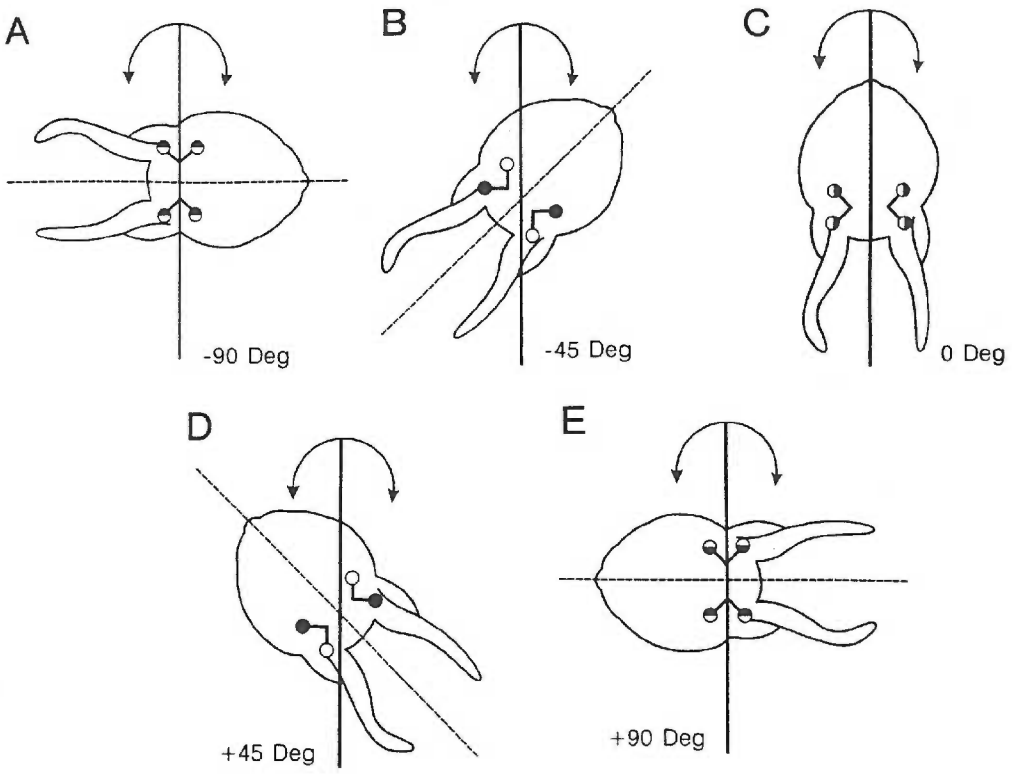

Fig. 1. Head angles and associated planes of vestibular stimulation. While the table was rotated about the longitudinal axis (heavy solid lines), the orientation of the longitudinal axis of the head of the rabbit (dotted lines) was varied by changing the orientation of the head about the vertical axis, effectively changing the plane of stimulation. A-E. Longitudinal axes and axes of rotation are shown for planes of stimulation at head angles of -90 deg, -45 deg, 0 deg, +45 deg and + 90 deg. A and E indicate pitch stimulation, while C indicates roll stimulation. In B, the plane of stimulation was in the plane of the right anterior-left posterior semicircular canals. In D, the plane of stimulation corresponded to the plane of the left anterior-right posterior semicircular canals, respectively. The locations of the vertical semicircular canals are indicated in each figurine. The filled circles indicate canals optimally stimulated, while the open circles indicate canals in the null plane. The half filled circles indicate canals which are partially stimulated.

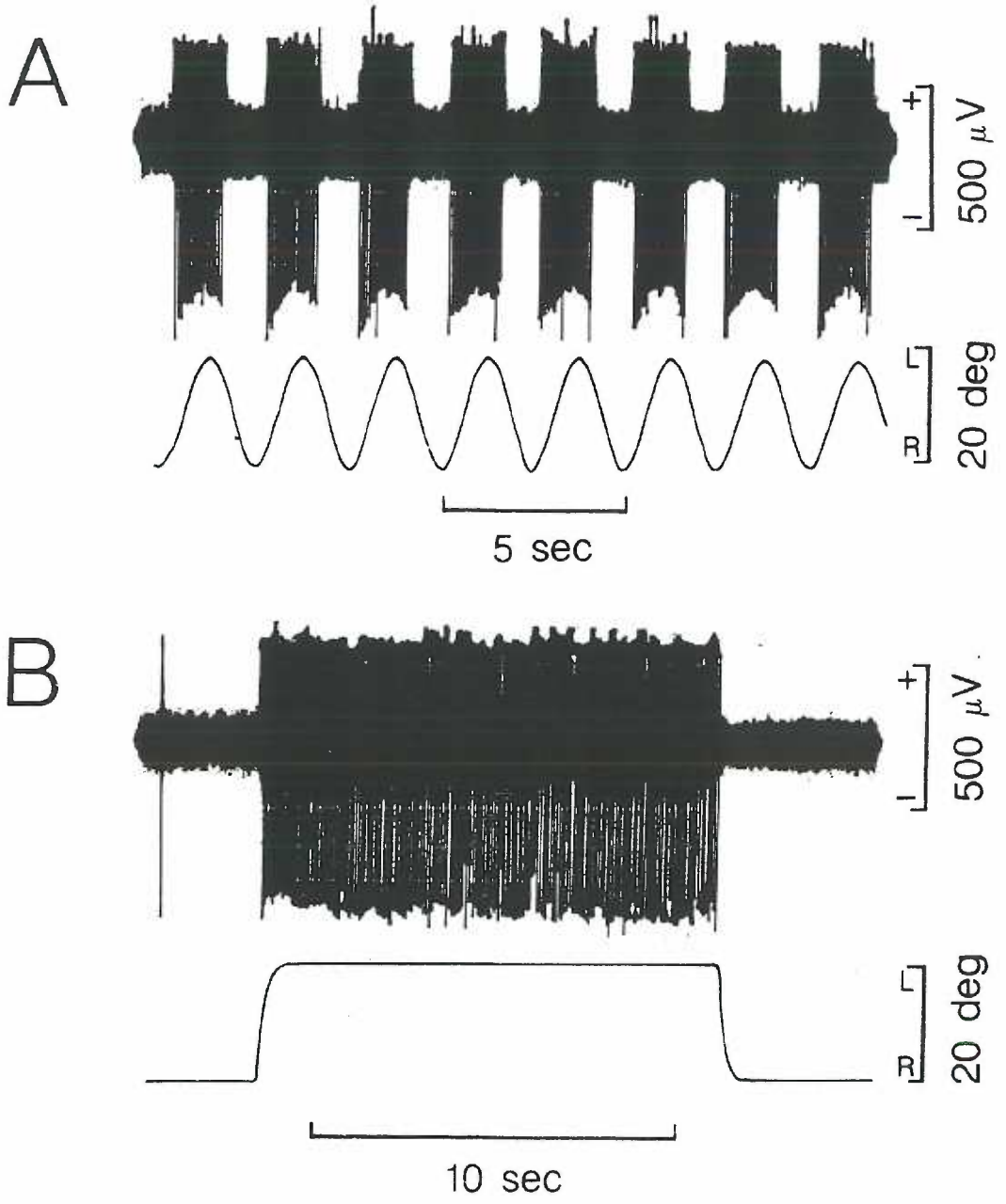

FIG. 2. Responses of an NRGc neurons evoked by sinusoidal and "step" vestibular stimulation. A. Responses to sinusoidal roll stimulation, 0.4 Hz, ± 10 deg. B. Responses to low frequency exponential "step" stimulation, 0.04 Hz, ± 10 deg.

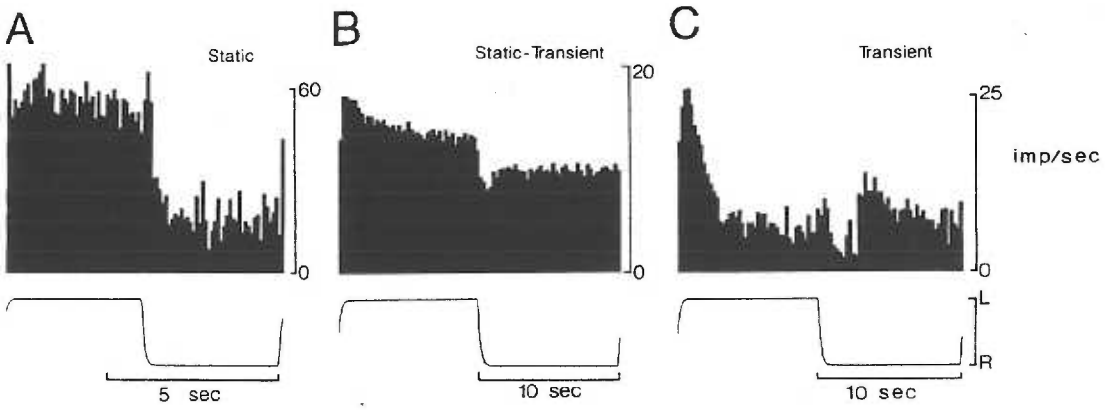

FIG. 3. Responses of three different neurons to exponential "step" vertical vestibular stimulation. A. Response of a neuron classified as "static." B. Response of a neuron classified as "static + transient." C. Response of a neuron classified as "transient." The traces below the histograms indicate the degree of head tilt. Tilt onto the contralateral side is indicated when the trace is up; tilt onto the ipsilateral side when the trace is down.

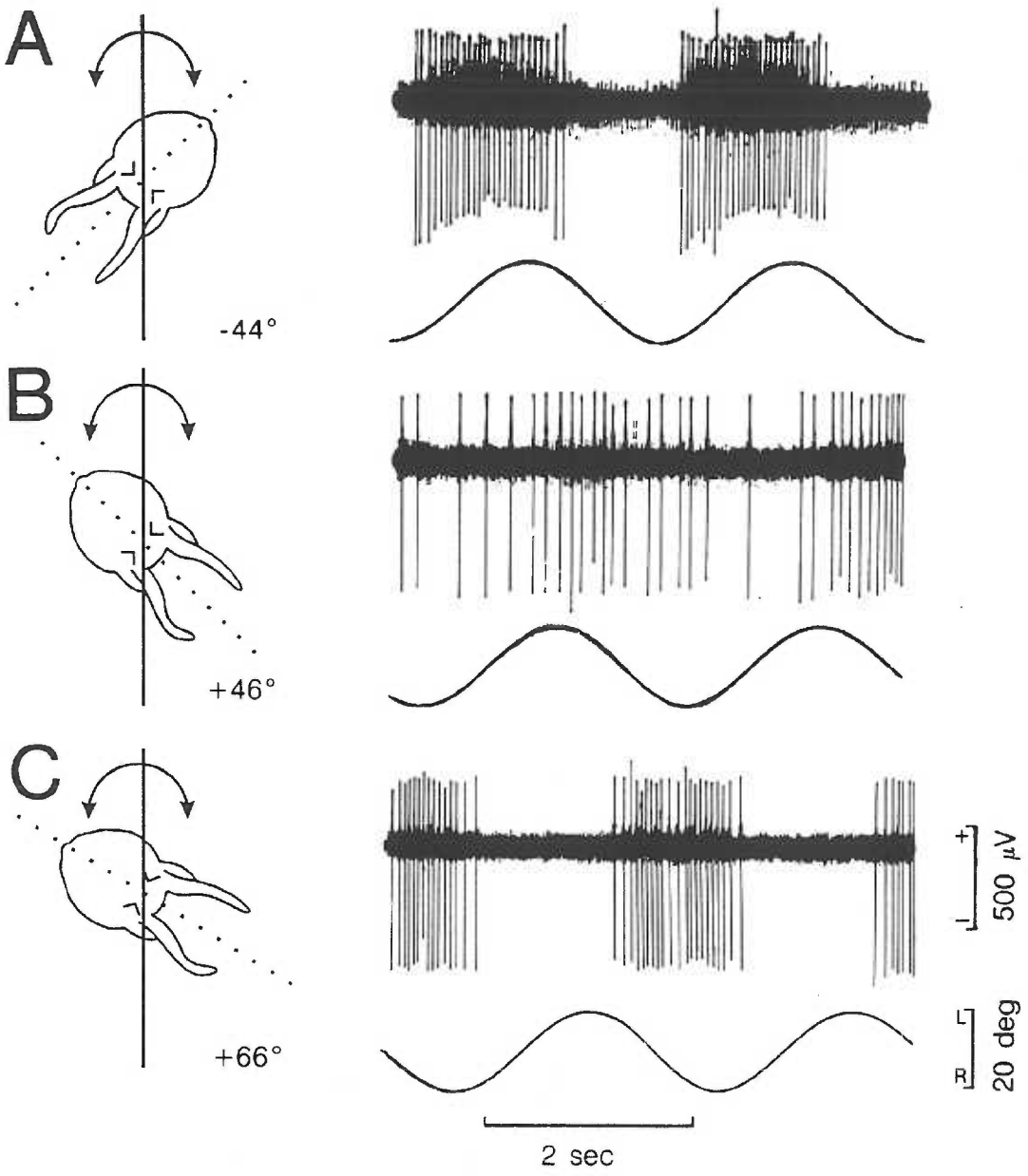

FIG. 4. Influence of plane of vestibular stimulation on activity of an NRGc neuron. During sinusoidal vertical vestibular stimulation (0.4 Hz, $\pm$ 10 deg), the rabbit was systematically shifted about the vertical axis. A. Responses near the optimal "response plane" at a head angle of -44 deg. B. Responses at the "null plane," at a head angle of +46 deg . C. At a head angle of +66 there was a 180 deg phase shift of the response. The plane of stimulation which corresponds to each response is shown to the left.

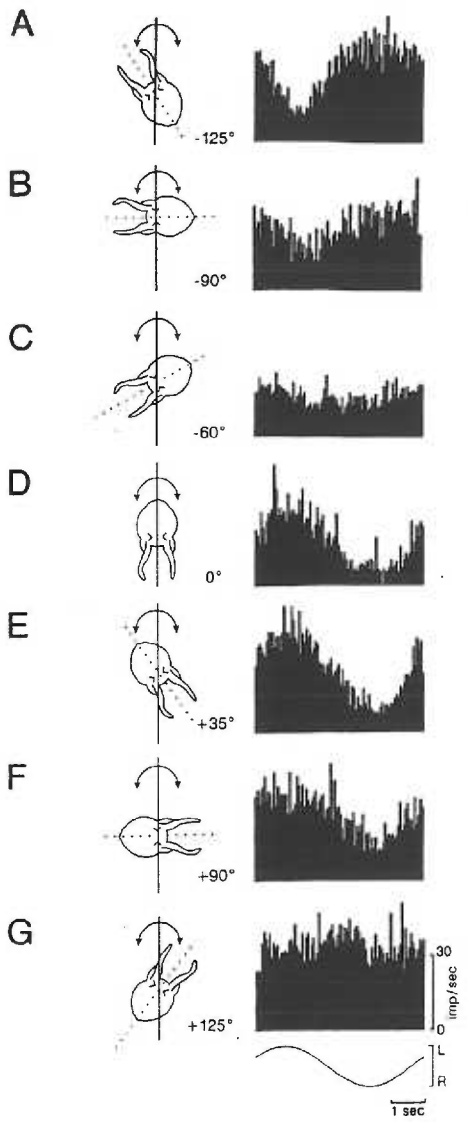

FIG. 5. Null plane of a "fixed phase" neuron. A-G. The orientation of the head about the vertical axis was shifted systematically from -125 deg (A) to +125 deg (G) as the rabbit was sinusoidally rotated about the longitudinal axis (0.2 Hz, $\pm$ 10 deg). C. The null plane was near a head angle of -45 deg. The gain of the response at this angle was near zero. D, E and F. At a head angles of 0, +35 and +90 deg, the neuron responded in phase with **contralateral** side down. A and B. At a head angles of -125 and -90 deg, the neuron responded in phase with **ipsilateral** side down. A second null plane (G) was identified at a head angle of approximately +125 deg (almost 180 deg from the head angle in C). E. Responses at a head angle of +35 deg, which was near the optimal response plane for this neuron (+45). The sinusoidal curve below the set of histograms shows the degree of head tilt about the rotational axis. An upward deflection indicates tilt onto the contralateral side and a downward deflection indicates tilt onto the ipsilateral.

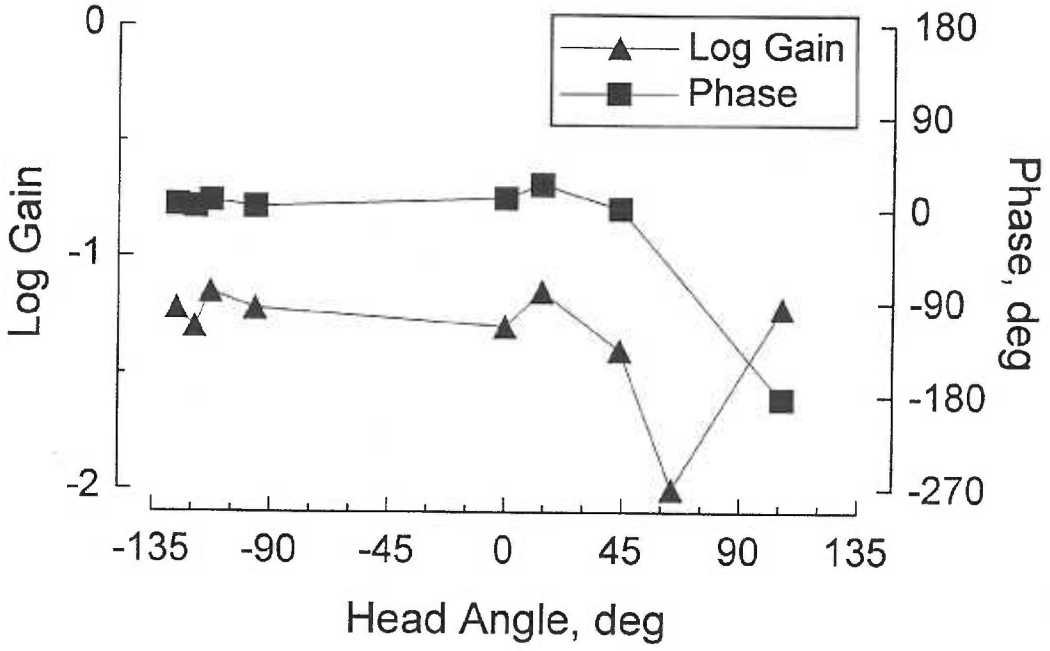

FIG. 6. The relationship of phase and gain for a "fixed phase" neuron. A phase of 0 deg indicates that the cell responded in phase with contralateral side down.

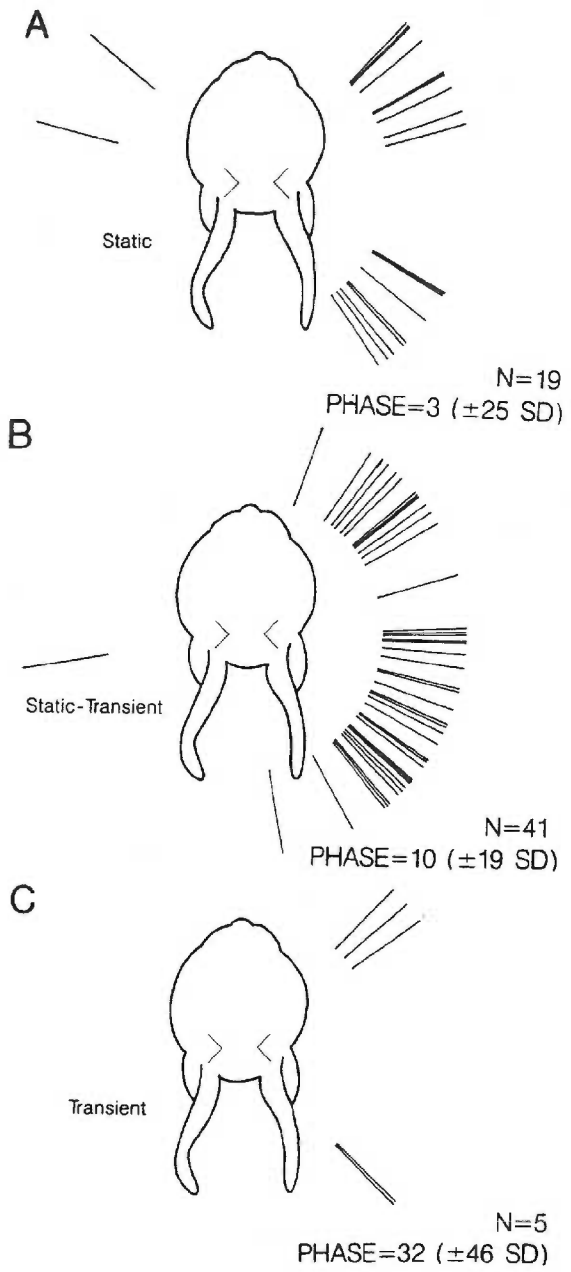

FIG. 7. Orientation of optimal "response planes" of NRGc neurons and phase during stimulation in the optimal "response plane." The orientations of the optimal "response plane" for each neuron are shown for each group: A, "static," B, "static + transient" and C, "transient." Each line radiating from a rabbit head indicates the plane of vestibular stimulation that evoked a maximal gain. For reference the positions of the vertical semicircular canals are indicated. For each group, the mean phase with respect to position (contralateral side down) during stimulation at frequencies of 0.1 to 0.5 Hz is indicated.

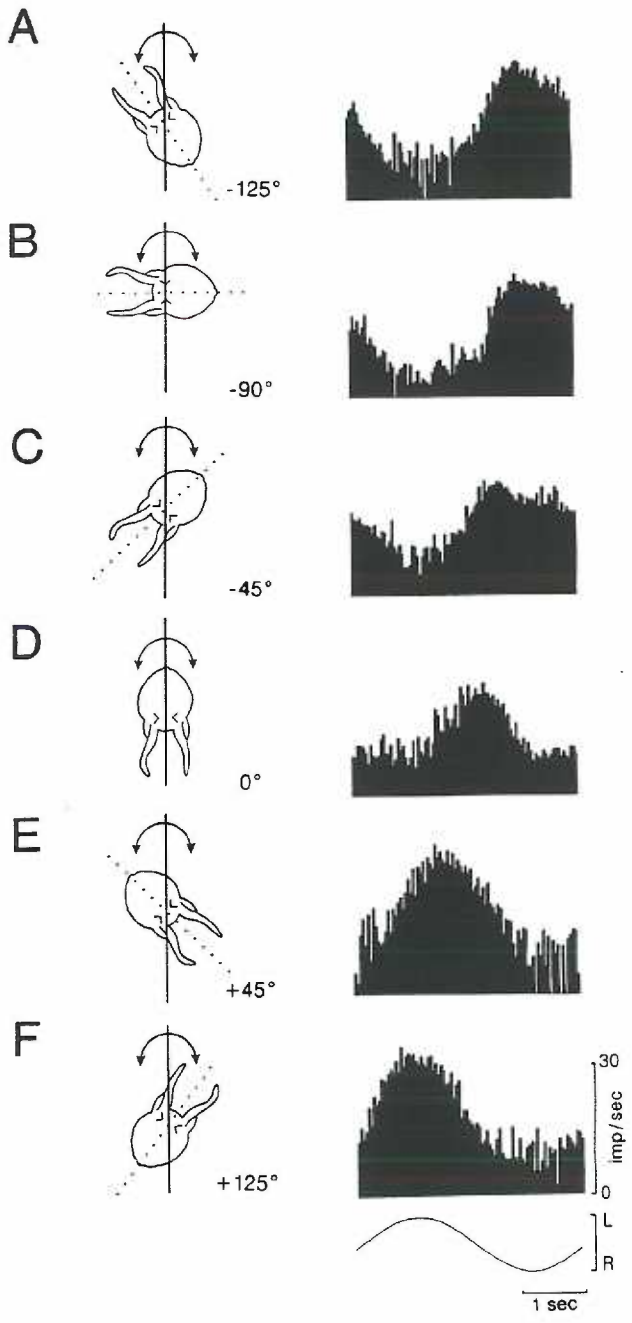

FIG. 8. Responses of a "variable phase" neuron. A-F: The orientation of the head about the vertical axis was shifted systematically from -125 deg (A) to +125 deg (F) as the rabbit was sinusoidally rotated about the longitudinal axis. The gain of the response was never zero and the response shifted gradually from in phase with ipsilateral side down (A and B) to in phase with contralateral side down (F).

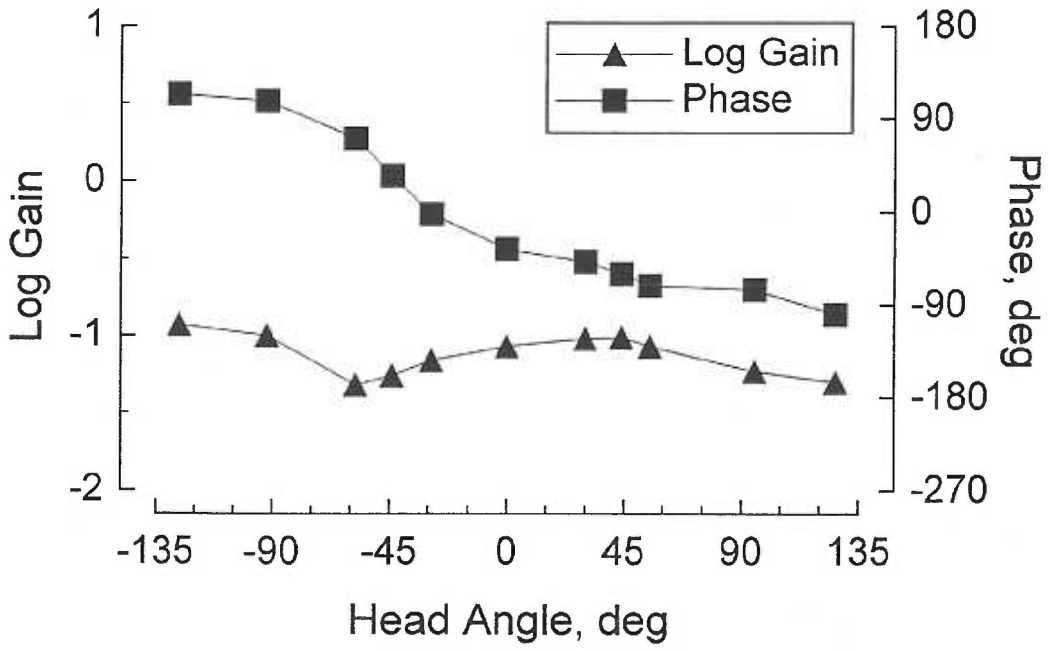

FIG. 9. Phase and gain of a "variable phase" neuron as a function of head orientation. The phase of the response of each neuron to sinusoidal vertical vestibular stimulation shifted gradually as the angle of the head with respect to the axis of rotation was changed. A phase of 0 deg indicates that the cell responded in phase with contralateral side down.

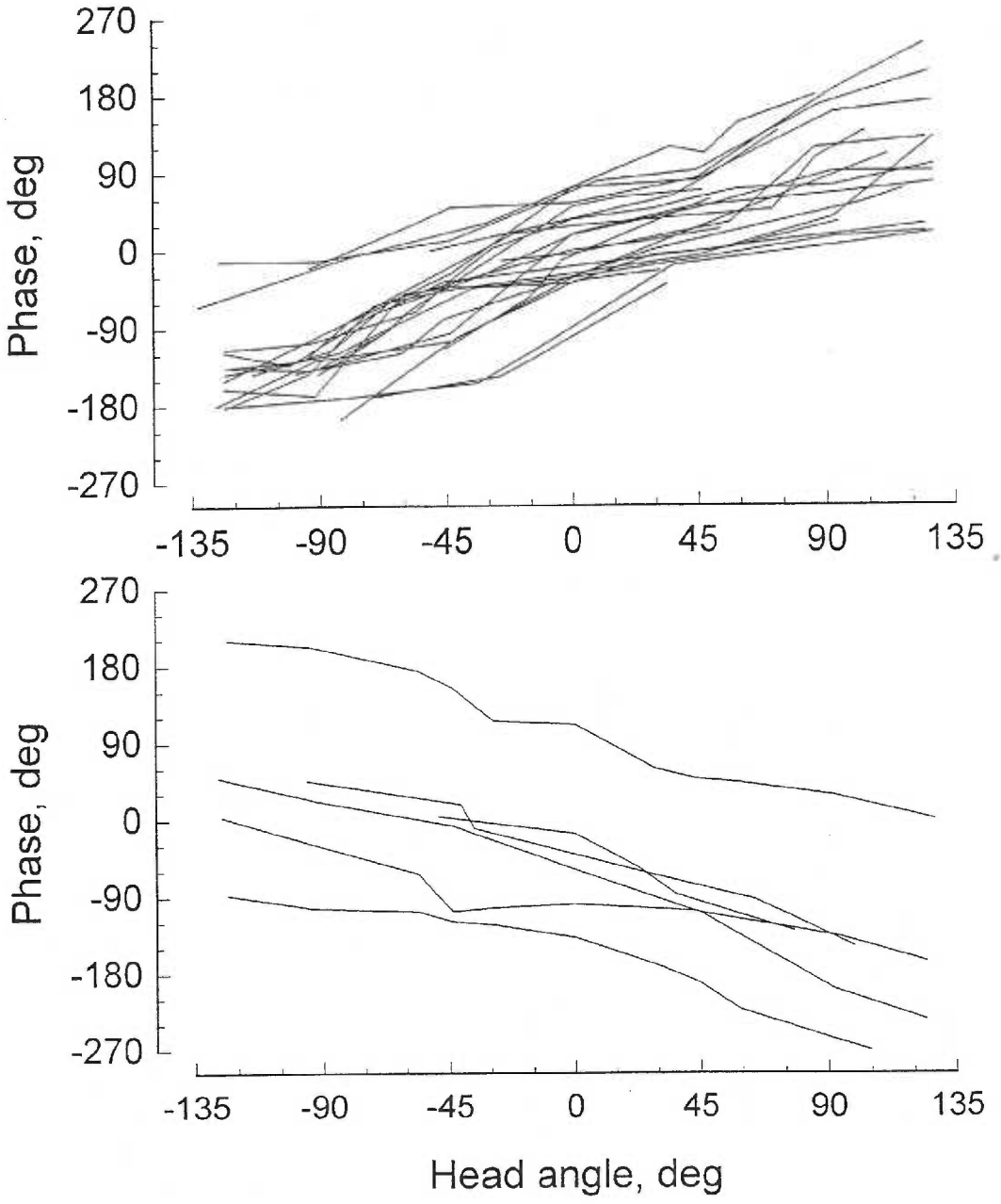

FIG. 10. The phase of the responses of "variable phase" neurons as a function of head orientation. For clarity the phases of neurons whose phase increased as head angles of the plane of stimulation increased from negative to positive values are plotted separately (A) from those of neurons whose phase decreased (B).

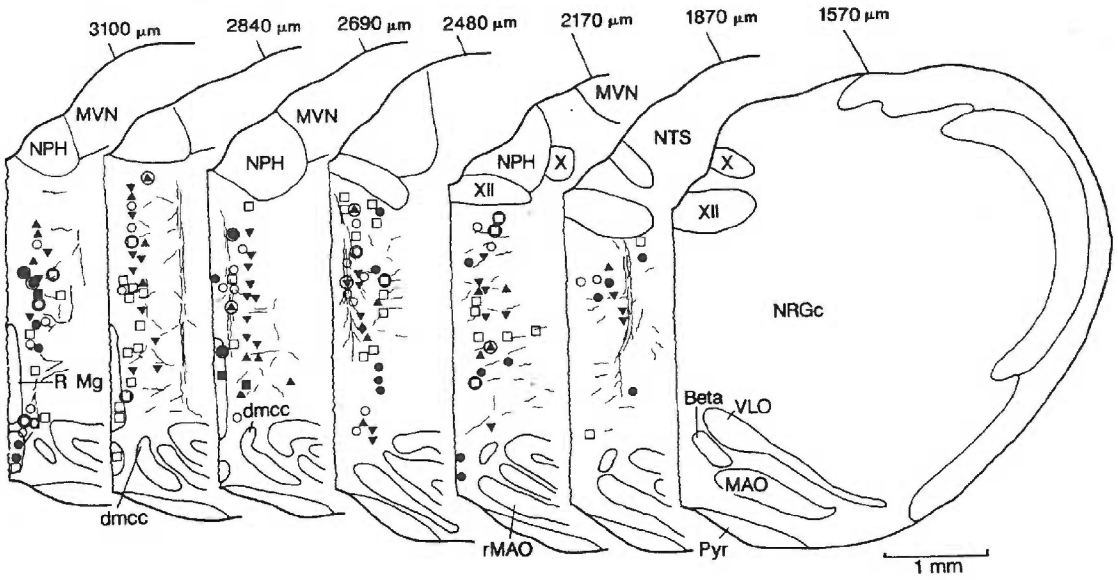

FIG. 11. Location of NRGc neurons responsive to vertical vestibular stimulation. The neurons, identified by extracellular recordings and whose locations were determined from marking lesions, were located in a region 500 μm on either side of the midline extending in the dorsocaudal direction from just ventral to the nucleus of the XIIth nerve and the nucleus prepositus hypoglossi to just dorsal and medial to the inferior olive. The fiber tracks shown in the figure are projections from the caudal vestibular nuclei which we have identified by orthograde transport of phaseolus leucoagglutinin (Fagerson and Barmack, unpublished observations). The distances shown on the sections indicate distances from the caudal pole of the inferior olive. The symbols on the schematic drawing indicate the location of each neuron and its classification. "Fixed phase" neurons (filled symbols) were grouped by their optimal "response plane" orientations into 5 groups: -90 deg to -60 deg (filled diamond), -59 deg to -25 deg (filled inverted triangle), -24 deg to +34 deg (filled circle), +35 deg to +59 deg (filled triangle), +60 deg to +90 deg (filled square). "Variable phase" neurons and unclassified neurons are indicated by open circles and open squares, respectively. Neurons which also responded to horizontal vestibular stimulation are indicated by large circles surrounding the symbol that indicates the response to vertical vestibular stimulation.

FIG. 12. Responses of a "fixed phase" neuron in all vertical planes of stimulation. A. Neuronal activity as the head was tilted down 10 deg. B. Neuronal activity as the head returned from a 10 deg tilt to the upright position. The responses of the neuron during sinusoidal stimulation in each plane of stimulation are separated into those during the downward tilt portion of the stimulus cycle (A) and those during the return to upright portion of the stimulus cycle (B). Thus, contiguous responses in one plane are alternately plotted in Figs. 12A and 12B. The responses in each plane are plotted in polar coordinates with a head angle of 0 deg indicating right side down, -90 deg nose down, $\pm$180 deg left side down, and +90 deg tail down. The vertical axis indicates impulses/sec (maximum for this neuron, 21 imp/sec). The radius indicates both degree of head tilt from 0 deg at the origin to 10 deg on the perimeter and the phase of the response with respect to head position from 90 deg at the origin to 0 deg on the perimeter of the figures. The phase of the response in each plane of stimulation are indicated by the filled circles on the horizontal plane below the surface representing the response. The filled circles in Fig. 12A (downward tilt) for each neuron indicate phase lead. Since these plots are constructed from responses during sinusoidal stimulation, the radius also represents velocity and acceleration of stimulation with maximum velocity at the origin and maximum acceleration at the perimeter. A surface was fit to the data using a distance weighted least squares smoothing method. (See APPENDIX for a description of the construction of this plot.)

FIG. 13. Responses of a "variable phase" neuron in all vertical planes of stimulation. A. Neuronal activity as the head was tilted down 10 deg. B. Neuronal activity as the head returned from a 10 deg tilt to the upright position. The filled circles in Fig. 13A (downward tilt) for each neuron indicate phase lead; that in Fig. 13B (return to upright) indicate phase lag. See Fig. 12 legend for explanation.

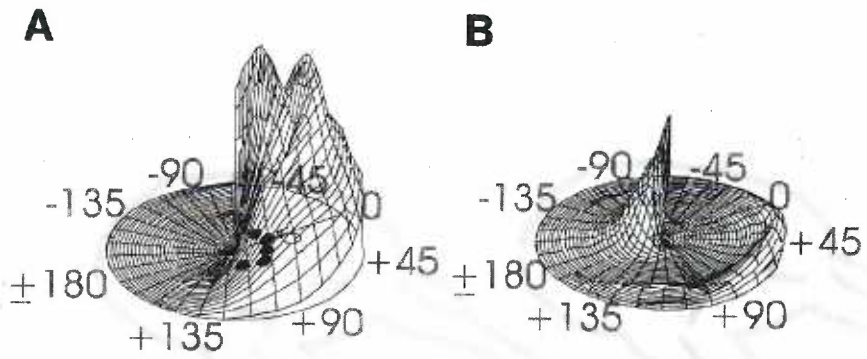

FIG. 12

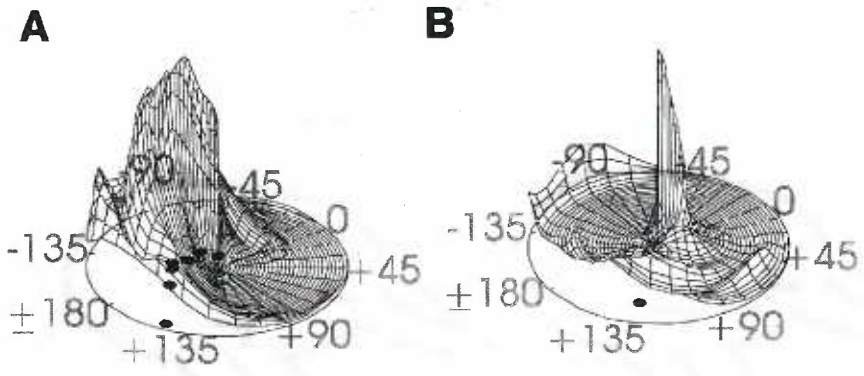

FIG. 13

JOURNAL OF NEUROPHYSIOLOGY

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PATHWAYS TO AND FROM VESTIBULARLY RESPONSIVE NEURONS IN THE MEDIAL NUCLEUS RETICULARIS GIGANTOCELLULARIS OF RABBIT

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ABSTRACT

Stabilization of the head in space during movement is controlled in part by vestibular information conveyed to neck motor neurons by reticulospinal neurons. Since neurons in the medial aspect of the caudal nucleus reticularis gigantocellularis (NRGc) are responsive to vestibular stimulation, we examined the source and destinations of this vestibular information.

Injections of Phaseolus leucoagglutinin (PHA-L) were made into the medial and descending vestibular nuclei (MVN, DVN) of rabbits. These injections labeled fibers in two separate fiber tracts that terminated in the medial NRGc. An immunohistochemical stain for glutamic acid decarboxylase (GAD) labeled terminals clustered along the descending fiber tracts and in the fiber networks of the medial NRGc. Injections of horseradish peroxidase (HRP) into the medial NRGc retrogradely labeled cells bilaterally in the MVN, DVN and the cerebellar fastigial nucleus with ipsilateral predominance in the DVN, contralateral predominance in the MVN and contralateral predominance in the fastigial nucleus.

Injections of HRP into the MVN and the nucleus prepositus hypoglossi (NPH) labeled neurons bilaterally in the medial NRGc. HRP injections into the cerebellar vermis, lobules 9 and 10, and the flocculus retrogradely labeled two spatially distinct groups of cells in the medial NRGc. Injections into the posterior vermis retrogradely labeled cells dorsally in the same region as the medial fiber tract identified by PHA-L injections as well as cells dispersed laterally and ventrally in the caudal NRGc.

Unilateral injections of HRP into the flocculus labeled cells bilaterally in a small dorsal region of the NRGc close to the midline.

We conclude that neurons in the medial aspect of the caudal NRGc receive GABAergic vestibular input from the contralateral MVN and the ipsilateral DVN as well as from the contralateral fastigial nucleus. This information is conveyed to the vestibular regions of the cerebellum providing tertiary vestibular mossy fiber input to these regions. These pathways may be involved in the adjustment of neck and postural reflexes in response to changes in the relationship of the head to gravity.

Gravitational forces acting on the head during movement require opposing mechanical forces in neck muscles in order to maintain stability of the head in space. Vestibular information is transmitted to neck motoneurons and contributes to the control of head position (Wilson and Maeda, 1974; Schor and Miller, 1981; Baker et al. 1985). Neurons in the nucleus reticularis gigantocellularis (NRGc) have monosynaptic connections with neck motor neurons (Peterson et al. 1978). Neurons in the reticular formation respond to vestibular stimulation of semicircular canal (Duensing and Schaefer, 1960; Orlovsky and Pavlova, 1972a; Spyer et al. 1974; Manzoni et al. 1983; Bolton et al. 1992) and otolith receptors (Duensing and Schaefer, 1960; Fukushima et al. 1977; Peterson et al. 1980; Manzoni et al. 1983; Bolton et al. 1992). In separate experiments, we have recorded the activity of neurons in the medial aspect of the caudal nucleus reticularis gigantocellularis (NRGc) in rabbits (Fagerson and Barmack, 1995). More than 85% of neurons in this region of the NRGc responded to natural vestibular stimulation. A majority of these neurons are sensitive to otolithic stimulation. Stimulation of the medial NRGc in the dorsal medulla primarily activates neck motoneurons (Peterson et al. 1979). Limb motoneurons are activated more ventrally and rostrally (Peterson et al. 1979; Drew and Rossingnol, 1990b; Drew and Rossingnol, 1990a). Thus, the region from which we recorded vestibular responses is primarily involved in the control of neck muscles.

Previous studies of fiber degeneration in the reticular formation after lesions in the vestibular nuclei (Ladpli and Brodal, 1968) and studies of activation of NRGc neurons by stimulation of the contralateral vestibular nuclei (Peterson and Abzug,

1975) indicated that fibers from the vestibular nuclei terminate in the NRGc. Since our recordings of vestibular responses in the NRGc were limited to the medial 500 μm of the caudal NRGc, we were interested in examining in more detail the vestibular projections to this region of the NRGc that might be responsible for the vestibular sensitivity of neurons of the medial aspect of the caudal NRGc.

Ninety-six percent of the neurons in our experiments had decreased activity as the rabbit was tilted onto the ipsilateral side and increased activity when tilted onto the contralateral side. This type of response is referred to as a β response. Vestibular neurons, however, are more likely (by a ratio of 4:1) to have decreased activity with contralateral tilt and increased activity with ipsilateral tilt (α response) (Adrian, 1943; Duensing and Schaefer, 1959; Fujita et al. 1968; Peterson, 1970; Kubo et al. 1977). Therefore, it is possible that the predominantly β response of the NRGc neurons is a result of inhibitory input from vestibular neurons. The MVN and DVN transmit vestibular information to the β-nucleus mediated by the transmitter, γ-aminobutyric acid (GABA) (Walberg, 1974; Saint-Cyr and Courville, 1979; Nelson et al. 1986; Nelson et al. 1989; Fredette and Mugnaini, 1991). The axons of these vestibular neurons, that terminate in the β-nucleus of the inferior olive, extend through the NRGc. This raises the possibility that vestibular inputs to the medial aspect of the caudal NRGc may also be GABAergic.

Projections from the NRGc terminate in most regions of the cerebellum (Brodal, 1953; Brodal, 1957; Kotchabhakidi et al. 1980). Vertical vestibular stimulation evokes simple spike responses in the cerebellar nodulus and uvula (Marini et al. 1976; Precht

et al. 1976; Shojaku et al. 1991; Barmack , and Shojaku, 1992; Barmack and Shojaku, 1993). Both the uvula/nodulus and the flocculus participate in vestibularly modulated reflexes (Fernandez and Fredrickson, 1964; Singleton, 1967; Ito et al. 1982; Ito, 1982; Waespe et al. 1985; Mason and Baker, 1989). Neurons in the uvula/nodulus receive both primary and secondary vestibular input and floccular neurons receive predominantly secondary vestibular input (Precht et al. 1976; Kotchabhakdi and Walberg, 1978; Korte and Mugnaini, 1979; Yamamoto, 1979; Epema et al. 1985; Magras and Voogd, 1985; Barmack et al. 1989; Barmack et al. 1992a; Barmack et al. 1992c; Barmack et al. 1993a). If the medial aspect of the caudal NRGc projects to these cerebellar regions, it may provide tertiary vestibular input.

In the present experiment, we address three questions: 1) Do projections from the vestibular nuclei terminate in the medial aspect of the caudal NRGc? Are these uni- or bilateral projections? 2) Is there GABAergic input to this nucleus that might account for the decrease in neuronal activity with tilt onto the ipsilateral side? 3) Do vestibularly responsive regions of the NRGc project to vestibular regions of the cerebellum and thus provide a third source of vestibular mossy fiber input to the cerebellum?

We have used the orthograde tracer, phaseolus leucoagglutinin (PHA-L) and the retrograde tracer, horseradish peroxidase (HRP), to identify terminals, fibers and soma of neurons projecting from the MVN and DVN and soma of neurons projecting from the fastigial nucleus to the medial aspect of the caudal NRGc. Using an immunohistochemical stain for glutamic acid decarboxylase (GAD), we demonstrated

that the input from the MVN/DVN to the NRGc is likely to be GABAergic. We have also demonstrated that NRGc neurons located in the region of vestibularly responsive neurons in the NRGc project to the vestibulocerebellum. These findings are discussed in the context of previous anatomical and physiological studies of the NRGc and the control of neck muscles.

METHODS

Fifteen rabbits and seven rats were used in these experiments. Some of these animals were used in previous investigations of projections from the inferior olive to the cerebellum (Barmack and Errico, 1993). The anatomical nomenclature for rabbit brainstem sections used in this report follows the atlas of Meesen and Olszewski (1949), that for rat brainstem sections follows the atlas of Paxinos and Watson (1986). There is considerable variation in the boundaries of cell groups implied by the nomenclature for subdivisions of the medullary reticular formation. The medial boundary of the NRGc of rabbits is near the midline (Meessen , and Olszewski, 1949). In rats, the medial boundary of the NRGc, dorsal to the middle third of the inferior olive, is the XIIth nerve and the region below the XIIth nerve is designated as the paramedian reticular nucleus (PRN) (Paxinos , and Watson, 1986). In rats, the ventral boundary of the PRN is the inferior olive. In rabbits, however, only the dorsal half of the region medial to the XIIth nerve is included in the PRN (Brodal, 1953). In this study, we use the terminology for rabbits. Thus, "NRGc" refers to the larger region

which extends laterally beyond the XIIth nerve and ventrally to the inferior olive. "PRN" refers to a subdivision of the NRGc.

Orthograde transport of phaseolus leucoagglutinin (PHA-L)

Three rabbits weighing 1-1.2 kg were anesthetized with intramuscular injections of ketamine hydrochloride (50 mg/kg), xylazine (6 mg/kg) and acepromazine maleate (1.2 mg/kg). The brainstems of rabbits were exposed by making an opening through the dura mater overlying the cisterna magna. A micropipette containing a solution of 2.5% PHA-L in 0.1 M phosphate-buffered saline (PBS), pH=7.4, was inserted under direct vision into the MVN. PHA-L was iontophoretically injected into the brainstem by means of 10 μA cathodal pulses, 10 sec on-10 sec off (Gerfen and Sawchenko, 1984; Ter Horst et al. 1984). Each injection lasted 10 minutes. Several (3-5) injections were made along the mediolateral and rostro-caudal extent of the brainstem. After a 3-6 day survival period, the animals were anesthetized and transcardially perfused. A brief saline flush was followed by a 4% paraformaldehyde, 0.15% picric acid solution in 0.1 M PBS, pH=7.2. The brain was blocked, cryoprotected in buffered sucrose solutions (10-30%), frozen and cut into 30-40 μm sections. A double peroxidase-antiperoxidase (PAP, diluted 1:50) immunohistochemical procedure, with diaminobenzidine (DAB, 1 mg/ml in 0.1 M Tris-buffered saline (TBS), pH=7.6, containing 0.01% H_2O_2) as the chromogen, was used to identify orthogradely transported PHA-L.

GAD immunocytochemistry

Two rabbits weighing 1-1.2 kg were deeply anesthetized with an intramuscular injection of a mixture of ketamine hydrochloride (50 mg/kg), xylazine (6 mg/kg), and acepromazine maleate (1.2 mg/kg). Each rabbit was transcardially perfused with a saline rinse followed by a slow (1 hr) flush with an axon terminal fixative (4% paraformaldehyde, 0.2% zinc salicylate in 0.9% NaCl, pH 4.5) and a final flush with 15% sucrose in 0.9% NaCl at 4°C (Oertel et al. 1981). The brain was then dissected and dehydrated in graded sucroses. Serial sections (20μm) were collected. Rabbit brainstem sections were processed for GAD immunocytochemistry using a peroxidase-antiperoxidase (PAP) method. The GAD antiserum was used at a dilution of 1:2,000. The rinsing solution and diluent for all immunoreagents was 0.5 M Tris-HCl buffer, pH=7.6.

HRP retrograde transport

Eight rabbits weighing 1.0-1.2 kg and four rats weighing 300 g were anesthetized either with intramuscular injections of ketamine hydrochloride (50 mg/kg), xylazine (6 mg/kg), and acepromazine maleate (1.2 mg/kg) or with intravenous α-chloralose (50mg/kg) urethan (500 mg/kg). For four rabbits, the brainstem was exposed by making an opening through the *dura mater* overlying the *cisterna magna*. A micropipette containing a 13% HRP solution in 0.1 M TBS, pH=8.6 was inserted under direct vision into the medial aspect of the caudal NRGc. The solution was either pressure injected at several locations along a single electrode track (0.5-1 μl) or

iontophoretically injected by 300 nA cathodal pulses lasting 2-3 sec with a 50-75% duty cycle. Several iontophoretic injections were made in the electrode track each lasting 30 min.

To ensure that the pipette was located in the region of vestibularly responsive neurons, we made extracellular recordings from neurons in this region during vestibular stimulation. This stimulation consisted of sinusoidal oscillation of the rabbit about the longitudinal axis (roll) or interaural axis (pitch) ($\pm$10 deg, 0.02 - 0.4 Hz) as previously described (Fagerson and Barmack, 1995).

In four rats, the brainstem was exposed and a micropipette containing a 30% HRP solution was inserted under direct vision into the medial aspect of the caudal MVN. At one to three locations, pressure injections of 0.1-0.3 μl were made through a glass micropipette connected via a flexible polyethylene tubing to a 1 μl syringe (Hamilton).

In four rabbits, the posterior cerebellum was exposed surgically by removing part of the overlying occipital bone. A 30% solution of horseradish peroxidase (HRP) was pressure-injected into the left posterior vermis. In two rabbits, a total of 4 μl of HRP was injected in four penetrations into the posterior vermis lobules 8, 9a, 9b and 10. In two rabbits, the flocculus was approached surgically through the middle ear. A 1 mm opening was made immediately rostral to the anterior semicircular canal (Barmack and Pettorossi, 1985). A micropipette containing HRP could be inserted through this opening directly into the flocculus. In flocculus penetrations, a total of 2 μl of HRP was injected.

Following a 24-48 hour postoperative survival, animals were deeply anesthetized with sodium pentobarbital (60 mg/kg). They were subsequently perfused transcardially with 0.9% saline, followed by a 2.0-2.5% paraformaldehyde, 0.15% picric acid, and 0.05-0.20% glutaraldehyde in 0.1 M sodium phosphate (PBS), pH = 7.2, lasting 20-30 minutes. The perfusion was terminated with a rinse of 0.1 M PBS, pH = 7.4. The blocked brainstems were cryoprotected in 10-30% saline solutions, frozen and cut in 30-40 μm sections. A DAB-stabilized, $CoCl_2$-intensified (Rye et al. 1984) tetramethylbenzidine (TMB) reaction (Rye et al. 1984; Olucha et al. 1985) was used to demonstrate the presence of retrogradely transported HRP.

RESULTS

Projection from the MVN/DVN to the NRGc

We injected phaseolus leucoagglutinin (PHA-L) iontophoretically into the left MVN and DVN of three rabbits. In the case illustrated in Figure 1, the injection site included most of the rostrocaudal extent of the MVN and extended into the most rostral portion of the DVN (Fig. 1A). Both coarse (1-4μm) and fine (less that 1.0μm) PHA-L labeled fibers were labeled by these PHA-L injections.

Fine PHA-L labeled fibers from the MVN/DVN descended in one of two fiber tracts through the medial aspect of the caudal NRGc, a medial tract approximately 75 μm from the midline and a lateral tract 125-400μm from the midline (Fig. 1B). Fibers

in the more medial fiber tract extended from the dorsal surface near the midline ventrally through the region of the medial longitudinal fasciculus. Fibers in the lateral tract entered the NRGc along the ventral surface of the nucleus prepositus hypoglossi (NPH). They coursed in a ventromedial direction. The lateral tract varied from animal to animal. In some animals it extended along a linear trajectory (as shown in Figure 1B). In others it divided into several parallel tracts. The fibers branched forming finer networks both ventral and lateral to the fiber tracts.

From the injection site some, fine fibers also extended in an arc crossing the midline at many dorsoventral levels. Some fine fibers also crossed the midline in the vestibular commissure 800-1000 μm caudal to the injection site. Other fibers turned ventrally at the midline and crossed the midline approximately 200 μm ventral to the vestibular commissure.

Varicose fibers in the fiber tracts descended both ipsilaterally and contralaterally. Contralaterally, the staining was nearly as dense as that on the ipsilateral side. Since some PHA-L may have diffused across the midline, it is possible that this diffusion was the source of the bilateral labeling. However, a much smaller injection, restricted to one side nevertheless labeled both ipsilateral and contralateral varicose fibers.

The fine fibers were punctuated with frequent varicosities. Within the dorsal NRGc, the fiber tracts contained many fibers with varicosities, giving a densely stained appearance along the fiber tracts (Fig. 2A). The PHA-L labeled fiber tracts were present in most sections throughout the caudal NRGc, but were not present in the

rostral NRGc or the nucleus reticularis parvocellularis (NRPc). Ventrally and laterally in the caudal NRGc the fibers branched into finer networks. The staining was less dense in these regions because there were fewer fibers. The varicosities on these PHA-L labeled fine fibers may be terminals suggesting that neurons in the MVN/DVN project to the NRGc. The PHA-L labeling in both the ipsi- and contralateral NRGc suggests that this projection is bilateral.

The coarse fibers (arrowheads Fig. 1B) were short segments extending in a caudal and ventral direction through the section. These coarse fibers were observed predominantly in the ipsilateral medial longitudinal fasciculus (MLF) at the level of the injection site and in sections caudal to the injection.

GABAergic projection to the NRGc

We processed rabbit brainstems using GAD-immunochemistry. The fixative was adjusted to pH 4.5 to provide maximum sensitivity for axon terminal staining (Oertel et al. 1981). and an axon terminal fixative resulting in a staining pattern similar to that seen with the PHA-L injections. GAD-labeled varicosities resulted in a densely stained pattern in the region of the fiber tracts (Fig. 2B). Unstained cell bodies were observed along the fiber tracts at different depths of focus in the section. These are shown schematically in Fig. 2C. Most but not all neurons along or near the fiber tracts were surrounded by GAD-labeled varicosities. In some sections, there were clusters of GAD-labeled varicosities in a small densely stained network branching out from the medial tract. GAD-labeling laterally in the region of the lateral fiber tract in these

animals was divided into a branching network extending ventrally. These results suggest that the labeled varicosities clustered along the fiber tracts are terminals contacting dendrites of the adjacent NRGc neurons and that the projection from the MVN and DVN to the NRGc is GABAergic.

HRP labeling after injections into the NRGc

We made HRP injections into the medial NRGc of six rabbits to determine the laterality of the projection from the MVN/DVN. Five rabbits received pressure injections and one received an iontophoretic injection. The rabbit shown in Figure 3 received several pressure injections, totaling 0.85 μl, along a single electrode track (Fig. 3A3). To ensure that the injection was made in the region of previously identified vestibularly responsive neurons (Fagerson and Barmack, 1995), we made extracellular recordings of neuronal responses to vestibular stimulation prior to the injection using the HRP filled microelectrode. A peristimulus histogram of the responses of a neuron, adjacent to the electrode track and prior to injection, shows decreased activity during tilt onto the ipsilateral side (in this case the left) and increased activity during tilt onto the contralateral side (Fig. 3B).

This injection resulted in a large area of dense staining focused in the NRGc but spreading to the caudal NPH, and the rostral hypoglossal nucleus. HRP-labeled cells were identified bilaterally in the MVN, DVN, and the fastigial nucleus (Fig. 3A and C). There were a total of 212 labeled cells in the ipsilateral DVN and 123 in the contralateral DVN. In the MVN, there were 14 labeled cells on the ipsilateral side and

80 labeled cells on the contralateral side. Within each of these nuclei, the neurons were located in several distinct subgroups (Fig. 3A). In the region of the HRP injections, clusters of terminals were also labeled in a pattern similar to that observed along the fiber tracts in animals with PHA-L injections into the MVN/DVN suggesting that the uptake of HRP by MVN and DVN occurred in terminals along fibers in the fiber tracts (Fig. 3A, open arrows). However, the HRP labeling was so dense that individual fibers could not be identified within the fiber tracts. Short segments of HRP labeled coarse fibers were also present primarily in the ipsilateral MLF (Fig. 3A, filled arrows).

The HRP pressure injection into the medial NRGc of rabbit also resulted in retrograde labeling of neurons in the fastigial nucleus (Fig. 3C). Fifty-one fastigial neurons were observed on the contralateral side and eleven on the ipsilateral side, indicating that the projection is primarily contralateral.

In another rabbit, we made two small iontophoretic injections into the dorsal NRGc (Fig. 5C). Some HRP was also deposited in the NPH at the beginning of the electrode track resulting in a small area of staining in the NPH. Retrogradely labeled neurons were identified in both the ipsi- and contralateral MVN and DVN (Fig. 5) indicating that the bilateral labeling in the pressure injected animals (Fig. 3) was not a result of spread of HRP across the midline.

In both the pressure injected and iontophoretically injected animals, labeled fibers were observed crossing the midline. Some fibers crossed the midline in the vestibular commissure (Figs. $3A_1$, 5D and 4A). Other fibers crossed the midline at the

level of the injection (Figs. 5C and 4B) and extended in an arc toward the contralateral MVN or DVN. These results indicate that the projection from the MVN/DVN is bilateral. However, the numbers of labeled cells in the MVN and DVN demonstrate that the projection from the DVN is predominantly ipsilateral while that from the MVN to the NRGc is predominantly contralateral.

Projection from the medial NRGc to the MVN in rat

Four rats received HRP injections into the MVN and/or NPH. A small injection into the medial aspect of the MVN and lateral NPH resulted in labeling of three groups of neurons in the NRGc. The first group was located dorsally on either side close to the midline in the NRGc [1] (Fig. 6). Scattered labeled neurons were also identified in the ventral NRGc (Fig. 6). A third group of labeled neurons was located ventrolateral to the NPH and lateral to the hypoglossal nerve in both the ipsi- and contralateral NRGc. Other structures that contained labeled neurons included raphe obscurus and raphe pallidus as well as both the ipsi- and contralateral MVN.

An HRP injection in the NPH and the hypoglossal nucleus (XII) in a third rat resulted in a different pattern of labeled neurons (not shown). In this rat, labeled neurons were located primarily ventrolateral to the NPH and extended ventrally in

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[1] The region that has been identified as the NRGc in rabbits Meesen and Olszewski (1949) has been subdivided in the rat into three regions Paxinos and Watson (1986). The most medial and caudal region is designated as the paramedian reticular nucleus (PMn) while the region lateral and rostroventral to this is designated as the NRGc. The rostrodorsal region is designated as the dorsal paragigantocellular nucleus (DPGc). In the text we use the rabbit terminology. However, we have used the rat terminology in Fig. 6.

more rostral sections. Only a few cells were located in the medial NRGc and PMn. Neurons in the medial aspect of the NRGc are likely to project bilaterally to the MVN while neurons in the lateral NRGc are likely to project to the NPH.

Projections from the NRGc to the cerebellum

To examine whether the vestibularly responsive neurons in the medial aspect of the caudal NRGc project to vestibular regions of the cerebellum, we injected HRP into the left posterior vermis of the cerebellum of two rabbits and the left flocculus of two rabbits. The injection site in one rabbit included lobules 9 and parts of 8 and 10 of the cerebellar vermis (Fig. 7A). Filled cells were noted in many locations within the brainstem (Fig. 7B). In particular, neurons in the medial aspect of both the ipsi- and contralateral NRGc were labeled. In the dorsal NRGc, filled neurons were located medially, clustered along fiber tracts similar to those identified by PHA-L and HRP injections and GAD immunohistochemistry (Fig. 8A, arrows). Fewer HRP-labeled neurons were observed in the ventral NRGc. The uvula/nodular injections spread across the midline so the laterality of the projections could not be determined.

HRP injections into the left flocculus resulted in bilateral labeling of neurons in a small area very close to the midline in the dorsal NRGc (Figs. 7C and D and 8B). HRP filled neurons were not present as far caudally as those in vermis injected animals. The labeled neurons were clustered primarily on either side of the midline with a few in more rostral sections extending dorsolaterally from this midline group of cells.

DISCUSSION

Projection from the MVN/DVN to vestibularly responsive neurons in the NRGc

Cells in the MVN/DVN project bilaterally to the medial NRGc. This projection pattern is confirmed by both orthograde PHA-L and retrograde HRP tracer experiments. This vestibular projection, reaches the NRGc in one of two fiber tracts that descend in the caudal NRGc. These vestibular projections terminate on neurons adjacent to the fiber tracts or neurons widely distributed ventral and lateral to the fiber tracts. Retrograde transport of HRP after injections into the medial aspect of the caudal NRGc confirmed the bilateral nature of this projection but also indicated that the projection from the DVN was predominantly ipsilateral while that from the MVN was predominantly contralateral.

Coarse fibers in the MLF were labeled both in rabbits that received PHA-L injections in the MVN/DVN and in those that received HRP injections into the NRGc. Some of these fibers may be from spinal projecting vestibular neurons. Others appear to project to the inferior olive.

The presence of a bilateral projection to the NRGc implies that there are at least two populations of neurons on each side: one that would have decreased activity with tilt onto the contralateral side and another that would have decreased activity with tilt onto the ipsilateral side. This was not the case in our experiments in which neurons responded primarily with decreased activity when the animal was rotated onto the ipsilateral side (Fagerson and Barmack, 1995). Only 4% of these neurons had decreased activity when tilted onto the contralateral side. An explanation for this

uniformity of responses in spite of both ipsilateral and contralateral projections is that the fibers originating from secondary vestibular neurons which cross the midline may receive primary vestibular afferent projections that are innervated by hair cells peripheral to the striola on the utricular macula. These hair cells have a functional polarity opposite to those medial to the striola (Goldberg et al. 1990). This would result in inputs to one side of the NRGc that arise from both the ipsi- and contralateral MVN/DVN having the same polarity. It is possible that the neurons in the MVN that project to the contralateral NRGc receive input from the macular region peripheral to the striola while those in the DVN that project to the ipsilateral NRGc receive input from the medial portion of the utricular macula. The fact that there were fewer retrogradely labeled cells in the MVN than in the DVN is consistent with this hypothesis since the region peripheral to the striola is smaller than that medial to the striola (Lindeman, 1973)

Inhibitory GABAergic input to the NRGc

GABAergic fibers terminate in the medial aspect of the caudal NRGc. These terminals are numerous along the medial fiber tract and may form synapses with dendrites of neurons adjacent to these tracts. The locations of these GAD-positive terminals are nearly identical to the locations of the PHA-L filled fibers and terminals following injections into the MVN/DVN. This similarity of staining patterns supports the hypothesis that the projections from the MVN and DVN to the NRGc are GABAergic. The decreased activity of NRGc neurons in response to ipsilateral tilt is

likely to be mediated by inhibitory GABAergic input from neurons in the MVN and DVN that have increased activity during ipsilateral tilt.

Projection from the fastigial nucleus to the NRGc

The results of the HRP injections into the NRGc confirm previous studies showing that the medial pontomedullary reticular formation receives input from the fastigial nucleus (Allen, 1924; Thomas et al. 1956; Carpenter et al. 1958; Walberg et al. 1962; Noda et al. 1990; Sugita and Noda, 1991). The fastigial nucleus receives input from the uvula/nodulus (Angaut and Brodal, 1967) as well as primary and secondary vestibular input from the semicircular canals and otoliths (Dow, 1936; Ghelarducci, 1973; Gherlarducci et al. 1974; Furuya et al. 1975; Gardner and Fuchs, 1975). There is also somatosensory input to the fastigial nucleus (Eccles et al. 1974a; Eccles et al. 1974b; Eccles et al. 1974c). The rostral half of the fastigial nucleus responds to roll tilt (Ghelarducci, 1973; Gherlarducci et al. 1974) and projects to contralateral vestibulospinal neurons (Brodal et al. 1962). It is not known whether the projection to the contralateral NRGc terminates on reticulospinal or other reticular neurons. Output from the fastigial nucleus is excitatory (Orlovsky and Pavlova, 1972b; Wilson and Yoshida, 1969). Thus, the NRGc receives inhibitory vestibular information from the contralateral MVN and ipsilateral DVN and excitatory vestibular input from the contralateral fastigial nucleus. Whether these two projections converge onto the same cells or terminate on different cells in the NRGc is unknown.

Outputs from the medial NRGc

HRP labeling after injections into the MVN/NPH confirm previous studies indicating that the medial NRGc projects bilaterally to the caudal MVN and NPH (Norvaja et al. 1979; Matsuyama et al. 1988; Grottel and Jakielska-Bukowska, 1993). The function of the input from the medial NRGc to the MVN and NPH is unknown.

Our results indicate that neurons in the medial aspect of the caudal NRGc also project to the uvula/nodulus and to the flocculus. These results confirm previous studies using lesions in the cerebellum to demonstrate that neurons in the medial NRGc project to the uvula and pyramis (Brodal and Torvik, 1954). In these experiments, no lesions were placed in the nodulus. In the present study, although no injections into the posterior vermis excluded the uvula, the inclusion of the nodulus in the injection site suggests that the nodulus also receives input from the medial NRGc. The uvula/nodulus receives primary and secondary vestibular afferent mossy fiber projections, whereas the flocculus receives primarily a secondary vestibular mossy fiber projection (Precht et al. 1976; Kotchabhakdi and Walberg, 1978; Korte and Mugnaini, 1979; Epema et al. 1985; Magras and Voogd, 1985; Smit et al. 1987; Simpson et al. 1988; Barmack et al. 1989; Barmack et al. 1992a; Barmack et al. 1992c; Barmack et al. 1993a; Yamamoto, 1979; Rubertone and Haines, 1981; Barmack et al. 1993a). Thus, this study demonstrates that the uvula/nodulus and the flocculus also receive a tertiary vestibular input from the NRGc.

Anatomy of the medial aspect of the caudal NRGc

Early studies of the anatomy of the reticular formation in cat identified the region medial to the hypoglossal nerve, lateral to the raphe nuclei, ventral to the hypoglossal nucleus and dorsal to the middle third of the inferior olive as the paramedian reticular nucleus (Brodal, 1953; Brodal and Torvik, 1954). The nucleus was identified by cell loss after cerebellectomy. The region from which we recorded vestibular responses included this region but continued rostrally to the rostral pole of the inferior olive (Fagerson and Barmack, 1995). This definition of the paramedian reticular nucleus is different from that used in the rat (Paxinos and Watson, 1986). In this discussion, we will distinguish Brodal's paramedian reticular nucleus from that used by Paxinos and Watson by using the abbreviation PRN for Brodal's and PMn for Paxinos and Watson's paramedian reticular formation.

Brodal identified three cell groups in the PRN based on cell loss following cerebellar lesions: dorsal, ventral and accessory (1953). We have identified fiber tracks and dense clusters of terminals adjacent to cell groups in the dorsal NRGc. It is likely that the cell groups adjacent to the fiber tracts are the previously identified dorsal and accessory cell groups and that the cell groups can be defined by the fiber tracts that project to them. Neurons adjacent to the medial fiber tract, identified in this study by HRP injection into the cerebellum, particularly those that project to the flocculus, and the most medial group of cells that project to the MVN, identified by HRP injection into the MVN and NPH, appear to be part of the accessory group. Those neurons adjacent to the dorsal part of lateral fiber tract are likely to be part of the dorsal group. The previous studies mentioned that there was individual variation in cell position and

density within the groups (Brodal, 1953; Brodal and Torvik, 1954). Our PHA-L injection and GAD immunohistochemistry experiments also reveal variation in the fiber tracts. Figures 1 and 2 show the linear arrangement of the lateral fiber tract. However, some animals had a looser group of neurons in the region of the lateral fiber tract. In these cases the lateral fiber tract divided into several parallel paths extending between neurons in the dorsal or ventral cell group.

Circuitry involved in conveying vestibular information through the NRGc

Vestibular information is conveyed to the medial aspect of the caudal NRGc by GABAergic projections from the contralateral MVN and the ipsilateral DVN (Fig.9). The NRGc also receives a second vestibular input from the contralateral fastigial nucleus (Orlovsky and Pavlova, 1972c). Neurons in the caudal NRGc project bilaterally as mossy fibers to the uvula/nodulus and to the flocculus. (For simplicity only the pathway to the uvula/nodulus is shown in Fig. 9.) The uvula/nodulus projects to the fastigial nucleus (not shown) (Angaut and Brodal, 1967).

A majority (78%) of the vestibularly responsive neurons in the medial aspect of the caudal NRGc respond to static tilt (Fagerson and Barmack, 1995). Since cerebellectomy reduces the number of neurons in the NRGc that respond to roll tilt by one half (Orlovsky and Pavlova, 1972a) and since the rostral fastigial nucleus responds to static tilt (Ghelarducci, 1973; Gherlarducci et al. 1974), otolithic input may be transmitted to the NRGc by fastigial as well as vestibular neurons. It is likely that the

vestibular input to the NRGc from the two sources terminates at least in part on different cell groups within the nucleus.

One might speculate that vestibular information from the contralateral MVN and the ipsilateral DVN is transmitted by cerebellar projecting neurons in the NRGc to granule cells in the uvula/nodulus and that the fastigial nucleus may transmit information from the uvula/nodulus, as well as from primary and secondary vestibular afferent input, to the reticulospinal neurons.

Alternatively the pathways through the cerebellum may originate from and terminate on reticulospinal neurons. Anatomical evidence indicates that the PRN does not project to the spinal cord and that the only overlap of cerebellar and spinal projecting neurons occurs in the perihypoglossal nuclei (nucleus of Roller, nucleus intercalatus of Staderini and NPH) (Brodal, 1957). Spinal projecting neurons were identified just lateral to the PRN and ventral to the rostral PRN (Brodal, 1957; Tohyama et al. 1979). However, physiological evidence demonstrates that neurons in the PRN can be antidromically activated by stimulation of the spinal cord (Peterson et al. 1980; Manzoni et al. 1983; Bolton et al. 1992). This implies that some cerebellar projecting neurons may also project to the spinal cord.

The bilateral projection from the NRGc to the MVN and NPH may be from cerebellar projecting NRGc neurons, reticulospinal neurons or from a third group of neurons. The location of HRP labeled neurons following injection into the MVN/NPH suggests that the accessory cell group of Brodal's PRN contributes to this projection.

The role of this projection in the transmission of vestibular information by NRGc neurons is unknown.

If there are several groups of neurons in the medial NRGc that receive vestibular input from different sources, one might expect that the responses of these groups might differ. However, our previous study of vestibular responses of NRGc neurons found relatively similar responses throughout the medial aspect of the caudal NRGc (Fagerson and Barmack, 1995).

There is a large overlap of regions of the NRGc that activate the neck, forelimbs and hindlimbs (Drew and Rossingnol, 1990b; Drew and Rossingnol, 1990a). This overlap allows the activation of different combinations of neck and limb muscles to produce different and possibly complex patterns of activity. The different cell groups may have similar responses to vestibular stimulation but may activate different muscle groups. Thus, one role of the fastigial input may be to provide the coordination required for activating the appropriate groups of postural muscles.

Neurons in the medial aspect of the caudal NRGc receive vestibular information from the MVN and DVN as well as the fastigial nucleus. These projections contribute information about the position of the head relative to gravity. This information is used by reticulospinal neurons to adjust postural reflexes to account for changes in the position of the head relative to gravity (Peterson et al. 1978; Schor and Miller, 1981; Bolton et al. 1992; Schor and Miller, 1995). Further study is necessary to identify the cell groups within each of the nuclei and the connections

between the cell groups before the roles of the two vestibular inputs to the medial NRGc in the control of neck muscles can be established.

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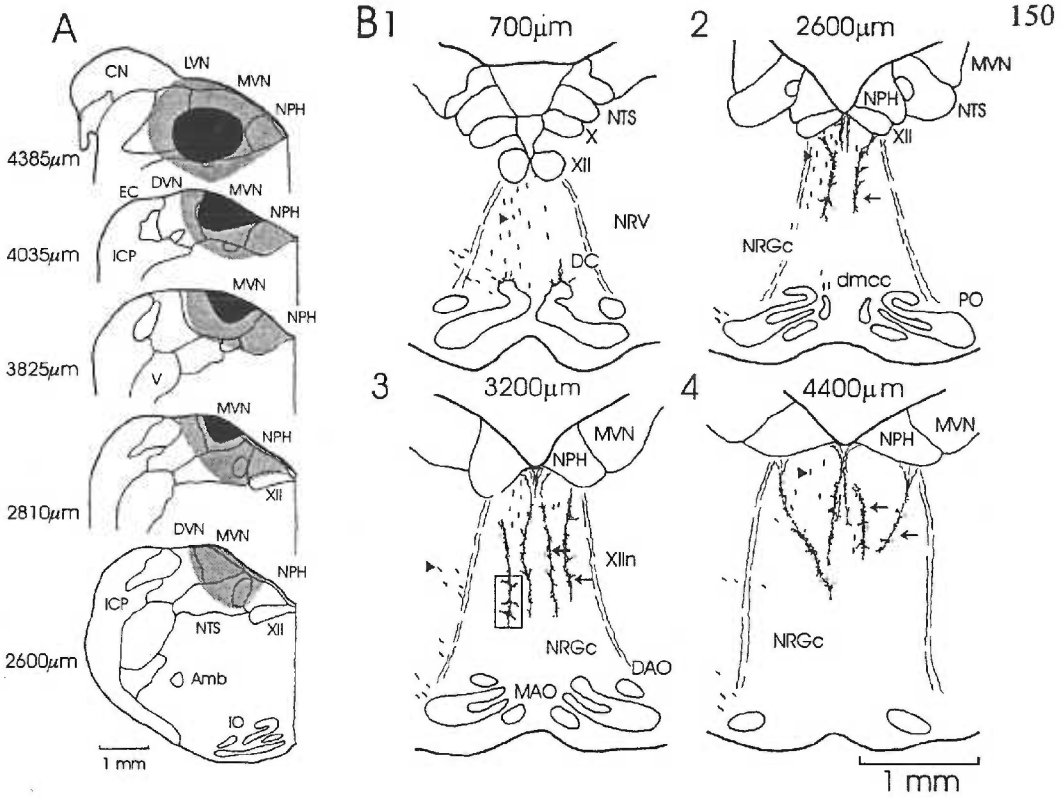


Fig. 1. Projections to the medial aspect of the NRGc following PHA-L injection into the medial and descending vestibular nuclei. **A,** Schematic serial coronal sections through the brainstem showing the injection site of PHA-L. The solid black indicates the areas in which the PHA-L was taken up into cell bodies while the shaded areas indicate the areas of diffusion of PHA-L. The brainstem cross sections are arranged from rostral (top) to caudal (bottom). The distances shown indicate the distance from the caudal pole of the inferior olive. **B,** Schematic diagram of brainstem coronal sections showing labeled fine fibers in medial and lateral tracts in the medial aspect of the NRGc as well as varicosities along fibers in the tracts (arrows). The location of labeled coarse fiber segments is also indicated (arrowheads). The distances shown indicate the distance from the caudal pole of the inferior olive. The box in **B3** indicates the location of the photomicrograph in Fig. 2A. Abbreviations: Amb, nucleus ambiguous; CN, cochlear nucleus; Cu, nucleus cuneatus; DAO, dorsal accessory olive; DC, dorsal cap of the inferior olive; dmcc, dorsomedial cell column of the inferior olive; DVN, descending vestibular nucleus; EC, external cuneate nucleus; Gr, nucleus gracilis; ICP, inferior cerebellar peduncle; NPH, nucleus prepositus hypoglossi; NRGc, nucleus reticularis gigantocellularis; NRV, nucleus reticularis ventralis; NTS, nucleus tractus solitarius; MAO, medial accessory olive; MVN, medial vestibular nucleus; PO, principal olive; X, dorsal motor nucleus of the vagus; XII, hypoglossal nucleus; XIIn, hypoglossal nerve.

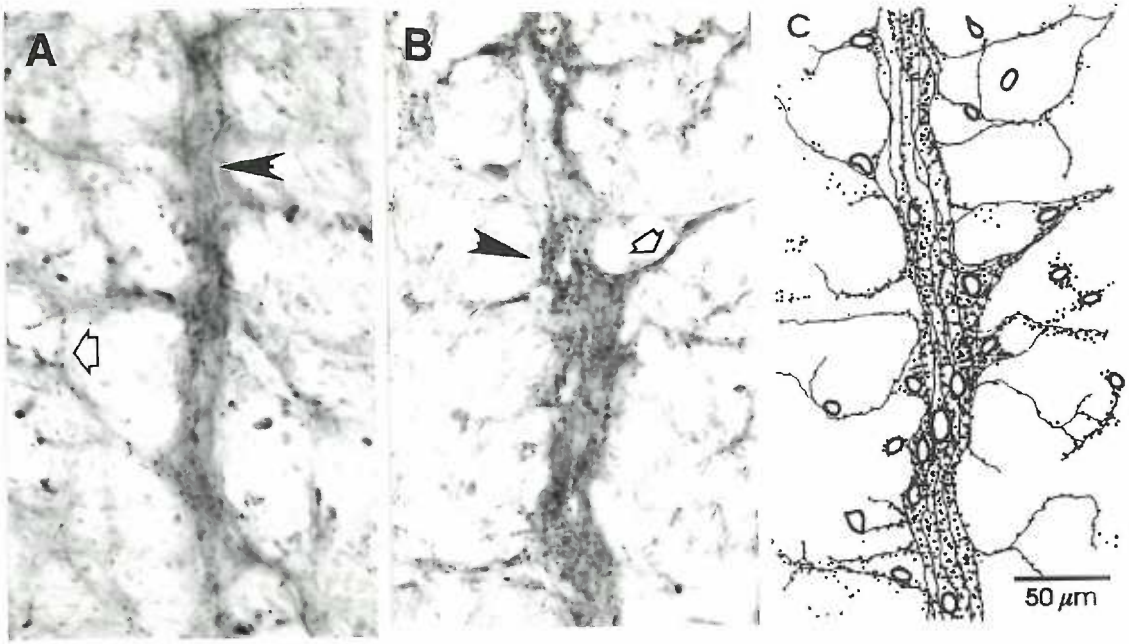
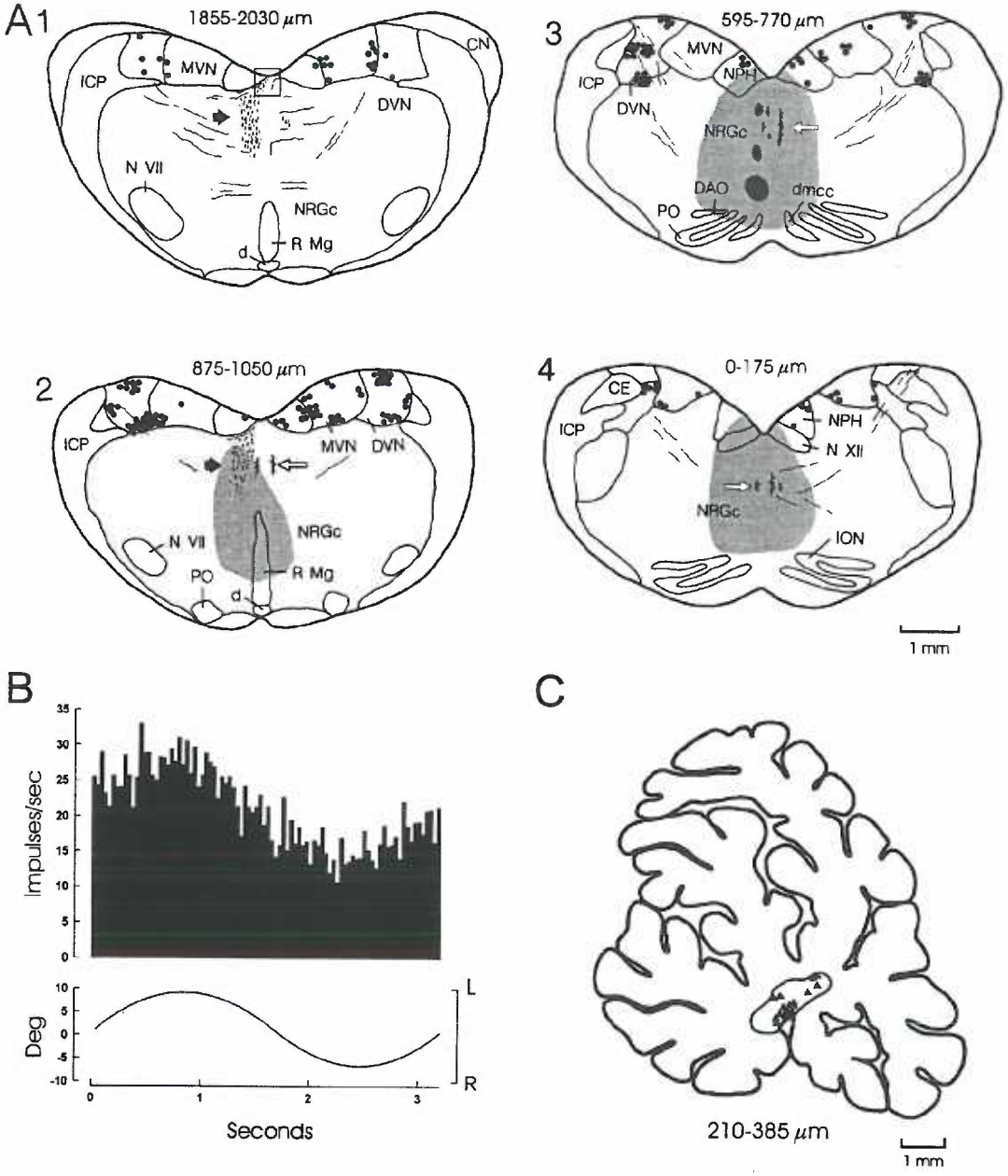


Fig. 2. Fiber tracts in the caudal NRGc. **A**, High magnification photomicrograph of PHA-L-labeled ipsilateral fiber tract, composed of many fibers with varicosities following injection into the MVN/DVN. The location of this photomicrograph is indicated in Fig. 1B. **B,** Photomicrograph of GAD-labeled fibers and varicosities along fibers in a fiber tract. The arrowhead indicates some GAD-labeled terminals in the fiber tract. This photomicrograph was taken 2600 μm rostral to the caudal pole of the inferior olive. **C**, Schematic diagram of fiber tract in **B** showing varicosities along fibers and cell bodies adjacent to the tract but out of focus in the photomicrograph. The arrowheads in each photomicrograph indicate some labeled fibers and/or labeled varicosities within the fiber tract. The open arrows indicate some fibers and/or labeled varicosities branching off from the fiber tract into the fiber network. The scale bar in **C** also applies to **A** and **B**.

Fig. 3. HRP-labeled neurons in the brainstem following three HRP pressure injections into the medial NRGc of rabbit. **A,** Diagram of HRP injection sites, labeled neurons and fibers after three injections totaling 0.85 μl injection. Regions of cellular uptake are indicated by the dark stippled areas. Areas of diffusion or uptake into terminals are indicated by the light stippled areas. Each filled circle indicates an HRP-labeled neuron in one of five consecutive 35μm sections. Labeled neurons in the NRGc are not shown since they may be a result of uptake into cell bodies rather than transport through axons. The open arrows indicate the locations of dense staining similar to the fiber tracts observed in PHA-L injected animals. The filled arrows indicate the location of short segments of labeled coarse fibers in the medial longitudinal fasciculus. The box in **A1** indicates the location of the photomicrograph in Fig. 4A. **B,** Responses evoked by sinusoidal vestibular stimulation of an NRGc. The histogram indicates the rate of firing of a neuron isolated along the track of the HRP-filled micropipette during sinusoidal vestibular stimulation (0.3 Hz, $\pm$10 deg). The trace below the histogram indicates the degree of head tilt. Tilt onto the contralateral side is indicated when the trace is up; tilt onto the ipsilateral side is indicated when the trace is down. **C,** HRP-labeled cells in the contralateral fastigial nucleus of the cerebellum after HRP pressure injection into the medial NRGc of rabbit. Each filled triangle indicates one cell in one of five consecutive sections. The distance from the midline is indicated below the diagram of the sagittal section of the contralateral vermis. Abbreviations: CE, external cuneate nucleus; CN, cochlear nucleus; d, cell group d; DAO, dorsal accessory olive; dmcc, dorsomedial cell column of the inferior olive; DVN, descending vestibular nucleus; ICP, inferior cerebellar peduncle; ION, inferior olive nucleus; MVN, medial vestibular nucleus; NPH, nucleus prepositus hypoglossi; N RGc, nucleus reticularis gigantocellularis; N XII, hypoglossal nucleus; PO, principal olive; RMg, nucleus raphe magnus.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N VII
PO
R Mg
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MVN
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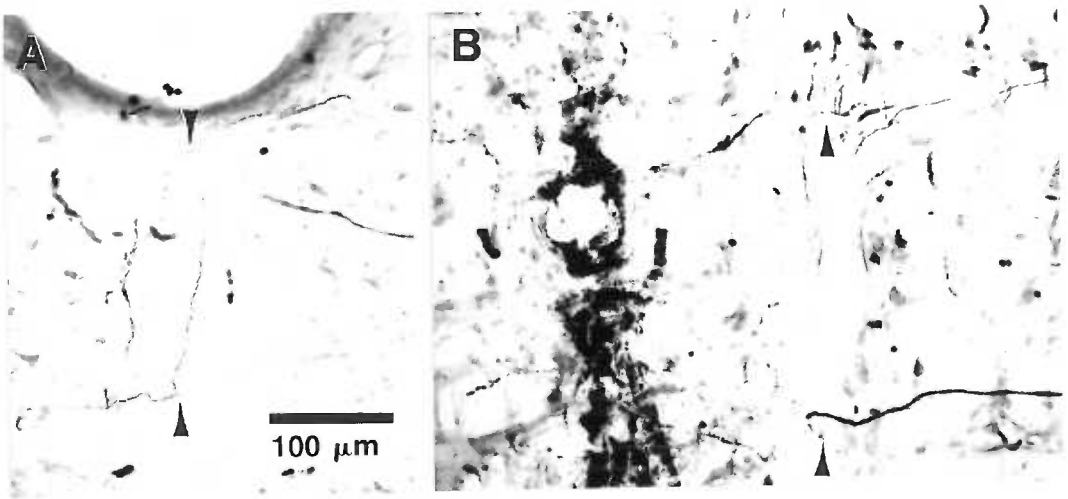

Fig. 4. HRP-labeled fibers crossing the midline in the brainstem following injection into the medial NRGc. **A,** HRP-labeled fibers crossing the midline in a pressure injected animal. The location of the photomicrograph is indicated in Fig. 3A1. **B,** HRP injection site and labeled fibers crossing the midline at the level of the injection site following an iontophoretic. The location of the HRP injection site is indicated in Fig. 5C. The arrowheads on the midline in **A** and **B** indicate fibers crossing the midline. The arrow in **B** indicates one of the injection sites. The scale bar in **A** also applies to **B**.

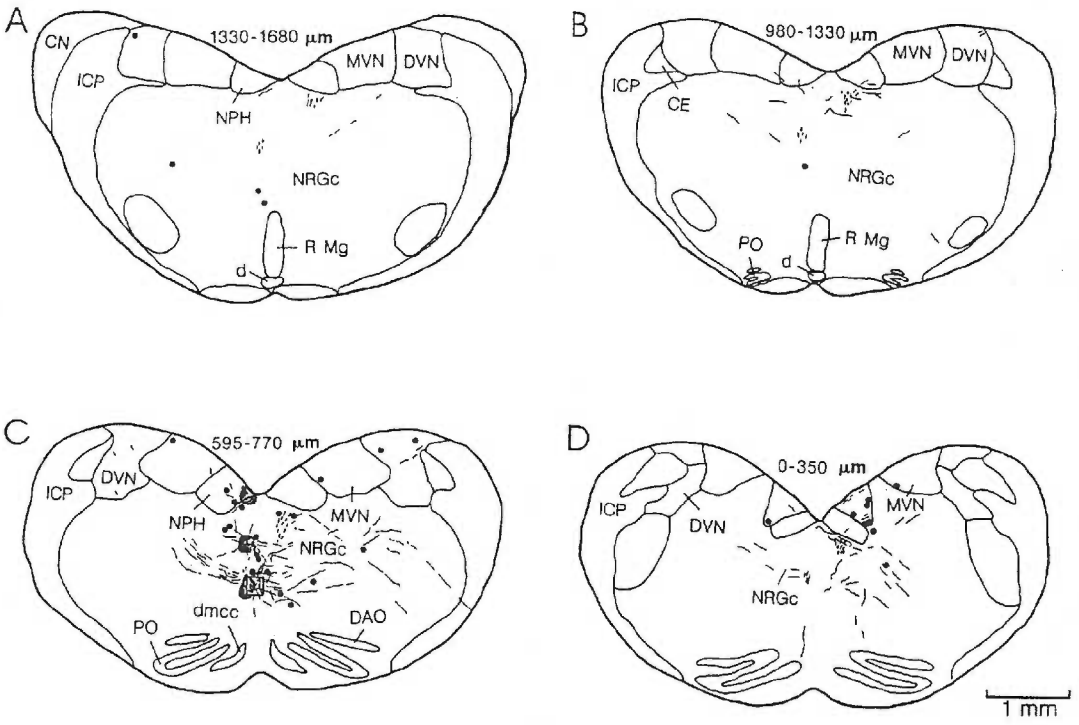

Fig. 5. HRP-labeled neurons and fibers in the brainstem following an iontophoretic HRP injection into the NRGc of rabbit. **A-D,** Schematic diagram of HRP labeling. The small injection sites are indicated in **C** by shaded areas that are exaggerated in size to distinguish them from filled cells. The location of HRP-labeled neurons and fibers in ten consecutive sections are indicated on each cross section diagram. Each filled circle indicates an HRP-labeled neuron. The box in **C** indicates the location of the photomicrograph in Fig. 3B. Abbreviations: CE, external cuneate nucleus; CN, cochlear nucleus; d, cell group d; DAO, dorsal accessory olive; dmcc, dorsomedial cell column of the inferior olive; DVN, descending vestibular nucleus; ICP, inferior cerebellar peduncle; MVN, medial vestibular nucleus; NPH, nucleus prepositus hypoglossi; NRGc, nucleus reticularis gigantocellularis; PO, principal olive; RMg, nucleus raphe magnus.

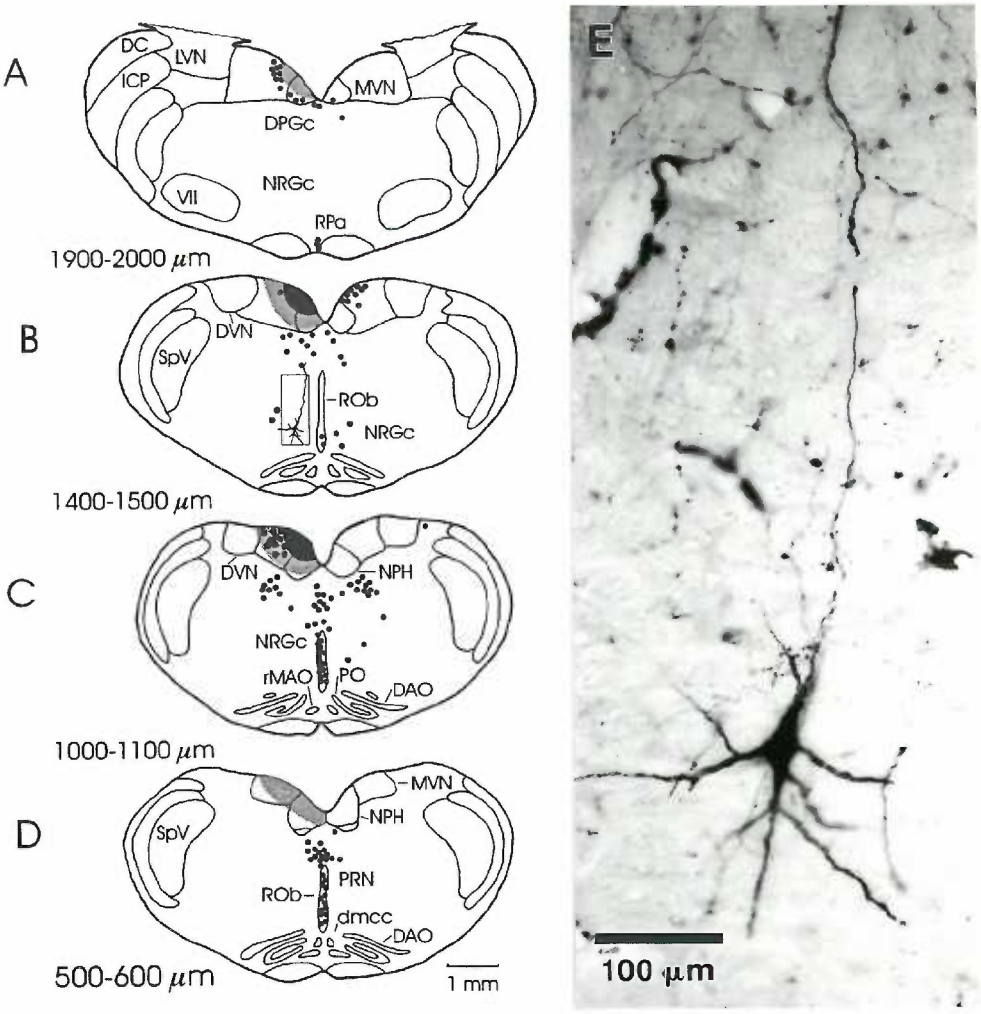

Fig.6. HRP-labeled neurons in a rat brainstem following HRP injections into the MVN/NPH. **A-D**, Schematic illustrations (rostral to caudal) of brainstem sections showing the injection site (dark shaded areas in **B** and **C**) and the location of HRP-labeled cells. Each filled circle indicates one labeled neuron in one of three consecutive histological sections. The single neuron in the box in **B** indicates the location of the photomicrograph in **E**. **E**, HRP-labeled neuron in the NRGc projecting to site of HRP injection in the MVN/NPH in **B**. Abbreviations: DAO, dorsal accessory olive; DC, dorsal cochlear nucleus; dmcc, dorsomedial cell column of the inferior olive; DPGc, dorsal paragigantocellular nucleus; DVN, descending vestibular nucleus; ICP, inferior cerebellar peduncle; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; NPH, nucleus prepositus hypoglossi; NRGc, nucleus reticularis gigantocellularis; PMn, paramedian reticular nucleus; PO, principal olive; rMAO, rostral medial accessory olive; RPa, raphe pallidus; ROb, raphe obscurus; SpV, spinal trigeminal nucleus.

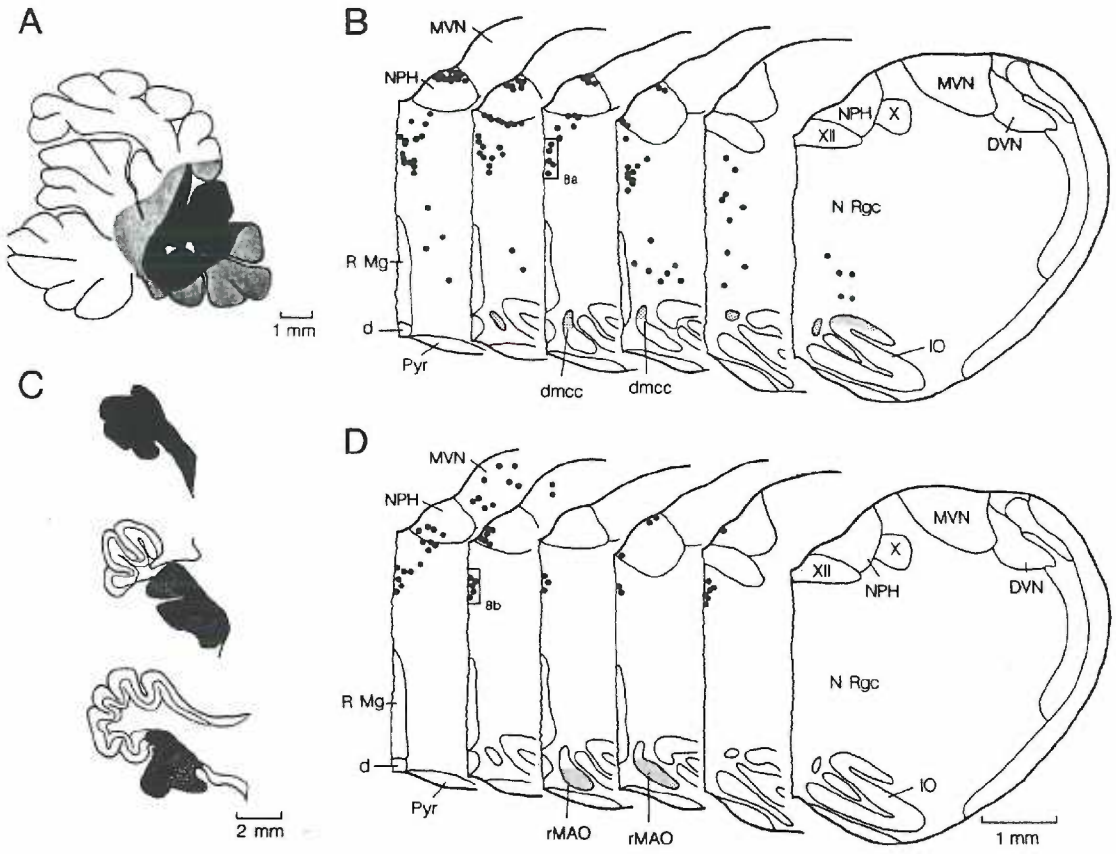

Fig. 7. HRP-labeled neurons in the brainstem following an HRP injection into the vestibulo-cerebellum. **A**, Schematic illustration of HRP injection site in lobules 9a and 10 of the vermis. The injection site crossed the midline. **B**, Retrogradely labeled brainstem neurons following HRP injection into the uvula/nodulus. Labeled neurons are indicated in schematic brainstem sections through the NRGc spaced about 300 μm. **C**, Schematic illustration of the HRP injection site into the flocculus. **D**, Retrogradely labeled brainstem neurons following HRP injection into the flocculus. Labeled neurons are indicated in schematic brainstem sections through the NRGc spaced about 300 μm apart. The solid black areas in **A** and **C** indicates the location of the injection and the shaded area indicates the area into which HRP diffused. The most rostral sections in **B** and **D** are shown at the left. Filled circles correspond to one HRP-labeled neuron in a single histological section. The boxes in **B** and **D** indicate the locations of the photomicrographs of the medial NRGc, shown in Fig. 8A and B, approximately 3000 and 3200 μm rostral to the caudal pole of the inferior olive, respectively. Abbreviations: d, cell group d; dmcc, dorsomedial cell column of the inferior olive; DVN, descending vestibular nucleus; IO, inferior olive; MVN, medial vestibular nucleus; NPH, nucleus prepositus hypoglossi; N Rgc, nucleus reticularis gigantocellularis; Pyr, pyramids; RMg, nucleus raphe magnus; X, dorsal motor nucleus of the vagus; XII, hypoglossal nucleus.

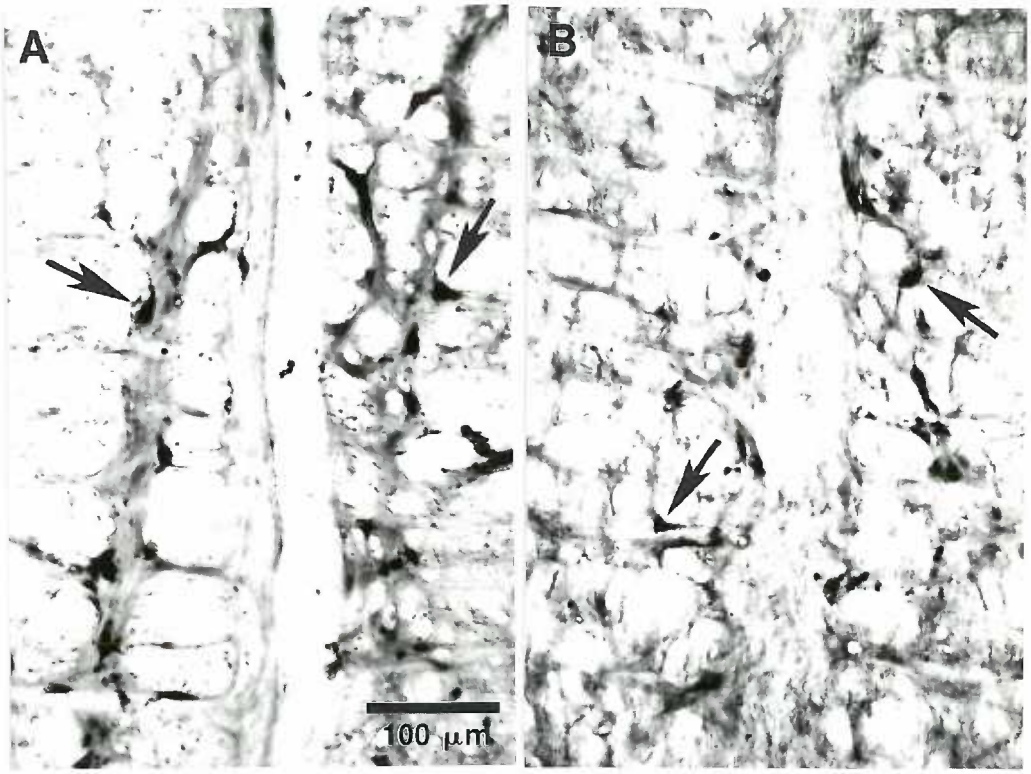

Fig. 8. HRP-labeled neurons following injection into the vestibulo-cerebellum. **A**, Photomicrograph of labeled neurons in the dorsal NRGc after HRP injection into lobules IX and X. Labeled neurons were clustered along fiber tracts **B**, Photomicrograph of labeled neurons after HRP injection into the flocculus. Labeled neurons were clustered in small groups along the dorsal midline. The locations of the photomicrographs in **A** and **B** are indicated in Fig. 7B and D, respectively. A few labeled neurons in each photomicrograph are indicated by arrows. The scale bar in **A** also applies to **B**.

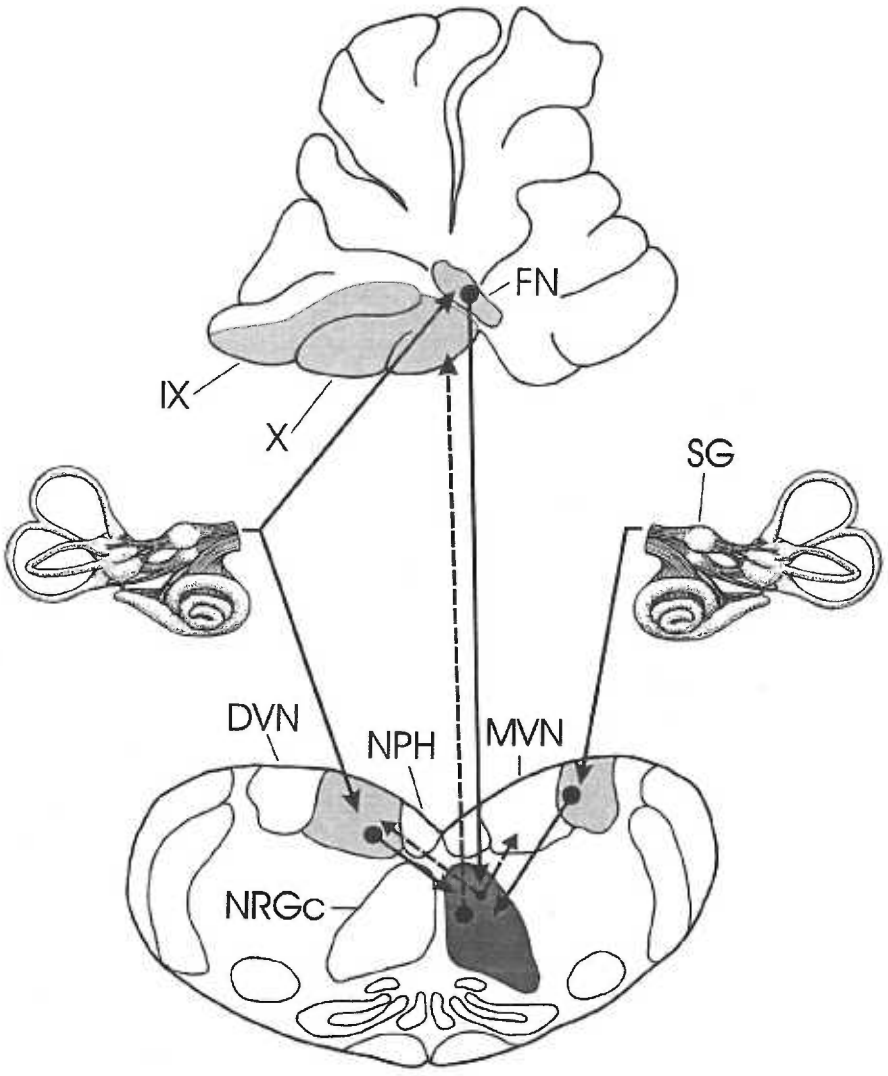

Fig. 9. Neuronal circuitry conveying vestibular information through the NRGc. Neurons in Scarpa's ganglion convey vestibular information to the ipsilateral medial and descending vestibular nuclei. Secondary vestibular information is transmitted to the NRGc by GABAergic neurons in the ipsilateral DVN and the contralateral MVN. Tertiary vestibular information from the NRGc is conveyed both to the uvula/nodulus and bilaterally to the flocculus (not shown). Neurons in the contralateral fastigial nucleus receive primary and secondary vestibular input as well as input from the uvula/nodulus (not shown). The fastigial nucleus also conveys secondary and tertiary vestibular information to the NRGc. The NRGc projects bilaterally to the MVN and to the NPH (not shown) and to the spinal cord (not shown). Abbreviations: DVN, descending vestibular nucleus; FN, fastigial nucleus of the cerebellum; MVN, medial vestibular nucleus; NPH, nucleus prepositus hypoglossi, NRGc, nucleus reticularis gigantocellularis; SG, Scarpa's ganglion; IX, cerebellar uvula; X, cerebellar nodulus.

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GUIDE FOR AUTHORS

Updated January 1995

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Abstract	Tables
Text	Figure legends
Literature Cited	

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Barbas, H., T.H. Haswell Henion, and C.R. Dermon (1991) Diverse thalamic projections to the prefrontal cortex in the rhesus monkey. J. Comp. Neurol. *313:*65–94.

Roland, P.E. (1993) Brain Activation. New York: Wiley-Liss.

Shaw, G. (1991) Neurofilament proteins. In R. Burgoyne (ed): The Neuronal Cytoskeleton. New York: Wiley-Liss, pp. 185–214.

Shering, A.F., and Lowenstein, P.R. (1994) Neocortex provides direct synaptic input to interstitial neurons of the intermediate zone of kittens and white matter of cats: A light and electron microscopic study. J. Comp. Neurol. *347:*433–443.

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GUIDE FOR AUTHORS

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The same abbreviations should be used in both the text and figures. If many neuroanatomical abbreviations are used, including those used repeatedly in the illustrations, they should appear as a separate list at the beginning of the paper.

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km	kilometer
m	meter
cm	centimeter
mm	millimeter
μm	micrometer (micron)
nm	nanometer
pm	picometer

Area

km^2	square kilometer
m^2	square meter
cm^2	square centimeter
mm^2	square millimeter
μm^2	square micrometer
nm^2	square nanometer

Volume

km^3	cubic kilometer
m^3	cubic meter
cm^3	cubic centimeter
mm^3	cubic millimeter
μm^3	cubic micrometer
nm^3	cubic nanometer

kl	kiloliter
	liter, always spell out
ml	milliliter
μl	microliter
nl	nanoliter
pl	picoliter

Weight

kg	kilogram
g	gram
mg	milligram
μg	microgram
ng	nanogram
pg	picogram

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DISCUSSION

One role of the medial NRGc in neck muscle reflexes may be to act as a modulator of neck muscle responses to account for the forces of gravity

More than 85% of neurons in the medial aspect of the caudal NRGc respond to vertical vestibular stimulation (Fagerson and Barmack, 1995b). The predominant response of NRGc neurons is to stimulation of the utriculus since 78% of the vestibularly responsive neurons respond to static tilt (Fagerson and Barmack, 1995b). Only a few neurons could be classified as either receiving input from the semicircular canals only or from phasic otolith neurons as evidenced by their response to transient vestibular stimulation. At least half of NRGc neurons had phasic-tonic responses indicating either convergence of input mediated by semicircular canals and tonic otolith neurons or input mediated by phasic-tonic otolith neurons. This sensitivity to the forces of linear acceleration could be useful in the adjustment of postural reflexes to account for variations in the magnitude of the moment arm of the gravitational force acting on the head during movement.

The medial NRGc receives GABAergic vestibular input from the contralateral MVN and the ipsilateral DVN

At least one source of vestibular input to the NRGc is conveyed by GABAergic fibers predominantly from the contralateral MVN and ipsilateral DVN (Fagerson and Barmack, 1995a). This inhibitory input reverses the polarity of the vestibular

responses conveyed from the vestibular nuclei (α responses) (Adrian, 1943; Duensing and Schaefer, 1959; Fujita et al. 1968; Peterson, 1970; Kubo et al. 1977) to produce the β responses observed in 96% of the vestibularly responsive population of neurons. The few ipsilateral MVN and contralateral DVN fibers may convey information from afferents innervating the peripheral region of the utricular striola that has hair cells with a polarity opposite to the majority of hair cells in the macula (Fernandez et al. 1972; Goldberg et al. 1990; Fernandez and Goldberg, 1976b; Loe et al. 1973; Vidal et al. 1971). This would also result in β type responses in the NRGc.

The role of the input from the contralateral fastigial nucleus is unclear

The fastigial nucleus may participate in a cerebellar loop from the NRGc. Neurons in the PRN of cat project to the cerebellum (Brodal, 1953; Brodal and Torvik, 1954). More specifically, we have demonstrated that neurons in the medial NRGc project to both the flocculus and the uvula/nodulus (Fagerson and Barmack, 1995a). The uvula/nodulus projects to the fastigial nucleus (Angaut and Brodal, 1967). The fastigial nucleus also projects to the pontomedullary reticular formation including the medial NRGc (Allen, 1924; Thomas et al. 1956; Carpenter et al. 1958; Walberg et al. 1962; Noda et al. 1990; Sugita and Noda, 1991; Fagerson and Barmack, 1995a). This implies that these pathways constitute a loop through the cerebellum that might modify the vestibular responses of NRGc neurons.

The projection from the fastigial nucleus to the medial NRGc may convey vestibular information, since the fastigial nucleus receives vestibular input from the

semicircular canals and the otoliths (Ghelarducci, 1973; Gherlarducci et al. 1974; Furuya et al. 1975; Gardner and Fuchs, 1975) and neurons in the nucleus respond to vestibular stimulation of the semicircular canals and otoliths (Ghelarducci, 1973; Gherlarducci et al. 1974; Fuchs et al. 1994; Guart and Delgado-Garcia, 1994), it is likely that vestibular information is conveyed from the fastigial nucleusto the NRGc. Neurons in the fastigial nucleus have increased activity with roll tilt to the ipsilateral side (α response) and projects to the contralateral medial reticular formation which responds to contralateral roll tilt. Thus, the two inputs would be synergistic. However, it is not known whether these two inputs converge on the same cells.

Any topographical organization of vestibular information in the medial NRGc exists at a more anatomically specific level than that which we examined

Since semicircular canal mediated responses in the β-nucleus and climbing fiber responses in the uvula/nodulus are topographically organized, it seems reasonable to assume that the input from the NRGc to this region of the cerebellum may also be topographically organized. However, the medial NRGc does not exhibit the clear topographical organization of semicircular canal information that is found in the β-nucleus or in climbing fiber responses in the uvula/nodulus (Leonard et al. 1988; Sato and Barmack, 1985; Barmack and Shojaku, 1992; Barmack et al. 1993). There was no apparent organization of vestibularly responsive neurons in the NRGc based on their optimal response plane orientation or their responses to exponential "step" stimulation (Fagerson and Barmack, 1995b). However, the orientation of the optimal response

planes of NRGc neurons mediated by the otoliths must be coordinated in some manner with the organization of climbing fiber responses in the uvula/nodulus.

Our explorations of the anatomy of the medial NRGc indicates that there may be an as yet unidentified organization within this region (Fagerson and Barmack, 1995a). The distinct segregation of projection fibers into the medial and lateral fiber tracts, one containing fibers that descend from the dorsal midline, the other containing fibers that begin their descent ventral to the hypoglossal nucleus and NPH, implies that these fibers may project from different cell groups.

The three cell groups identified by Brodal in the cerebellar projecting PRN appear to be located along the fiber tracts that we identified (Brodal, 1953; Brodal and Torvik, 1954; Fagerson and Barmack, 1995a). The accessory cell group correlates with neurons clustered along the medial fiber tract. The dorsal cell group correlates with neurons clustered among the fibers of the dorsal part of the lateral tract. The ventral group may be innervated by the fibers branching from the lateral tract in the ventral NRGc. Thus, the fiber tracts and the three cell groups may provide clues to a more localized topographical organization.

Some processing of vestibular information is likely to occur in the NRGc

Recent investigations of the vestibulocollic reflex have sought the neuronal substrate for the apparent convergence of right and left anterior or posterior semicircular canals as well as the substrate for convergence of spatially and temporally differing vestibular input with little success (Baker et al. 1984a; Baker et al. 1984b;

Wilson et al. 1990; Wilson et al. 1992; Peterson et al. 1992; Bolton et al. 1992; Graf et al. 1993; Endo et al. 1994). Our data indicate that contrary to previous reports at least some convergence may take place in the medial NRGc (Bolton et al. 1992; Fagerson and Barmack, 1995b). We identified a larger proportion of "variable phase" neurons or STC neurons in our experimental population than previous studies found in the NRGc or vestibular nuclei (25% as opposed to 9% in the NRGc or 10% in the vestibular nuclei).

The higher percentage of variable phase neurons in the NRGc than in the vestibular nuclei indicates that convergence of inputs with differing properties does occur in the NRGc. The distinct roles of the inputs from the fastigial nucleus and the MVN and DVN to the medial NRGc are yet to be determined. It remains to be seen whether inputs from the fastigial nucleus provide a spatially or temporally differing input from that of the MVN and DVN and whether these inputs provide the basis for the additional convergence in the NRGc. Convergence of inputs, however or wherever it occurs, may be a significant way in which the nervous system modifies vestibular information to mold motor neuron output to respond to the range of linear and angular accelerations measured by the vestibular organs and by the differing actions of the numerous neck muscles.

The significance of convergence of inputs

When two inputs with different properties converge on a neuron, the resultant response is likely to combine the characteristics of the two neurons' responses. Some

of the characteristics of input neurons that may vary include: the peripheral source of the response (semicircular canal or otolith), regularity of the neuronal discharge, the orientation of the optimal response plane as well as characteristics observed during sinusoidal stimulation such as gain and phase. When the frequency of sinusoidal vestibular stimulation is varied, the gain and phase of regular and irregular semicircular canal or otolith afferents can each be described by a distinct curve (Goldberg and Fernandez, 1971; Fernandez and Goldberg, 1976a). Thus, an almost infinite number of combinations of neuronal characteristics are possible. Many of these combinations may result in response properties that do not exist in peripheral afferent responses.

A simple type of convergence such as convergence of two inputs with similar phase and gain dynamics but with differing optimal response planes should provide a means of controlling three parameters of a neuron's response to vestibular stimulation. It would allow a modification of the orientation of the optimal response plane to a point midway between the optimal response planes of the two input neurons. Also depending on the difference between the optimal response planes of the two input neurons, it would allow a modification of either the amplitude or the breadth of the range of stimulus planes over which there is a maximal response. The rules that might govern the resultant response of the target neuron receiving this type of convergence are as follows: 1) During portions of the stimulus cycle if only one of the two neurons is responding, the amplitude of the response is determined by the neuron that is responding. 2) If both neurons are responding, the amplitude of the response is determined by the sum of the two responses. Thus, where the orientations of the

optimal response planes differ by more than approximately 90 deg, the sums of the two responses would be less than or equal to the amplitudes of the two neurons (assuming they have equal amplitudes). However, the maximal response is achieved over a range of stimulus planes. Where the optimal response plane orientations differ by less than 90 deg, the sums of the two responses would generally be greater than the maximal response of each of the input neurons. As the orientations of the optimal planes converge, the amplitude of the response approaches the sum of the two input neurons' responses. However, the optimal response plane would remain narrow.

If the convergent inputs differ in both orientation of optimal response planes and in phase dynamics, a fourth parameter is modified, the timing of the response. This is what we observed in the "variable phase" (or STC) neurons (Fagerson and Barmack, 1995b). The results of this type of convergence may be determined by 1) the rules that govern the convergence of two inputs with differing directional sensitivity orientations and 2) by rules that appear to govern convergence of inputs with differing phases. The resultant phase appears to be an average of the two input neuron phases. Thus, where only one neuron is responding the phase is determined by that neuron's phase. Where both neurons are responding, the phase is determined by the average of the two phases.

This type of convergence broadens the range of optimal response planes while retaining a maximal response. However, this maximal response during stimulation in the optimal response plane occurs somewhat later during tilt stimulation than a canal neuron would typically respond. Also, the response phase during stimulation in orientations surrounding the optimal orientation would vary from a maximal response

early in the stimulus cycle to late in the stimulus cycle depending on the degree of influence of the two inputs. Thus, a wide range of responses is possible through convergence of two inputs on a neuron providing a means to transform sensory inputs to meet the wide range of requirements imposed by muscle geometry and by the forces acting on the head.

CONCLUSION

The responses of neurons in the medial aspect of the caudal NRGc to static tilt and exponential "step" stimulation indicate that this nucleus must provide significant vestibular input for the adjustment of reflex responses to account for changes in the position of the head with respect to gravitational forces. This nucleus receives input from three distinct nuclei which may transmit vestibular information: GABAergic input from the contralateral MVN and the ipsilateral DVN and excitatory input from the fastigial nucleus.

Several anatomical findings of these experiments may provide some clues to the organization of the vestibular information in the medial NRGc. The distinct separation of the two fiber tracks and the associated cell groups may be a result of localized cell groups within which directionally selective inputs are organized. The locations of the floccular and uvula/nodular projecting neurons also suggest an organization of NRGc neurons by function. The relative roles of the cerebellar input from the fastigial nucleus and the inputs from the MVN and DVN in determining the response properties of these neurons are currently unknown.

Additional convergence of inputs beyond that in the vestibular nucleus occurs in the medial aspect of the NRGc as indicated by the higher proportion of STC neurons in our study. This convergence may provide the basis for the necessary transformations of vestibular information required for neck muscle control.

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