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OTOLITH-CANAL CONVERGENCE IN VESTIBULAR NUCLEI NEURONS
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PROJECT ABSTRACT

During manned spaceflight, acute vestibular disturbances often occur, leading to physical duress and a loss of performance. Vestibular adaptation to the weightless environment follows within two to three days, yet the mechanisms responsible for the disturbance and subsequent adaptation are still unknown. In order to understand vestibular system function in space and normal earth conditions, the basic physiological mechanisms of vestibular information coding must be determined. Information processing regarding head movement and head position with respect to gravity takes place in the vestibular nuclei neurons that receive signals from the semicircular canals and otolith organs in the vestibular labyrinth. These neurons must synthesize the information into a coded output signal that provides for the head and eye movement reflexes, as well as the conscious perception of the body in three-dimensional space. The current investigation will, for the first time, determine how the vestibular nuclei neurons quantitatively synthesize afferent information from the different linear and angular acceleration receptors in the vestibular labyrinths into an integrated output signal. During the second year of funding, progress on the current project has been focused on the anatomical orientation of semicircular canals and the spatial orientation of the innervating afferent responses. This information is necessary in order to understand how vestibular nuclei neurons process the incoming afferent spatial signals, particularly with the convergent otolith afferent signals that are also spatially distributed. Since information from the vestibular nuclei is presented to different brain regions associated with differing reflexive and sensory functions, it is important to understand the computational mechanisms used by vestibular neurons to produce the appropriate output signal.
PROJECT DESCRIPTION

A. SCIENTIFIC OBJECTIVES

The primary objective of this project was to determine the nature of information processing used by the central nervous system to encode neural signals regarding head movements, eye movements, and position of the head with respect to gravity. It is known that a fast conducting neural pathway contained within the vestibular system is responsible for the control of head and eye movements. Information provided to the central vestibular system arises from peripheral receptors of the semicircular canals and otolith organs which is carried through the primary afferent fibers. In order to determine precisely how the central neurons process afferent information from the different receptor endorgans into an appropriate neural output signal, the spatial properties of the afferents and their termination patterns within the central nervous system must be known. The data obtained by in the current project answered both of these questions.

B. ACCOMPLISHMENTS

Vestibular afferent and nucleus neuron responses elicited by electrical stimulation: Before I could begin to study the synthesis of information from the different linear and angular acceleration receptors by the central vestibular neurons, it was necessary to determine if selective types of afferents could differentially be activated by their thresholds to electrical stimulation. It has been demonstrated that small electrical currents applied to the labyrinth can selectively either silence or excite afferent fibers that have irregular discharge rates, while afferents with more regular firing patterns are little affected (Goldberg et al., 1984). It is currently believed that these regular and irregular firing afferents converge upon vestibular nucleus neurons in unique patterns to control different types of muscle movements. For example, there is evidence to suggest in monkeys that regular afferents are more involved in the control of eye movements, while irregular afferents primarily are involved in the control of head and/or postural movements (Minor and Goldberg, 1991). As an added tool in the current investigation of synthesis of converging inputs to vestibular nucleus neurons, I used electrical stimulation of the labyrinth to examine the effectiveness of selectively stimulating the regular and irregular afferent fibers in pigeons.

Electrical stimulating electrodes were implanted into the perilymphatic space of the labyrinth, while extracellular electrophysiological recordings were made from the ipsilateral afferent fibers. Recordings were obtained from both semicircular canal and otolith organ afferent fibers. Both anodal and cathodal electrical DC currents were utilized, with current magnitudes ranging between -100 μA to +100 μA. In addition, for many neurons, sinusoidal rotational (semicircular canal afferents) or off-vertical axis rotational (otolith afferents) stimulation was applied with and without simultaneous electrical stimulation, in order to determine if the responsiveness of the fiber changed during the electrical stimulation. These experiments are continuing, however, to date 93 afferents have been studied using electrical stimulation, with effects observed in both semicircular canal and otolith afferent fibers. Similar to the results reported in monkeys (Goldberg et al., 1984), pigeon afferent fibers can be selectively excited or inhibited based upon their discharge regularity. All afferent fibers were inhibited by cathodal (positive) currents and were excited by anodal (negative) currents, as shown in Figure 1. Irregular firing afferent fibers had substantially lower thresholds to electrical currents applied to the labyrinth than did regular afferent fibers. In fact, irregular afferent fibers could be selectively silenced with low amplitude (5 - 25 μA) anodal currents (Figure 1).
Regular firing afferents, however, could never be silenced even when administering large amplitude (100 µA) currents. The afferent's response magnitude was correlated with the electrical stimulus magnitude, again with the irregular afferents having the greatest sensitivity to the galvanic polarizations, as shown in Figure 1. Further, for most fibers, the sensitivity of the afferent to electrical stimulation was linearly related to the discharge regularity. Irregular firing semicircular canal fibers could be ablated (silenced) with electrical stimulation, even during sinusoidal rotations at 20 deg/sec peak velocity. Irregular firing otolith afferent fibers could also be ablated during off-vertical axis rotations of 30 deg/sec constant velocity. However, with both otolith and semicircular canal regular firing afferents, only the discharge rate was slightly changed and little or no effect was observed in the fibers sensitivity to rotation.

Once the studies regarding the effects of galvanic stimulation upon afferent discharge rate were completed, I began to record responses from vestibular nuclei neurons during sinusoidal rotation and off-vertical axis rotation paradigms. During the rotation sequences, nuclei neuron responses were obtained before, during, and after simultaneous galvanic polarization of the ipsilateral labyrinth, as shown in Figure 2. The current amplitude for all cells was varied between 10 to 100 µA. If signals from irregular and regular firing afferents converge upon single vestibular nuclei neurons, then the effects of silencing the irregular input can be observed. Rotation stimulation was varied across frequencies between 0.02 and 5 Hz, with the amplitude remaining constant at 20 deg/sec. Differences between the response dynamics for the before and during galvanic polarization conditions were noted for each cell. First, each recorded neuron was identified as to type of semicircular canal input by manipulating the animal with a series of manual yaw, pitch, and roll movements in the three major semicircular canal planes. Thus, each neuron could be classified as to
a Type I or II, with input from either the horizontal, left anterior - right posterior, or right anterior - left posterior canals. In addition, each neuron was identified as to whether it received monosynaptic or polysynaptic input from the ipsilateral labyrinth. As shown in Figure 3, the response dynamics for some neurons were affected by the elimination of the irregular afferent inputs, while other cells were not. In Figure 3, the response gain and phase values are plotted across stimulus frequency for three horizontal canal related vestibular nuclei neurons. The solid symbols and lines indicate the responses to sinusoidal rotational stimulation alone. Open symbols and dotted lines indicate responses to rotational and simultaneous galvanic polarization stimulation. For some neurons, galvanic polarization reduced the gain during rotational stimulation, particularly at the lower frequencies of stimulation. The phase of these affected cells sometimes changed and sometimes did not. Similar results have also been obtained for vertical canal related neurons. To date, only 11 vestibular neurons with complete protocols have been obtained during rotational and simultaneous electrical stimulation. These studies are still on-going and will be completed in the new NASA grant NAGW-4507. However the results so far suggest that many vestibular nuclei neurons receive convergent inputs from irregular and regular firing afferents. Further, the convergence in differing dynamic information from the afferents appears to be important in determining the response sensitivity of these cells to head movement stimulation.

Figure 2. Neural responses from a horizontal canal related vestibular nucleus neuron to a 1 Hz vertical axis rotation, before, during, and after a combined bilateral galvanic stimulation of both labyrinths. Top three histograms: responses for different levels of galvanic current amplitude. Bottom trace: rotation velocity (peak velocity of 20 deg/sec). Dark bar: galvanic stimulation period.
Spatial orientation of semicircular canals and afferent fibers: Once I began to study the effects of ablation of irregular afferent inputs upon vestibular nuclei neuron responses, it became clear that the convergence of irregular and regular afferent inputs may determine the spatial orientation of these cells. The spatial orientation of each neuron can be assessed by determining the maximum and minimum sensitivity responses to rotational stimulation about an earth vertical axis while differing the animal's head orientation. The head orientation that produces the maximum gain of the neuron can be considered to correspond to the spatial orientation vector of the cell, where the vector direction represents the axis about which the head must be rotated. One of the principal goals of the present project was to determine these vectors for the different vestibular neurons, particularly during rotational and linear acceleration stimuli. However, before this goal could be accomplished, it was first necessary to measure the anatomical orientation of the semicircular canals relative to the head in pigeons. Next, the rotation vectors for the different canal afferents were also determined in order to make comparisons with the derived vectors for vestibular nuclei neurons.

The anatomical planar orientations of the semicircular canals in five pigeons were measured bilaterally. The labyrinths of both ears were exposed and the bony surfaces the semicircular canals debrided of vessels and connective tissue. The heads were mounted in a stereotaxic device such that the horizontal semicircular canals were coplanar to the horizontal stereotaxic plane. Using a small needle attached to a micromanipulator, different points along the circumference of the exposed bony canal for each of the semicircular canals were measured in three dimensions. Between 17 and 65 points were obtained for each canal, with the number of points being dependent upon the size of canal. The measured points were then entered into a three-dimensional plotting program on a microcomputer, where a planar equation could be fit through the points for each canal using a least-squares algorithm, as shown in Figure 4. The best-fit planes for each canal bilaterally were then reconstructed in three-dimensional space and angles between the canal planes were calculated.

Figure 3  Response gain and phase values of horizontal canal related vestibular nuclei neurons to rotation before and during bilateral galvanic stimulation. Solid lines: control response values obtained before galvanic stimulation. Dashed lines: response values obtained during galvanic stimulation (+25μA). m = monosynaptic input from ipsilateral labyrinth; p = polysynaptic input; n = no response to orthodromic activation of ipsilateral labyrinth.
Figure 4. Anatomical measurements of left semicircular canals. Circles, triangles, and diamonds represent coordinate values from the horizontal, anterior, and posterior canals, respectively. Calculated canal planes are plotted through each set of canal points with the best-fit parameter values indicated above the plot. All axes are in stereotaxic coordinates, with point (0,0,0) corresponding to ear bar zero in the center of the head.

As shown in Figure 5, the results indicated that the horizontal canals were approximately orthogonal to the vertical canals and complementary vertical canals on opposite sides of the head were nearly coplanar. Still, significant deviations on the order of 5 to 20 degrees between complementary canal pairs were noted. Similar results have been reported for cats (Blanks et al., 1977) and monkeys (Reisine et al., 1988).
Once the anatomical canal planes had been determined, spatial orientation vectors from semicircular canal afferent fibers were obtained. The responses of afferents to sinusoidal rotational stimulation (0.5 Hz, 20 deg/sec peak velocity) about an earth vertical axis were obtained with the animal’s head placed in different orientations relative to the rotation axis. A minimum of five head orientations were used, identical to those stated above for nuclei neuron experiments. As long as the afferent remained isolated during recording, other head orientations were also used. The gain and phase values observed during rotational stimulation were then plotted versus head orientation and fit with a cosine function. The orientation that produced the maximum response gain from each afferent was thus calculated and served to define the spatial orientation vector for the cell.

Maximum sensitivity directions were obtained for 36 afferents, including fibers from each of the three semicircular canals. The rotation vectors for the afferents were plotted in three-dimensional space, with reference coordinates expressed relative to the pigeon’s head, as shown in Figure 6. A right-hand coordinate system was used, such that the positive X, Y, and Z directions corresponded to the animal’s nose, left ear, and vertex, respectively. Each spatial orientation vector represents one afferent, where the direction of the vector corresponds to the direction of the axis about which the animal must be rotated to produce the maximum response from the cell. Each vector is shown as a unity gain response (all vectors have equal magnitudes) for clarity.

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**Figure 6.** Spatial orientation vectors for semicircular canal afferent fibers. X, Y, and Z directions correspond to beak, left ear, and vertex, respectively. All vectors are shown as unity gain. Circles, triangles, and squares represent horizontal, anterior and posterior canal afferent rotation vectors, respectively. Dotted lines indicate measured anatomical canal orientations.
The vectors were classified by their canal innervation with the horizontal, anterior, and posterior canal afferents being represented by the circles, triangles, and squares, respectively. In addition, the measured anatomical planes for each canal are indicated by the dotted line vectors. One should note the close correlation between the measured anatomical canal planes and the calculated spatial orientation vectors for the innervating afferent fibers. It is also interesting that the spatial orientation vectors of the afferents have some degree of variability, particularly when comparing vectors of similar canal innervation. Thus, not all of the horizontal canal afferent vectors are oriented in a tight spherical group, even though a cluster of directions is apparent. This study is now complete, with a manuscript published (Dickman, 1996a).

**Vestibular afferent projections to the brainstem:** One of the stated objectives in the initial project was to determine the location of the vestibular nuclei neurons within the vestibular complex, as well as their axonal targets, then correlate morphological characteristics of the neurons with their gravitational response properties. In my initial experiments regarding vestibular nuclei neuron responses, fast green dye spots were placed at the recording site location for histological verification. As I began to examine the locations of the recorded neurons, it became increasingly clear that we did not have an adequate description of the anatomical distribution of the vestibular nuclei in pigeons, neither the cell types, borders, or relative locations of the afferent terminations with the brainstem.

Figure 7. Horizontal canal afferent terminations in the brainstem. Representative sections from one pigeon are shown, with each section separated by 200 μm. Thin lines: HRP filled afferent fibers. Filled circles: termination zone of afferent s. Cbl, lateral cerebellar nucleus; D, descending vestibular nucleus; dC, dorsal cochlear nucleus; IO, inferior olive; L, lateral vestibular nucleus; M, medical vestibular nucleus; S, superior vestibular nucleus; Sg, Scarpa's ganglion; SO, superior olive; vC, ventral cochlear nucleus; VIII, vestibulo-cochlear nerve; IX, glossopharyngeal nerve; X, vagus nucleus; Xn, vagus nerve; XII hypoglossal nucleus.
Thus, I initialized a neural tracing study using horseradish peroxidase (HRP), biocytin, and cholera toxin B conjugate HRP compounds in order to identify the afferent fiber termination patterns and extent of the vestibular nuclei in pigeons. The isolated branch of the VIIIth nerve that innervated a specific receptor neuroepithelium in the vestibular labyrinth was exposed, cut, and bathed in either tracer crystals or injected with tracer/saline (30%) solution. Separate animals were used for tracing the afferents innervating the anterior, posterior, and horizontal semicircular canals, as well as the utricle, saccule, and lagena otolith organs. At least three birds were used for each receptor organ, as well as two control birds where HRP crystals were placed within the endolymphatic membrane without transection of any nerve branch. The animals were allowed to survive for 48 to 96 hours following tracer implantation, then perfused with warm heparin/saline followed by cold aldehyde fixatives. The brains of each animal were stereotaxically marked, then removed and sectioned (50-100 µm) using a freezing microtome. Tissue sections were reacted using standard histochemical procedures for HRP and biocytin processing. Camera lucida drawings were made of the reacted tissue for each animal. As shown in Figure 6 for one animal where the horizontal canal nerve was traced with HRP, afferent fibers coursed into the ipsilateral brainstem and differentially terminated in a number of brainstem regions. For each receptor organ, the innervating afferent's cell bodies lie clustered within Scarpa's ganglion, with central projecting processes remaining bundled together until distributing to different brain regions. When viewing relative afferent termination patterns from the different canal and otolith receptor nerve branches, an
overlapping but distinguishable pattern appears to emerge, as shown in Figure 7. The distribution of afferent terminations in the brainstem of pigeons is similar to that reported for other vertebrate species. Generally, the semicircular canal afferents terminated in the more rostral and medial portions of the vestibular nuclei complex, with fibers from all three canals terminating in the superior, medial, lateral, and descending vestibular nuclei. Very few afferents from the semicircular canals projecting to the cerebellar nuclei were observed. The otolith organs, conversely, largely terminated in the more caudal and lateral portions of the vestibular nuclei. Utricular afferents, but not fibers from the lagena, were noted to be much more numerous than canal afferents in the cerebellar nuclei. Afferent terminations from both the semicircular canal and otolith organs were also observed in the reticular formation and prepositus hypoglossal nucleus. In addition to determining the afferent distribution, this study served to define the anatomical regions and borders of the vestibular nuclei in pigeons. The organization of the afferent input and the regional cytoarchitecture of the vestibular nuclei is important to understand, in order to make inferences regarding the locations of vestibular neurons. The information obtained in these initial experiments has appeared in short paper form (Dickman, 1996b), and is currently being completed for a larger publication as a full paper.

Publications resulting from project.

Articles

Abstracts
F. References


In order to determine how vestibular nuclei neurons process convergent information from semicircular canal and otolith afferent fibers regarding head movements, the spatial orientation vectors of the afferent fibers must be known. The present study examined the responses of semicircular canal afferent fibers to sinusoidal oscillations (0.5 Hz, 20 deg/sec) about an earth vertical axis, with the animal being oriented in a number of different head planes; including horizontal, nose up pitch, nose down pitch, left each down, and left anterior - right posterior nose down. The response gain and phase values obtained for each orientation were determined, with the maximum and minimum response vectors then being calculated for each fiber. In addition, the major anatomical plane for each of the semicircular canals, bilaterally, were measured in two animals for comparison. Although the response vectors are still being analyzed, preliminary evidence suggests that the horizontal and posterior semicircular canal afferents provide responses that are spatially oriented close to their respective membranous duct planes. However, anterior canal afferents appear to be aligned 15 - 35 degrees away from the major anterior canal plane. (Supported by NASA grant #NAG2-766).

CHANGES IN cFOS STAINING WITHIN THE RAT SUPRACHIASMATIC NUCLEUS FOLLOWING 2G EXPOSURE. C.A. Fuller, D.M. Murakami, T.M. Hanes-Higgins, and J-H Tang. Section of Neuroendocrinology, Physiology and Behavior, University of California, Davis, California 95616. USA.

This study examined the changes in rat body temperature and activity circadian rhythms caused by exposure to a hypergravity field. In addition, the gene activation marker, c-Fos, was used to reveal potential protein synthesis changes in the suprachiasmatic nucleus (SCN). Biotelemetry units were used to record body temperature and activity. Rats exposed to 2G exhibited a significant reduction in mean body temperature and activity, and a significantly depressed circadian amplitude. A second experiment examined changes in protein synthesis in a group of rats exposed to one hour of 2G, a centrifugal 1G control group, and a noncentrifugal 1G control group. All rats were sacrificed and stained for c-Fos. Hypothalamic sections revealed significant c-Fos staining differences between control and experimental rats in the SCN. The 1G control group of rats also exhibited a large number of intensely reactive neurons within the SCN. However, the SCN of the 2G experimental rats exhibited a significant reduction in c-Fos reactive neurons, with only a few lightly reactive neurons. These results suggest that 2G exposure has a direct effect on immediate early gene expression within the SCN neurons. These changes in protein synthesis function within the SCN may be responsible for the reduced circadian amplitude of body temperature and activity.

BEHAVIORAL REACTIONS OF TERRITORIAL VESPELS TO MICROGRAVITY. M. Peretz and R. Wassersug. Dalhousie University, Halifax, NS, B3H 4M7, Canada.

Vertebrates exposed to abrupt decreases in gravity respond in various ways. However, these responses appear to correlate with the animals' natural history. As part of an ongoing comparative investigation of vertebrate reactions to microgravity (μG), two lizards, the terrestrial Dipsas azurea (Crotaphyinae) and the arboreal Anolis carolinensis, were video taped aboard an aircraft in parabolic flight. We hypothesized that the two lizards would react differently based on their divergent ecologies.

In μG, the terrestrial lizard exhibited long axis rolling (previously seen in non-arboreal frogs and salamanders) in conjunction with tail flipping. This behavior is similar to the common vertebrate righting reflex performed when inverted in normal gravity. In contrast, the arboreal lizard did not torque its body in this manner. Instead, it rotated randomly while clawing the ventral substrate. After several parabolas, the Anolis succeeded in maintaining its hold on the side of the container in μG. Both species responded as expected, with the protective behaviors used when they lose their footing in 1G.

This is the first report on the behavior of lizards in microgravity.

RENAL CALBINDIN-D28K IN RATS EXPOSED TO MICROGRAVITY. W. Berry, Department of Physiology and Pharmacology, University of Georgia, Athens, GA 30602.

Calbindin-D28K is a 28 kilodalton calcium-binding protein found in avian intestine and in avian and mammalian brain, cerebellum, kidney, bone, vestibular hair cells, retina, and other tissues. This protein is thought to facilitate calcium transport in intestine, kidney, and bone, and apparently acts as a protective calcium buffer in cells of other tissues. Calcium flux, vitamin D and other hormones, tissue receptor levels, and cell turnover rates interact to determine calbindin levels. Therefore, microgravity-induced alterations in calcium and calcium homeostatic hormones may cause changes in tissue calbindin levels during space flight.

To examine the possibility that space flight could affect calbindin-D28K levels, kidney tissue was obtained from rats flown for 7 days on STS-54 (PAY2 experiment rats) and from control rats maintained on the ground under similar conditions. These tissues are being analyzed for calbindin-D28K content and 1,25 dihydroxyvitamin D receptor content including relative occupancy of receptors by endogenous dihydroxyvitamin D.

Specimens for this study were provided by the NASA Life Sciences Division Secondary Payloads Program.
Galley Proof

Association for Research in Otolaryngology

177 Semicircular canal and otochal afferent projections to the vestibular nuclei in pigeons

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As an initial step in determining the functional organization of the vestibular nuclei in pigeons, possible regional differences in afferent input to the nuclear complex were discerned using neural tracing techniques. For each animal, only one vestibular receptor organ in the left labyrinth was surgically exposed, with random selection of either a semicircular canal (SC) ampulla or otochial (OT) macula. For some animals, several cuts into the receptor neuroepithelium were made, followed by application of horseradish peroxidase (HRP) crystals and/or cholera-toxin conjugate horseradish peroxidase (CT-HRP) to the receptor area. For other animals, the VIII nerve branch supplying the specific receptor organ was exposed, cut, and HRP and/or CT-HRP was applied to the severed nerve stump. Following either a 24 or 48 hour survival time, the animal was euthanized and perfused with aldehyde fixatives. Frozen 50 μm sections were cut and reacted using a modified molybdate-teramethyl benzidine procedure.

To date, afferent projections from the SCs have been traced in six animals (two pigeons for each case) and from the utricle in one animal. For each receptor organ, the afferent cell bodies lie clustered in Scarpas ganglion, with central projecting processes remaining bundled together, even following bifurcation, until reaching the synaptic target. Generally, anterior SC afferents project heavily to the superior (SVN) and lateral (LVN) vestibular nuclei, with some projection to the medial vestibular nucleus (MVN) and cerebellum. Horizontal SC afferents project heavily to the SVN and LVN, with some projection to the SVN, the cerebellum, the vestibular formations, and the descending vestibular nuclei (DVN). Posterior SC afferents project heavily to the LVN, SVN, moderately to the DVN, and lightly to the cerebellum. Otochial afferent projections are still being investigated. The results indicate that regional differences in the distribution of the afferent inputs to the vestibular nuclei do exist, similar to those reported for most other species.

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The phase of FRNs leads to the phase of the FRN modulation. During sinusoidal rotation, the phase of FRN leads that of medial vestibular nucleus neurons not receiving floccular inhibition (nonFRNs). If the FRN phase lead is produced by signals from the flocculus, then the P-cells should functionally lead the FRNs. In the present study we recorded from floccular VA P-cells in awake rabbits that were sinusoidally rotated about the VA in the light and the dark at 0.05 - 0.8 Hz with different amplitudes. The phase of the simple spike (SS) modulation was calculated by determining the eye position sensitivity (k) and eye velocity sensitivity (r) using multivariate linear regression. The phase of the SS modulation was compared to the phase of the FRN modulation. The SS activity of nearly all VA P-cells increased with contralateral head rotation. During rotation in the light, the SS modulation showed a phase lead in reference to contralateral head position that increased with increasing frequency (median 56.9° at 0.05 Hz, 78.6° at 0.8 Hz). The SS modulation led the FRN modulation significantly at all frequencies. The difference of medians was greatest (19.2°) at 0.05 Hz and progressively decreased with increasing frequency (all p's < 0.005, Wilcoxon rank-sum test). During rotation in the dark, the SS modulation had a greater phase lead than in the light (median 110.4° at 0.05 Hz, 86.6° at 0.8 Hz). The phase of the SS modulation in the dark led that of the FRNs significantly at all frequencies (difference of medians varied from 24.2° at 0.05 Hz to 9.1° at 0.8 Hz, all p's < 0.005). The data are consistent with the hypothesis that the phase-leading characteristics of FRN modulation could come about by summation of VA P-cell activity with that of cells whose phase would otherwise be identical to that of nonFRNs. The floccular SS output appears to increase the phase lead of the net pre-oculomotor signal, which is in part composed of the FRN and nonFRN signals.

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Afferent Projections of Individual Vestibular Organs in Pigeons: J. David Dickman and Qian Fang, University of Mississippi Medical Center, Jackson, MS.

A central issue in determining the functional organization of the vestibular system in birds is to understand the anatomical substrate serving various types of movement. Differences in afferent input to the vestibular nuclei and other regions of the brainstem and cerebellum were delineated using neural tracer techniques for each vestibular end organ in pigeons. For each animal, a single branch of the vestibulocochlear nerve that innervated a specific receptor neuroepithelium was surgically isolated, cut, then traced by application of a horseradish peroxidase/cholera-toxin conjugate mixture (HRP) or biocytin (BT) crystals. Following a 24 - 48 hour survival, the animals were euthanized and perfused with aldehyde fixatives. Sections were cut in either the transverse or parasagittal planes, reacted using either a modified molybdate-tetramethyl benzidine or diamine benzidine procedure, and counterstained with neutral red.

Reliable data was obtained from 21 animals. Generally, vertical canal afferents projected heavily to medial regions of the superior (SVN) and descending (DVN) vestibular nuclei, and the A group, with lesser projections to the medial (MVN) and lateral (LVN) vestibular nuclei. Horizontal canal afferents projected primarily to the central regions of the SVN, LVN, and DVN, with many fibers terminating medially in the MVN. Utricular and sacular afferents generally terminated in the lateral regions of all vestibular nuclei and the B group. In addition, utricular and sacular afferents had separate projections that terminally overlapped with the horizontal and vertical canal terminal zones, respectively. All vestibular organs projected to the paraflocculus, deep nuclei, nodulus and uvula. Lagenar afferents projected to both cochlear and vestibular nuclei. Supported by funds from NIH/NIDCD grant #DC01092, NASA grant #NAG2-786, and the Whitaker Foundation.

As part of an effort to understand information channels in the vestibular nerve we are examining the branching patterns and synapses of individual nerve fibers made by the central axons of vestibular primary afferents. We labeled axons by extracellular injections of HRP into the posterior semicircular canal nerve (PAN), retrogradely labeled other axons, and quantified their diameters and branching patterns using a FastWeb morphometry system. In some cases we partially resected selected branches from the PAN axons to allow us to vary the length of central axon segments. We studied the effects of these axons on the central axons of vestibular primary afferents by labeling axons with HRP, inserting glass micropipettes into the vestibular nerve to record single unit activity of the vestibular nerve, and finally, labeling axons by retrograde transport of HRP. In the present study, we examined the branching patterns of the central axons of vestibular primary afferents and their effects on the central axons of vestibular primary afferents.
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