Vestibular Compensation: A spinovestibular mediated process

by

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Changes in posture are detected by the central nervous system through a number of sensory afferents. The vestibular labyrinths are one such sensor that discern rotational and accelerative movements of the head. The vestibular nuclei, the primary processor of labyrinthine input, coordinates several system outputs to maintain stable balance, visual gaze, and autonomic control in response to changes in posture. Following destruction of bilateral labyrinths, organisms are unable to effectively interact with their environment. Over time, these animals adapt due to some currently undefined process. It is our hypothesis that the observed behavioral recovery is due to a process that occurs within the vestibular nuclei. The nuclei regain their functional ability to sense changes in posture through substitution of sensory inputs from remaining non-labyrinthine afferents. Ascending spinovestibular afferents are ideal sources of plasticity, as they are ideally situated to convey this postural information.

Recordings were made from the vestibular nuclei of decerebrate cats that had undergone a combined bilateral labyrinthectomy and vestibular neurectomy 49–103 days previously and allowed to recover. Responses of neurons were recorded to tilts in multiple vertical planes at frequencies ranging from 0.05 to 1 Hz and amplitudes up to 15°. The firing of 27% of the neurons was modulated by tilt. These findings show that activation of vestibular nucleus neurons during vertical rotations is not exclusively the result of labyrinthine inputs, and suggest

that limb and trunk inputs may play an important role in graviception and modulating vestibular-elicited reflexes.

In the second portion of this work, we examined the spinal contributions to the vestibular nuclei in both labyrinthectomized and normal animals. The large majority (72%) of vestibular nucleus neurons in labyrinth-intact animals whose firing was modulated by vertical rotations responded to electrical stimulation of limb and/or visceral nerves; the activity of even more vestibular nucleus neurons (93%) was affected by limb or visceral nerve stimulation in chronically labyrinthectomized preparations. These data suggest that nonlabyrinthine inputs elicited during movement will modulate the gain of responses elicited by the central vestibular system, and may provide for the recovery of spontaneous activity of vestibular nucleus neurons following peripheral vestibular lesions.

Acknowledgements

It is easy to say that I am not the person that I thought I would be five years ago. It is most certain that the path that I have followed is considerably distant from the path that I charted so many years ago. From my undergraduate education to today, my interest in science and research have led me to learn a number of things about life. The accompanying dissertation is the tiniest fraction of them. Needless to say, these years in graduate school have been a learning experience like no other. It has been a test of will, determination, and commitment. I sought to gain knowledge of the truths of the world and of Science, instead I learned that Truth is slippery and elusive. Whether it was a product of a coming of age or that of environment, these past few years have taught me that communication, respect, and an overall appreciation of community are important factors in any individual's success. Because of this windfall, it is important that I mention a few of those people who have helped me along my path.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 Introduction

1.1 Clinical Consequences of Vestibular Disease

Approximately 20% percent of the people in the United States are currently afflicted with some type of vestibular disorder (Awan *et al.*, 2001; da Costa *et al.*, 2002; Mancini *et al.*, 2002). Common otological disorders of the inner ear include Meniere's disease, vestibular neuritis, benign paroxysmal positional vertigo (BPPV), and bilateral vestibular paresis. Typically, vestibular pathology occurs during the 4th and 5th decades of life causing individuals to become bed-ridden, resulting in significant loss of work hours and productivity (da Costa *et al.*, 2002). In elderly individuals reductions in balance can cause falls, lead to injuries, and compromise their quality of life, resulting in greater rates of morbidity and mortality with the associated costs to the healthcare infrastructure. Fifty percent of all the falls suffered by the elderly are reported to be the result of some type of vestibular pathology (Patel, 2000; Ishiyama *et al.*, 2001; Ballester *et al.*, 2002; Salles *et al.*, 2003; Pothula *et al.*, 2004). Despite the severity of symptoms, sudden onset, and the potential economic losses, research and public interest in vestibular dysfunction remains relatively low, perhaps because the majority of spontaneous vestibular pathologies subside over time.

It is generally accepted that the vestibular nuclei are a region adept at compensating for changes in incoming afferent signals (Precht, 1974; Gauthier & Robinson, 1975; Robinson, 1976; Dieringer & Precht, 1979b; Miles & Lisberger, 1981; Xerri *et al.*, 1983; Igarashi, 1984; Pompeiano *et al.*, 1984; Lacour *et al.*, 1985; Precht & Dieringer, 1985; Xerri *et al.*, 1985). Within hours to days the system can adapt to gross changes of incoming sensory signals. Compensation following unilateral or bilateral deafferentation of the primary sensory organs, the

vestibular labyrinths, has been well documented. Following unilateral labyrinthectomy, the contralesional vestibular nuclei return the entire system to near-normal function. However in the bilaterally labyrinthectomized animal, the source or mechanism of compensation remains unclear. The prevailing theory remains that non-labyrinthine sensory signals aid in the recovery of maintaining stable visual gaze and balance following bilateral labyrinthine destruction. It is our hypothesis that spinovestibular afferents provide the sensory input responsible for recovery of function following bilateral labyrinthine destruction. Alteration in spinovestibular signaling is an attractive potential mechanism-of-recovery following bilateral deafferentation of the vestibular labyrinths.

The following introduction will provide a short overview of the anatomy and basic function of the vestibular nuclei. The major structures of the vestibular system and their normal firing characteristics will be discussed to highlight the disturbances that occur following removal of this peripheral sensory organ, subsequently referred to as *labyrinthectomy*. Following the brief discussion of normal neuronal activity in the vestibular nuclei, a focused effort will be made to characterize the vestibular nuclei in the deafferented state.

In the bilaterally labyrinthectomized animal, recovery cannot occur through a rebalancing of signal from the intact labyrinth. Instead, recovery depends upon the rebalancing of remaining sensory drive. The spinal cord is a known source of non-labyrinthine input to the vestibular nuclei and is believed to be a participant in the recovery of the bilaterally deafferented organism (Castro & Smith, 1979; Clegg & Perachio, 1985; Newlands & Perachio, 1991a; Peterka & Benolken, 1995). Although the studies in this dissertation focus on the bilaterally labyrinthectomized model, much of the literature discussed in the following introduction will examine findings from experiments using unilaterally labyrinthectomized animals. This discussion is necessary because much of what is understood about vestibular compensation has

been gleaned from the unilateral labyrinthectomized animal. When it is of value in understanding the underlying principles of the system, distinctions and known differences between the two compensation models will be highlighted.

1.2 Anatomy and Physiology of the Vestibular System

Vestibular pathologies can affect any one of the system's three major components: the vestibular labyrinth, the eighth nerve, and the vestibular nuclei (see Figure 1). These three structures represent the sensor, the signal conduit, and signal processor, respectively, and perform the basic biological function of sensing an organism's position in space with respect to gravity. Similar to vision and hearing, gravitoinertial frame of reference is a requisite biological function for an animal's survival.

There are several major vestibular output pathways responsible for maintaining an animal's ability to interact with its environment. The vestibulocular system maintains stable retinal focus during rotational and translational movements of the head and body (Rubin *et al.*, 1978; Cazin *et al.*, 1980; Chubb *et al.*, 1984). The vestibulospinal system innervates extensor muscles of the limbs and is a major participant in an organism's righting reflexes during moments of imbalance. The vestibulo-autonomic system regulates internal homeostatic mechanisms during changes in body position with respect to gravity. This system has been demonstrated to maintain stable blood pressure during movements from the prone to vertical position (Doba & Reis, 1974; Jian *et al.*, 1999; Yates *et al.*, 1999a). In conjunction, these output systems receive inputs from the labyrinth to coordinate their respective motor tasks to produce dynamic systems that respond to changes in posture.

Anatomy of the Vestibular System

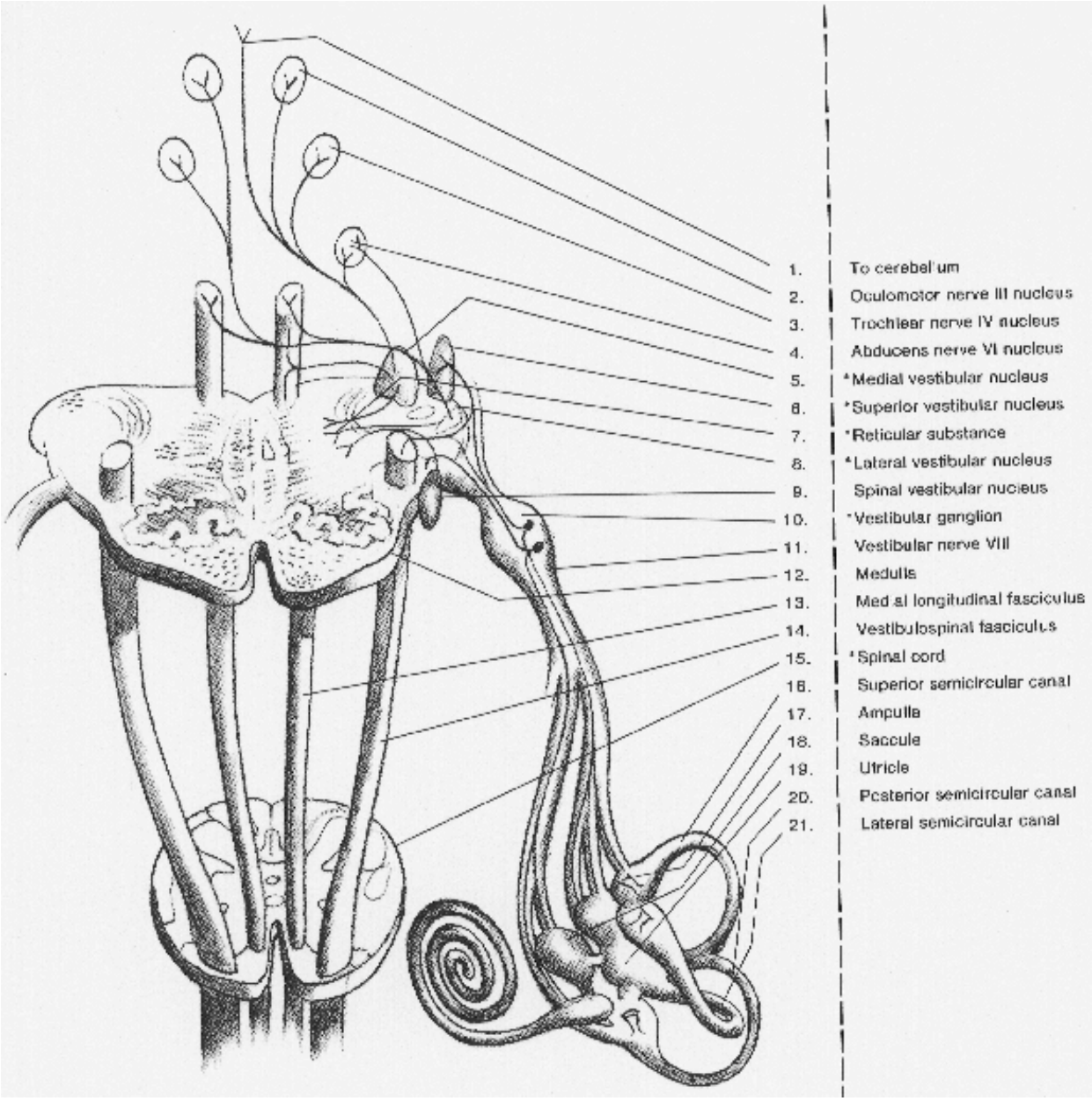

Figure 1.1. Drawing of the vestibular system and its major output pathways. Located on the bottom right (16-21) is the vestibular labyrinth. The eighth nerve (10,11) transmits signals trasnduced by the labyrinth to the vestibular nuclei (5,6,8,9) which are then used to coordinate one of the three systems major outputs: the vestibulo-ocular system (2-4), the vestibulospinal system (14), and the vestibulo-autonomic system (not shown).

http://soma.npa.uiuc.edu/labs/greenough/statements/rswain/tech/lect4.html

The vestibular labyrinth provides the major sensory input to the vestibular nuclei (Figure 2). The vestibular labyrinth is housed in the temporal bone of the skull and each is comprised of three orthogonal semicircular canals, and two otolith organs. Its semicircular canals and otoliths are oriented to identify movements of the head in three-dimensional space. The canals and the otoliths serve two separate functions, namely to encode three-dimensional head velocity and acceleration, respectively. These structures in tandem supply the vestibular nuclei with information necessary to identify position of the head during static and dynamic situations.

The eighth cranial, or vestibulocochlear, nerve provides the brainstem with excitatory glutamatergic input to the vestibular nuclei along with additional inputs from the cochlea to auditory brainstem centers (Evans, 1986; Klinke, 1986). Scarpa's ganglion, residing in the internal auditory meatus, contains the neurons that receive signals from the vestibular labyrinth and project axons to the vestibular nuclei. The vestibular nuclei are divided into 4 distinct subregions based upon neuronal morphology. The superior, inferior, lateral, and medial vestibular nuclei receive direct monosynaptic input from the labyrinths. However, inputs from the labyrinth are not homogenously distributed through all subregions of the nuclei. Some regions are devoid of labyrinthine input altogether whereas other regions receive either canal, otolith, or input from both sensory structures. This anatomic segregation correlates with the functional segregation defining the vestibulo-ocular-vestibulospinal, and vestibulo-autonomic systems.

The predominant labyrinthine input to the superior vestibular nucleus is from the semicircular canals (Shimazu & Precht, 1965; Markham, 1968; Gacek, 1969; Curthoys & Markham, 1971; Abend, 1977). The superior vestibular nucleus projects mainly to extraocular motor nuclei. Its afferent and efferent circuitry is well suited for controlling movements of the

Anatomy of the Vestibular Labyrinth

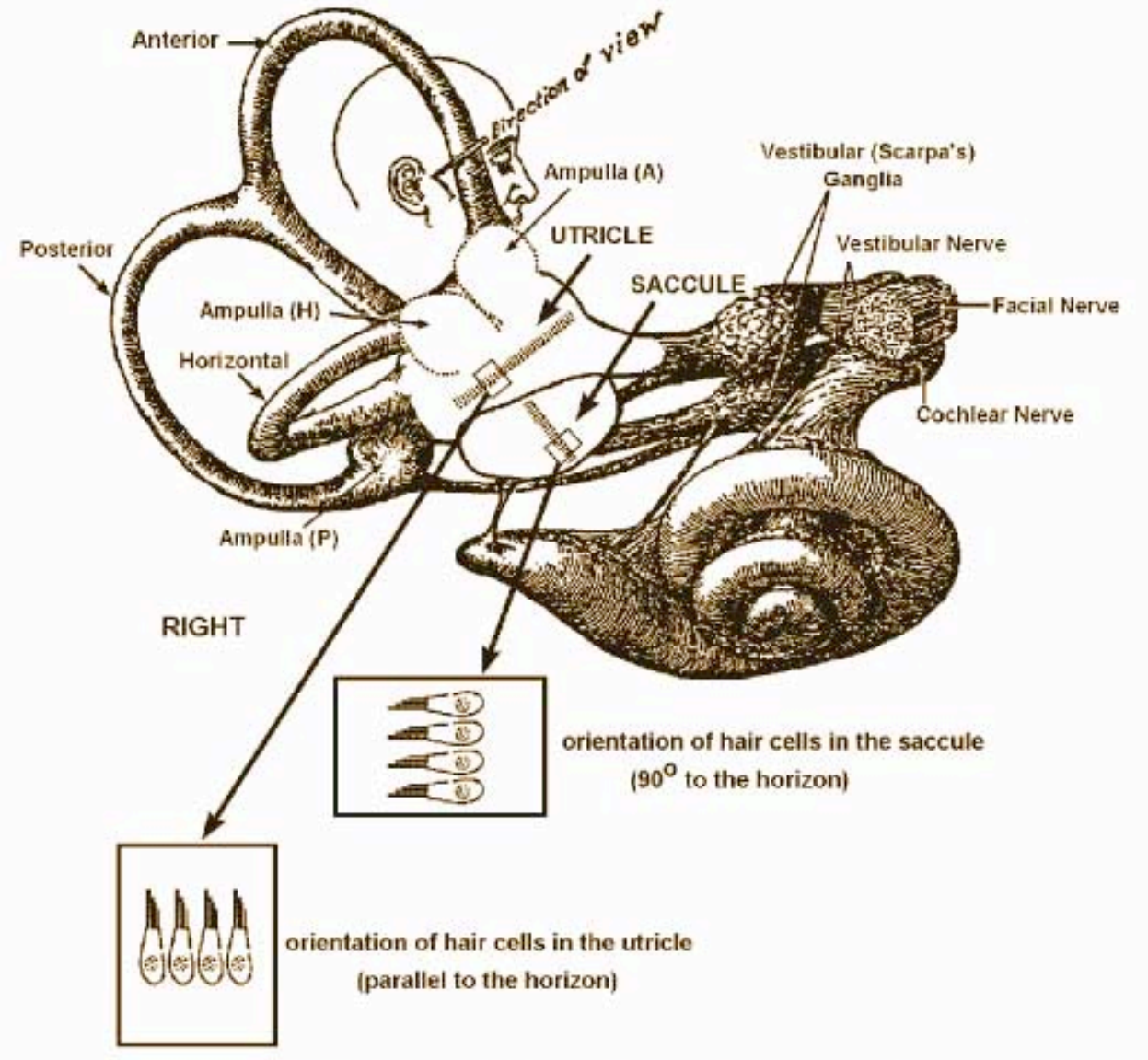

Figure 1.2: Drawing of right vestibular labyrinth and cochlea. Semicircular canals and otolith sensory structures are labeled. The orientation of the hair cells for the utricle and saccule are depicted in the insets. Hair cells for the semicircular canals are located in their respective ampullae. The vestibular nerve transmits signals that are transduced by the hair cells to the vestibular nuclei. (From Howard, 1986a)

http://www.hitl.washington.edu/projects/vestibular/dis1.html

eye during rotational movements of the head. The superior vestibular nucleus is also thought to receive signals from the accessory optic system (Montgomery *et al.*, 1981; McCrea & Baker, 1985; Giolli *et al.*, 1988; Barnes, 1993), further supporting the role of the superior vestibular nuclei in maintaining stable vision.

The medial vestibular nucleus receives a large amount of canal input from the labyrinths (Carpenter, 1967; Gacek, 1969). The medial vestibular nucleus also receives input from the utricle, the horizontal acceleration-sensing organ of the labyrinth. Rostrally, where the majority of canal input projects (Carpenter, 1967) the medial vestibular nucleus controls the vestibulo-ocular reflex during horizontal rotations of the head. Caudally the nuclei maintain reciprocal connections with the vestibulocerebellum and are the origins of the medial vestibulospinal tract. In addition to participating in control of balance, the caudal nuclei also have been shown to serve as a region that participates in the control of vestibulo-autonomic control (Yates, 1996a; Yates, 1996b; Porter & Balaban, 1997; Stocker *et al.*, 1997; Yates & Miller, 1998; Yates *et al.*, 1998b). Portions of the medial vestibular nucleus also have reciprocal connections with the parabrachial nucleus (Balaban & Porter, 1998; Balaban & Thayer, 2001). This vestibulo-parabrachial connection is thought to mediate a large proportion of the vestibulo-autonomic interactions that have been observed in both human and animals (Balaban *et al.*, 2002; Balaban, 2004).

Caudal portions of the lateral vestibular nucleus have similarly been shown to participate in the control of the vestibulo-autonomic system (Yates *et al.*, 1995; Yates *et al.*, 1998a; Yates *et al.*, 1999b) as well as the vestibulospinal system (Carleton & Carpenter, 1983). Histologically the neurons in the lateral vestibular nuclei contain large motoneurons that distinguish the nucleus from the other vestibular sub nuclei. Labyrinthine innervation of the lateral vestibular nucleus is the most complex of the structures. The nuclei receive input from the canals, the otoliths and also significant contributions from the cerebellum (Pompeiano, 1972; Carleton & Carpenter,

1983). The heterogeneous distribution of inputs to the lateral vestibular nucleus suggests of its role in several of the output pathways. Control of vision, balance, and autonomic function is distributed throughout this nucleus (Wilson, 1972; Wilson & Felpel, 1972).

The inferior vestibular nucleus also participates in several of the vestibular nuclei's output systems. Rostrally the region receives monosynaptic input from the labyrinth (Peterson, 1970) and aids in vestibulospinal as well as vestibulo-autonomic control. Ascending monosynaptic input from the spinal cord (Carleton & Carpenter, 1983; McKelvey-Briggs *et al.*, 1989; Matsushita *et al.*, 1995) terminate throughout the inferior vestibular nucleus. Dense afferent input from the neck's central cervical nucleus aids in identification of head-on-neck movements versus movements of the head via rotations at the waist.

1.2.1 Normal Function

Neurons in the vestibular nuclei alter their firing activity in response to changes in body position. Specifically a vestibular neuron will fire maximally in response to changes along a specific directional axis and minimally in response to movements along the orthogonal axis of direction (Shimazu & Precht, 1965; Wilson *et al.*, 1967; Schor *et al.*, 1984b; Schor *et al.*, 1985; Wilson *et al.*, 1990; Schor & Angelaki, 1992; Schor & Yates, 1995; Schor *et al.*, 1998b, a). The reason for this apparent directional sensitivity is determined by the primary input from afferents of the labyrinth. A subset of neurons do exist however that do not have these characteristic firing response properties due to a convergence of signals from varied otolith and canal input. These broadly tuned spatio-temporal convergent cells (STC) are thought to thought to provide convergent sensory information of otolith canal and fastigial information (Kleine 1999; Angelaki 1992; Siebold 2001).

The commissural inhibitory system is a major regulator of neuronal activity in the vestibular nuclei (Newlands *et al.*, 1989). Its apparent functional role is to reduce the intrinsic

activity of neurons in the contralateral vestibular nuclei during ipsilateral movements of the head that stimulate ipsilateral labyrinthine receptors. This system plays a vital role in vestibular modulation and is considered a primary participant in recovery following unilateral labyrinthectomy. Clearly this system has no obvious role in the recovery process following *bilateral* labyrinthectomy (Curthoys, 2000). Consequently, examining vestibular compensation using the bilateral labyrinthectomized model requires the analysis of other sensory afferents to the vestibular nuclei.

1.2.2 Normal Resting Rate

Neurons in the vestibular nuclei maintain an intrinsic firing rate that typically ranges between 30-40 spikes/sec depending upon the species examined (Ris & Godaux, 1998b, a; Him & Dutia, 2001; Ris *et al.*, 2001). Although the functional significance of the baseline activity is unknown, it is believed that it allows for a greater degree of accuracy in calculating position in space. Neurons in the vestibular nuclei exhibit tonic active firing, due to continuous input from the canals and otolith afferents and nonlabyrinthine inputs (Clegg & Perachio, 1985; Newlands & Perachio, 1991b; Ris & Godaux, 1998b). Tonic firing is believed to be characteristic of all vestibular neurons, not just neurons that receive input predominantly from the otoliths (which are constantly sending a gravitational signal except in the cases of microgravity). This resting rate increases and is inhibited by various movements of the head and limbs. Following labyrinthectomies the resting activity of vestibular neurons cease. The sudden loss of activity is considered to be the biological basis behind the resulting symptoms. Return in resting rate is considered to be tantamount to behavioral recovery (discussed in Chapter 1.5).

1.3 Nonlabyrinthine input to the Vestibular Nuclei

The vestibular nuclei receive signals from a number of other sources. The nuclei receive a heterogeneous distribution of input from cerebellar, visual, spinal, visceral and reticular pathways (see Figure 1.3). These inputs are thought to further aid in determination of gravitoinertial frame of reference in conjunction with the labyrinths.

1.3.1 Cerebellar input to the Vestibular Nuclei

The cerebellum constantly modulates neuronal spiking activity of the vestibular nuclei. It is also generally thought to play a significant role in mediating plasticity in the vestibular nuclei. The cerebellum can be divided into two main regions with respect to the vestibular nuclei, the vestibulocerebellum and the fastigial nucleus.

The vestibulocerebellum consists of the flocculus, nodulus, uvula, and ventral paraflocculus (Angaut & Brodal, 1967; Brodal & Brodal, 1985). It routinely regulates the system's various motor outputs including the vestibulo-ocular and vestibulospinal system. The vestibulocerebellum receives primary afferent input from the labyrinths through the mossy fiber pathway (Kaufman *et al.*, 1996). These cortical cerebellar structures project to various regions of the vestibular nuclei (Angaut & Brodal, 1967). These inhibitory Purkinje neurons modulate the vestibulospinal and vestibulo-ocular pathways by inhibiting activity in neurons in the medial, inferior and superior vestibular nuclei (Ito & Yoshida, 1966 ; Llinas *et al.*, 1967). The fastigial nucleus, although not generally considered part of the vestibulocerebellum, maintains

Model of Input and Output Systems of Vestibular Nuclei

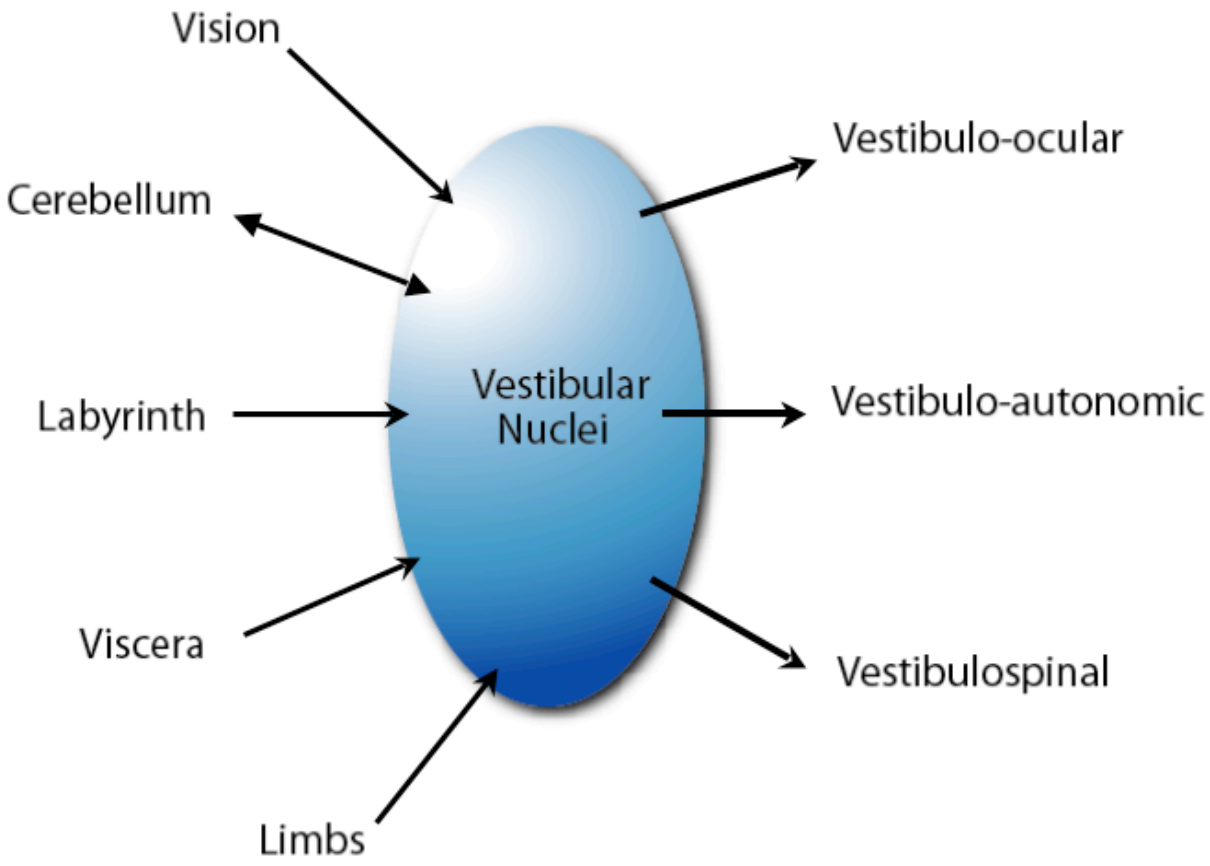

Figure 1.3 The vestibular nuclei receive signals from the vestibular labyrinth, visual system, viscera, and limbs. These converging signals are relayed to the cerebellum and the resulting outputs are used to coordinate visual gaze, autonomic regulation, and balance during changes in posture.

connections with portions of the vestibular nuclei including the lateral vestibular nucleus. Its predominant role is thought to participate in descending control of the vestibulospinal system through excitatory regulation of vestibular neuronal activity.

Inhibitory input from the vestibulocerebellum and excitatory modulation via the fastigial nucleus in tandem supply dual control of both vestibulospinal and vestibulo-ocular systems. The modulation of activity of both systems through these pathways is thought to be the mechanism by which the cerebellum modulates plasticity in the vestibular nuclei. Lesion studies have demonstrated reduced recovery of balance (Igarashi & Ishikawa, 1985; Kitahara *et al.*, 1997; Kitahara *et al.*, 1998a) and autonomic homeostasis (Holmes *et al.*, 2002) in animals that have undergone ablation of both the cerebellum and vestibular labyrinth. The inability for animals to recover is thought to be a result of the loss of inhibitory modulation from the cerebellum. Evidence also exists, however, that implicates excitatory modulation of vestibular plasticity through the fastigial nucleus (Xu *et al.*, 1998).

1.3.2 Visual Input to the Vestibular Nuclei

The visual system has long been known to affect vestibular function (Guedry, 1978; Waespe & Henn, 1978; Lacour *et al.*, 1979). Patients with vestibular damage have been shown to rely more heavily on vision to maintain balance (Lacour *et al.*, 1997a, b; Anand *et al.*, 2003). These signals are likely to be mediated through a number of pathways including the nucleus of the optic tract(Magnin *et al.*, 1989; Vargas *et al.*, 1996), medial terminal accessory nucleus (Giolli *et al.*, 1988), and prepositus hypoglossi (Magnin *et al.*, 1983; Sato *et al.*, 1996). In conjunction signals from the visual system interact with the vestibular nuclei to provide for a more accurate determination of sense in space. Recent evidence suggests that visual information is sufficient to coordinate autonomic responses during vertical changes in posture (Jian *et al.*, 1999) 24 hours after bilateral vestibular lesions. In the same data set animals required over 1

week to coordinate autonomic responses when both visual and vestibular cues were absent. Consequently visual input to the vestibular nuclei is a likely significant participant in the immediate and long-term recovery of the animal. Other sensory inputs require approximately a week before they can coordinate vestibulo-autonomic responses.

1.3.3 Spinal Input to the Vestibular Nuclei

Anatomical studies have identified direct monosynaptic connections of the cervical and lumbar spinal cord with caudal portions of the vestibular nuclei (McKelvey-Briggs *et al.*, 1989). However electrophysiological studies suggest that the *polysynaptic* afferent input to the vestibular nuclei is more prominent than the anatomical studies have proposed. Studies characterizing spinal input to the vestibular nuclei using HRP have demonstrated connections to only exist with the caudal portions of the vestibular nuclei (Neuhuber & Zenker, 1989). Functional studies, on the other hand, suggest that spinal input to the vestibular nuclei reaches rostral regions of the vestibular nuclei, including the superior vestibular nucleus. Innervation of the vestibular nuclei by spinal afferents will be discussed later in this chapter in regard to its potential to mediate recovery following bilateral labyrinthectomy.

1.3.4 Visceral Input to the Vestibular Nuclei

There are only a few lines of evidence that suggest that visceral afferent signals impinge on the vestibular nuclei. Although few lines of evidence exist that suggest that the vestibular nuclei receive signals from the viscera, there are several efferent pathways that the vestibular nuclei use to control autonomic circuitry. Electrical stimulation of the vestibular nerve in the cat elicits robust changes in activity of sympathetic nervous system efferents, including those innervating vascular smooth muscle (Yates, 1992; Kerman & Yates, 1998). Nose-up whole-body rotations of labyrinthectomized animals causes deficits in blood pressure (Woodring *et al.*, 1997; Jian *et al.*, 1999) and respiratory regulation(Cotter *et al.*, 2001b; Yates *et al.*, 2002). Centrifugation of

humans while minimizing vestibular input has also revealed potential visceral signals of motion (Mittelstaedt, 1995, 1996; Mittelstaedt & Mittelstaedt, 1996). Data gathered from these studies suggest that vagus nerve activation could alter vestibular neuronal activity. No studies to date have effectively characterized the functional significance or distribution of vagal information to the four subregions of the vestibular nuclei

1.3.5 Reticular Input to the Vestibular Nuclei

Although the reticular system cannot be easily classified into its own separate sensory input, it is a brain region that receives sensory signals from a number of 1st order sensory afferents, such as visual, auditory, somatosensory, and gustatory information. This incoming sensory network situates the reticular system as a potential region for integration of multiple sensory signals before the signals reach the vestibular nuclei. Consequently visual, visceral, and spinal input to this region may provide an additional novel set of information to the vestibular nuclei that the respective senses do not transmit alone (Zemlan *et al.*, 1984; Elisevich *et al.*, 1985; McCrea & Baker, 1985; Giolli *et al.*, 1988; Bankoul & Neuhuber, 1990; Grottel & Jakielskabukowska, 1993; Watanabe *et al.*, 1993; Stocker *et al.*, 1997; Lai *et al.*, 1999; Collet *et al.*, 2000; Sarkisian, 2000; Yakushin *et al.*, 2000; Feroah *et al.*, 2002; Yates *et al.*, 2002).

1.4 Physiology of the Vestibular Nuclei after Labyrinthine Loss

Vestibular compensation has been observed in the three major efferent systems, which suggest that plasticity is occurring in all four subnuclei (Wilson & Melvill Jones, 1979). As mentioned above, the vestibular nuclei contain a considerable commissural system, primarily involved in inhibiting activity in the contralateral nuclei. Modulation of contralateral vestibular nuclei activity is an important factor in understanding vestibular compensation following unilateral labyrinthectomy. The significance of this system is lost, however, following bilateral

destruction of the labyrinths. After bilateral labyrinthectomy neurons in the vestibular nuclei become silent. The return in spiking activity is thought to occur as a result of two general mechanisms. Acute alterations in the intrinsic excitability of vestibular neurons are thought to aid recovery in the first 48 hours. Chronic adaptation, however, is thought to occur through a number of processes that include reorganization of remaining sensory sources described previously in this chapter. These two mechanisms participate in the varied recovery observed in the vestibulo-ocular, vestibulospinal, and vestibulo-autonomic systems.

1.4.1 Resting Activity after Labyrinthectomy

Following labyrinthectomy, neurons in the vestibular nuclei become devoid of their predominant glutamatergic input. The resulting loss of excitatory drive produces an immediate cessation of resting activity of vestibular neurons. Consequently neurons in the vestibular nuclei do not modulate their activity in response to changes in body position. The termination of resting activity in the vestibular nuclei is believed to be the biological basis behind some of the deficits that are observed following unilateral and bilateral labyrinthectomies. After an ipsilateral labyrinthectomy, neurons in the ipsilesional vestibular nuclei first cease to fire -- but recover their previous discharge over a period of days (Ris *et al.*, 2002). Following *bilateral* destruction of the labyrinths (labyrinthectomy) or eighth nerve transections central to Scarpa's ganglion, neurons in *both* vestibular nuclei lose their background firing activity, due to loss of the primary excitatory glutamatergic input. The silence that occurs following labyrinthectomy occurs for a protracted period of time, depending upon species.

In guinea pigs the spontaneous nystagmus, caused by the imbalance in neuronal activity following unilateral labyrinthectomy, gradually disappears over the course of 1-2 days (Smith *et al.*, 1986; Smith & Curthoys, 1988; Smith & Darlington, 1988). Immediately following bilateral labyrinthectomy the ipsilesional and contralesional vestibular nuclei are silent but within a few

hours there is a progressive return of resting activity in these neurons. During this period the animal is unable to walk, maintain stable head control, or foveate on a target in its environment. Despite this, the animal does not exhibit signs of spontaneous nystagmus. The lack of spontaneous nystagmus in the bilaterally labyrinthectomized animal evinces the consequence of a symmetrical drop in resting activity in the vestibular nuclei. Ris et al (Ris *et al.*, 1997) have noted that spontaneous nystagmus may be abolished while there is still a substantial asymmetry of average resting activity between the two vestibular nuclei. The remaining asymmetry observed could potentially explain the behavioral imbalances that still remain.

The normal vestibular neuronal response is due to the combined effects of excitation from the ipsilateral periphery and disinhibition from the contralateral side. Following unilateral labyrinthectomy, rotations to the lesioned side produce no ipsilateral excitatory drive, only disinhibition from the contralateral side. The inadequate horizontal vestibulo-ocular reflex for ipsilesional rotations is due to this substantially reduced source of activation. In the bilaterally labyrinthectomized animal there is no resulting asymmetry in neuronal activity since both sides suffer from loss of their primary excitatory drive (Ris & Godaux, 1998a). As expected some deficits that occur from the imbalance in activity in unilaterally labyrinthectomized animals, do not occur in the animal with symmetrically lesioned labyrinths.

1.4.2 Visual Gaze after Labyrinthectomy

Vestibular compensation is a complex process that underlies the observed behavioral recovery. The degree of recovery for each vestibular efferent system varies across time and in magnitude. One sign, spontaneous nystagmus, shows very fast and almost complete recovery after unilateral labyrinthine loss. However the deficits observed in the vestibulo-ocular reflex (VOR) rarely recover following bilateral labyrinthectomy. During normal function movements of the eye are coordinated to move in the opposite direction of rotational movements of the head.

This coordinated motor reflex (VOR) is regulated by the vestibulo-ocular system in order to provide a stable retinal image during movement. Following labyrinthectomy, this system does not recover adequately to maintain stable visual tracking during movements of the head (Fetter & Zee, 1988; Vibert *et al.*, 1993; Angelaki & Hess, 1996; Angelaki *et al.*, 1996). Although there have been reports of recovery with various components of the system (Angelaki *et al.*, 1999), the overall recovery observed is relatively less than recovery observed in the vestibulospinal and vestibulo-autonomic pathways.

1.4.3 Balance after Labyrinthectomy

Ascending and descending vestibular pathways may involve different vestibular compensatory mechanisms due to the heterogeneous nature of their sensory inputs (Black *et al.*, 1989). Compensation of vestibulospinal function does not necessarily parallel the recovery of vestibulo-ocular function. In patients with unilateral vestibular damage, compensation plays an indispensable role in reorganizing and returning the vestibulospinal reflex (VSR) to normal function. Discordance between results of rotation tests (evaluating the vestibulo-ocular reflex) and posturographic test results (evaluating compensation of the VSR) are observed in 50% of the patients with unilateral vestibular damage (Norre *et al.*, 1987). In baboons the rebalancing of asymmetric electromyographic (EMG) responses following unilateral labyrinthectomy illustrates the temporal aspect of recovery. By three weeks post labyrinthectomy, unilaterally labyrinthectomized but not bilaterally labyrinthectomized animals had normal EMG responses following unexpected drops (Lacour & Xerri, 1980). This indicates that the remaining contralateral labyrinthine afferents are necessary for full compensation of postural stability. However even in the bilaterally labyrinthectomized animal, a degree of compensation does occur such that the animal is able to regain its ability to locomote about its environment (Macpherson & Inglis, 1993).

1.4.4 Autonomic Regulation after Labyrinthectomy

In animal models, the vestibular system appears to elicit changes in blood pressure through influences on the sympathetic subdivision of the autonomic nervous system, with minimal effects on parasympathetic outflow to the heart (Tang & Gernandt, 1969; Uchino *et al.*, 1970 ; Yates *et al.*, 1991; Yates *et al.*, 1992; Yates *et al.*, 1993; Yates, 1996b; Kerman & Yates, 1998). Natural vestibular stimulation, produced by head rotations in animals with extensive denervations (including transection of the upper cervical dorsal roots and cranial nerves IX and X) to remove nonlabyrinthine inputs elicited by this movement, also elicits change in sympathetic nervous system activity (Yates & Miller, 1994) and blood pressure (Woodring *et al.*, 1997). In particular, nose-up vestibular stimulation, but not roll or yaw rotations, produces cardiovascular responses in cats. This response pattern serves to stabilize blood pressure in a quadruped along its longitudinal axis and requires an increase in sympathetic nerve activity to maintain constant blood pressure during nose-up body rotations (as during vertical climbing). As would be expected from these findings, bilateral removal of vestibular inputs through transection of the VIIIth cranial nerves results in blood pressure lability during nose-up rotations in anesthetized (Doba & Reis, 1974) and awake animals (Jian *et al.*, 1999). However, these effects are only observed during the first week following labyrinthectomy, as the animal compensates over time.

1.5 Return of Resting Activity and Potential Recovery Mechanisms

There are two potential explanations for the return in resting activity. The first is that the vestibular nuclei regain an intrinsic (pacemaker-like) neuronal activity. Cessation of spiking activity may stimulate the intrinsic mechanisms of vestibular neurons to return the resting rate to prelesion levels. Spontaneous discharges in a neuron may result from the activity of an

endogenous pacemaker or from synaptic drive, or a combination of both. Evidence from several studies suggest that there exists some pacemaker-type activity in the vestibular nuclei (Ris & Godaux, 1998b; Him & Dutia, 2001; Darlington *et al.*, 2002). Perfusion of the vestibular nuclei with solutions containing low calcium and high magnesium concentrations that block transmitter release does not suppress all spontaneous activity in the vestibular nuclei. These intrinsic --non-calcium-dependent—mechanisms impart a degree of neuronal activity to the resting rate activity of the vestibular nuclei (Smith *et al.*, 1990; Carpenter & Hori, 1992; Dutia *et al.*, 1992; Johnston *et al.*, 1994). However the majority of neurons do become silent following ipsilateral labyrinthectomy (Ris *et al.*, 1995; Ris *et al.*, 1997). The second possible explanation to recovery of resting activity is reorganization of remaining sensory inputs. These neurons likely rely on synaptic contacts to produce the resting rate observed in normal and compensated animals. Additionally it has been observed that neurons in the vestibular nuclei of labyrinthectomized animals have a discharge rate that is highly variable, implicating synaptic drive as the potential mediator of background activity rather than a consistent pacemaker-like property driving the resting rate (Ris & Godaux, 1998c).

A reasonable explanation for the increase in synaptic drive strength is the sprouting of synaptic contacts to remaining vestibular neurons. While axonal sprouting does occur in the mammalian central nervous system, it is too slow to adequately explain the earlier signs of recovery. In the following studies we examined the responses of vestibular neurons of animals that had undergone labyrinthectomy at least 1 month prior. This time period is sufficient in length to allow for synaptic reorganization of remaining nonlabyrinthine afferents. Alterations in connections between spinal and vestibular neurons may be one of the mediating pathways.

1.6 Data Supporting a Spinovestibular Mediated Recovery

The vestibular nuclei contain a heterogeneous mixture of neurons that receive signals from various regions. These regions are thought to act as feedback signals for the systems aforementioned efferent pathways, as well as forms of efferent copies from other synergistic systems, such as the ocular system. Furthermore, cortical and diencephalic signals are thought to aid in higher cortical modulation of brainstem "reflex" pathways. The vestibular nuclei receive signals from other primary sensory sources. In addition to known visual afferents received through the accessory optic system, the vestibular nuclei have also been shown to receive monosynaptic input from the cervical, thoracic, and lumbar spine (Wold, 1979; Rubertone & Haines, 1982; Carleton & Carpenter, 1983; McKelvey-Briggs *et al.*, 1989; Matsushita *et al.*, 1995). Signals from the forelimbs as well as the hindlimbs reach the vestibular nuclei and modulate vestibular neurons. Electrical stimulation, limb manipulation, as well as standing maneuvers have been shown to exert influence on vestibular neuronal activity, cells exhibiting baseline-firing activity demonstrate altered responses following these various stimulation paradigms (Kasper *et al.*, 1986a). The observation that physiologic stimuli, as well as electrical stimulation, influences vestibular nuclei activity is strong evidence that these inputs participate in normal function in the awake behaving animal.

The importance of the spinovestibular afferents become clear when attempting to understand vestibular compensation in the bilaterally labyrinthectomized animal. In this paradigm, destruction of both peripheral endorgan sensors and the resulting loss of activity in the commissural inhibitory system results in a model system that inadequately explains the observed behavioral recovery when considering the activity of the vestibular nuclei. Although evidence suggests that the return in baseline activity alone is not sufficient to explain observed improvements in behavioral function (Ris *et al.*, 1997; Ris & Godaux, 1998b, a), it is accepted as

a requisite part of the recovery process. Once baseline activity has returned it is likely that remaining sensory signals -- such as the spinal system – return the vestibular nuclei to its once positional-sensitive state.

Various afferents from the limbs have been shown to alter activity of neurons in the vestibular nuclei in response to electrical and manual stimulation. Spinovestibular afferents have long been known to project to the vestibular nuclei. Early degeneration studies in the rat (Castro & Smith, 1979; Matsushita *et al.*, 1995), primate (Rubertone & Haines, 1982), cat (Pompeiano & Brodal, 1957; Brodal & Angaut, 1967), human (Loken & Brodal, 1970), and more recent conventional monosynaptic tracing studies (Wiksten, 1979; Wold, 1979; Carleton & Carpenter, 1983; McKelvey *et al.*, 1989) have shown direct projections from spinal afferents to the medial, lateral, and inferior vestibular nuclei. More recent electrophysiologic studies have demonstrated that these connections project to various regions of the subnuclei, including the more rostral superior vestibular nucleus (Rubin *et al.*, 1979b; Kasper *et al.*, 1986b). Electrical stimulation of peripheral limb nerves and physiologic stimuli of fore- and hindlimbs have been shown to alter the modulation of cells in all four subregions of the vestibular nuclei.

Regions of the reticular formation (e.g. gigantocellularis, and lateral reticular formation) that project to the vestibular nuclei have also been shown to receive spinal input from pelvic regions and pain pathways (Hoddevik *et al.*, 1975; Ito *et al.*, 1982; Carleton & Carpenter, 1983; Suzuki, 1985; Matsuyama *et al.*, 1988; Grottel & Jakielskabukowska, 1993). These connections may prove to aid in hip stability and in accurate pain localization, both of which are important for survival. The dorsal column nuclei, which receive spinal inputs from the limbs, can act as an important relay center for the ascending spinovestibular afferents (Jasmin & Courville, 1987; Neuhuber & Zenker, 1989; Arvidsson & Pfaller, 1990). The inferior olive receives and sends projections from the spinal cord (Spence & Saint-Cyr, 1988), the cerebellum (Fredette &

Mugnaini, 1991), and the vestibular nuclei (Balaban, 1988), thus making it an ideal participant in coordinating movements controlled by the cerebellum and vestibular nuclei. The information contained in these afferents and the signals from the vestibular labyrinths may be integrated to accurately determine an organism's position in space. Thus the vestibular nuclei could be viewed as a potential processing center not only of afferent volleys from the vestibular labyrinths, but also as an integrative center that serves the function of determining self-position using a number of sensory cues.

Spinovestibular and other sensory inputs are integrated in the vestibular nuclei. Few studies have systematically examined the effects of bilateral removal of vestibular inputs on behavior and vestibular function. While some responses typically attributed to the vestibular system, such as horizontal vestibulo-ocular and vestibulo-collic reflexes (Dutia & Hunter, 1985; Waespe & Wolfensberger, 1985), are abolished following bilateral vestibular neurectomy, postural stability is not profoundly impaired (Thomson *et al.*, 1991; Macpherson & Inglis, 1993). The latter data emphasize the large role that somatosensory inputs may play in signaling and controlling body position in space. Mittelstaedt provided further evidence that inputs from the trunk, including visceral graviceptors, can contribute to a sense of spatial orientation (Mittelstaedt, 1992, 1995, 1996). As might be expected from these findings, somatosensory and proprioceptive signals have been shown to acquire a larger role in controlling postural stability in humans following loss of vestibular inputs (Pyykko *et al.*, 1991; Lacour *et al.*, 1997a).

Inputs from the neck have long been known to affect the firing of vestibular nucleus neurons (Brink *et al.*, 1980; Boyle & Pompeiano, 1981; Kasper *et al.*, 1988b). Both anatomical and physiological studies have suggested that receptors in the limbs and trunk also provide inputs to cells in the vestibular nuclei (Wilson *et al.*, 1968; Wylie & Felpel, 1971; Rubin *et al.*, 1979a; McKelvey-Briggs *et al.*, 1989), although the effects of these inputs on the processing of

labyrinthine signals have not been investigated. The observation that the parabrachial nucleus, which integrates visceral inputs, has projections to the vestibular nuclei (Balaban, 2004) suggests that graviception derived from visceral receptors could be mediated through the vestibular system. Other visceral influences on the activity of vestibular nucleus neurons could be relayed through the reticular formation. However, the possibility that visceral inputs may affect signal processing in the vestibular nuclei has not been explored experimentally.

It seems likely that at least some integration occurs within the vestibular nuclei, and that modulation of vestibular nucleus neuronal activity during body tilt is the result of the convergence of a variety of inputs. Therefore, vestibular neuronal activity may regain the ability to respond to vertical body rotations following chronic bilateral removal of vestibular inputs, as these cells still receive considerable information regarding spatial orientation.

1.7 The Specific Aims of this Thesis

One of the aims of this work is to determine if the behavior termed as "vestibular compensation" is a product of a reorganization that occurs in the vestibular nuclei following bilateral removal of the peripheral sensory endorgans. We examined the activity of vestibular neurons in chronically labyrinthectomized animals to determine if cells in the nuclei respond to changes in body position, despite lacking both labyrinths. It is our hypothesis that in order for the observed compensation to truly be a vestibular process, a portion of cells in the vestibular nuclei should regain their sensitivity to changes in body position. Neurons in the vestibular nuclei of animals that have undergone bilateral ablation of their labyrinths and eighth nerves were studied during whole-body tilt stimulation. Neurons were first examined to determine if they modulated their activity in response to changes in table position. Responsive neurons were then characterized by classic gain and phase analysis to determine if the neurons that still

modulated their activity in response to tilt stimulation maintained response properties that were similar to neurons characterized previously in the literature. Data gathered from this analysis would shed light on whether the recovery was due to a selective reorganization or a process that involved neurons that received both canal and otolith input. In order to determine if the spinal cord or vagus nerves were the likely contributing inputs to the neurons that modulated to changes in body position, in a subset of animals the spinal cord and vagus nerves was acutely transected to determine the proportion of the recovery that was due to ascending spinal and visceral information.

In Chapter 3 we examined the role of the spinal cord in the return of function. To determine if spinal inputs to the vestibular nuclei mediate recovery, we examined the responses of vestibular neurons to limb stimulation in both the intact and chronically labyrinthectomized animal. Based on the commonly held belief that changes in behavioral function are a result of changes in neuronal weighting at the synapse, we examined the sensitivity of neurons in the vestibular nuclei to limb stimulation in normal animals and compared them to the population of neurons of the vestibular nuclei in chronically labyrinthectomized animals. Potential differences in the response characteristics to electrical stimulation are likely to reveal underlying differences in spinovestibular circuitry between the normal and compensated animal.

2 Responses of vestibular nucleus neurons to tilt following chronic bilateral removal of vestibular inputs

2.1 Introduction

Many studies have considered the effects of *unilateral* labyrinthectomy or vestibular neurectomy on vestibular-elicited reflexes and activity of vestibular nucleus neurons (Clegg & Perachio, 1985; Newlands & Perachio, 1991a) (Ried et al. 1984; Yagi and Markham 1984; Hamann and Lannou 1987). However, few studies have systematically considered the effects of *bilateral* removal of vestibular inputs on an animal's behavior or on activity in the vestibular system. Curiously, although some responses typically attributed to the vestibular system, such as horizontal vestibulo-ocular and vestibulo-collic reflexes (Dutia & Hunter, 1985; Waespe & Wolfensberger, 1985), are abolished following bilateral vestibular neurectomy, postural stability is not profoundly impaired (Thomson *et al.*, 1991; Macpherson & Inglis, 1993). The latter data emphasize the large role that somatosensory inputs may play in signaling and controlling body position in space. Mittelstaedt provided further evidence that inputs from the trunk, including visceral graviceptors, can contribute to a sense of spatial orientation (Mittelstaedt, 1992, 1995, 1996; Mittelstaedt & Mittelstaedt, 1996). As might be expected from these findings, somatosensory and proprioceptive signals have been shown to acquire a larger role in controlling postural stability in humans following loss of vestibular inputs (Pyykko *et al.*, 1991; Lacour *et al.*, 1997a).

Although the labyrinth provides the dominant input to vestibular nucleus neurons, the activity of these cells is influenced additionally by other signals during movement. Inputs from the neck have long been known to affect the firing of vestibular nucleus neurons (Brink *et al.*, 1980; Boyle & Pompeiano, 1981; Kasper *et al.*, 1988b). Both anatomical and physiological

studies have suggested that somatosensory receptors in the limbs and trunk also provide inputs to cells in the vestibular nuclei (Wilson *et al.*, 1968; Wylie & Felpel, 1971; Pompeiano, 1972; Rubin *et al.*, 1979b; McKelvey-Briggs *et al.*, 1989), although the effects of these inputs on the processing of labyrinthine signals have not been investigated. The observation that the parabrachial nucleus, which integrates visceral inputs, has projections to the vestibular nuclei (Balaban & Porter, 1998; Balaban *et al.*, 2002) suggests that graviception derived from visceral receptors could be mediated through the vestibular system. Other visceral influences on the activity of vestibular nucleus neurons could be relayed through the reticular formation. However, the possibility that visceral inputs may affect signal processing in the vestibular nuclei has not been explored experimentally.

The goal of the current work was to ascertain whether activity of vestibular nucleus neurons is modulated during whole-body tilts in the absence of vestibular and neck inputs. A second aim was to determine the spatial and dynamic properties of vestibular nucleus neuron responses to these stimuli, which presumably activated somatosensory receptors in the trunk and limbs as well as visceral receptors. To achieve these aims, extracellular recordings were made from the medial, inferior, and lateral vestibular nuclei during whole-body sinusoidal tilts in animals whose vestibular inputs were removed bilaterally (through both a labyrinthectomy and a vestibular neurectomy) 1.5–3 months previously. These experiments were conducted in decerebrate cats whose vertebral column was clamped to eliminate neck movement during tilt; in two cases, the C1–C3 dorsal roots were cut bilaterally on the day of the experiment to ensure that any responses were *not* due to neck inputs. Data recorded in animals lacking vestibular inputs were compared with those previously observed in vestibular-intact animals.

2.2 Methods and materials

All experimental procedures used in this study were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee, and were consistent with the National Institutes of Health Guidelines.

2.2.1 Overview of procedures

Acute experiments were performed on eight adult female cats (weight 3.2–4.7 kg) whose vestibular inputs were eliminated 49–103 days (median 89 days) previously. These animals had been used in studies examining the effects of bilateral removal of vestibular inputs on control of blood pressure (Jian *et al.*, 1999) or respiratory muscle activity (Cotter et al. 1999). As part of these prior experiments, alert animals were tilted at amplitudes up to 60° in a lighted room for hundreds of trials following the vestibular lesion. These manipulations ensured that animals employed in the current study had experienced movement-related non-labyrinthine sensory signals after vestibular inputs were removed.

2.2.2 Procedures for bilateral removal of vestibular inputs

Recovery surgeries to eliminate vestibular inputs were performed using aseptic procedures in a dedicated operating suite. Animals were initially anesthetized by using an intramuscular injection of ketamine (20 mg/kg) and acepromazine (0.2 mg/kg). Subsequently, an endotracheal tube and intravenous catheter were inserted. Anesthesia was continued by using 1–1.5% isoflurane vaporized in O_2; the concentration was adjusted to maintain a constant heart rate (determined by monitoring the electrocardiogram) and areflexia. Ringer's lactate solution was infused intravenously to replace fluid loss during the surgery, and a heating pad was used to maintain rectal temperature near 38°C.

To eliminate vestibular inputs, the tympanic bulla on each side was exposed ventrolaterally and opened to expose the cochlea and vestibular labyrinth. A drill was then used to remove the otic capsule and expose the vestibule and ampullae of the semicircular canals. The vestibular neuroepithelium was removed by dissection and suction to ensure that the labyrinthectomy was complete. Further bone removal provided access to the eighth cranial nerve, which was transected under microscopic observation within the internal auditory canal, central to Scarpa's ganglion. Thus, two independent lesions affecting the vestibular system were made on each side to ensure that vestibular inputs were completely removed. In no case did nystagmus or deviation in eye position occur after the surgery, confirming that the peripheral lesions were complete bilaterally (Baloh and Halmagyi 1996). Immediately following the surgery, animals exhibited poor postural control and head instability, and thus were fed by hand and hydrated by infusing ~100 ml Ringer's lactate solution subcutaneously each day. After a few days, animals recovered sufficiently to eat and drink without assistance. Within a month following the surgery, animals could locomote efficiently, although they were unable to walk in a straight line without being provided a fixation point. Head stability was adequate to allow normal behaviors (e.g., grooming) but was obviously worse than in a vestibular-intact animal.

2.2.3 Surgical preparation for acute recordings from the vestibular nuclei

In order to perform acute recordings, animals were initially anesthetized with the use of 1–2% halothane vaporized in N_2O and O_2. Blood pressure was monitored from a femoral artery using a Millar Mikro-Tip transducer. Intravenous catheters were inserted, and if necessary blood pressure was maintained over 100 mmHg by infusing Ringer's lactate solution or metaraminol bitartrate (Aramine, Merck, Sharpe & Dohme, 80 μg/ml). A DC-powered heat lamp and heating blanket were used to maintain body temperature near 38°C throughout the experiment.

The animal was placed in a modified stereotaxic frame, with the head pitched down 30° to align the horizontal semicircular canals with the earth horizontal plane. As described below, this stereotaxic frame was mounted on a tilt table capable of simultaneous rotations in the roll and pitch planes. The animal's body was secured with the use of hip pins and a clamp placed on the dorsal process of the T1 vertebra. These procedures fixed the neck in place during whole-body rotations, minimizing the possibility that inputs from neck receptors affected the firing of vestibular nucleus neurons in these experiments. A midcollicular decerebration was performed after ligation of the carotid arteries and aspiration of the portion of cerebral cortex overlying the brainstem on the left side. Subsequently, a craniotomy was performed to expose the caudal cerebellum, and the caudal most 2–3 mm of the cerebellar vermis was aspirated to expose the caudal brainstem. This procedure revealed landmarks that allowed us to accurately target the vestibular nuclei for recording. In two experiments, the C1–C3 dorsal roots were transected to remove inputs from neck receptors to the central nervous system.

At least 1 h before the beginning of the recording session (and after the decerebration was complete), anesthesia was stopped, and the animal was paralyzed with the use of an intravenous injection of 10 mg/kg gallamine triethiodide (Sigma), which was supplemented by hourly injections of 5 mg/kg. While paralyzed, animals were artificially respired with the use of a positive-pressure ventilator, and end tidal CO_2 was maintained near 4%. At the end of the recording session, animals were perfused transcardially with 1l saline and 2l of the paraformaldehyde fixative developed by McLean and Nakane (1974). The head of each animal was removed, postfixed in the paraformaldehyde solution, and the brainstem and temporal bone were removed for histological analysis (see below).

In three of the animals, the spinal cord was transected at the atlanto-occiptal joint to remove all ascending spinal information. During these surgeries artificial positive pressure

ventilation was initiated immediately after transection of the cord. In addition to spinal transection, the vagus nerves were isolated bilaterally posterior-lateral to the cricoid cartilage. These nerves were transected during the recording procedure if a neuron was encountered that modulated to vertical tilt stimulus.

2.2.4 Production of whole-body tilts

Vertical tilts were produced in the roll and pitch axes using a servo-controlled hydraulic tilt table (NeuroKinetics Inc., Pittsburgh, PA). The hydraulics of the tilt table were driven by sinusoidal stimuli delivered by a Cambridge Electronic Design (CED) 1401-plus data collection system interfaced with a Macintosh Quadra 800 computer. To characterize neuronal responses to vertical rotations, the "wobble" stimulus, a constant-amplitude tilt whose direction moves around the animal at constant speed, was first employed (Schor *et al.*, 1984b). Clockwise wobble stimuli were generated by driving the pitch axis of the tilt table with a sine wave while simultaneously driving the roll axis with a cosine wave; during this stimulus, the animal's body, viewed from above, appeared to wobble, having in succession nose down, right ear down, nose up, and left ear down. When the signal to the pitch axis of the tilt table was inverted, the stimulus vector rotated in the counterclockwise direction. The direction of the response vector orientation lies midway between the maximal response directions to clockwise and counterclockwise wobble stimulation, because the phase differences between stimulus and response are reversed during the two directions of stimulation (Schor *et al.*, 1984b). Thus, by considering both responses, these phase differences can be accounted for. Wobble stimuli were delivered at frequencies ranging from 0.05 to 0.5 Hz (always including 0.1 Hz) and at amplitudes up to 15°.

Once response vector orientation was obtained, stimuli in a fixed vertical plane at or near this orientation were used to study the dynamics of the response to tilt (i.e., response gain and

phase across stimulus frequencies). Planar stimuli were generated by applying sine waves to the roll axis, the pitch axis, or simultaneously to both axes of the tilt table, so that during one half-cycle one side of the body was tilted down and during the second half-cycle the opposite side was tilted down. Driving both the pitch and roll axes simultaneously with sine waves produced tilts in a plane oriented between the pitch and roll planes; the orientation was determined by the ratio of the signal sent to the pitch and roll axes. Planar stimuli were delivered at frequencies ranging from 0.05 to 1 Hz (and occasionally at 0.02 Hz and 2 Hz), and at amplitudes up to 10° at frequencies <0.5 Hz. At frequencies $\geq$0.5 Hz, it was difficult to hold units when conducting large-amplitude tilts; the maximal-amplitude rotations used were 7.5° at 0.5 Hz and 5° at 1 Hz.

2.2.5 Recording and analysis of unit activity

Electrode penetrations were made 4–7 mm rostral to the obex and 3–5 mm to the left side of the midline, using epoxy-insulated tungsten microelectrodes with an impedance of 12 MW (A-M Systems). Neural activity was amplified by a factor of 10^3–10^4, 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 the CED 1401-plus data collection system and Macintosh computer; the sampling rate was 10,000 Hz. Electrolytic lesions were made in the vicinity of some recording sites (by passing a 20-μA negative current for 30 s).

When a unit was encountered, spontaneous activity in the absence of stimulation was monitored for approximately 1 min, and spontaneous firing rate and coefficient of variation of firing rate (CV; standard deviation of interval between spikes divided by mean interval between spikes) were determined. Subsequently, responses to body tilt were recorded; neural activity was binned (500 bins/cycle) and averaged over the sinusoidal stimulus period by the Macintosh computer. The approximate numbers of sweeps averaged at each frequency are as follows: 10 at

0.05 Hz, 15 at 0.1 Hz, 25 at 0.2 Hz, 50 at 0.5 Hz, and 100 at 1 Hz. However, when modulation of activity was particularly strong, fewer sweeps were collected.

Sine waves were fitted to responses with the use of a least squares minimization technique (Schor et al. 1984). The response sinusoid was characterized by two parameters: phase shift from the stimulus sinusoid (subsequently referred to as phase) and amplitude relative to the stimulus sinusoid (subsequently referred to as gain). Gain and phase measurements were then corrected for the dynamics of the tilt table. Responses were considered significant if the signal-to-noise ratio (see Schor et al. 1984 for method of calculation) was >0.5 and there was only one obvious first harmonic (see Fig. 2.3A for examples of significant responses). Only units whose activity was significantly modulated during multiple runs and at least two frequencies of rotation were considered to respond to tilt. Similar criteria have been used in many previous studies to determine whether a neuron responds to tilt (Kasper *et al.*, 1988b; Wilson *et al.*, 1990; Woodring *et al.*, 1997). The response vector orientation was expressed using a head-centered coordinate system, with 0° corresponding to ipsilateral ear-down roll, 90° to nose-down pitch, 180° to contralateral ear-down roll, and –90° to nose-up pitch.

2.2.6 Histological analysis

In all animals, sections of the brainstem (100 μm thick) were made in the transverse plane and stained with thionine. Locations of recorded neurons were reconstructed on camera lucida drawings of sections with reference to placement of electrolytic lesions, relative locations of electrode tracks, and microelectrode depth. In three animals, the temporal bone was removed, decalcified using a solution of ethylenediaminetetraacetic acid (EDTA) and hydrochloric acid, embedded in either 12% celloidin (n=2) or paraffin (n=1), cut in the coronal plane (30 μm thickness for celloidin embedding; 8 μm thickness for paraffin), and stained using hematoxylin.

The temporal bone sections were then inspected histologically to determine the extent of damage to the eighth nerves and vestibular labyrinth.

2.3 Results

Sixty seven units whose firing could be faithfully discriminated during whole-body tilts were included in our analysis. These neurons were distributed evenly across all 8 animals. Many more neurons were encountered during tracking, but the level of spontaneous activity in the vestibular nuclei was so high that it was often difficult to discriminate single units. Thus, our data sample may be biased toward large cells, whose spikes were large enough to be cleanly separated from background firing. Of these neurons, the activity of 18 cells (27% of the sample) was significantly modulated during tilts. In the two animals in which the C1–C3 dorsal roots were transected, 5 of 21 neurons responded to tilt. Neurons whose activity was modulated during whole-body rotations were detected in every experiment. A total of 90 units were recorded from the subgroup of animals that had undergone an acute spinal transection to remove ascending spinal input. Of these neurons, 1 cell (1%) was found to respond to vertical tilt stimulation despite lacking both labyrinths and ascending spinal and vagal input. No inter-animal differences were detected despite their varied survival times following labyrinthectomy. Responses from the animal recorded 49 days post-labyrinthectomy was not statistically different than the animal that was recorded from 103 days post lesioning.

Figure 2.1 shows the spontaneous firing rate for all neurons included in this study. The mean firing rate was 43±5 (SEM) spikes/s for the entire population, 55±15 spikes/s for cells whose activity was modulated by tilt, and 38±4 spikes/s for cells whose firing did not respond significantly to tilt stimuli. The spontaneous firing rates for neurons that were classified as being

affected and unaffected by tilt were not significantly different (Mann-Whitney test, P=0.50). A caveat in these findings, however, is that we did not sample silent units, and thus firing rates that were determined for neurons exclude cells with no spontaneous activity. Figure 1 also illustrates that the CV of firing rate was similar for the two populations of cells: 0.80±0.11 for neurons whose activity was modulated by tilt and 0.89±0.19 for neurons that were not demonstrated to respond to rotations. For the population as a whole, no significant correlation between spontaneous neuronal firing rate and CV of firing rate was noted (Spearman rank test, P=0.93).

Response vector orientation was determined for all neurons that responded to tilt with the use of the wobble stimulus. At least two frequencies of wobble rotations (typically 0.1 and 0.5 Hz) were employed for all but one cell. The direction of tilt that produced the maximal response was similar for all frequencies of rotation; the variability in response vector orientation across the frequencies employed for a neuron was typically less than 25° (median 20°, range 7°–38°). Figure 2.2 is a plot of the response vector orientations determined at 0.1 Hz. Fourteen of the 18 response vector orientations (78%) were within 25° of the pitch plane; of these, half were near nose-up pitch, and half were near nose-down pitch. Only two of the neurons (11%) had response vector orientations that were closer to roll than to pitch. The preferred direction of tilt for modulating the activity of neurons in the inferior and lateral vestibular nuclei of cats with intact labyrinthine function is considerably different: over two thirds of the neurons have

Figure 2.1

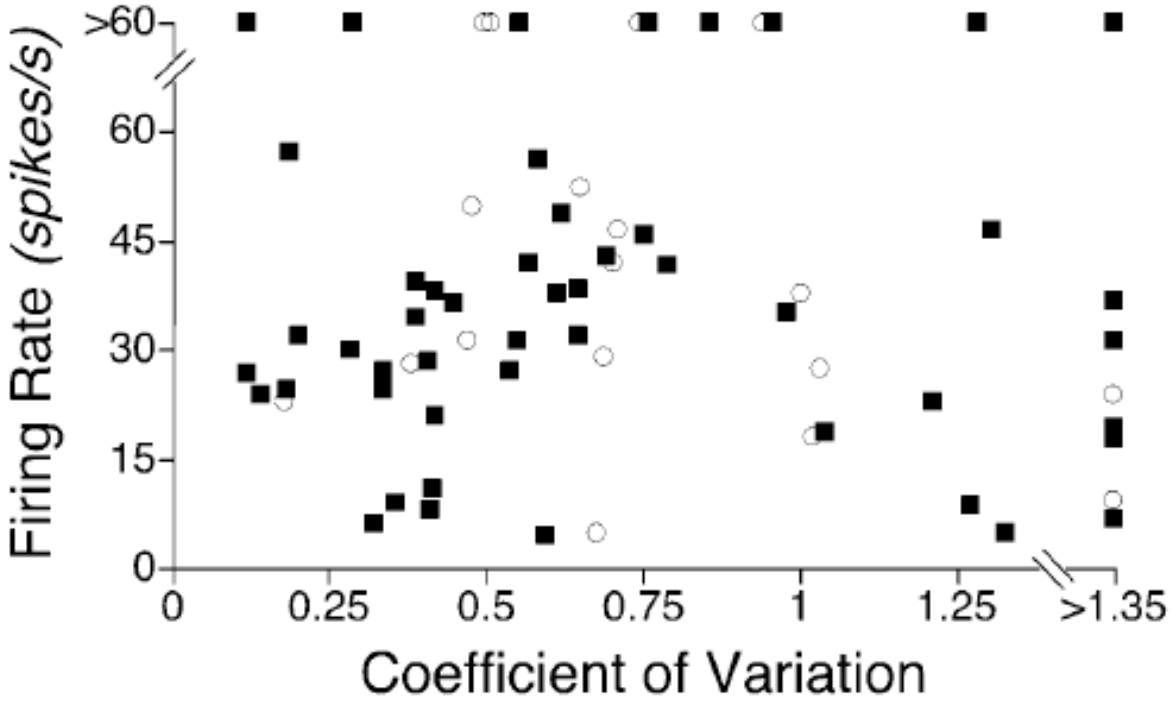

Figure 2.1 Plot showing coefficient of variation of spontaneous firing and spontaneous firing rate for all neurons included in this study (*Empty* neuron whose activity was modulated by tilt, *Black* neuron whose activity was *not* modulated by tilt)

Figure 2.2

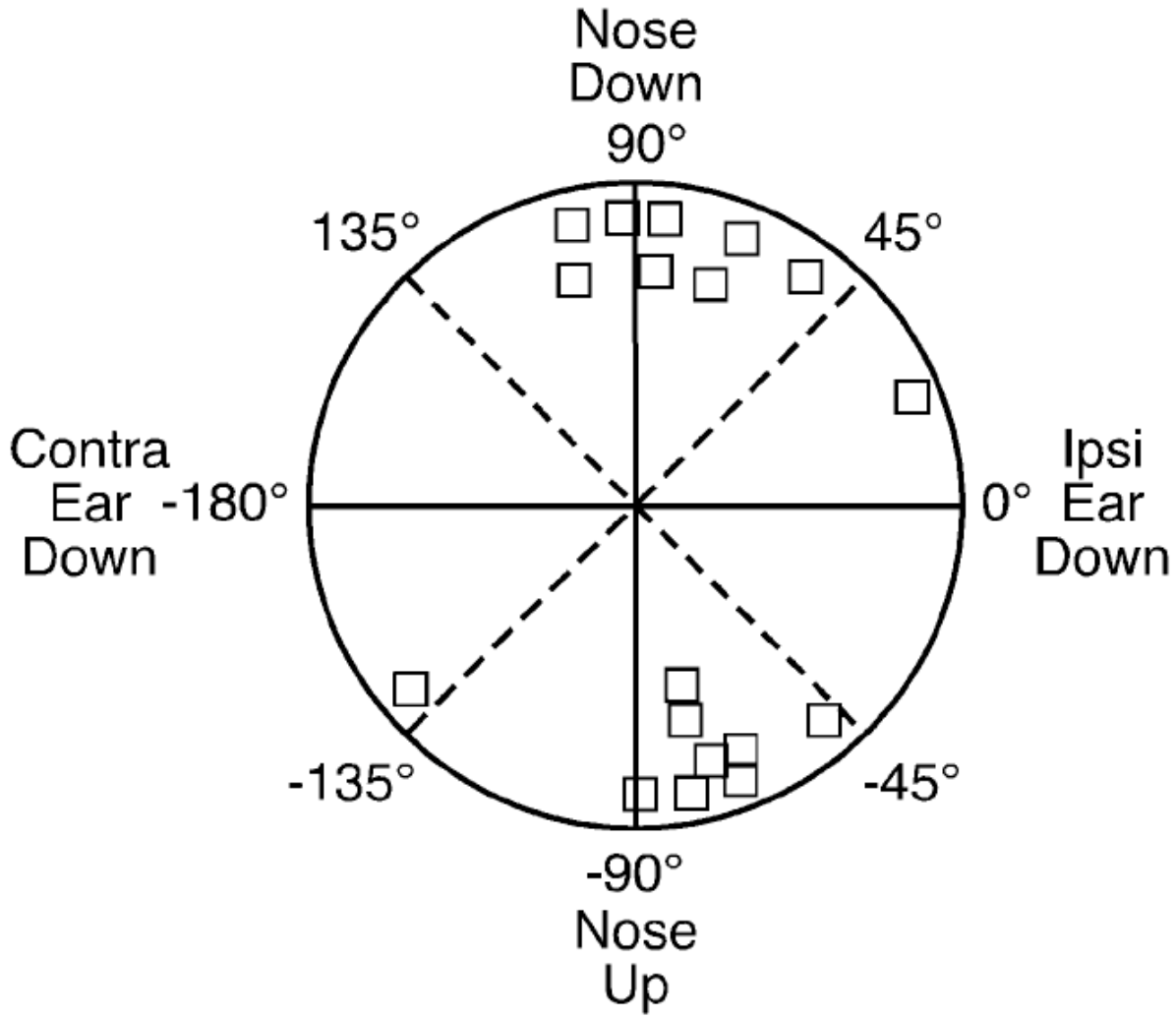

Figure 2.2 Plot of response vector orientations of neurons from bilaterally labyrinthectomized animals, determined at 0.1 Hz, whose activity was modulated by tilt.

response vector orientations closer to roll than to pitch (Kasper *et al.*, 1988b; Wilson *et al.*, 1990). Thus, although some neurons in the vestibular nuclei respond to vertical rotations in the absence of vestibular inputs, the spatial properties of these responses are considerably different than in a labyrinth-intact animal.

After response vector orientation was determined, sinusoidal stimuli were delivered in a fixed plane near that which elicited the maximal response. Typically, rotations in the pitch plane were employed. Planar stimuli were usually delivered at frequencies ranging from 0.05 to 1 Hz, and occasionally at 0.02 Hz and 2 Hz. However, neurons were sometimes lost before the entire range of stimuli could be delivered. Figure 2.3A illustrates responses of one neuron to three frequencies of rotation, and the Bode plots in Fig. 2.3B show the gain and phase (relative to stimulus position) of the responses of this neuron and another cell to rotations in the pitch plane. These two neurons were typical of the entire lesioned population, in that the response gain was relatively constant across stimulus frequencies, and response phase was near stimulus position at low frequencies but developed a moderate phase lag at higher frequencies. A mean Bode plot for all neurons that responded to tilt is illustrated in Fig. 2.4. As indicated in Fig. 2.4A, the mean response gain with respect to stimulus position was approximately 1 spike/s/° at all frequencies, which is approximately equal to that of normal vestibular nucleus neurons whose predominant inputs are presumed to come from otolith organs (Kasper *et al.*, 1988b; Wilson *et al.*, 1990). Figure 2.4B shows that the mean response phase for the population was near stimulus position at low frequencies, but developed a modest phase lag as stimulus frequency increased. These dynamic properties are similar to those of many normal vestibular nucleus neurons classified as receiving dominant otolith inputs (Kasper *et al.*, 1988b; Wilson *et al.*, 1990).

Figure 2.3

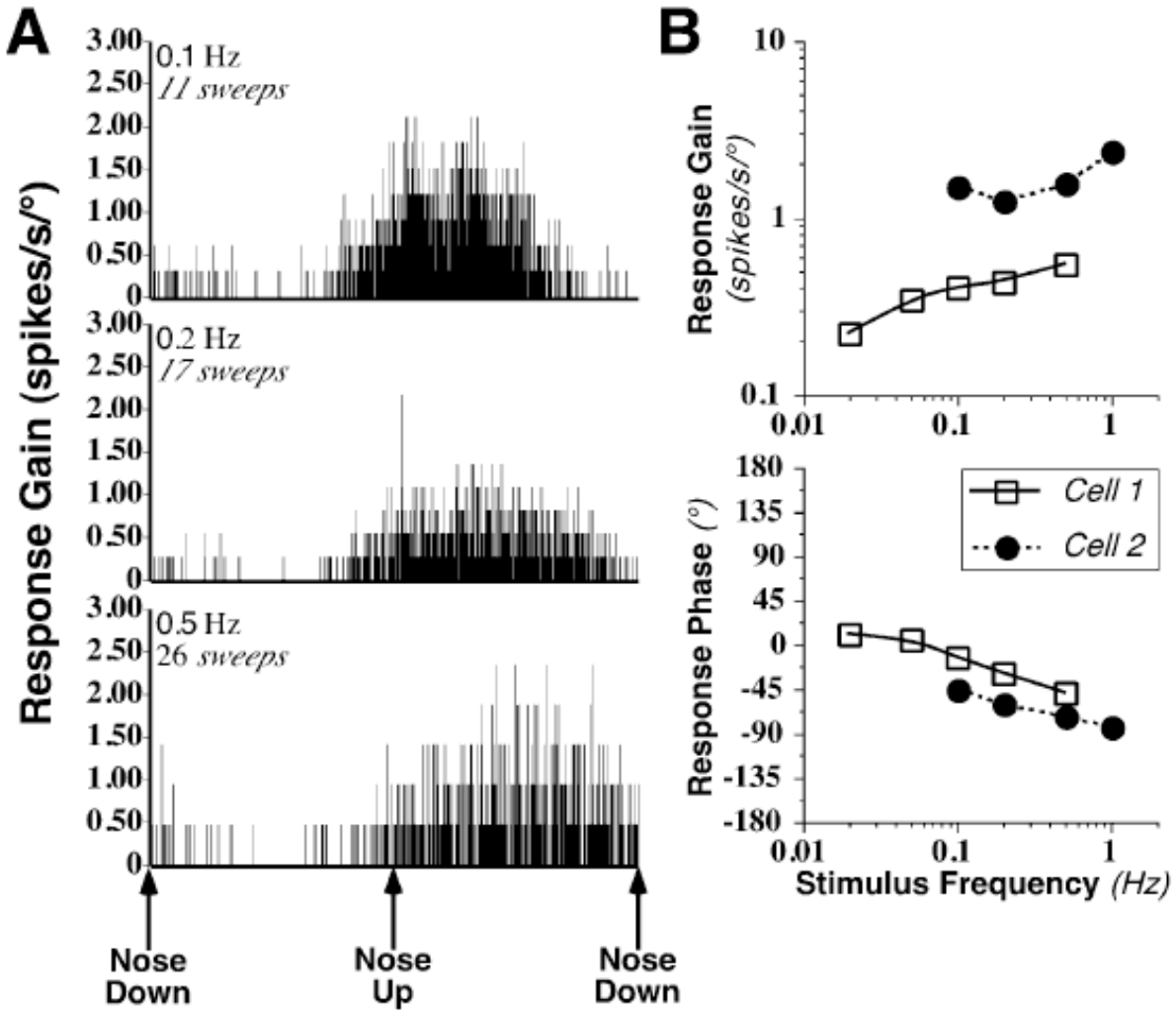

Figure 2.3 **A** Histograms showing the responses of a single neuron to pitch rotations at three different frequencies. The stimulus frequency employed and the number of sweeps averaged for each trace are indicated.

B Bode plots indicating the dynamics of responses of two neurons to pitch rotations at multiple frequencies. Both response gain and phase are plotted with respect to stimulus position. Responses recorded from cell 2 in this Bode plot are illustrated in **A**.

Figure 2.4

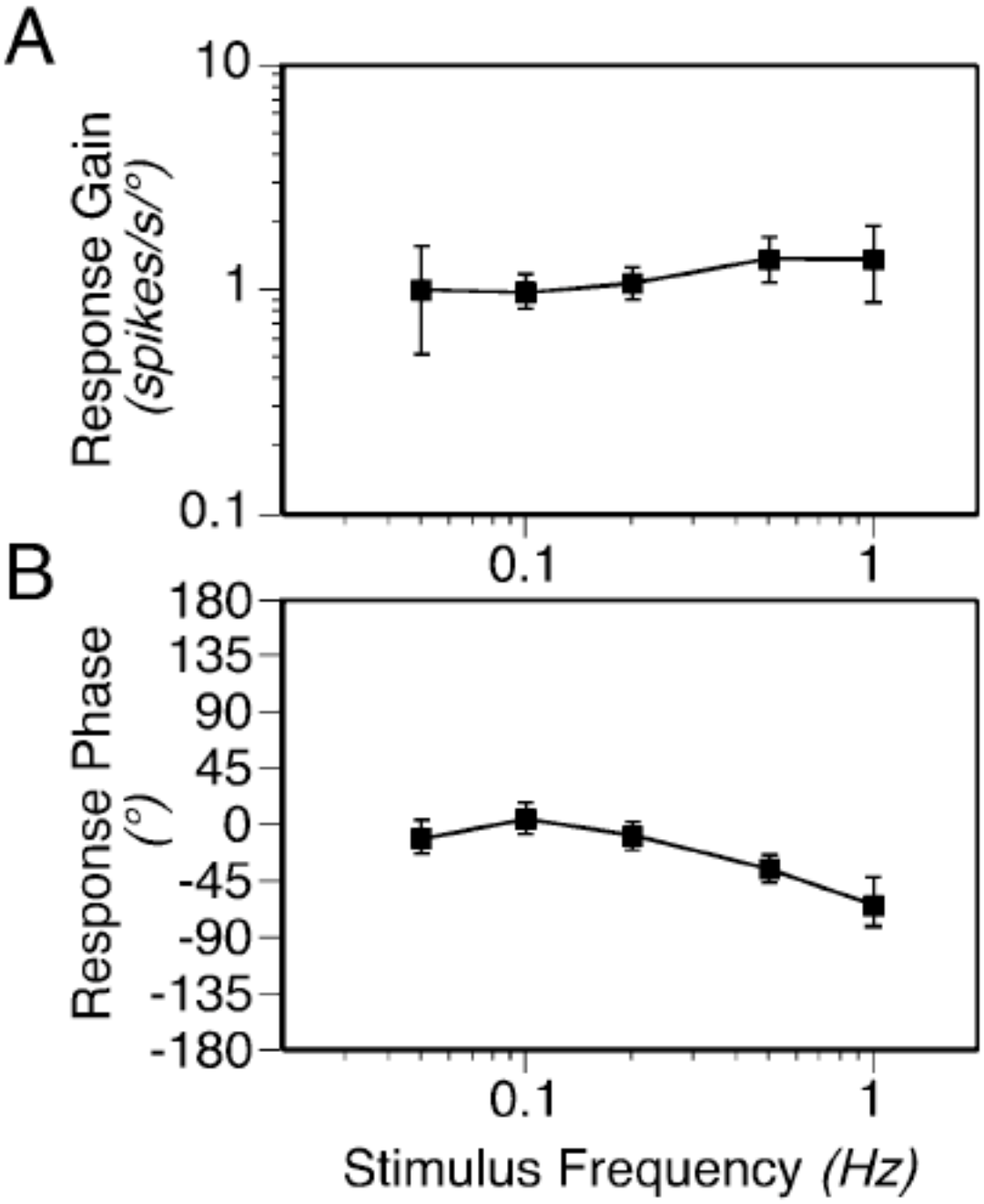

Figure 2.4 Bode plot indicating mean gain (**A**) and phase (**B**) of responses to tilt for all neurons whose activity was affected by this stimulus. Both response gain and phase are plotted with respect to stimulus position. *Error bars* indicate one SEM.

2.3.1 Neurons in spinal transected subgroup do not modulate to vertical tilt

Figure 2.5 illustrates findings from all recorded neurons in the spinal intact and transected subgroups. Of 90 recorded neurons only 1 (1%) neuron was found to respond to vertical tilt stimulation, in contrast to 27% modulation encountered in the spinal intact animals. Chi square analysis of the two populations resulted in a significance value of $p<.0001$. As expected spinal transection eliminated almost all modulating neurons in the vestibular nuclei. Of particular interest, however, is the one neuron that was encountered that still showed evidence of modulation. This neuron continued to respond to movements of the tilt table even following transection of both vagus nerves. Average phase response properties of this neuron were similarly tuned to pitch sensitivity similar to neurons in animals in spinal intact subgroup. Likewise response gain dynamics remained relatively flat across stimulus frequency.

2.3.2 Location of recorded neurons

The locations of recording sites were reconstructed based on the locations of lesions and are shown in Fig. 2.6. The recording sites were distributed mainly in the lateral vestibular nucleus, the rostral half of the inferior vestibular nucleus, and the adjacent portions of the medial vestibular nucleus. Neurons whose activity was modulated by tilt did not appear to be clustered separately from neurons whose firing was unaffected by body rotation.

Because both a labyrinthectomy and a vestibular neurectomy were performed bilaterally in each animal, it is highly unlikely that the responses recorded in this study were due to vestibular stimulation. Furthermore, nystagmus was not observed in any of the animals, but should have occurred if an incomplete lesion was made on one side (Baloh and Halmagyi 1996). The spatial properties of responses to tilt were also considerably different than in vestibular-intact animals. To provide further evidence that vestibular inputs were eliminated in these

Figure 2.5

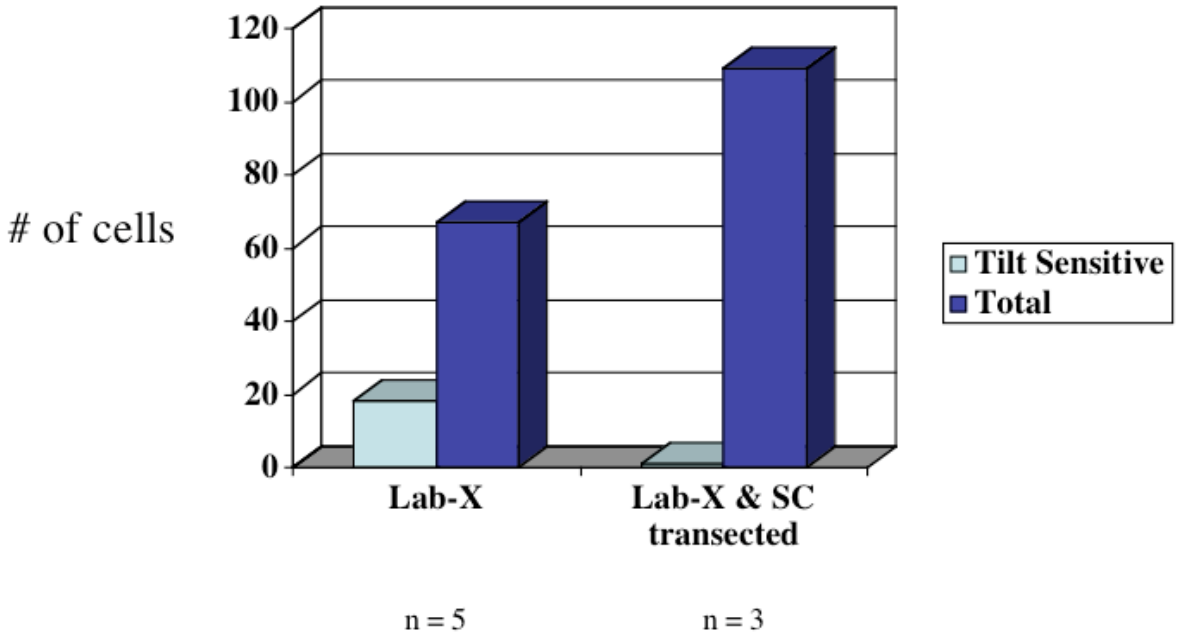

Figure 2.5 Number of vestibular neurons that responded to vertical tilt stimulus in labyrinthectomized (Lab-X) animals and animals that had undergone an acute spinal transection in addition to labyrinthectomy (Lab-X & SC transected)

Figure 2.6

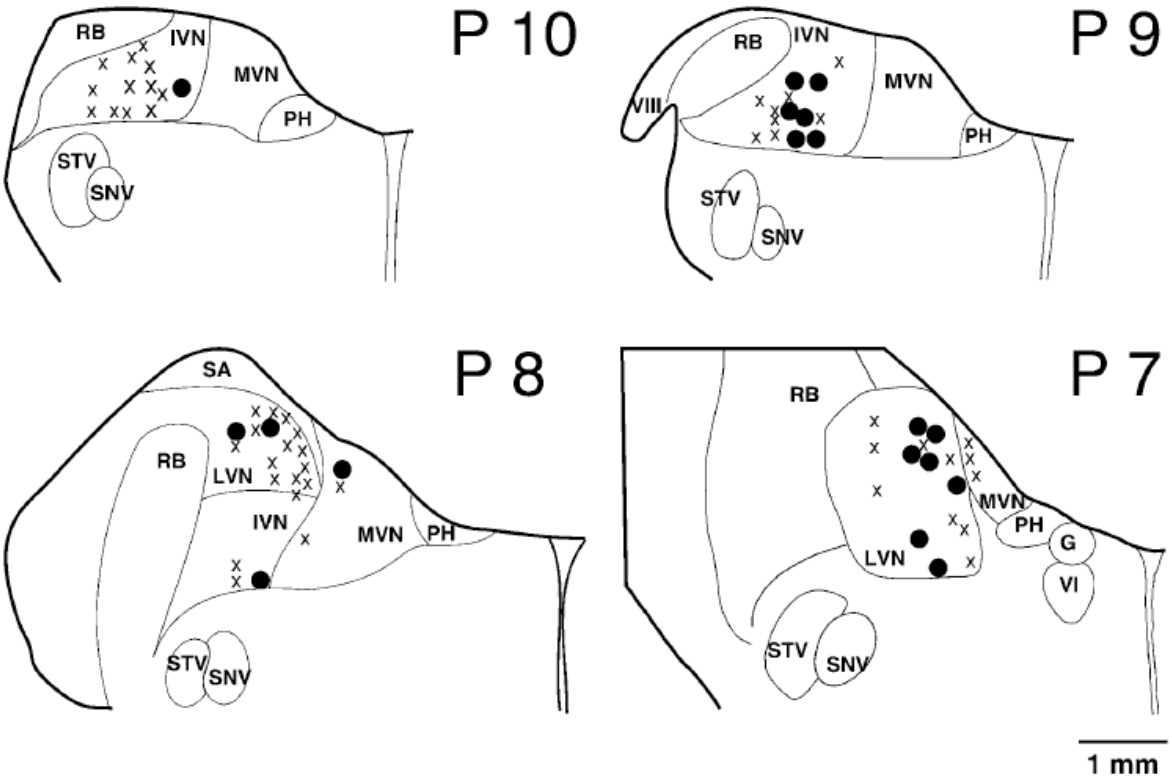

Figure 2.6 Location of all neurons whose activity was recorded in this study. Neuron locations are plotted on transverse sections of the brainstem (closed circles represent neurons that responded to tilt, X represents neurons whose activity was unaffected by tilt). *Values to the right of each section* indicate relative distance (in millimeters) posterior to stereotaxic zero; level of obex was at ~P13.5 (G genu of facial nerve, IVN inferior vestibular nucleus, LVN lateral vestibular nucleus, MVN medial vestibular nucleus, PH prepositus hypoglossi, RB restiform body, SA stria acoustica, SNV spinal trigeminal nucleus, STV spinal trigeminal tract, VI abducens nucleus, VIII vestibulocochlear nerve)

animals, temporal bone sections from three animals were analyzed histologically. In all cases, we confirmed that a complete bilateral labyrinthectomy had been performed. Furthermore, in all of the cases, both eighth nerves were found to be completely severed; the cut ends of the nerves were degenerated and surrounded with glial scars. Thus, we are confident that vestibular inputs were removed in the animals employed in these experiments.

2.4 Discussion

The current study revealed that following chronic bilateral loss of vestibular inputs neurons in the vestibular nuclei are spontaneously active and that about one-fourth of these cells respond to sensory inputs elicited by 5°–10° vertical tilts. Presumably, if these experiments had been conducted in awake animals or vertical tilts of greater amplitude had been employed, the activity of even a larger fraction of vestibular nucleus neurons may have been modulated. Visual information in the awake animal could participate in modulating vestibular neuronal activity, as well as other potential sensory and higher cortical processes. Three previous studies conducted in anesthetized cats (Ryu and McCabe 1976), alert monkeys (Waespe *et al.*, 1992), and alert guinea pigs (Ris & Godaux, 1998a) demonstrated that following chronic bilateral labyrinthectomy or vestibular neurectomy, the spontaneous activity of vestibular nucleus neurons is approximately equal to that in labyrinth-intact animals. The present data extend this observation to the decerebrate cat preparation. However, the resting discharge of vestibular nucleus neurons that we determined in animals lacking vestibular inputs was more irregular (mean CV 0.86, median CV 0.62) than that previously reported in labyrinth-intact decerebrate cats (median CV 0.4; Schor et al. 1984). Thus, although removal of vestibular inputs does not appreciably alter the mean level of resting activity in the vestibular nuclei, the characteristics of the background activity appear to be changed.

Data gathered from labyrinth intact animals (Schor *et al.*, 1984b; Schor *et al.*, 1984a) suggest that the majority of spontaneously active neurons in the vestibular nuclei are sensitive to vertical tilt stimuli. The most surprising finding in this study is that some neurons in the vestibular nuclei of cats that have been chronically bilaterally labyrinthectomized still respond to moderate-amplitude tilts. Although the source of the sensory inputs responsible for modulating vestibular nucleus activity during vertical body rotations was not established in this study, it seems *unlikely* that these inputs came from neck proprioceptors. Five lines of evidence support this conclusion. First, because the head was fixed in a stereotaxic frame and the T1 vertebra was clamped, movement of the neck during body rotations was minimal. Second, because the animals were paralyzed, thereby eliminating gamma motoneuron drive to muscle spindles, the sensitivity of neck proprioceptors to movement should have been low (Richmond and Abrahams 1979). Third, vestibular nucleus neurons still responded to tilt in animals whose neck inputs were eliminated by a C1–C3 dorsal root rhizotomy. Fourth, most of the vestibular nucleus neurons in this study that responded to tilt were activated better by pitch than by roll tilt. In contrast, most neurons in the lateral and inferior vestibular nuclei respond better to rotations of the neck in the roll plane than in the pitch plane (Kasper *et al.*, 1988b). Fifth, the responses of vestibular nucleus neurons recorded in these experiments exhibited a modest phase lag at high stimulus frequencies, whereas responses of such neurons to neck rotation develop a moderate phase lead with advancing stimulus frequency (e.g., mean of 61° at 1 Hz) (Kasper *et al.*, 1988b). These data collectively suggest that modulation of vestibular nucleus neuronal activity in bilaterally labyrinthectomized animals is due to activation of receptors in the viscera, trunk or limbs, but not the neck.

A large variety of receptors in the trunk and limbs could potentially have been activated by vertical tilts in this study. These inputs could have contributed to the modulation of firing of

vestibular nucleus neurons. For example, the animal's limbs were in contact with the stereotaxic frame, and sliding of the limbs during tilt could have stimulated cutaneous receptors. Although the animals were paralyzed, muscles in the limbs could have been sufficiently stretched during tilts to provide spindle inputs to the vestibular nuclei. Signals from visceral graviceptors have been shown to contribute to the sense of body position in space (Mittelstaedt, 1992, 1995, 1996; Mittelstaedt & Mittelstaedt, 1996), and it is possible that inputs from visceral receptors could elicit alterations in firing of vestibular nucleus neurons during vertical body rotations. The dynamics of responses of vestibular nucleus neurons to tilt in this study differed from the classical behavior of simple high- and low-pass filter systems. Vestibular neurons normally have responses that increase in gain with increasing stimulus velocity, if they are neurons that receive primarily semicircular canal input. Likewise they have typically flat gains if their predominant input is from otolith afferents. The neurons from the lesioned animals had both flat responses as well as responses that increased with stimulus velocity, raising the possibility that these responses were due to the convergence of several spinal and/or visceral afferent inputs, or to the complex processing of these signals. Additional experiments will be required, however, to delineate which non-labyrinthine receptors contribute to modulating vestibular nucleus neuronal activity during whole-body tilt.

Further work also will be needed to determine whether the modulation of vestibular nucleus neuronal activity during whole-body tilt in animals lacking labyrinthine inputs has functional significance. Most neurons that responded to vertical rotations in this study had response vector orientations near the pitch plane. Thus, these neuronal responses could contribute to physiological processes that must be modified during sagittal plane movements. Previous studies have shown that neurons in the medial and inferior vestibular nuclei are components of a vestibulosympathetic reflex arc that adjusts blood distribution in the body

during movement (Yates, 1996a). Because cats have a long longitudinal axis, this species is susceptible to blood pressure disturbances during body rotations in the pitch plane; it was previously shown that after bilateral removal of vestibular inputs, cats experience lability in blood pressure during pitch tilts (Doba & Reis, 1974; Jian *et al.*, 1999). However, this deficit recovers over a period of days to weeks after the vestibular lesion, suggesting that these animals can learn to use other sensory inputs to trigger compensatory changes in blood pressure during movement (Jian *et al.*, 1999). The current findings raise the possibility that adaptive plasticity in control of blood pressure following vestibular lesions could be mediated through the vestibular nuclei and that the ability to rapidly adjust blood pressure during movement could return as vestibular nucleus neurons begin to respond again to pitch tilts. This possibility remains to be explored.

One caveat that must be raised is the possibility that plastic changes, such as synaptic remodeling, collateralization, or receptor alterations, in the central vestibular system following vestibular lesions could result in an amplification of spinal and visceral afferent influences on vestibular nucleus neuronal activity. Thus, the functional role of inputs from the viscera, trunk, and limbs to the vestibular nuclei of animals that have normal vestibular function remains to be determined. These inputs could potentially modify pitch-elicited reflex responses mediated by the vestibular nuclei, including vestibulosympathetic reflexes, vertical vestibulo-ocular reflexes, and postural adjustments during movements in the sagittal plane. Signals from receptors in the limbs or trunk may also be partly responsible for producing complex responses of some vestibular nucleus neurons, such as spatiotemporal convergence (STC) behavior (Baker et al. 1984). Although the functional significance of inputs from receptors outside of the labyrinth to the vestibular nuclei is as yet only speculative, it is now clear that vestibular nucleus neuronal responses to vertical tilt cannot be assumed to be due solely to stimulation of labyrinthine

receptors. Instead, the vestibular nuclei appear to integrate a number of sensory inputs which are important in eliciting the responses of vestibular nucleus neurons during movement.

3 Convergence of limb, visceral, and vertical vestibular inputs onto vestibular nucleus neurons

3.1 Introduction

It is well established that nonlabyrinthine inputs influence the activity of vestibular nucleus neurons. For example, the integration of signals from the labyrinth and neck by these neurons presumably provides for the discrimination of whole body from head on body movements (Wilson & Melvill Jones, 1979). Besides inputs from the neck, both anatomical (McKelvey *et al.*, 1989) and physiological (Fredrickson *et al.*, 1966; Wilson *et al.*, 1966a; Wilson *et al.*, 1966b; Pompeiano, 1972; Rubin *et al.*, 1977a; Rubin *et al.*, 1977b; Rubin *et al.*, 1978; Rubin *et al.*, 1979b; Kasper *et al.*, 1986a) (Barnes and Pompeiano 1971; D'Ascanio et al. 1986) studies have also demonstrated that the vestibular nuclei receive afferent signals from the entire length of the spinal cord. Furthermore, physiological experiments have demonstrated that signals from the limbs influence the activity of vestibular nucleus neurons that receive labyrinthine inputs (Fredrickson *et al.*, 1966; Wilson *et al.*, 1966b; Rubin *et al.*, 1977b). Additionally, work by Mittlestaedt has shown that visceral inputs modulate vestibular-elicited responses, raising the possibility that the vestibular nuclei receive visceral signals (Mittelstaedt, 1995, 1996). Although the functional significance of the presence of limb and visceral inputs to the vestibular nuclei remains largely unknown, these signals presumably provide for a more precise, unambiguous determination of body position in space than is available from consideration of vestibular inputs alone.

Recent studies have also suggested that nonlabyrinthine inputs may participate in recovery of function subsequent to peripheral vestibular lesions. For example, following complete bilateral removal of vestibular inputs, vestibular nucleus neurons regain spontaneous

activity, presumably due in part to the presence of ascending signals from the spinal cord (Waespe *et al.*, 1992; Ris & Godaux, 1998a; Yates *et al.*, 2000). Furthermore, approximately one-fourth of vestibular nucleus neurons of decerebrate cats that had previously undergone a bilateral labyrinthectomy responded to vertical tilts < 10° in amplitude (Chapter 2); these neuronal responses could contribute to physiological processes that must be modified during movements in vertical planes. It also seems likely that in labyrinth-intact animals, vestibular nucleus neurons with vertical vestibular inputs (i.e., signals from vertical semicircular canals or otolith organs) are more likely to receive convergent inputs from the limbs or viscera than neurons that respond to horizontal rotation, because movements in vertical planes should be particularly effective in stimulating peripheral receptors. Evidence to support this hypothesis lies in the observation that, whereas over 70% of vestibular nucleus neurons that were activated by electrical stimulation of the entire vestibular nerve responded to stimulation of limb nerves (Fredrickson *et al.*, 1966; Wilson *et al.*, 1966a), only approximately 30% of the subset of units that responded to horizontal rotation received inputs from peripheral nerves (Rubin *et al.*, 1977a; Kasper *et al.*, 1986a). Nonetheless, the prevalence of convergence of limb and visceral signals onto vestibular nucleus neurons that receive vertical vestibular inputs has not been directly examined.

The present study had two major goals. The first objective was to determine the patterns of convergence of somatic and visceral inputs onto vestibular nucleus neurons that respond to vertical rotations. As part of this aim, we also ascertained whether neurons with different dynamic and spatial responses to vertical rotation, or which were located in different regions of the vestibular nuclei, received similar nonlabyrinthine inputs. The second objective was to compare the effectiveness of nonlabyrinthine inputs in modulating the activity of vestibular nucleus neurons in labyrinth-intact animals and in animals with chronic bilateral removal of

vestibular inputs. Through the consideration of these data, we also sought to propose a more definite role for the processing of limb and visceral inputs by the vestibular nuclei than has been provided by previous studies.

3.2 Materials and methods

All experimental procedures used in this study were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee, and were consistent with National Institutes of Health Guidelines. Experiments were conducted on 19 adult cats of either sex weighing 2.7-6.3 Kg. Of these animals, four had undergone a combined bilateral labyrinthectomy and vestibular neurectomy 34-94 days previously and allowed to recover. All four of these animals had been employed in another study considering the effects of removal of vestibular inputs on activity of respiratory muscles, and details concerning the surgical procedures for eliminating labyrinthine signals as well as the techniques used to assess the completeness of these lesions are discussed in a previous publication (Cotter *et al.*, 2001a). No interanimal differences were detected in the responses with respect to days following bilateral labyrinthectomy. Consequently it is not likely that environmental factors or training regimens effect spinal reorganization after 34 days of recovery.

3.2.1 Surgical procedures

Anesthesia was induced and maintained with either 1-2% halothane (Fluothane, Ayerst Laboratories) or isoflurane (IsoFlo, Abbott Laboratories) vaporized in N_2O and O_2. Blood pressure was monitored from a femoral artery with a Mikro-Tip® transducer (Millar Instruments), a femoral vein and a jugular vein were cannulated to permit intravenous injections, and rectal temperature was maintained between 36 and 38°C with an infrared lamp and heating pad. If necessary, an intravenous infusion of lactated Ringer solution or metariminol bitartrate

(Aramine, Merck, 80 μg/ml) in lactated Ringer solution was used to keep blood pressure > 100 mm Hg. The animal was placed in a modified stereotaxic frame, with the head pitched down 30° to align the horizontal canals with the earth horizontal plane. As described below, this stereotaxic frame was mounted on a tilt table capable of simultaneous rotations in the roll and pitch planes. The animal's body was secured in place with the use of hip pins and a clamp placed on the dorsal process of the T_1 vertebra. A midcollicular decerebration was performed after ligation of the carotid arteries and aspiration of the portion of the cerebral cortex overlying the rostral brainstem. Approximately 1 cm of brain tissue rostral to the midcollicular transection was aspirated to ensure that the decerebration was complete. A craniotomy was performed to expose the caudal cerebellum, and the caudalmost 4-5 mm of the cerebellar vermis was retracted or aspirated to allow access to the caudal brainstem. A laminectomy was performed to expose the cervical (C_6-T_1 segments) and lumbar (L_5-L_7 segments) enlargements of the spinal cord, and the dura mater covering the exposed spinal cord was opened.

The following nerves were isolated and either mounted on a pair of bipolar silver hook electrodes or on a pair of implanted electrodes that were subsequently insulated from surrounding tissues using Kwik-Cast® silicone sealant (World Precision Instruments): vagus, triceps (branches to all heads combined), musculocutaneous, deep radial, superficial radial, sural, tibialis anterior, gastrocnemius (branches to medial gastrocnemius and lateral gastrocnemius/soleus combined), and tibial (portion just distal to exit of gastrocnemius). Afferent fibers in the vagus nerve mainly arise from the viscera, those in the triceps nerve arise from a muscle that extends the elbow, those in musculocutaneous from both muscle and skin of the forearm, those in deep radial from extensor muscles of the forearm and the supinator muscle of the forepaw, those in superficial radial from skin and muscles of the wrist and paw, those in sural from skin of the hindpaw, those in tibialis anterior from a flexor muscle of the foot, those in

gastrocnemius from extensor muscles of the foot, and those in tibial from both skin and muscle of the lower leg (Crouch, 1969). Typically, musculocutaneous and all hindlimb nerves were isolated on the same side that recordings took place, whereas the superficial and deep radial and triceps nerves were isolated on the contralateral side. The vagus nerves were stimulated bilaterally.

At least 1 h before the beginning of the recording session (and after decerebration), anesthesia was stopped, and the animal was paralyzed with an intravenous injection of 10 mg/kg gallamine triethiodide (Sigma), which was supplemented by hourly injections of 5 mg/kg. While paralyzed, animals were artificially respired with the use of a positive pressure ventilator; end-tidal CO_2 was monitored and maintained near 4%.

At the end of the recording session, animals were killed with an overdose of pentobarbital sodium (120 mg/kg injected intravenously), and the brainstem was removed for histological determination of recording sites.

3.2.2 Recording procedures.

Epoxy-insulated tungsten microelectrodes with an impedance of 12 MΩ (A-M Systems) were used to make recordings from neurons in the vestibular nuclei during whole-body rotations in vertical planes and electrical stimulation of isolated nerves. Neural activity was amplified by a factor of 1000 or 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, United Kingdom) and Macintosh G4 computer (Apple Computer, Cupertino, CA); the sampling rate was 10,000 Hz. Electrolytic lesions were made in the vicinity of recording sites in each animal so that recording locations could be reconstructed.

When a unit was encountered, we initially recorded its responses to vertical vestibular stimulation, which was produced by tilting the entire animal about the pitch (transverse) and roll (longitudinal) axes using a servo-controlled hydraulic tilt table (NeuroKinetics, Pittsburgh, PA). The hydraulics of the tilt table were driven sinusoidal stimuli delivered by the Cambridge Electronic Design data collection system. We first determined the plane of tilt that produced maximal modulation of the unit's firing rate (response vector orientation). Response vector orientation was calculated from responses to the "wobble" stimulus, a constant-amplitude tilt whose direction moves around the animal at constant speed (Schor *et al.*, 1984b). Clockwise wobble stimuli were generated by driving the pitch axis of the tilt table with a sine wave while simultaneously driving the roll axis with a cosine wave; during this stimulus, the animal's body (viewed from above) appeared to wobble, having in succession nose down, right ear down, nose up, and left ear down. When the signal to the pitch axis of the tilt table was inverted, the stimulus vector rotated in the counterclockwise direction. The direction of the response vector orientation lies midway between the maximal response directions to clockwise and counterclockwise wobble stimulation, because the phase differences between stimulus and response are reversed during the two directions of rotation (Schor *et al.*, 1984b). Thus, by consideration of both responses, these phase differences can be accounted for. Wobble stimulation was delivered at 0.2 and 0.5 Hz, and sometimes at lower frequencies, typically at amplitude of 5°.

Subsequently, the response vector orientation was confirmed by comparing the gain of responses to tilts in a variety of fixed vertical planes, typically delivered at 0.5 Hz and at an amplitude of 5°. These tilts always included the roll and pitch planes, as well as planes oriented midway between roll and pitch (i.e., the approximate planes of the vertical semicircular planes). Planar stimuli were generated by applying sine waves to the roll axis, the pitch axis, or

simultaneously to both axes of the tilt table, so that during the first half-cycle one side of the body was tilted down, and during the second half-cycle the opposite side was tilted down. Driving both the roll and pitch axes simultaneously produced tilts in a plane oriented between the roll and pitch planes; the orientation was determined by the ratio of the signal sent to the two axes.

After response vector orientation was established, planar tilts at or near this orientation were used to study the dynamics of the vestibular response (i.e., response gain and phase across stimulus frequencies). Response dynamics were routinely determined over the frequency range of 0.05-1 Hz; for some units rotations at 0.02 and 2 Hz were also delivered. The amplitude of these stimuli was usually 5°, although in some cases 7.5° tilts were delivered at frequencies ≤ 0.2 Hz.

The final step of data collection was to characterize the unit's responses to stimulation of each of the nerves that was mounted on electrodes. Limb nerves were stimulated at increments of the threshold (T) required for eliciting field potentials recordable from the cord dorsum of the cervical or lumbar spinal cord. Spinal cord field potentials were monitored using silver ball electrodes positioned near the dorsal root entry zone and were led into an AC amplifier, amplified by a factor of 10,000, filtered with a bandpass of 10-10,000 Hz, and sampled at 1,000 Hz using an A-D channel of the 1401-plus data collection system. Nerves were stimulated using trains of 5 shocks of 0.2 ms duration with an interpulse interval of 3 msec that were delivered at a rate of once per second. Typically, we first determined whether a unit responded to stimuli that were 5 times the threshold required to elicit a spinal cord field potential. If this stimulus intensity was effective, we subsequently delivered stimuli at a variety of weaker intensities until the minimal stimulus strength required to modulate the neuron's firing rate was determined. However, if a neuron failed to respond to a stimulus that was 5 times that required to elicit a

spinal cord field potential, a 10T stimulus was then delivered. Units that failed to respond to a 10T stimulus were classified as receiving no inputs from a particular nerve. Because the afferents in the vagus nerve are mainly unmyelinated (Gwyn *et al.*, 1982; Jammes *et al.*, 1982; Asala & Bower, 1986), this nerve was stimulated using high current intensities of 1 mA-5mA, and if a neuron did not respond to vagus nerve stimulation using a five-shock train at 5 mA intensity, it was classified as being unaffected by that input. The brief stimulus trains applied to the vagus nerve had no obvious effects on either blood pressure or heart rate.

3.2.3 Analysis of responses to vertical vestibular stimulation.

Neural activity recorded during vertical vestibular stimulation was binned (500 bins/cycle) and averaged over the sinusoidal stimulus period. The approximate number of sweeps typically averaged at each frequency was as follows: 5-10 at frequencies ≤ 0.1 Hz, 15-25 at 0.2 Hz, 25-50 at 0.5 Hz, and 50-100 at 1.0 Hz. Sine waves were fitted to responses with the use of a least-squares minimization technique (Schor *et al.*, 1984b). The response sinusoid was characterized by two parameters: phase shift from the stimulus sinusoid (subsequently referred to as phase) and amplitude relative to the stimulus sinusoid (subsequently referred to as gain). Gain and phase measurements were then corrected for the dynamics of the tilt table. Responses were considered significant if the signal-to-noise ratio (see (Schor *et al.*, 1984b) for method of calculation) was > 0.5 and there was only one obvious first harmonic (see Fig. 1 for examples of significant responses).

The vertical vestibular response vector orientation was expressed using a head-centered coordinate system, with 0° corresponding to ipsilateral ear-down roll, 90° to nose-down pitch, 180° to contralateral ear-down roll, and –90° to nose-up pitch. In this coordinate system, the plane of the ipsilateral anterior semicircular canal is near 45°, the plane of the contralateral

anterior semicircular canal is near 135°, and the planes of the ipsilateral and contralateral posterior semicircular canals are near –45° and –135°, respectively.

Labyrinthine input to vestibular neurons can be determined by characterizing a vestibular neuron's dynamic responses to various frequency tilt stimuli. Neurons that have increasing gain responses to increased table velocity typically have a large phase lag and are characteristic of canal cell afferent input. Neurons that have flat gains with phase responses that correspond with table position typically receive otolith input. Neurons were divided into groups according to the dynamics of their responses to sinusoidal rotations. In particular, this classification was based on the increase in response gain that occurred per stimulus decade, typically over the range of 0.1-1.0 Hz. Neurons whose response gain increased little (< 3-fold) per stimulus decade were placed in one group, those whose response gain increased 3 to 7-fold per stimulus decade were placed in a second group, and neurons whose response gain increased sharply (>7-fold per stimulus decade) over the frequency range employed were placed in a third group. For the sake of this classification, we included responses whose signal-to-noise ratio was less than 0.5, as some neurons that were activated robustly by higher-frequency tilts failed to have significant responses to rotations at 0.1 Hz. A fourth group of neurons was comprised of cells for which incomplete data were gathered to determine the increase in response gain per stimulus decade, that had inconsistent responses from trial to trial, or had complex response dynamics that did not fit the other categories.

3.2.4 Analysis of responses to electrical stimulation of nerves

Post stimulus histograms were generated from neural activity recorded during electrical stimulation of nerves; approximately 50 sweeps were averaged to generate each histogram. The presence of responses to nerve stimulation was determined by qualitatively and quantitatively comparing neural activity following the shocks to that immediately before the stimuli. When the

onset of responses was apparent, the response latency was determined relative to the beginning of the stimulus train. During muscle nerve stimulation, we classified effects with thresholds between 1 and 2 times those required to elicit cord dorsum field potentials as being due to activation of large-diameter afferents (Group I or perhaps Group II); responses with thresholds between 2 and 5 T were considered to be elicited by group II afferents, and those with higher thresholds were presumed to be due to activation of group III/IV afferents (Edgley & Jankowska, 1987a, b). Similarly, responses to cutaneous nerve stimulation at thresholds < 5 times those necessary to evoke a cord dorsum potential were classified as being the result of activation of large fibers (group I/II), and those with higher thresholds were considered to be the result of activation of group III/IV fibers (Willis & Coggeshall, 1991). If a neuron did not respond to limb nerve stimulation using a 5-shock train at 10T intensity, it was classified as not receiving inputs from that nerve. Similarly, if a neuron did not respond to vagus nerve stimulation using a 5-shock train at 5 mA intensity, it was classified as being unaffected by that input. We realize that the smallest-diameter afferents may not have been activated by these stimuli, but it is unlikely that "nonspecific signals" carried by such afferents could be useful in signaling body position in space.

3.2.5 Histological procedures

After the animals were killed, the brainstem was removed and fixed by immersion in 10% formaldehyde solution. Sections (100 μm thick) were made in the transverse plane and stained using thionine. Locations of recorded neurons were reconstructed on standard sections with reference to placement of electrolytic lesions, relative locations of electrode tracks, and microelectrode depth.

3.3 **Results**

The neurons sampled for this study included 81 units in labyrinth-intact animals whose responses to both rotations in vertical planes and electrical stimulation of limb and visceral nerves were recorded. Furthermore, the responses of an additional 53 units to electrical stimulation of nerves were recorded in cats with chronic bilateral labyrinthectomies. Although we have previously demonstrated that activity of some vestibular nucleus neurons in bilaterally labyrinthectomized animals is modulated by vertical tilt (see Chapter 2), we did not perform rotations in vertical planes in labyrinthectomized animals during the present study, because a number of the nerves mounted for stimulation were cut distally, limiting the nonlabyrinthine inputs that might be elicited by whole-body movement.

In order to compare effects of electrical stimulation of limb nerves among experiments, we expressed stimulus intensities as multiples of the threshold required to elicit field potentials recordable from the cord dorsum near the dorsal root entry zone of the stimulated afferents. The threshold intensity for a particular nerve was similar across experiments. The median values for each nerve are as follow: superficial radial, 30 μA; deep radial, 40 μA; musculocutaneous, 38 μA; triceps, 45 μA; tibial, 40 μA; sural, 20 μA; gastrocnemius, 20 μA; tibialis anterior, 25 μA.

Examples of responses of one neuron in a labyrinth-intact animal to rotations in vertical planes and stimulation of visceral and limb nerves are shown in Figure 3.1. For every unit, we first determined responses to wobble stimuli typically delivered at 0.2 and 0.5 Hz and sometimes at lower frequencies as well. Figure 3.1A illustrates modulation of the neuron's activity during 0.5 Hz wobble stimulation at 5° amplitude. The response vector orientation determined for the

Figure 3.1

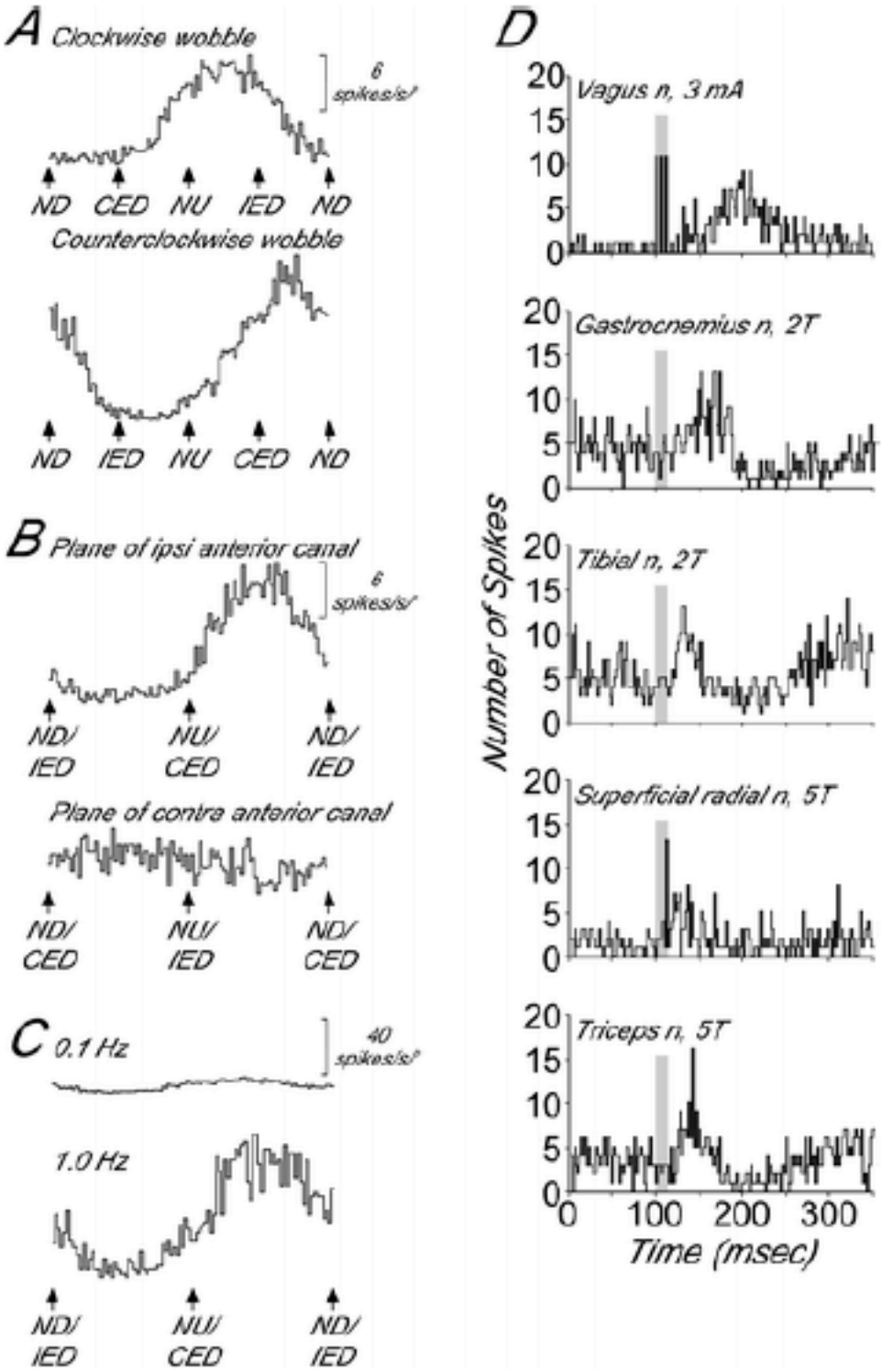
A Clockwise wobble
6 spikes/s
ND CED NU IED ND
Counterclockwise wobble
ND IED NU CED ND
B Plane of ipsi anterior canal
6 spikes/s
ND/ IED
NU/ CED
ND/ IED
Plane of contra anterior canal
ND/ CED
NU/ IED
ND/ CED
C 0.1 Hz
40 spikes/s
1.0 Hz
ND/ IED
NU/ CED
ND/ IED
D
Vagus n, 3 mA
Gastrocnemius n, 2T
Tibial n, 2T
Superficial radial n, 5T
Triceps n, 5T
Number of Spikes
0 5 10 15 20
0 100 200 300
Time (msec)

Fig. 3.1A–D Examples of data collected from one neuron, illustrating the methods used in this study. **A** Averaged responses to five clockwise and counterclockwise wobble stimulation delivered at 0.5 Hz. **B** Averaged responses to rotations (0.5 Hz, 5°) in the planes of the ipsilateral anterior canal/contralateral posterior canal as well as the contralateral anterior canal/ipsilateral posterior canal. **C** Averaged responses to 5° rotations at 0.1 Hz and 1.0 Hz in the plane of the ipsilateral anterior canal and contralateral posterior canal. To allow comparison of magnitude, the two traces are shown at the same gain in terms of spikes/s per degree. Because the bins for the 0.1-Hz trace reflect an average over ten times the time period as those for the 1.0-Hz trace (due to the fact that both waveforms contain the same number of bins), the noise in the 0.1-Hz trace appears smaller than at 1.0 Hz. **D** Poststimulus histograms indicating the responses of the unit to stimulation of several different nerves; all waveforms are the average of approximately 50 sweeps. For all nerves except for the vagus, the stimulus intensity employed is indicated relative to the intensity required to evoke a field potential recordable from the cord dorsum of the cervical or lumbar enlargements. Nerves were stimulated using a train of five shocks with an interpulse interval of 3 ms; a *shaded area* indicates the time at which the shocks were delivered. *CED* Contralateral ear down, *IED* ipsilateral ear down, *ND* nose down, *NU* nose up, *T* threshold

neuron (47°) was near the plane of the ipsilateral anterior canal, whereas the calculated response phase relative to stimulus position was approximately 90°. After wobble stimulation was performed, the effects of rotations in the roll and pitch planes as well as the planes of the vertical canals were determined. Figure 3.1B shows responses to 0.5 Hz, 5° tilts in the approximate planes of the semicircular canals. Note that whereas rotations in the plane of the ipsilateral anterior canal produced robust modulation of neuronal activity (signal-to-noise ratio of 1.45), rotations in the orthogonal plane failed to produce a response (signal-to-noise ratio of 0.27). These observations confirmed that the response vector orientation determined using the wobble stimulus was accurate. Subsequently, rotations were performed at several frequencies in a plane near that of the response vector orientation (in this case the plane of the ipsilateral anterior canal and contralateral posterior canal). The unit's responses to 5° planar tilts at 0.1 and 1.0 Hz are illustrated in Figure 3.1C. The response gain at 1.0 Hz was 9.8 times larger than that at 0.1 Hz, although at both frequencies the response phase was near stimulus velocity (the response phase led stimulus position by 111° at 0.1 Hz and 82° at 1.0 Hz). Following the characterization of responses to vertical vestibular stimulation, each nerve mounted on electrodes was stimulated in turn using a train of 5 shocks at a variety of intensities. Figure 3.1D shows examples of responses to electrical stimulation of nerves. Note that for all limb nerves stimulated, the unit's response consisted of an increase in activity, followed by inhibited firing and sometimes late rebound excitation. In general, all neurons whose activity was altered by nerve stimulation in this study expressed similar responses to stimulation of every effective nerve. Furthermore, stimulation also sometimes produced long-lasting changes in the unit's excitability, as reflected as an alteration in its background firing rate, but these effects did not appear to be related to changes in blood pressure or heart rate.

Figure 3.2 illustrates the dynamics of responses of another neuron to vertical vestibular stimulation (Panel A), as well as the unit's activity before and after stimulation of four limb nerves (Panel B). Note that response gain relative to stimulus position was relatively flat across stimulus frequencies (top of Figure 3.2A), and that response phase remained within 45° of position at all frequencies (bottom of Figure 3.2A). Unlike the unit whose responses are illustrated in Figure 3.1, this neuron's firing was inhibited at short latency by stimulation of limb nerves; this inhibition was typically followed by rebound excitation. Stimulation of the sural nerve also elicited a tonic elevation in the cell's baseline firing rate.

One goal of this study was to determine whether the presence of limb or visceral inputs to a neuron was related to the dynamics of its responses to vertical rotations. For this purpose, neurons recorded in labyrinth-intact animals were classified according to the increase of their response gain per decade change in stimulus frequency. Typically, we considered the change in response gain that occurred over the frequency range of 0.1-1.0 Hz for this analysis. Neurons were categorized as having a response gain that increased less than threefold per decade (29 cells, including the unit whose responses are illustrated in Fig. 3.2), three- to sevenfold per decade (21 cells), or more than sevenfold per decade (15 cells, including the unit whose responses are illustrated in Figure 3.1). A fourth group of 17 neurons was comprised of cells for which insufficient data were gathered to determine the increase in response gain per stimulus decade, that had inconsistent responses from trial to trial, that had inconsistent response vector orientations at different frequencies of rotation, or had complex response dynamics that did not fit the other categories. Figure 3.3 shows the mean response dynamics for neurons classified in different groups. To facilitate comparison of the data, the gain of responses recorded at each frequency for each neuron was expressed as a percent of the maximal response gain determined

Figure 3.2

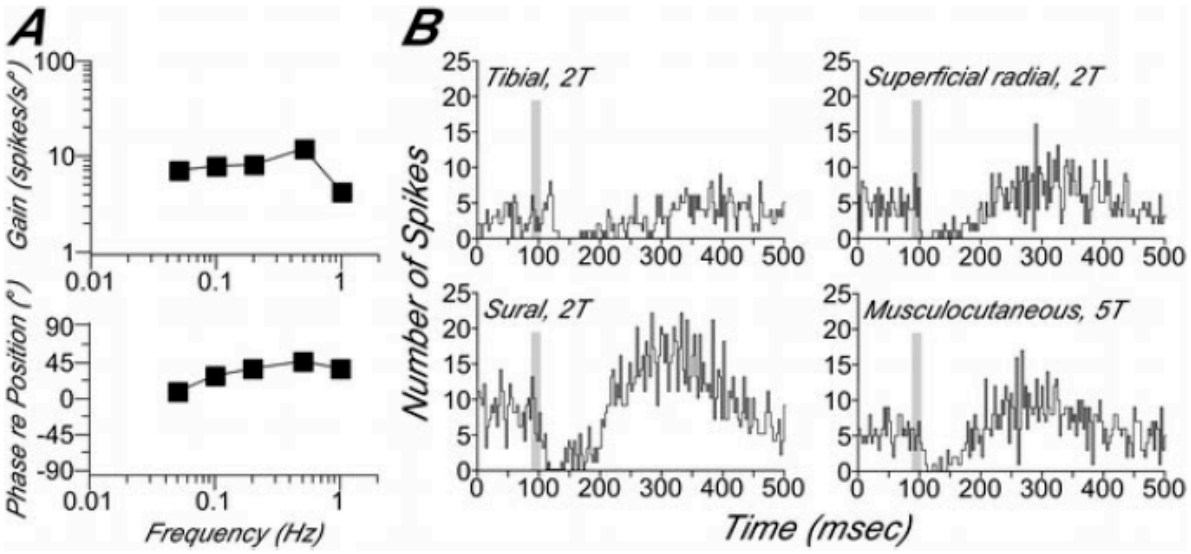

Fig. 2. A Dynamics of responses of another unit to vertical vestibular stimulation. The response vector orientation was 54° at 0.5 Hz, and the Bode plot was generated from data collected during tilts delivered at a 45°. Both response gain and phase are relative to stimulus position. **B** Averaged responses to electrical stimulation of four limb nerves; each trace reflects the average of approximately 50 sweeps. A *shaded area* indicates the latency at which the five-shock stimulus train was delivered; stimulus intensities are indicated relative to the threshold required to elicit field potentials recorded from the cord dorsum

Figure 3.3

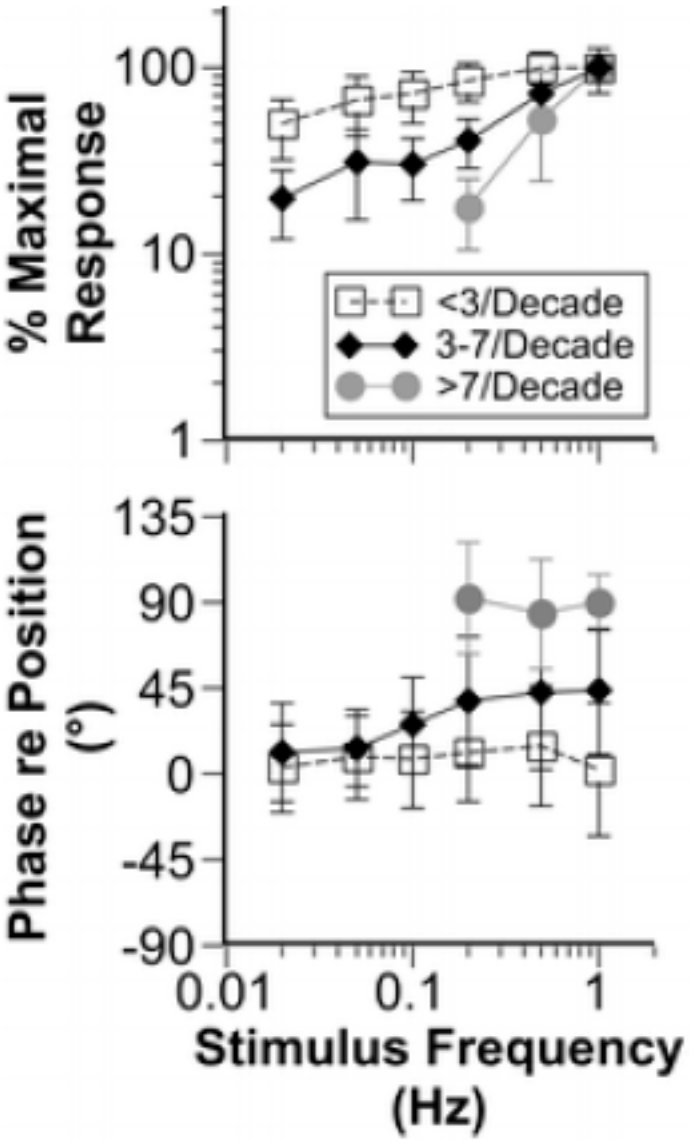
% Maximal Response
100
10
1
<3/Decade
3-7/Decade
>7/Decade
Phase re Position (°)
135
90
45
0
-45
-90
0.01
0.1
1
Stimulus Frequency (Hz)

Fig. 3.3 Bode plots illustrating mean response dynamics for neurons classified as having gain increases less than threefold per decade three- to sevenfold per decade, and greater than sevenfold per decade. Both response gain and phase are indicated relative to stimulus position; *error bars* represent one standard deviation. To facilitate comparison of the data, the gain of responses recorded at each frequency for each neuron was expressed as a percent of the maximal response gain determined for that cell at any frequency. Subsequently, the percent maximal gains determined for all neurons in a group were averaged. Many neurons classified as having large gain increases per stimulus decade did not respond significantly to rotations at frequencies less than 0.2 Hz (i.e., responses had signal-to-noise ratios less than 0.5 at these frequencies). Although such insignificant responses were considered in classifying a neuron into a group, they were not included in the data used for generating Bode plots. For this reason, we have excluded data points at frequencies less than 0.2 Hz for neurons whose response gain increased more than sevenfold per decade

for that cell at any frequency. Subsequently, the percent maximal gains determined at a particular frequency were averaged for all neurons in a group. In addition, the mean response phase with respect to stimulus position was determined at each frequency.

3.3.1 Patterns of convergence of limb and visceral inputs onto vestibular nucleus neurons whose firing rate is modulated by vertical vestibular stimulation.

A large majority of neurons whose firing rate was modulated by rotations in vertical planes also responded to stimulation of at least one of the nerves that was mounted on electrodes. As shown in Table 3.1, of the subset of 76 neurons in labyrinth-intact animals with responses to vertical tilt that were tested for both forelimb and hindlimb inputs, the firing of 55 was altered by stimulation of one or more limb nerves. Stimulation of limb nerves was even more effective in labyrinthectomized animals: 37 of 40 units tested for both forelimb and hindlimb inputs responded to stimulation of at least one nerve. A two-sided Fisher's exact test confirmed that limb nerve stimulation altered the firing of more neurons in labyrinthectomized than in labyrinth-intact animals ($p=0.015$). Vagus nerve stimulation altered the firing rate of 24 out of 76 tested units in labyrinth-intact animals, and 16 out of 33 tested neurons in labyrinthectomized preparations. However, a two-sided Fisher's exact test did not reveal that vagal inputs were more prevalent to vestibular nucleus neurons in labyrinthectomized that in labyrinth-intact animals ($p=0.13$).

Each nerve considered in this study provided inputs to the vestibular nuclei, although stimulation of some nerve altered the firing rate of more units than did stimulation of others (chi-squared test, $P=0.003$). Stimulation of mixed nerve such as superficial radial and tibial was more effective than stimulation of nerves innervating a single target such as a particular muscle. For example, in labyrinth-intact animals stimulation of the superficial radial nerve altered the firing of 55% of tested units, whereas stimulation of the triceps nerve only affected the firing of 30% of

the tested neurons. To evaluate the extent of convergence of inputs onto individual neurons, we determined the number of units that responded to stimulation of more than one muscle nerve. In labyrinth intact animals, 27 neurons were tested for inputs from triceps, gastrocnemius, and tibialis anterior. Of these neurons, the activity of 5 units was altered by stimulation of all three muscle nerves, whereas the activity of 6 cells was affected by stimulation of two of the nerves and 5 received inputs from only one of the muscles. The other 11 neurons did not respond to stimulation of any of the nerves. In labyrinthectomized animals, 10 neurons were tested for inputs from triceps, gastrocnemius, and tibialis anterior. None of these units responded to stimulation of all three muscle nerves, only one unit was affected by stimulation of two of the three nerves, 7 cells responded to stimulation of one of the nerves, and 3 were unaffected by muscle nerve stimulation. It is also useful to evaluate whether neurons received inputs from both tibialis anterior and gastrocnemius, as these two muscles are antagonists and rarely contract together. Of 43 units in labyrinth-intact animals tested for both gastrocnemius and tibialis anterior inputs, 11 responded to stimulation of the nerves innervating both muscles, 10 were affected by stimulation of only one of the two muscle nerves, and the firing of 22 cells was unaltered by stimulation of either nerve. Similar data were noted in labyrinthectomized animals. Thus, although some vestibular nucleus neurons receive widespread inputs from a number of peripheral sources, and their activity is likely to be altered by the limb and visceral inputs elicited during a wide range of movements, other cells receive more limited inputs and may only be affected by these signals during particular behaviors.

We also determined the stimulus intensities delivered to limb nerves, relative to the current strengths required to produce spinal cord field potentials, that elicited responses of

TABLE 1.

Unit Type	# Units Tested for Inputs from Both Limbs	Input from Both Limbs	Input from Forelimb Only	Input from Hindlimb Only	No Limb Input
Labyrinth-Intact Animals					
<3/Decade	27	10 (37%)	4 (15%)	1 (4%)	12 (44%)
3-7/Decade	19	12 (63%)	2 (11%)	1 (5%)	4 (21%)
>7/Decade	13	7 (54%)	2 (15%)	1 (8%)	3 (23%)
Unclassified	17	8 (47%)	4 (24%)	3 (18%)	2 (12%)
Total	76	37 (49%)	12 (16%)	6 (8%)	21 (28%)
Labyrinthectomized Animals					
	40	23 (58%)	14 (35%)	0 (0%)	3 (8%)

Table 1 Number of units responding to stimulation of nerves in the forelimb, hindlimb, or both limbs, in both labyrinth-intact and labyrinthectomized animals. Units from labyrinth-intact animals were subclassified as having gain increases less than threefold per decade, three- to sevenfold per decade, more than sevenfold per decade, or as being unclassifiable based on the criteria.

vestibular nucleus neurons. The interpretation of these data is clearest for responses to muscle nerve stimulation (i.e., triceps, gastrocnemius, or tibialis anterior), since there is a well-established correlation between stimulus strengths applied to these nerves (in relation to the threshold for producing spinal cord field potentials) and the diameters of afferents that are activated (Edgley and Jankowska 1987a, b). Of the 79 neurons recorded in labyrinth-intact animals that were tested for inputs from one or more particular muscles, 13 (16%) responded to stimulation of at least one muscle nerve at a threshold less than twice that required to elicit a spinal cord field potential. Such units are likely to receive muscle inputs from group I or II afferents (Edgley and Jankowska 1987a, b). In labyrinthectomized animals, of the 40 cells tested for inputs from at least one muscle nerve, 25 (63%) received inputs at thresholds less than twice the minimal intensity that produced spinal cord field potentials. A two-sided Fisher's exact test ($P<0.0001$) confirmed that a higher fraction of neurons in labyrinthectomized than in labyrinth-intact animals received inputs from group I or II muscle nerve fibers. These data raise the possibility that plasticity that occurs in the central vestibular system subsequent to removal of labyrinthine inputs increases the probability that firing of vestibular nucleus neurons will be modulated by inputs from large diameter muscle afferents.

The data presented in Table 3.1 are subdivided into groups based on the dynamics of responses of vestibular nucleus neurons to rotations in vertical planes. A similar fraction of neurons whose response gain increased less than threefold per stimulus decade, three to sevenfold per stimulus decade, and more than sevenfold per stimulus decade responded to stimulation of limb nerves or the vagus nerve. Furthermore, there were no obvious differences in the pattern of convergence of inputs onto vestibular nucleus neurons with different dynamic responses to vertical tilt. As illustrated in Figs. 3.1 and 3.2, each neuron typically exhibited similar response patterns (i.e., excitation, inhibition, or a combination of excitation and

inhibition) to stimulation of any effective nerve. Approximately half of the neurons examined in both labyrinthectomized and labyrinth-intact animals were mainly excited by nerve stimulation, whereas the firing of the other half was primarily inhibited. There was also no apparent correlation between the dynamics of a neuron's responses to vertical tilt and whether its firing was mainly excited or inhibited by nerve stimulation. These data suggest that during body movements that activate receptors in the limbs and viscera, non-labyrinthine inputs will increase the firing rate of some vestibular nucleus neurons but depress the firing rate of others.

3.3.2 Latency of vestibular nucleus neuronal responses to nerve stimulation

Table 3.2 lists the mean response latencies of vestibular nucleus neurons to stimulation of different nerves. All latencies were determined relative to the onset of the five-shock stimulus train. Because the minimal number of effective shocks was not determined, it is impossible to precisely determine the central conduction time required for peripheral inputs to reach the vestibular nuclei. Furthermore, in a few cases although the presence of a response to stimulation of a nerve was obvious, the onset latency of the response was not clear. Thus the "*n*" for the latency calculations is slightly lower than the number of units shown to receive inputs from a particular nerve. Nonetheless, because the difference between the latency of the last shock of trains delivered to limb nerves and mean latency of responses was 12–20 ms, it seems likely that multisynaptic pathways were mainly responsible for relaying peripheral inputs to the vestibular nuclei.

3.3.3 Responses of vestibular neurons are lengthened to limb stimulation

The average time to the 50% spike (T50) was determined for each nerve that was stimulated to see if duration of response was altered in labyrinthectomized animals. The T50s for

TABLE 2.

Latency of neuronal responses to nerve stimulation.

Latencies were determined from the onset of the stimulus train. Values represent mean ± one standard deviation.

Nerve	Latency, msec	
	Labyrinth-Intact Animals	Labyrinthectomized Animals
Triceps	33.3±23.6 (n=15)	41.1±35.9 (n=20)
Musculocutaneous	26.8±19.6 (n=30)	26.9±18.8 (n=32)
Superficial Radial	24.0±17.7 (n=41)	25.9±22.7 (n=27)
Deep Radial	23.1±16.0 (n=38)	32.8±21.2 (n=15)
Tibial	28.2±15.8 (n=41)	36.7±28.8 (n=22)
Gastrocnemius	32.8±21.3 (n=21)	35.9±19.8 (n=15)
Tibialis Anterior	26.5±19.5 (n=25)	28.8±21.8 (n=5)
Sural	28.1±10.0 (n=25)	52.2±83.3 (n=19)
Vagus	41.7±50.0 (n=20)	33.4±18.6 (n=13)

Table 2 Latency of neuronal responses to nerve stimulation. Latencies were determined from the onset of the stimulus train. Values represent mean $\pm$1 SD.

labyrinthectomized and normal animals were average and compared using an unpaired t-test with Welch correction. Of the nerves tested, responses were significantly longer in runs stimulating the musculocutaneous ($p=0.0179$), sural ($p<.0001$), tibial ($p=0.0122$), tricep ($p=.0082$), and vagus nerves ($p<.0088$). Figure 3.4 shows the averaged responses of vestibular neurons to electrical stimulation of the musculocutaneous (A), tibial (B) and tricept (C) nerves. The responses of these neurons were determined to be significantly different between the two groups. The left column are averaged responses of vestibular neurons in labyrinth intact animals, and the right column are the responses chronically labyrinthectomized animals. As evident of the figures, responses of vestibular neurons following the 5 train shock stimulus (50 msec) were either longer in duration (Fig 3.4a,b) or had altered excitatory/inhibitory properties (Fig 3.4c). Limb nerves that showed altered response properties represent a combination of cutaneous, pure muscle, mixed proprioceptor, visceral nerves, forelimb and hindlimb nerves. Likewise, stimulated nerves that were determined to *not* be significantly different from controls also represented these subtypes. The lengthening of duration of response to limb stimulation could allow for greater modulation of vestibular activity by ascending afferent input.

3.3.4 Response vector orientations of vestibular nucleus neurons that responded to peripheral nerve stimulation

The response vector orientations of vestibular nucleus neurons tested for the presence of non-labyrinthine inputs are shown in Fig. 3.5, and these orientations are displayed in three different panels based on the dynamics of responses of the neurons to rotations in vertical planes. The vertical vestibular response vector orientation was expressed using a head-centered coordinate system, with 0° corresponding to ipsilateral ear-down roll, 90° to nose-down pitch, 180° to contralateral ear-down roll, and –90° to nose-up pitch. In this coordinate system, the plane of the ipsilateral anterior semicircular canal is near 45°, the plane of the contralateral anterior

semicircular canal is near 135°, and the planes of the ipsilateral and contralateral posterior semicircular canals are near –45° and –135°, respectively. The response vector orientations shown in Fig. 3.5 were determined using wobble stimuli delivered at 0.5 Hz, although most neurons were also tested using lower frequencies of wobble rotations and the variability in vector orientation across frequencies was typically less than 10°. Approximately 75% of the neurons were better activated by roll than by pitch rotations, and about two-thirds of the units had response vector orientations ipsilateral to the direction of tilt. These data are similar to those reported in previous studies (Kasper *et al.*, 1988a; Wilson *et al.*, 1990). There were no obvious differences in the presence of peripheral inputs to vestibular nucleus neurons with different response vector orientations. Thus, inputs from the limbs and viscera modulate the activity of a majority of vestibular nucleus neurons that respond to vertical head movement in any plane.

3.3.5 Locations of vestibular nucleus neurons that responded to peripheral nerve stimulation

The locations of neurons tested for the presence of peripheral inputs are plotted in Fig. 3.6. The units were distributed across the entire rostral-caudal extent of the vestibular nuclei, and there was no obvious relationship between a cell's location and the likelihood that it would respond to limb or vagus nerve stimulation. Of the 134 neurons whose activity was recorded in these experiments (in both labyrinth-intact and labyrinthectomized animals), 42 of 57 (74%) units in the inferior vestibular nucleus, 30 of 38 (79%) units in the medial vestibular nucleus, 16 of 20 (80%) units in Deiters' nucleus, and 14 of 19 (74%) units in the superior vestibular nucleus responded to stimulation of at least one nerve. Because different regions of the vestibular nuclei have diverse physiological roles (Wilson and Melvill Jones 1979), these data suggest that inputs

Figure 3.4

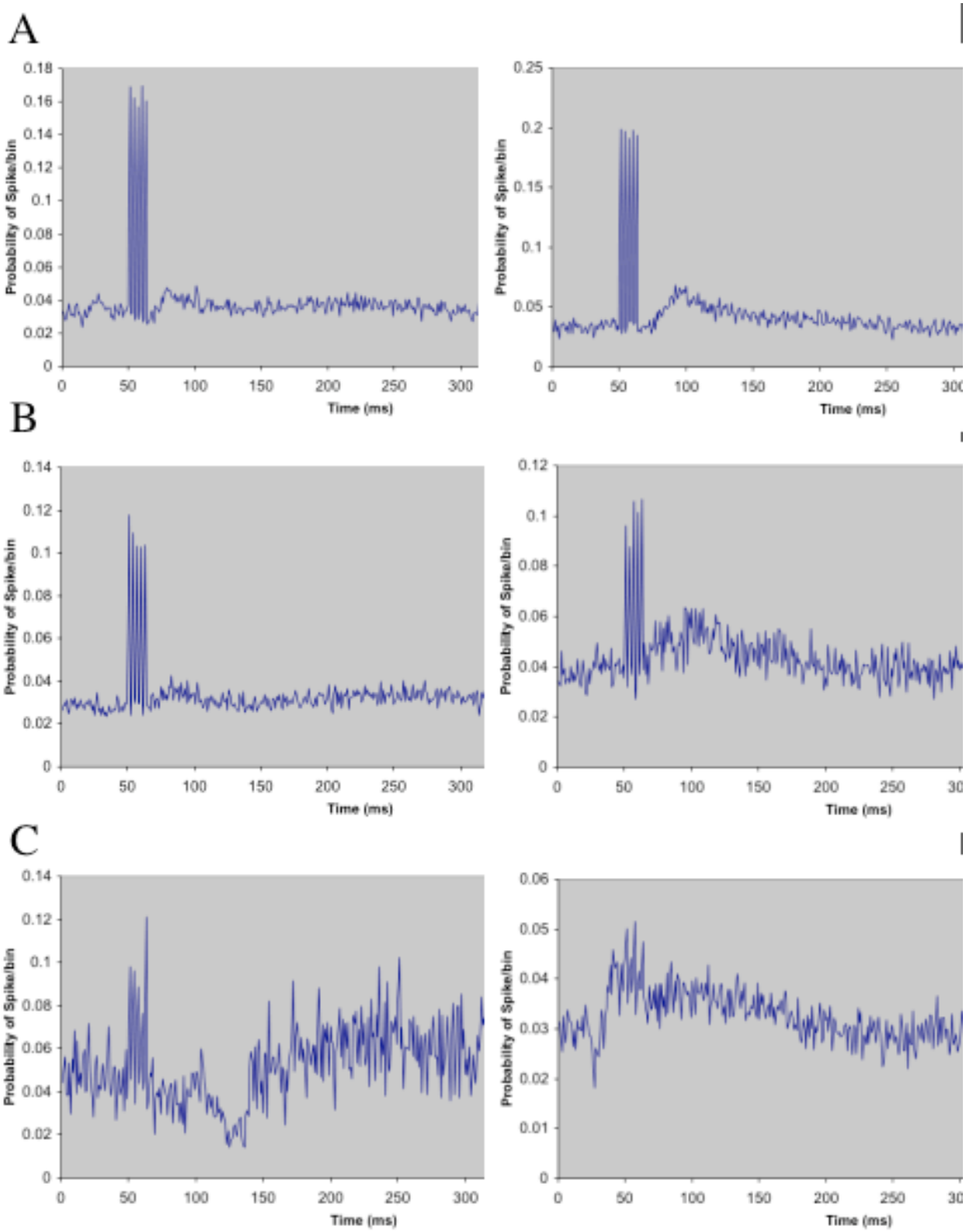

Figure 3.4 Averaged responses of vestibular neurons to stimulation of musculocutaneous (A), tibial (B), and tricep (C) nerves. Traces on the left are from normal animals, traces on the right are averaged responses from chronically labyrinthectomized animals, The five train spike stimulus was given at 50 milliseconds.

Figure 3.5

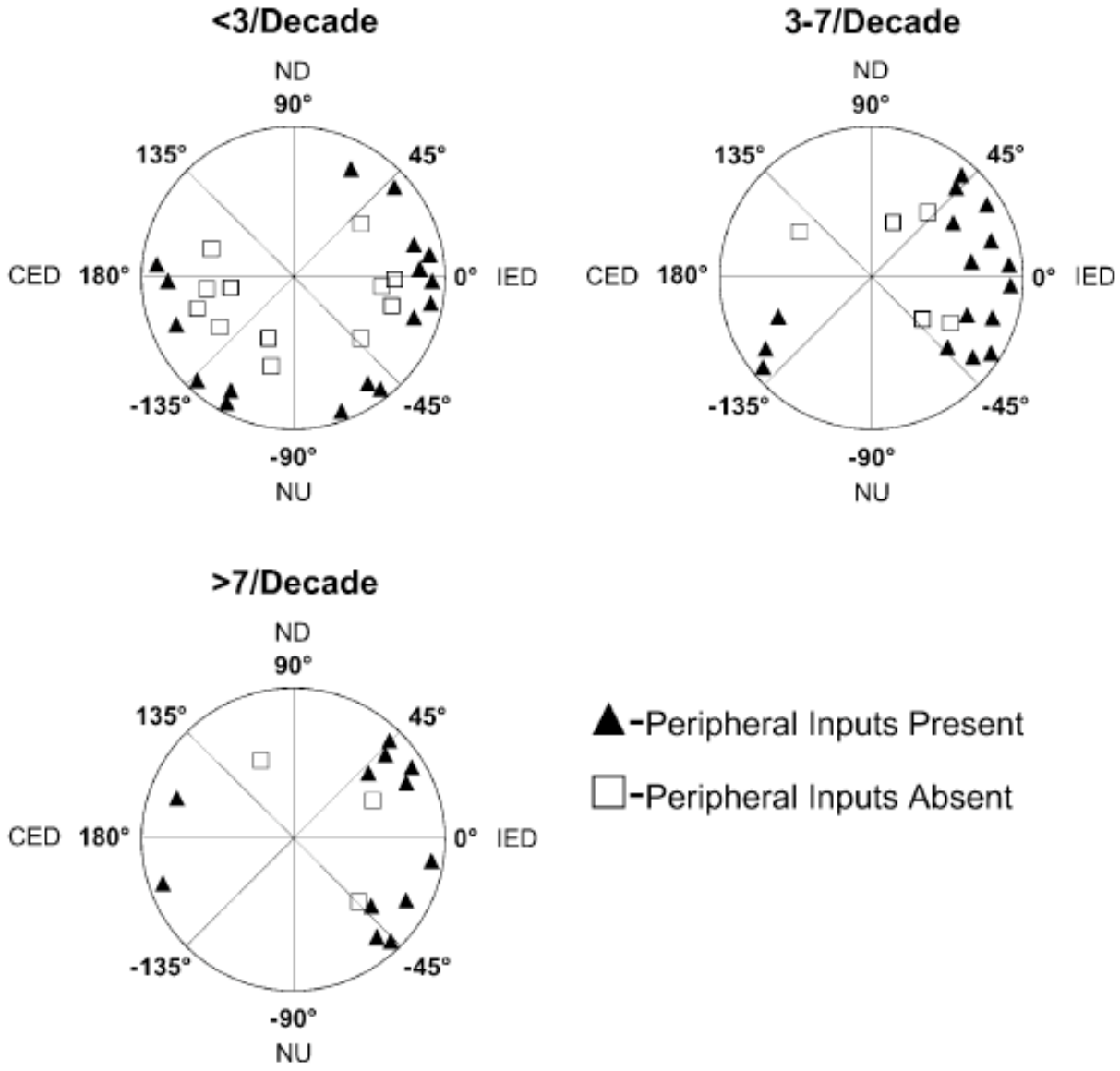
<3/Decade
ND
90°
135°
45°
CED
180°
0°
IED
-135°
-45°
-90°
NU
3-7/Decade
ND
90°
135°
45°
CED
180°
0°
IED
-135°
-45°
-90°
NU
>7/Decade
ND
90°
135°
45°
CED
180°
0°
IED
-135°
-45°
-90°
NU
▲-Peripheral Inputs Present
□-Peripheral Inputs Absent

Figure 3.5 Plot of response vector orientations, determined at 0.5 Hz, for neurons tested for the presence of inputs from the limbs and viscera. Response vector orientations are subdivided into three groups: those determined for neurons whose gain of responses to vertical tilts increased < 3-fold per decade, those for neurons whose response gain increased 3 to 7-fold per stimulus decade, and those for neurons whose response gain increased > 7-fold per decade. Response vector orientations for units whose response dynamics could not be classified are not shown. Abbreviations: CED, contralateral ear down; IED, ipsilateral ear down; ND, nose down; NU, nose up.

Figure 3.6

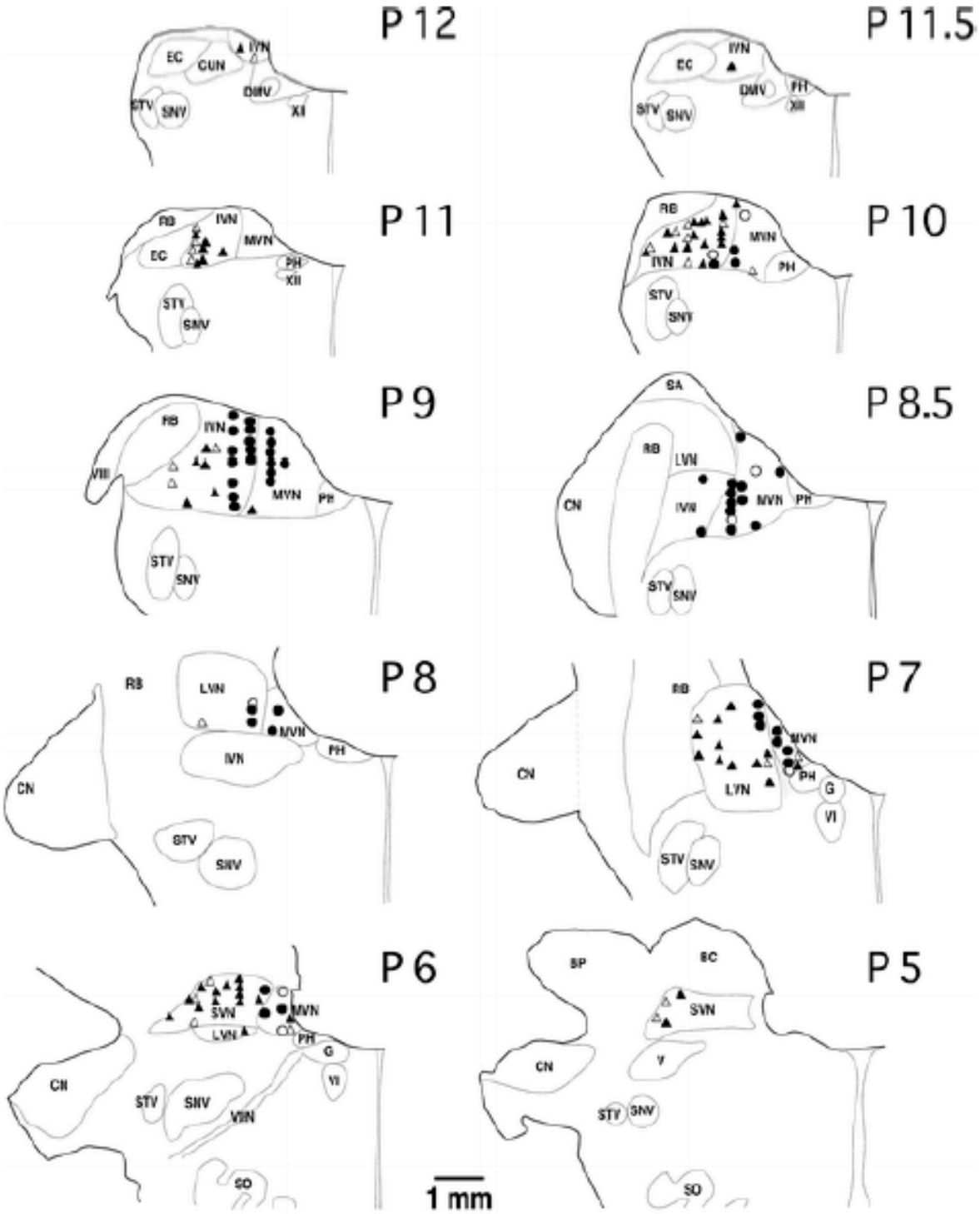
P 12
EC
CUN
IVN
DMV
STV
SNV
XII
P 11.5
IVN
EC
DMV
FH
XII
STV
SNV
P 11
RB
IVN
MVN
EC
PH
XII
STV
SNV
P 10
RB
MVN
IVN
PH
STV
SNV
P 9
RB
IVN
VIII
MVN
PH
STV
SNV
P 8.5
SA
RB
LVN
CN
IVN
MVN
PH
STV
SNV
P 8
RB
LVN
MVN
IVN
PH
CN
STV
SNV
P 7
RB
MVN
CN
PH
LVN
G
VI
STV
SNV
P 6
SVN
MVN
LVN
PH
G
CN
VI
STV
SNV
VIIN
SO
P 5
SP
SC
SVN
CN
V
STV
SNV
SO
1 mm

Figure 3.6 Location of all neurons whose activity was recorded in this study. Neuron locations are plotted on transverse sections of the brainstem. *Triangles* Neurons recorded in labyrinth-intact animals, *circles* units recorded in labyrinthectomized preparations. *Filled Symbols* indicate neurons that received non-labyrinthine inputs, whereas *open symbols* indicate cells that did not respond to nerve stimulation. *Values to the right of each section* indicate the relative distance (in mm) posterior to stereotaxic zero; the level of the obex was ~ P13.5. Abbreviations: BC, brachium conjunctivum; BP, brachium pontis; CUN, cuneate nucleus; DMV, dorsal motor nucleus of the vagus; EC, external cuneate; IVN, inferior vestibular nucleus; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus PH, prepositus hypoglossi; RB, restiform body; SA, stria acoustica; CN, cochlear nucleus; SNV, spinal trigeminal nucleus; SO, superior olive; STV, spinal trigeminal tract; SVN, superior vestibular nucleus; V, trigeminal nucleus; VI, abducens motor nucleus; VIIG, genu of facial nerve; VIIN, facial nerve; VIII, vestibulo-cochlear nerve; XII, hypoglossal nucleus.

from the limbs and viscera influence the majority of functions subserved by the central vestibular system.

3.4 Discussion

The present data demonstrate that the majority (72%) of vestibular nucleus neurons whose activity is modulated by vertical tilt also receive inputs from the limbs. Furthermore, we showed that vagus nerve stimulation alters the firing rate of vestibular nucleus neurons, thereby supporting the hypothesis that visceral inputs modulate physiological processes mediated by the central vestibular system (Mittelstaedt, 1996; Mittelstaedt & Mittelstaedt, 1996). In addition, we found that the activity of vestibular nucleus neurons in chronically labyrinthectomized animals is affected by stimulation of peripheral nerves, which bolsters the presumption that nonlabyrinthine inputs provide for the recovery of spontaneous activity of these neurons following elimination of vestibular inputs, as well as modulation of firing of some cells during vertical rotations (Chapter 2). In combination, the present data support the notion that whole-body movements in vertical planes elicit a variety of sensory inputs that are integrated by the central vestibular system.

Previous studies showed that only a small fraction of vestibular nucleus that respond to horizontal rotation receive inputs from the limbs (Rubin *et al.*, 1977a; Kasper *et al.*, 1986a), which is in contrast to the findings of the present experiments showing that the majority of vestibular nucleus neurons responding to vertical tilt were affected by stimulation of limb nerves. Although we did not examine the prevalence of nonlabyrinthine projections to horizontal rotation sensitive vestibular neurons, a comparison of the present data with previous data sets raises the possibility that spinal input to horizontal and vertical sensitive vestibular neurons is differentially distributed. In studies examining spinal input to neurons that responds to horizontal rotations (Fredrickson *et al.*, 1966), a lower preponderance of spinal input was observed compared to the

present study. However, for the neurons that responded to tilts in vertical planes, there was no apparent relationship between the plane of tilt that produced maximal activation and the likelihood that the cell received limb or vagal inputs. Furthermore, neurons whose response gains increased < 3-fold per decade, 3 to 7-fold per decade, and > 7-fold per decade received similar nonlabyrinthine inputs. Although previous studies have made conclusions regarding the vestibular end organs (i.e., otolith organs or specific semicircular canals) providing inputs to a neuron based on the dynamics of the cell's responses to vertical rotations (Kasper *et al.*, 1988b; Wilson *et al.*, 1990; Bolton *et al.*, 1992; Endo *et al.*, 1994), recent data have suggested that this approach can be problematic as otolith organ inputs can be transformed by the central nervous system to resemble signals from semicircular canals (Angelaki & Dickman, 2000). Nonetheless, because a majority of units with a wide range of response dynamics to vertical tilt were affected by limb and vagus nerve stimulation, it seems likely that neurons with either vertical semicircular canal or otolith organ inputs receive nonlabyrinthine inputs.

Some units received inputs from multiple sources, including both agonist and antagonist muscles acting at the same joint, as was noted in previous studies considering convergence of inputs onto vestibular nucleus neurons (Fredrickson *et al.*, 1966; Wilson *et al.*, 1966a; Rubin *et al.*, 1977b; Rubin *et al.*, 1978; Rubin *et al.*, 1979a). It seems unlikely that these inputs conveyed specific information regarding body position in space, but rather served to alter neuronal firing during a variety of whole-body movements. Thus, the effects of labyrinthine inputs to neurons receiving convergent inputs from a variety of peripheral sources may either be amplified or reduced during whole-body movements depending on whether the nonlabyrinthine inputs to the cells are excitatory or inhibitory. Furthermore, the extent to which widely convergent nonlabyrinthine inputs influence the firing of vestibular nucleus neurons is likely to be graded based upon the speed and magnitude of ongoing movements, as large, rapid movements should

provide more signals than small, slow ones. However, some neurons received inputs from more limited sources, suggesting that vestibular-elicited responses produced through these cells would be modulated by nonlabyrinthine signals only during selective behaviors. Thus, inputs from the limbs and viscera to the vestibular nuclei may play a variety of roles, depending on the extent of convergence of signals to a particular neuron.

Another finding of this study is that a majority of neurons in all four vestibular nuclei that responded to vertical tilts received inputs from the limbs or viscera. Although we did not determine the projections and physiological roles of the neurons that were recorded, it is well established that neurons in different regions of the vestibular nucleus complex subserve somewhat different functions (Wilson & Melvill Jones, 1979). Thus, it seems likely that nonlabyrinthine inputs modulate a variety of responses produced or influenced by the central vestibular system.

The present data also demonstrated that vestibular nucleus neurons in animals with chronic bilateral labyrinthectomies can respond to inputs from the limbs and viscera and that they may be more likely to receive nonlabyrinthine inputs (particularly from large-diameter muscle afferents) than vestibular nucleus neurons in labyrinth-intact animals. One caveat to this conclusion is that we tested all spontaneously active neurons in labyrinthectomized animals for inputs from the limbs and viscera, whereas we only studied neurons in labyrinth-intact animals that responded to vertical tilts. Nonetheless, these data show that nonlabyrinthine inputs can modulate vestibular nucleus neuronal activity following elimination of vestibular signals and may participate in the recovery of spontaneous activity of these cells (Waespe *et al.*, 1992; Ris & Godaux, 1998a). Furthermore, as noted above, the activity of vestibular nucleus neurons receiving nonlabyrinthine inputs from limited sources is likely to only be affected by particular body movements. Thus, inputs from the limbs and viscera to vestibular nucleus neurons of

bilaterally labyrinthectomized animals may underlie the observation that activity of approximately one-fourth of these cells is modulated during rotations in specific vertical planes (Chapter 2).

A final issue to be considered while interpreting the current data is that they were collected from decerebrate cats; translating these findings to awake primates should be done cautiously. There is current debate in the literature concerning the extent of influences of proprioceptive inputs from the neck on the activity of vestibular nucleus neurons in awake primates (Gdowski *et al.*, 2000), and there are few data concerning inputs from the limbs and viscera to the vestibular nuclei in primate species. Nonetheless, because the present experiments demonstrate that many feline vestibular nucleus neurons receive signals from cutaneous and muscle receptors throughout the body as well as from visceral receptors, it is difficult to imagine that these inputs do not play some role in modulating signals from the labyrinth in all mammals, at least under some circumstances. Furthermore, our observation that the effects of limb and visceral inputs on the activity of vestibular nucleus neurons may be amplified following loss of labyrinthine signals raises the possibility that these signals participate in recovery of function following damage to the inner ear or VIIIth nerve. For example, previous studies have shown that although removal of vestibular inputs compromises the ability to make adjustments in blood pressure (Jian *et al.*, 1999) and respiratory muscle activity (Cotter *et al.*, 2001b) during changes in posture, recovery occurs over time. The present data are consistent with the notion that the central vestibular system participates in this compensation, as it still receives nonlabyrinthine inputs that can signal when the body is changing position in space. Furthermore, vestibular nucleus neurons that integrate limb or visceral signals from limited body regions may still be able to encode movements in particular planes and trigger compensatory responses during movements that require adjustments in blood distribution in the body or in respiratory muscle

activity, even following the complete loss of vestibular inputs. If these suppositions are correct, then lesions of the vestibular nuclei would be expected to produce more severe and prolonged autonomic disturbances during movement than bilateral labyrinthectomies. Testing this hypothesis should provide considerable insights regarding the functional significance of the processing of nonlabyrinthine inputs by the vestibular nuclei.

4 General Discussion

The studies in this dissertation sought to evaluate the role of the vestibular nuclei and spinovestibular afferents in the role of recovery following bilateral eighth nerve and labyrinth lesions. Normally the vestibular nuclei receive signals from several sensory sources. The predominant afferent signal arises from the vestibular labyrinth and is responsible for the characteristic firing activity of vestibular neurons during movement. The resulting sensitivity to changes in posture is then used to coordinate responses to several motor output systems. The vestibulo-ocular, vestibulospinal, and vestibulo-autonomic systems are the major output paths of the vestibular nuclei. Following bilateral labyrinthectomy spiking activity in the vestibular nuclei ceases. Over a period of time the vestibular nuclei reorganize and the nuclei regain the ability to discriminate movements of the body. The reorganization process is considered to be a mechanism that incorporates the strengthening of connections between nonlabyrinthine inputs and the vestibular nuclei.

In Chapter 2 we demonstrated that approximately one quarter of the neurons in the vestibular nuclei of bilaterally labyrinthectomized animals modulate their activity in response to changes in body position. This modulation occurs despite the absence of the vestibular nuclei's primary source of positional information, the vestibular labyrinths. The work from Chapter 2 is the first evidence that demonstrates that neurons in the vestibular nuclei -- following recovery -- do not need labyrinthine input in order to detect changes in body position. This data set supports the theory that behavioral recovery occurs as a result of the vestibular nuclei regaining their sensitivity to changes in body position. The return of function in the vestibular nuclei suggests a reorganization of remaining sensory inputs that promote the behavioral recovery process.

In Chapter 3, the spinovestibular pathways of chronically labyrinthectomized animals were characterized by a battery of stimuli to various forelimb and hindlimb nerves. Spontaneously active neurons in the vestibular nuclei of chronically labyrinthectomized animals were found to have a greater probability of responding to stimulation of distal limb nerves than normal animals. In a subset of these responses significant lengthening of the average duration of response to electrical stimulation was also observed to occur. These findings agree with the general supposition that connections between the limbs and neurons in the vestibular nuclei are altered following bilateral labyrinthectomy. Vestibular neurons of chronically labyrinthectomized animals have increased sensitivity to limb nerve stimulation. This alteration supports our general hypothesis that reorganization of connections in the vestibular nuclei leads to increased control by spinal afferents of activity of vestibular neurons.

4.1 Reorganization is reflected by modulation of neuronal activity

Evidence of a return in resting activity of the vestibular nuclei following unilateral and bilateral labyrinthectomy has been well documented. Evidence from Chapter 2 has added to this body of literature by demonstrating that the return in resting activity is later followed by a return in sensitivity to changes in body position following *bilateral* labyrinthectomy. Average resting rate and variability of spiking activity of neurons in the bilaterally labyrinthectomized animals are similar to control animals. These spiking characteristics are likely to be important in the function of vestibular neurons. The modulation of vestibular neuronal activity is reflective of a reorganization that occurs following bilateral deafferentation of the labyrinths.

The spinovestibular reorganization that occurs in the vestibular nuclei results in altered vector orientations of neurons in the vestibular nuclei. This observation is most evident in the vector orientation plots (see Figure 2.2, page 36) demonstrating a population of neurons that

preferentially code towards the pitch planes. Although it is unclear as to why the chronically labyrinthectomized animal population directional sensitivities code preferentially to the pitch plane, it is likely a result of the need for the organism to determine exact position of the body in the pitch plane. The remaining non-labyrinthine afferents tuning sensitivities for pitch movements is of considerable interest, since pitch related movements would have the greatest impact on autonomic control (Yates, 1996a; Yates *et al.*, 1998b). Given that the vestibulo-autonomic control regains near-complete recovery following bilateral labyrinthectomy, unlike the vestibulo-ocular system (Curthoys & Halmagyi, 1999; Curthoys, 2000), is likely not of a coincidental finding. The fact that the compensated animal is more suited to sensing changes in posture in the pitch direction predisposes the animal to combating orthostatic challenges at the expense of adapting to changes in horizontal posture, such as visual tracking of targets in its environment. Consequently if recovery of vestibular function was a limited process, it is of considerable value that vestibulo-autonomic recovery would take precedent before vestibulo-ocular control.

4.2 Evidence that spinal inputs alter vestibular neuronal activity

Spinovestibular modulation of vestibular neuronal activity is more prevalent in the chronically labyrinthectomized animal. This increased preponderance of inputs supports the notion that the recovery of directional sensitivity observed in Chapter 2 is mediated by limb input. Transections of the spinal cord, vagus nerves, optic pathways, and cerebellum in our procedural setup identify spinal input as the mediator of vestibular neuronal firing.

4.2.1 Reorganization of connections between spinal afferents and neurons in the vestibular nuclei.

Ninety three percent of spontaneously active neurons in chronically labyrinthectomized animals maintain some type of spinovestibular connection (see Chapter 3). This percentage is likely to be an underestimate of the actual relationship between spontaneously firing vestibular neurons and spinal innervation, because only eight limb nerves were stimulated to examine potential spinal innervation. The seven percent of spontaneously firing neurons that did not respond to our battery of stimuli could have received dominant spinal innervation from other sources such as the trunk or girdle muscles. This possibility is further supported by anatomical studies that identify the majority of possible monosynaptic (McKelvey-Briggs *et al.*, 1989; Matsushita *et al.*, 1995) and polysynaptic (Jian, Acernese, Lorenzo, Card Yates unpublished findings) connections that project to the vestibular nuclei are from spinal cord regions that typically control limb and trunk muscles. Findings from these studies suggest that distal limb input to the vestibular nuclei is of lesser magnitude than proximal proprioceptive or cutaneous information from the trunk of hips. Consequently, studies examining limb innervation of the vestibular nuclei are likely to underestimate the proportion of spinal innervation of the vestibular nuclei. This conclusion is in accord with the general notion that proximal muscle information is more informative of body posture then distal limb information (Mittelstaedt, 1992, 1996). Future studies will need to determine the extent of control proximal proprioceptive information has on the modulation of vestibular neuronal activity.

4.3 Reorganization of other non-labyrinthine afferents may mediate recovery

Several lines of evidence exist that also implicate other nonlabyrinthine afferents in the recovery of vestibular neuronal function. Inputs from vision, the cerebellum, and the viscera are capable of sensing motion and relaying the signals to the vestibular nuclei for processing.

4.3.1 Data supporting a visceral mediated recovery of function

In a subset of animals in chapter 2, the spinal cord was acutely transected in chronically labyrinthectomized animals prior to recording vestibular activity in response to tilts. In this subset, only one neuron (1%) was determined to modulate its activity in response to vertical tilt stimulus. This data set is a bit surprising given the data gathered in chapter 3 that demonstrated that approximately 50% of neurons in the vestibular nuclei respond to electrical stimulation of the vagus. This discrepancy could be potentially explained by the relatively small amplitude stimulus (15°) that was used to invoke the responses. Electrical stimulation, on the other hand, would effectively stimulate all potential vagovestibular connections and give a more accurate representation of visceral projections to the vestibular nuclei. In chapter 3 data gathered from both chronically labyrinthectomized and normal animals demonstrate that vagal input to the vestibular nuclei remains unchanged following labyrinthectomy. We may infer that visceral information to the vestibular nuclei is important in relaying vertical movements of large amplitudes, such as upright posture, climbing, and falling but not for compensation of routine movements.

Anecdotal and personal accounts of movements of the internal organs during falls, such as during amusement park rides, agree with the data presented in these chapters. Theoretical demonstrations (von Gierke & Parker, 1994; Mittelstaedt, 1996) of visceral signals being mediated by stretch receptors in the walls of the intestines (Drewes 2003, Berthoud 2001, Zagorodnyuk 2000) further substantiate our claims of visceral modulation of vestibular neuronal activity. Given the current data set, it is difficult to argue for a significant role of visceral signaling of posture during normal locomotion and posture. Furthermore it is unlikely that these inputs are used in the chronically labyrinthectomized animal to return the vestibular nuclei to function. Future studies will need to be carried to out determine the role of the viscera in modulating neuronal activity in the bilaterally labyrinthectomized animals. Fast accelerative,

large amplitude natural stimulations should determine if these inputs are functionally active in the labyrinthectomized condition.

4.3.2 Data supporting a visually mediated recovery of function

As previously mentioned in chapter 1, there is considerable evidence that implicates visual modulation of activity of vestibular neurons (Smith *et al.*, 1986; Giolli *et al.*, 1988; Eyeson-Annan *et al.*, 1996; Sato *et al.*, 1996; Anand *et al.*, 2003). Visual input is likely to be a considerable source of afferent input in the awake chronically labyrinthectomized animal. Evidence exists to suggest that visual input can modulate vestibulo-autonomic and vestibulospinal responses only 24 hours after labyrinthectomy (Park *et al.*, 1995; Jian *et al.*, 1999). Consequently it is likely that visual information to the vestibular nuclei does not need to undergo as much synaptic reorganization in order to modulate activity of vestibular neurons. This is in contrast to data gathered from this work that suggest spinovestibular synaptic reorganization needs to occur before it regains control of vestibulo-autonomic control (Jian *et al.*, 1999). This recovery process is likely to occur over a period of a week, since regulation of vestibulo-autonomic control has been observed to return at this time.

Studies (Takeda *et al.*, 1995; DiZio *et al.*, 1997; Reschke *et al.*, 1998; Previc *et al.*, 2000) have linked visual sensory information with the perception of movement and position in space. Although the sensation of gravito-inertial frame of reference is rarely fooled in the normal animal, in patients with bilateral vestibular damage visual information is more heavily relied upon (Maurer *et al.*, 2000; Suarez *et al.*, 2001). These patients suffer from imbalance, and unstable autonomic regulation in situations with limited visual cues for spatial reference. The modulation of vestibular neuronal activity observed in Chapters 2 and 3 are not a result of this descending source of visual information. Data obtained from both studies carried out in Chapters 2 and 3 occurred in decerebrate animals. This procedure removes all potential visual

pathways to the vestibular nuclei. Consequently, modulated activity observed in the vestibular nuclei could not be explained by modulation via the visual system. Similar studies carried out in awake behaving animals are likely to find that a greater proportion of neurons in the vestibular nuclei sense change in body position. Afferents from the accessory optic system are likely sources of visuo-vestibular modulation (Barnes, 1993; Sato *et al.*, 1996; Vargas *et al.*, 1996; Severac Cauquil *et al.*, 1997; Yakushin *et al.*, 2000).

4.3.3 Data supporting a cerebellar mediated recovery of function

The last major source of afferent input to the vestibular nuclei is from the cerebellum. The cerebellum coordinates cortical, spinal, and vestibular signals in order to produce a coordinated organism. Patients with cerebellar strokes suffer from severe incoordination and balance. It is generally accepted that cerebellar and vestibular function are highly integrated and that vestibular plasticity is dependent upon proper cerebellar function. Animals that have undergone damage of portions of the vestibulocerebellum and fastigial nucleus prior to destruction of the labyrinths have shown limited recovery (Holmes *et al.*, 2002). In contrast, recovery is observed in these same autonomic systems when the cerebellar lesion is performed some time *after* labyrinthectomy. This phenomenon suggests that the cerebellum modulates vestibular activity in response to changes in afferent input or motor learning. Its role in vestibular modulation during normal function is likely to be limited.

In the works in this chapter, we did not precisely control cerebellar input. In both studies portions of the cerebellum were removed in order to gain access to vestibular nuclei. Unfortunately a systematic process was not carried out in order to effectively evaluate our results in relation to the varied amount of cerebellum removed across experiments. Consequently in order to effectively evaluate the cerebellar control of vestibular neuronal activity in the labyrinthectomized animal future studies will need to be performed. Regardless of these results,

the cerebellum is a large source of modulation and plasticity of the vestibular nuclei. Its general function is a requisite factor in vestibular compensation following labyrinthine insult.

4.4 Physiologic relevance of vestibulcochlear nerve afferents

"No study, however, has correlated any physiologic properties beyond spontaneous discharge patterns to anatomical distribution." (Newlands & Perachio, 2003)

4.5 Is vestibular compensation a product of reorganization of spinal or vestibular neurons?

Determining the neurochemical makeup of the spinovestibular pathways should allow a further understanding of the relationship between spinal afferents and the vestibular nuclei following labyrinthectomy (Clegg & Perachio, 1985). Stimulation of limb and visceral nerves is known to produce varied excitatory and inhibitory responses in the vestibular nuclei. It is clear that portions of these pathways are direct connections from neurons in the cervical and lumbar spinal segments (McKelvey-Briggs *et al.*, 1989; Matsushita *et al.*, 1995). However based on latency data, a greater proportion of inputs appear to be polysynaptic in nature (see Table 3.2, page 70). Hence, the responsible final-order neuron that synapses on the neuron in the vestibular nuclei could be one of a number of cell types releasing any number of known neurotransmitters (cholinergic (Kirsten & Sharma, 1976; Tohyama *et al.*, 1979; Matsuoka *et al.*, 1984; Perachio & Kevetter, 1989; Takeshita *et al.*, 1999), glutamatergic (de Waele *et al.*, 1990; Walberg *et al.*, 1990; Kevetter, 1992; Smith & Darlington, 1994; Soto *et al.*, 1994), serotonergic (Kevetter, 1992; Licata *et al.*, 1992; Johnston *et al.*, 1993; Licata *et al.*, 1993; Sachanska, 1996), dopaminergic (Matsuoka *et al.*, 1984; Cransac *et al.*, 1996), noradrenergic (Pompeiano, 1989; Andre *et al.*, 1991; Steinbusch, 1991; Pompeiano, 1994; Podda *et al.*, 2001) and GABAergic (Walberg *et al.*, 1990; Fredette & Mugnaini, 1991; Calza *et al.*, 1992; Luccarini *et al.*, 1992). Alterations in the synthesis, release, binding, reuptake, or receptor distribution of anyone of these systems, could lead to recovery. Anatomic identification of these potential polysynaptic pathways is currently underway to determine candidate brainstem and cerebellar relay centers of ascending spinal information (unpublished results). Current lines of evidence have examined the roles of glutamatergic and GABAergic transmission following labyrinthectomy.

Although multiple mechanisms and potential structures may be involved in vestibular compensation, it is evident that plasticity does occur in the spinovestibular system. The cellular mechanisms underlying vestibular compensation, however, are still not thoroughly understood. Many studies provide evidence for synaptic or neurochemical changes in the vestibular nuclei and related central structures ((Smith & Curthoys, 1989; Curthoys & Halmagyi, 1995; Dieringer, 1995). It has been suggested that presynaptic changes, such as substitution of non-vestibular sensory inputs via synaptogenesis, as well as postsynaptic modifications take place during vestibular compensation. Some authors have proposed an up-regulation of N-methyl-D-aspartate (NMDA) receptors within ipsilesional MVN ((de Waele *et al.*, 1990; Smith *et al.*, 1991; Smith & Darlington, 1992; Sans *et al.*, 1997) de Waele et al., 1994, 1995) or amplification of intrinsic membrane properties ((Precht & Dieringer, 1985; Smith & Curthoys, 1989; Smith & Darlington, 1991) de Waele et al 1988; Flohr et al 1989). However, recent studies have determined that glutamate receptor synthesis, degradation, and subunit composition are not likely mechanisms of recovery following labyrinthine deafferentation (Kitahara *et al.*, 1998a; Horii *et al.*, 2001) Other authors have shown a down regulation of the functional efficacy of GABA postsynaptic receptors in the ipsilesional MVN and re-balancing of the excitability of the lesioned and intact sides, as occurs after unilateral labyrinthectomy (Yamanaka *et al.*, 2000). Studies administering GABA analogues and antagonists have demonstrated altered properties following labyrinthectomy (Magnusson *et al.*, 2000; Yamanaka *et al.*, 2000; Him *et al.*, 2001; Johnston *et al.*, 2001). These findings agree with theories that explain vestibular compensation as a mechanism that is regulated by changes in inhibitory modulation by cerebellar efferents (Castro & Smith, 1979; Dieringer & Precht, 1979a; Furman *et al.*, 1997; Goto *et al.*, 1997; Kitahara *et al.*, 1997; Kitahara *et al.*, 1998b; Darlington & Smith, 2000)

Despite the findings that glutamate receptor synthesis and degradation is likely unaffected following labyrinthectomy, a number of potential secondary pathways allow for modification of synaptic transmission. It is likely that other secondary cascade systems are being regulated to alter the response properties of these receptors. Accompanying this growing body of evidence of glutamatergic transmission, it is evident that within the vestibular nuclei long term potentiation (LTP) and depression (LTD) occur following peripheral labyrinthine destruction (Grassi *et al.*, 1995a; Grassi *et al.*, 1995b; Grassi *et al.*, 2001; Grassi *et al.*, 2002). Most knowledge of LTP and LTD mechanisms has been derived from advances in the field of glutamate receptor biology, examining behavioral memory. These findings are of a great deal of potential value, since a wealth of information has been gleaned from studies characterizing synaptic reorganization in the hippocampus. Advancements in the understanding of the mechanisms underlying memory and synaptic reorganization brought to the vestibular field should prove to expand our knowledge of the cellular and molecular mechanisms behind vestibular compensation.

4.6 Is recovery of the functional activity vestibular neurons requisite for behavioral recovery?

Evidence demonstrating the vestibular nuclei are a necessary component of the recovery process remains scant. Recovery of function may also occur through other brain regions, including the cortex as well as cerebellar control (Precht, 1974; Castro & Smith, 1979; Dieringer & Precht, 1979a; Cirelli *et al.*, 1996; Kitahara *et al.*, 1997; Smith, 1997; Kitahara *et al.*, 1998b; Giardino *et al.*, 2002). The observed recovery could potentially be completely substituted by a network of other brainstem, cerebellar, or cortical pathways. Recent investigations using ibotenic acid injections into the vestibular nuclei are underway to determine if recovery of

vestibulo-autonomic regulation occurs in animals devoid of bilateral vestibular nuclei (Mori et al, unpublished). Careful controlled lesions should determine if the vestibular nuclei are necessary components for recovery. Data gathered thus far, suggest that the nuclei are a necessary component of the recovery process. Findings from this study will demonstrate that vestibular nuclei function is required for maintaining autonomic homeostasis during changes in posture. Similar studies could determine if compensation of spinovestibular and other vestibular output systems require vestibular nuclei function.

4.7 Conclusions

Although more attention needs to be focused on understanding the role of the spinovestibular system in vestibular recovery following bilateral labyrinthine insult, it is clear that the vestibular nuclei are active members of the recovery process. Findings from these studies demonstrated that the nuclei are sensitive to changes in body position, despite lacking primary labyrinthine input, bilaterally. Furthermore it is likely that the increased sensitivity of the vestibular nuclei to limb stimulation is due in part to reorganization of the spinovestibular system. The work of this thesis provides more evidence supporting the importance of ascending spinal input to the vestibular nuclei in the bilaterally labyrinthectomized animal. Based upon the findings of this thesis it has been demonstrated that the neurons in the vestibular nuclei of bilaterally labyrinthectomized animals rely heavily upon spinal input to sense changes in body position. Although the aforementioned studies did not carefully characterize other remaining inputs, such as vision, the control of these inputs through decerebration, spinal transection and bilateral vagotomies supported our hypotheses. However, what is not completely clear is if spinovestibular reorganization is also a prevalent process that occurs in the unilaterally labyrinthectomized animal. This question is not only a scientific pursuit but has considerable

clinical ramifications, since unilateral peripheral end organ loss is much more common than pathologies that affect the labyrinths bilaterally.

The fact that *bilateral* vestibular disorders are extremely rare and extraordinarily incapacitating –when they do occur— speaks enormously of the high selective pressure that proper vestibular function placed on the evolving animal. Of further interest is the observation that when vestibular dysfunction does occur, the system is adaptive in returning the organism to functional levels. It is therefore not surprising to observe that the vestibular system is highly conserved through many levels of phylogeny. These characteristics of the vestibular system make an excellent model for examining systems level plasticity in the central nervous system.

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