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RESPONSES OF ROSTRAL FASTIGIAL NUCLEUS NEURONS OF CONSCIOUS CATS TO ROTATIONS IN VERTICAL PLANES

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Abstract—The rostral fastigial nucleus (RFN) of the cerebellum is thought to play an important role in postural control, and recent studies in conscious nonhuman primates suggest that this region also participates in the sensory processing required to compute body motion in space. The goal of the present study was to examine the dynamic and spatial responses to sinusoidal rotations in vertical planes of RFN neurons in conscious cats, and determine if they are similar to responses reported for monkeys. Approximately half of the RFN neurons examined were classified as graviceptive, since their firing was synchronized with stimulus position and the gain of their responses was relatively unaffected by the frequency of the tilts. The large majority (80%) of graviceptive RFN neurons were activated by pitch rotations. Most of the remaining RFN units exhibited responses to vertical oscillations that encoded stimulus velocity, and approximately 50% of these velocity units had a response vector orientation aligned near the plane of a single vertical semicircular canal. Unlike in primates, few feline RFN neurons had responses to vertical rotations that suggested integration of graviceptive (otolith) and velocity (vertical semicircular canal) signals. These data indicate that the physiological role of the RFN may differ between primates and lower mammals. The RFN in rats and cats in known to be involved in adjusting blood pressure and breathing during postural alterations in the transverse (pitch) plane. The relatively simple responses of many RFN neurons in cats are appropriate for triggering such compensatory autonomic responses. © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.

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The fastigial nucleus of the cerebellum comprises two distinct regions, both of which include neurons that respond robustly to vestibular stimulation (Gardner and Fuchs, 1975; Büttner et al., 1991; Shaikh et al., 2005). The caudal fastigial nucleus contains neurons with eye movement–related activity, and coordinates oculomotor responses (Gardner and Fuchs, 1975; Büttner et al., 1991; Robinson and Fuchs, 2001; Brettler and Fuchs, 2002; Shaikh et al., 2005). In contrast, neurons in the rostral fastigial nucleus (RFN) lack firing related to eye movements, and are believed to participate in the control of gait and posture (Büttner et al., 1991; Thach et al., 1992; Siebold et al., 1997; Mori et al., 1998, 2004), presumably through the extensive projections of these cells to the lateral vestibular nucleus and the medial medullary reticular formation (Batton et al., 1977; Carleton and Carpenter, 1983). Furthermore, recent studies in nonhuman primates indicate that the RFN plays an important role in computing body motion in space and determining spatial orientation (Kleine et al., 2004; Shaikh et al., 2004, 2005). In addition, evidence from experiments in rats, cats, and goats suggests that the RFN participates in regulating breathing (Huang et al., 1977; Lutherer and Williams, 1986; Xu and Frazier, 1995, 1997, 2000, 2002; Martino et al., 2006a,b) and blood distribution in the body (Doba and Reis, 1972, 1974; Huang et al., 1977) during postural alterations.

The first experiments considering the responses of RFN neurons to vestibular stimulation were conducted using the decerebrate cat preparation (Ghelarducci, 1973; Ghelarducci et al., 1974; Erway et al., 1978; Favilla et al., 1980; Stanojevic et al., 1980; Stanojevic, 1981). About a quarter of the cells responded to static ear-down tilt (Ghelarducci, 1973), although the activity of two-thirds of the units was modulated by low-frequency sinusoidal roll rotations that presumably were more effective in activating otolith afferents and additionally provided a minor stimulus to the vertical semicircular canals (Stanojevic et al., 1980). In addition, 65% of RFN cells were activated by horizontal rotations (Favilla et al., 1980). However, since the variety of rotational stimuli used during these studies was limited (e.g. pitch rotations were not employed) and the animals were not conscious, it is difficult to draw firm conclusions regarding the processing of vestibular signals by feline RFN neurons from these data.

A more extensive analysis of the responses of anterior vermis Purkinje cells to vestibular stimulation has been conducted in decerebrate cats (Manzoni et al., 1995; Pompeiano et al., 1997); many Purkinje cells in the anterior vermis have projections to the RFN. These studies employed off-vertical axis rotations that selectively activate otolith organs (Manzoni et al., 1995) or constant-amplitude

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Abbreviations: CCW, counterclockwise; CV, coefficient of variation; CW, clockwise; EOG, electrooculogram; IED, ipsilateral ear down; NU, nose up; RFN, rostral fastigial nucleus; STC, spatiotemporal convergence.

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tilts whose direction moves around the animal at a constant speed ("wobble" stimuli), which stimulate both the otolith organs and vertical semicircular canals (Pompeiano et al., 1997). Anterior vermis Purkinje cells responded to a wide array of tilt directions, and a majority of the neurons were only activated by rotations in the clockwise (CW) or counterclockwise (CCW) direction or had unequal responses to the two directions of movement. These data suggest that the neurons receive vestibular inputs with different spatial and temporal properties, and thus can be classified as spatiotemporal convergence (STC) units. However, it is yet to be determined whether neurons in the feline RFN display analogous STC behavior.

RFN neuronal responses to vestibular stimulation have recently been characterized extensively in conscious nonhuman primates (Büttner et al., 1991, 1999, 2003; Siebold et al., 1997, 1999, 2001; Kleine et al., 1999, 2004; Zhou et al., 2001; Wilden et al., 2002; Shaikh et al., 2004, 2005). Many RFN neurons in this animal model were determined to receive otolith organ inputs, in accordance with their responses to vertical tilts (Siebold et al., 1997; Büttner et al., 1999) or linear translation (Zhou et al., 2001; Shaikh et al., 2004, 2005). Furthermore, based on the dynamics of RFN neuronal responses to vertical rotations (Siebold et al., 1997, 2001) or translations combined with yaw rotations (Shaikh et al., 2005), it was concluded that convergence of semicircular canal and otolith inputs onto the cells is common. Moreover, many RFN neurons exhibited STC behavior in response to vestibular stimulation (Kleine et al., 1999; Siebold et al., 1999; Zhou et al., 2001; Wilden et al., 2002). During vertical rotations, most units in the primate RFN had response vector orientations near the roll plane or the planes of the vertical semicircular canals; only 13% were best activated by oscillations in the transverse (pitch) plane (Siebold et al., 1997). In particular, few cells that responded to pitch rotations were characterized as receiving otolith inputs, based on having responses synchronized with stimulus position (Siebold et al., 1997). Studies using linear translation as a stimulus also showed that only a small fraction of RFN neurons in monkeys is best activated by fore–aft accelerations (Shaikh et al., 2005).

Nonhuman primates differ from other mammals in their typical postural orientation: monkeys usually remain semierect in a vertical stance, whereas other species such as cats are normally in a horizontal position. Thus, comparing vestibular processing by the RFN in nonhuman primates and felines is likely to provide insights about the physiological role of this nucleus. The goal of the present study was to determine the dynamic and spatial responses to rotations in vertical planes of RFN neurons in conscious cats, and to contrast these findings to those previously obtained in monkeys (e.g. Siebold et al., 1997).

EXPERIMENTAL PROCEDURES

All procedures on animals performed in this study were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee, and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were conducted on three purpose-bred adult female cats obtained from Liberty Research (Waverly, NY, USA). Animals were spayed prior to being included in this study to eliminate cyclic changes in hormonal levels. The number of animals employed in the study was reduced to the lowest required to provide valid results, and pain and distress were minimized.

Animals underwent an aseptic surgery that employed standard techniques and incorporated anesthetic and post-surgical procedures we have employed in many previous studies (e.g. Wilson et al., 2006; Arshian et al., 2007). A fixation plate was mounted on the skull so that the head could subsequently be immobilized during recordings. Silver/silver-chloride electrodes were implanted adjacent to each eye for monitoring the electrooculogram (EOG). A craniotomy with a diameter of 1 cm was performed at the midline of the posterior aspect of the skull, and a recording chamber (David Kopf Instruments, Tujunga, CA, USA) was lowered using a microdrive to stereotaxic coordinates that would permit access to the RFN, and attached to the skull adjacent to the craniotomy using Palacos® bone cement (Zimmer, Warsaw, IN, USA) and stainless steel screws. The chamber was tilted at an 8° angle relative to the frontal stereotaxic plane so that electrodes would course slightly rostrally as they were lowered.

Prior to recordings, the animals were trained over a period of 1–2 months to be restrained on a tilt table during sinusoidal rotations in vertical planes at frequencies of 0.02–2 Hz and maximal amplitudes ranging from 5° at high frequencies to 20° at low frequencies. The head was immobilized by inserting a screw into the nut fixed to the animal's skull; the head was pitched 15° down from the stereotaxic plane to bring the plane of the vertical canals close to vertical and minimize horizontal canal stimulation during vertical rotations. The torso was enclosed in a cylindrical tube, and straps placed around the animal's body ensured that its position on the table did not change during rotations.

All recordings were conducted in a dimly lit room; the visual field available to the animal was rotated with its body, such that no visual cues regarding body position in space were available. An X–Y positioner was attached to the recording chamber and used to maneuver an 8–10 MΩ epoxy-insulated tungsten microelectrode (Frederick Haer, Bowdoin, ME, USA), which was inserted through the dura via a 25-gauge guide tube, and lowered into the RFN using a David Kopf model 650 hydraulic microdrive. Neural activity was amplified by a factor of 10,000, filtered with a bandpass of 300–10,000 Hz, and led into a window discriminator for the delineation of spikes from single units. The output of the window discriminator was led into a 1401-plus data collection system (Cambridge Electronic Design, Cambridge, UK) and Macintosh G4 computer (Apple Computer, Cupertino, CA, USA) running Spike-2 software (Cambridge Electronic Design); the sampling rate was 10,000 Hz.

When a unit was encountered, its spontaneous firing was recorded along with the EOG, which was amplified by a factor of 1000 and sampled at 1000 Hz. After a unit was verified to lack activity correlated with voluntary eye movements, we recorded its responses to tilting the entire animal about the pitch and roll axes using a servo-controlled hydraulic tilt table (Neurokinetics, Pittsburgh, PA, USA), as in our previous studies (e.g. Jian et al., 2002). The plane of tilt that produced maximal modulation of a unit's firing rate (response vector orientation) was first determined with the use of the wobble stimulus, a constant-amplitude tilt whose direction moves around the animal at a consistent speed (Schor et al., 1984). The direction of the response vector orientation lies midway between the maximal response directions to CW and CCW wobble stimulation, because the phase differences between stimulus and response are reversed during the two directions of rotation (Schor et al., 1984). Thus, by consideration of both responses, these phase differences could be accounted for. Subsequently, the response vector orientation was confirmed by comparing the gain of responses to tilts in the roll and pitch planes. After a unit's response vector orientation was established, planar tilts at or near this orientation were used to study the dynamics of