Coordination of Eye and Head Movements in *Cavia Porcellus*

by

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To my grandmothers, Ana and Lydia

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List of Acronyms

VOR	Vestibuloocular reflex
VCR	Vestibulocolic reflex
GVS	Galvanic vestibular stimulation
PWBR	Passive whole body rotation
VO	Vestibular-only
PVP	Position-vestibular-pause
CCR	Cervicocolic Reflex
ES	Eye-movement-sensitive
OKN	Optokinetic Nystagmus
OKAN	Optokinetic Afternystagmus
EIS	Eye-in-Space
EIH	Eye-in-Head
HIS	Head-in-Space
HOB	Head-on-Body
BIS	Body-in-Space
EMG	Electromyography
VEMP	Vestibular evoked myogenic potentials

Abstract

The present work provides novel insights into the coordination of vestibular reflexes in the head-unrestrained guinea pig. Eye and head movements were recorded during passive whole body rotations and active head movements. Vestibular signals were subsequently manipulated using galvanic vestibular stimulation to determine the extent to which certain responses are dependent on vestibular afference. The results of this work can be divided into three parts: 1. Guinea pigs exhibit compensatory eye and head movements to passive whole body rotations similar to those in other species. Unlike primates, however, the responses are only weakly compensatory, allowing for significant motion of the eyes relative to space. This difference suggests a lesser need for complete visual stability in the guinea pig and implies therefore, a different purpose for vestibular reflexes. Although there is a high degree of variability in head movement responses to passive whole body rotations, eye movements are always tightly coupled to the resulting movement of the head relative to space. 2. Guinea pigs exhibit anticipatory, compensatory eye movements during voluntary head motion. The response timing and amplitude imply that they are not reflexive in nature and occur in animals with and without an intact vestibular system. This behavior has not been previously reported in animals with an intact vestibular system, and implies different (partially extra-vestibular) neural mechanisms for its generation. 3. Galvanic vestibular stimulation affects both compensatory eye and head movements in the guinea pig. This finding is in contrast to previous ones in primates and implies differences between guinea pig and primate in how

afferent vestibular signals are weighted and processed centrally to produce compensatory responses.

This work supports the guinea pig as an important animal model in the study of the vestibular system. Additionally, it demonstrates the need for an experimental approach that examines eye and head movements simultaneously as they are reliant on each other for successful vestibular compensation. Finally, the results presented in this work suggest a number of future experiments to improve our understanding of how vestibular afferent signal and extra-vestibular influences interact to produce compensatory eye and head movements.

Chapter 1

Introduction

The vestibular system plays a singular role in orchestrating eye, head and body movements to maintain balance, visual acuity and spatial orientation. This important sensorimotor system is called upon during active movements – those caused by the subject, for example walking, looking around or even breathing; and during movements passively applied to that subject, such as the sudden jerk of a departing train or an accidental push by a stranger.

In foveate animals, such as primates, the vestibular system is a key player in keeping the fovea – the area of highest retinal acuity – on the object of interest during either active or passive motion. For example, a tennis player is able to track the ball almost perfectly while also running, or even jumping, to return a serve. When the system is not properly functional, movements as small as breathing can be noticeable and can impede simple everyday activities like reading (Crawford 1964).

More generally, vestibularly-driven movements reduce the blurring of the world across the retina during self motion and allow an animal to detect external motion, such as that of a creeping predator or escaping prey. The alignment of the retina relative to the horizon can also inform the animal regarding its terrain and orientation.

1.1 Compensatory Mechanisms of Eye and Head

The coordination of eye, head and body movements is important for the stabilization of an individual's body in space (*e.g.*, posture) and the stabilization of that individual's gaze upon the world. Gaze can be defined as the combination of the individual's head position relative to the world and the position of that individual's eyes within the head. The vestibuloocular reflex (VOR) is responsible for counter-rotating the eyes to compensate for motion of the head and/or body. The VOR is essential for maintaining strong visual acuity and is enhanced at low frequencies, in primates, when visual feedback is available (monkey: Minor et al. 1999; humans: Larsby et al. 1984), although gains in afoveate animals tend to be lower and less affected by vision (mouse: Kimpo and Raymond 2007; guinea pig: Serafin et al. 1999, Escudero et al. 1993).

The vestibulocolic reflex (VCR) is the head movement counterpart to the VOR, serving to counter-rotate the head in response to perturbations of the body. The reflex is also important for maintenance of visual acuity and is synergistic with the VOR. During directed head movements, such as a change in head orientation or direction of regard, the VCR must be suppressed. Otherwise, the reflex would tend to oppose the voluntary head movement in order to reduce the motion of the head and therefore the vestibular signal (Cullen et al. 2009). This action is, therefore, counterproductive to the intention of the animal. During motion, the VCR plays a key role in the control of posture and head stability. Classification of head movements as purely VCR-driven is difficult, however, as the head is also subject to inertial forces, muscle tone and other neck reflexes such as the cervicocolic reflex (CCR, Goldberg and Peterson 1986).

Because of this complexity, technical difficulty of *in vivo* recording during motion, and the robust nature of the VOR, much of vestibular research has traditionally been done in animals with restrained heads and bodies. Consequently, eye movements have been the primary measure of vestibular function (*e.g.,* Benjamins 1918: orienting eye movements in fish). The mechanics of the VOR itself were described in detail early in the study of the vestibular system and brain anatomy as a whole by Lorente de Nó (1933) in his characterization of the three-neuron arc associated with the VOR. The arc consists of the afferent fibers that provide connections between the vestibular sensors in the ear, neurons in the vestibular nucleus, and the extraocular motoneurons corresponding to the appropriate ocular muscles (Figure 1.1).

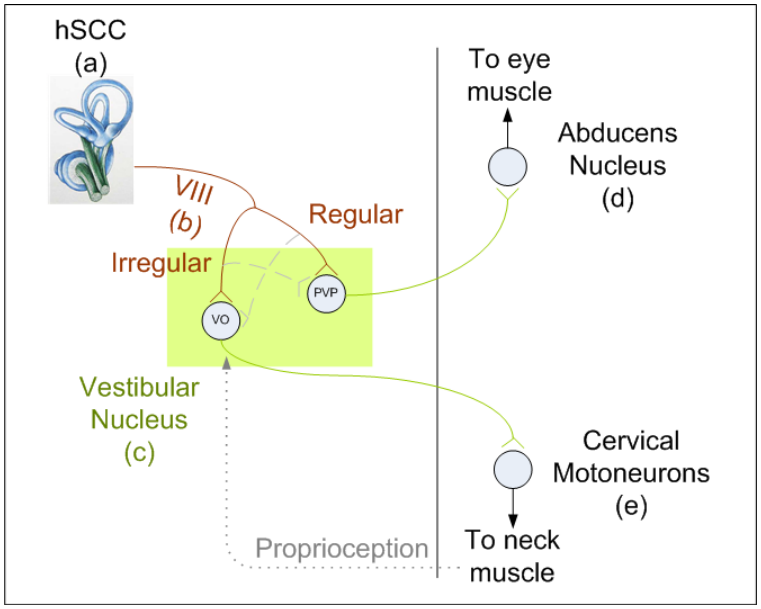

Figure 1.1: Simplified vestibular pathways featuring the three-neuron arc of the VOR.

Vestibular pathways processing information in the horizontal plane. 1. Vestibular afferents (**B**) transmit information from the vestibular sense organs (**A**) to the vestibular nucleus (**C**). 2. Eye movement related cells in the vestibular nucleus project to nuclei that are responsible for eye movements (e.g. abducens for horizontal eye movements, **D**). 3. Motoneurons project to the appropriate eye muscles to drive the eye movements associated with the VOR.

The arc was further characterized by Szentágothai (1950) who described the functional aspects of the arc and called it the "'skeleton' of the vestibulo-ocular reflex mechanism," having shown that the putative three neuron arc only accounted for the slow phase of the VOR and therefore additional pathways were required to describe the reflex as a whole. The mechanics of the VOR pathways were further described by Robinson (*e.g.,* 1975) and others based on the firing characteristics of the neurons involved in the arc and physical characteristics of the vestibular system components. Based on this work, other neuronal inputs could be identified and studied (for review, see Baker et al. 1981). These early studies were important as they characterized the reflex and elucidated the basic neural connections necessary for the VOR. Later, these experiments would be extended to the key brain regions that are responsible for not only controlling the VOR response but also fine-tuning it (*e.g.,* Lisberger and Pavelko 1988).

However, the vestibular system naturally relies on an interplay and coordination of eye, head and body movements and therefore a more global approach is necessary to gain an understanding of the system's performance and possible failures of function. The importance of more natural, head-unrestrained methods has been shown for other sensory systems (*e.g.,* auditory, Populin, 2006). Specifically in the vestibular system, head unrestrained approaches have allowed researchers to probe pathways mediated by different behaviors and behavioral goals, and to examine the natural coordination between eye and head movements (Bizzi et al. 1971; Gresty 1975; Gdowski and McCrea 1999; Roy and Cullen 1998, 2002).

1.2 Guinea Pig Studies of the Vestibular System

Coordination of voluntary eye and head movements with vestibular reflexes has been frequently studied in non-human primates because of their similarity to humans (Bizzi et al. 1971; Dichgans et al. 1974; Tweed et al. 1995; Gdowski and McCrea 1999; Roy and Cullen 2001, 2002; Cullen and Roy 2004; Freedman 2008). Although lateral-eyed and lacking a fovea, the guinea pig is also an important animal model for studies of vestibular reflexes and eye–head coordination. For example, it is often used to study recovery from peripheral vestibular lesions (Ris et al. 1995, 1997; Ris and Godaux 1998; Beraneck et al. 2003; Curthoys et al. 1995; Gilchrist et al. 1998; Vibert et al. 1993), as an animal model for active and passive regeneration of the vestibular periphery (Forge et al. 1998; Kopke et al. 2001; Walsh et al. 2000; Yamane et al. 1995); and to evaluate aminoglycoside ototoxicity (Forge and Li 2000; Pettorossi et al. 1986; Bamonte et al. 1986; Jones et al. 2003). It is somewhat surprising, therefore, that only a few studies of normal vestibular function have been reported in the guinea pig: the vestibulo-ocular reflex, VOR (Escudero et al. 1993; Serafin et al. 1999); vestibular-controlled head movements (Gresty 1975; Escudero et al. 1993); and posture (Graf, et al. 1995).

Unlike primates, guinea pigs do not initiate rapid gaze shifts using eye movements (voluntary saccades). Guinea pigs do, however, produce anti-compensatory (in the direction of head motion) rapid eye movements during active head movements. The pattern of eye-head coordination during rapid gaze shifts appears to be similar to that of primates (*e.g.*, Bizzi et al. 1971) except that the rapid eye movement follows the head movement. Eye movements that compensate for self-generated head rotations as well as passive stimulus were reported previously (Gresty 1975) as two behaviors mediated by

the VOR. No concerted efforts have been made, however, since Gresty's report (1975) to determine how eye movements are coordinated with passive or active head and body movements in the guinea pig.

1.3 Gaze Shifts and Voluntary Head Movements

Along with gaze stabilization and vestibular compensation, an equally important and interesting behavior is shifting the direction of regard (or redirecting gaze). Just as individuals are often subject to external perturbations that require compensatory movements to reduce retinal slip and postural instability, they often initiate self-generated movements geared towards shifting the line of sight or reorienting themselves in space. In these cases, the vestibular reflexes are in conflict with an individual's intentions and must be attenuated to allow for the planned behavior to occur. Most behaviors are not so clear-cut, however, and require varying combinations of gaze redirection and gaze stability to provide the synergy required to achieve the subject's ultimate goal. Moreover, the vestibular system needs to be able to distinguish between signals that are generated due to active versus passive head movements in order to properly adjust its output and correctly process environmental changes associated with motion (for review see Angelaki and Cullen, 2008). This modulation of the vestibular output with respect to behavioral context is an important area of study that has allowed further elucidation of the vestibular pathways and neurons implicated in different behaviors (for review, see Cullen and Roy, 2004). These insights have broadened the basic view of the vestibular reflexes (VOR, VCR) as 3-neuron arcs (Figure 1.1).

Stated differently, gaze shifts consist of coordinated rapid eye and head movements that shift the line of sight into a new direction and ocular counter-rotations that stabilize

the retinal image during the head movement. The vestibulo-ocular reflex (VOR) is usually assumed to produce the ocular counter-rotations during gaze shifts since it is the main mechanism by which retinal image stability is achieved during passive perturbations of head position. Several studies have described coordination of eye and head movements with vestibular reflexes during gaze shifts and passive perturbations of head position (Bizzi et al. 1971; Dichgans et al. 1973; Dichgans et al. 1974; Collewijn et al. 1983; Jell et al. 1988; Barnes and Grealy 1992; McCrea and Cullen 1992; Tweed et al. 1995; Crawford et al. 1999; Roy and Cullen 2002; Cullen and Roy 2004; Freedman 2008). However, compensatory eye movements with an extravestibular origin were also reported by Dichgans et al. (1973) in monkeys with bilateral vestibular lesions. They interpreted the eye movements to be a learned compensatory mechanism to restore retinal image stability during active gaze shifts. Although the study provided a number of interesting insights, most importantly it created more questions about this remarkable behavior to be addressed in future studies.

1.4 Studies Using Galvanic Vestibular Stimulation

One of the approaches used to probe the vestibular system is the use of galvanic vestibular stimulation (GVS) to either suppress (anodal GVS) or excite (cathodal GVS) the firing rates of vestibular afferents (Goldberg et al. 1984). The technique has proven to be a powerful tool both in the laboratory, looking at neuronal signals and connections within vestibular pathways (for review, see Goldberg 2000), and in the clinic, providing a highly controlled stimulus for diagnostic and research purposes (*e.g.*, looking at effects of orientation on vestibular signals, Fitzpatrick et al. 2006).

Since the technique was first introduced, its effects on the vestibular periphery have been thoroughly investigated. Application of galvanic currents to the vestibular periphery has been shown to affect a subpopulation of afferent fibers innervating vestibular hair cells in all of the vestibular sense organs (guinea pig: Kim and Curthoys 2004; for review across multiple species, see Goldberg 2000). Specifically, irregularly firing axons show a high degree of sensitivity to weak galvanic currents, whereas regular afferents are relatively unaffected (Goldberg et al. 1984; Baird et al. 1988). An afferent's sensitivity to electrical stimulation is one of several properties used to distinguish between the two types (for review see Goldberg 2000). Afferents vary in their anatomy, response dynamics, firing regularity and response gains (Baird et al. 1988). Irregular afferents are usually characterized by calyceal endings (but may be dimorphic), are associated with more centrally located (on the crista ampullaris) Type I hair cells (Wersäll 1956), and respond to sinusoidal head movements with irregular firing patterns and relatively low gains and large phase advances across a broad range of stimulus frequencies (Baird et al. 1988; Goldberg 2000). Regular vestibular nerve fibers are characterized by bouton endings, synapse onto more peripherally located Type II hair cells and have high-gain, tonic responses with low sensitivities to angular and linear acceleration. Furthermore, because of their dynamics, the regular afferents have been implicated in generation of the VOR (Hullar and Minor 1999). Despite this marked distinction within the vestibular periphery, the trend does not continue into the vestibular nucleus, where the projections are only partially segregated (Goldberg et al. 1987; Highstein et al. 1987).

Previous experiments have shown that GVS does not affect the vestibulo-ocular reflex (VOR) during sinusoidal motion or transient velocity steps in primates (Minor and

Goldberg 1991; Angelaki and Perachio 1993; Chen-Huang et al. 1997), thereby implying that the irregular afferent fibers may not contribute to activity in pathways that produce the VOR. This finding is surprising, as experimenters have also shown that there is no preferential innervation of second-order vestibular neurons by the different afferent types (Highstein et al. 1987; Boyle et al. 1992; Chen-Huang et al. 1997). One possible explanation for this apparent paradox is the influence of extra-vestibular factors such as target distance or attention (Chen-Huang et al. 1997; Chen-Huang and McCrea 1998) on the responses of neurons within the vestibular nucleus. Alternatively, irregular afferents might predominantly influence vestibular control of head stability (Angelaki and Perachio 1993; Bilotto et al. 1982) rather than the VOR, although this idea has never been directly tested in head-unrestrained animals.

1.5 Goals and Significance

The interplay of GVS with vestibular afferents and vestibular reflexes is just one example of the complexity of eye and head movement interaction. Although it is tempting to explain the segregation of vestibular afferents into two types as necessary for independent control of eye and head movements, at the level of the vestibular nuclei in the brainstem the distinction seems to disappear due to the complex connections between both types of afferents and neurons of the vestibular nucleus that project to eye or neck muscles. Furthermore, at the level of the vestibular nuclei, neurons must rely not only on the vestibular afferent input but additional pathways to differentiate between passive and voluntary motion, in order to suppress compensatory eye and/or movement responses. These are only two of the many examples of the complexity involved in eye-head coordination.

Understanding how eye and head movement behaviors are mediated by the vestibular system is particularly important in the clinical context. Vestibular-induced eye movements are commonly used as the main tool for diagnosis of vestibular deficits. However, the underlying neural components of the vestibular system are diverse and damage to various elements may influence different aspects of vestibular performance. For this reason, looking at a single, albeit very important, functional component of the vestibular system may lead to an incorrect or incomplete diagnosis and obscure full understanding of the vestibular deficit.

Thus, the objective of this study is to further elucidate coordination of eye and head movements in an animal model that can naturally move its head. For a full description of this coordination, three sets of experiments are designed to probe eye and head movements when the demands on the two are systematically varied. The first set of experiments will look at compensatory eye and head movements during passive whole body rotation around the earth-vertical axis. In these experiments, interaction between eye and head movements will be examined in a situation where both have the matching goal of compensation for the passively imposed perturbation. We predict that eye and head movements will both be compensatory, although the eyes will provide a large portion of the compensation and head movements will exhibit other influences, for example inertia. The second set of experiments will examine compensatory eye movement responses during voluntary head motion. In this condition, the eyes and the head have conflicting goals – where the head is moving and the VCR is suppressed, but the eye movements are compensatory and VOR is maintained. We predict that eye movements responses will be similar to those recorded in response to passive whole body

rotations, although will likely exhibit a higher degree of variability due to differences in the animal's behavioral state. In the third and last set of experiments we will attempt to decouple vestibular eye and head movements during passive whole body rotations using GVS. In agreement with the literature, we expect low current GVS to affect head, but not eye movements.

The first two experiments will provide information regarding eye and head movement coordination during two naturally-occurring situations of voluntary motion and passive perturbation. The last experiment will bear upon what can happen when the vestibular system is altered and natural patterns of coordination can no longer be applied. For example, the effects of GVS on irregular afferents are redolent of the effects of some aminoglycosides on the vestibular periphery, and specifically Type I hair cells (Jones et al. 2003). If these cells do, in fact, preferentially control head movements then better treatments for patients receiving aminoglycosides can be developed.

Chapter 2

Methods

Experimental and surgical procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Michigan's University Committee on Use and Care of Animals.

14 male guinea pigs (10 pigmented, 4 albino) between 500 and 1200 grams were used. Both pigmented and albino animals were used to establish if the known differences in visual processing (Vingrys and Bui 2001; Bui and Vingrys 1999) would be correlated to differences in vestibular behavior. Since there were no differences in eye and head movements in response to whole body angular rotations between the two groups, all of their data were combined for this report.

2.1 Behavioral Testing

During the experiment, fully awake animals were restrained and placed on a servo-controlled turntable (Neurokinetics, Inc, Pittsburgh, PA). The restraint consisted of a polycarbonate box, which had an adjustable width that could be adjusted to comfortably fit the animals' trunk. The animal's body position was fixed relative to the turntable via the restraint box, but its head was able to move freely. Guinea pigs were located so that

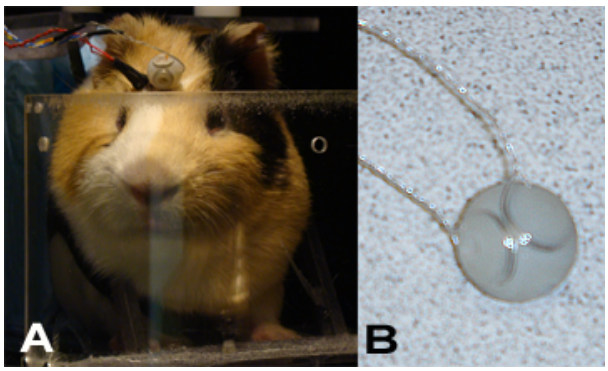

Figure 2.1: Head movement recording.

A Animal in the restraint box with search coils **(B)** used to detect head movement attached.

their heads were centered above the axis of rotation. Data sets of eye and head movements were collected in both light and dark conditions. Video camera recordings using infrared illumination were used to ensure the animals remained alert and to confirm that average head position in the pitch and roll planes remained upright and aligned with the body axis.

Eye and head movements were recorded using the electromagnetic search coil technique (*e.g.* Eye: Robinson 1963 in human; Fuchs and Robinson 1966 in monkey; Stahl et al. 2000; Head: Baker 2005 and Takemura and King 2005 in mouse). Each animal was implanted with a search coil in the right eye (Zhou et al. 2003; Judge et al. 1980), and an implanted titanium head post supported a lightweight plastic ball containing a second search coil to record head position (Figure 2.1). A Primelec search coil system (D. Florin, Ostring, Sw; model CS681) generated three orthogonal electromagnetic fields around the guinea pig. The Primalec field coils were stationary relative to the world and the animals were rotated within the generated fields. In this configuration, measured eye and head movement signals were eye-in-space and head-in-space relative to the earth fixed coordinate frame established by the field coils.

Eye position, head position and body velocity data were each sampled at 500-1000 times per second by a dedicated data acquisition system (CED Power 1401, Cambridge Electronic Design, Cambridge, UK). Data were analyzed offline using custom software written in the Spike2 (Cambridge Electronic Design) and MATLAB (The MathWorks, Inc, Natick, MA) environments. The recordings provided body-, head- and eye-in-space position, which could in turn be used to calculate the relative positions of the head on the body and the eye in the head. A smoothing filter with a 0.005 or 0.01 second time constant was applied to all acquired position channels and eye and head velocity were computed by differentiating the position data (FIR Differentiator, Spike2).

2.1.1 Surgical Procedures

To ready the animals for eye and head movement recordings, each guinea pig underwent two surgical procedures: head bolt/holder and eye coil implantation surgeries. The surgical site was shaved and prepared using 3 Nolvasan scrubs, followed by alcohol and sterile saline. Animals were anesthetized using an intramuscular cocktail of Ketamine (0.40 ml/kg) and Xylazine (0.50 ml/kg) and were administered saline solution (20 cc) and atropine (0.125 ml/kg) subcutaneously for each surgical procedure. A heating pad was used to maintain the animals' body temperature. Vital signs were monitored until the animal became mobile and could stand upright.

For head holder implantation, a 1x2 cm titanium bolt was placed head-down, stereotactically on the midline at AP 0.0. A midline incision was made centered about the interaural line and the skin, subcutaneous tissue and periosteum were reflected back exposing the calvarium. Two different approaches were used. In the first, three small stainless steel screws (8 mm) were attached to the skull, spaced on either side of the

midline. For each screw a T-shaped opening was made in the skull using a dental drill. The head of the screw was slid beneath the bone via the T-shaped opening so that the screw shaft emerged through and above the skull. Each screw was secured using a stainless steel nut and any remaining bone defects were sealed with sterile bone wax. The screws were linked together using dental acrylic to form a rectangular platform. The titanium bolt was placed head-down, centrally between the screws and secured to them using the dental acrylic. The scalp was then sutured around the assembly to minimize exposure and form a tight apposition to the implant. For the second approach, the titanium bolt was directly adhered to the skull using bone cement (C&B Metabond, Parkell, Inc.) along with two small stainless steel screws to provide a basis for a small acrylic platform on which the eye coil and stimulating electrode connectors could be attached. The skin around the incision area was sutured (Ethicon, 3-0/4-0) to form a tight apposition to the new implant.

The eye coil was typically implanted in the right eye of each animal (two animals underwent replacement coil surgeries where a new coil was implanted in the left eye). An eye coil, consisting of 3 loops (9 - 11 mm diameter) of gas sterilized insulated wire, was implanted beneath the conjunctiva of an eye. The wire is 7−strand, teflon coated stainless steel with an outside diameter of 0.0110 +/− 0.00015 in. The conjunctiva was opened about the limbus using a fine scalpel, blunt dissected from the sclera, and reflected back. The eye coil was placed on the eye beneath the reflected conjunctiva. At the superior temporal margin of the eye, the ends of the wire were led subcutaneously from the orbit using a surgical needle to guide the wire. A small (2−3 mm) skin incision was made dorsolateral to the outer canthus of the eye, and the wire from the orbit was brought out

from this incision. The conjunctiva was replaced over the coil, and was drawn together with 8−0 absorbable Vicryl. The exposed eye coil leads were led subcutaneously to the skull and brought out at the base of the head holder implant where they were attached to a connecter and mounted on the head bolt implant using dental acrylic. The skin incision was closed with 1 or 2 Ethicon (3−0) interrupted sutures.

Postoperatively, the wound margin was cleaned during recovery and Yohimbine (Xylazine reversal agent, 0.50 ml/kg) and analgesics Acetominophen (0.625 ml/kg PO bid 3−5 days) or Metacam (0.2 ml/kg) were administered immediately after the procedure and further in consultation with veterinary staff. Sutures were removed 7−10 days after surgery. Animals were placed on antibiotics (Sulfamethoxazole Trimethoprim, 0.60 ml/kg) starting two days prior to surgical procedure, for a total of ten days.

2.1.2 Passive Whole Body Rotation

2.1.2.1 Vestibular Stimulation

To simulate head movements over a range of frequencies and accelerations, several types of rotational stimuli were used. The animals were rotated around an earth vertical axis in a sinusoidal motion with frequencies ranging from 0.05 to 15 Hz. The peak velocities of rotations were 20, 30, 40, 60, 80 or 90 deg/s, with maximum, accelerations up to 5000 deg/s/s. To simulate more natural head turns, animals were also rotated abruptly in a transient manner, where each rotation had a Gaussian acceleration profile that lasted approximately 90 msec with accelerations up to 2500 deg/s/s and final velocity of 20, 30, 40, 60, 80 or 90 deg/s. These accelerations were comparable to those produced by the animals themselves during active head movements. The animals were randomly

rotated to the right or left on successive trials. Up to 300 randomly interleaved trials in each direction were obtained in each experimental session.

2.1.2.2 Data Analysis

For sinusoidal data, rapid eye movements were removed using a computer algorithm tuned to each frequency. The algorithm used both velocity and acceleration threshold criteria to detect the onset and offset of rapid eye movements. Each set of removed eye movements was verified. De-saccaded cycles were averaged and fit with a sinusoid using a least squares fit. Each fit was checked by the experimenter. Head-on-body and eye-in-head velocities were computed by subtracting body velocity from the directly obtained head-in-space velocity, and head-in-space velocity from the directly obtained eye-in-space velocity. To analyze transient data, we chose a time point at 90% of the total rise time of the body velocity. This time point occurred 80-90 milliseconds after the onset of body acceleration and was thus within the open loop interval (before any visual feedback, Lisberger et al. 1981; Zhou et al. 2003) and typically before the occurrence of any quick phases. Values for the body-, head- and eye-in-space velocities were used to calculate the animals' response on each trial. Data from multiple trials were aggregated for statistical analyses. The latency of the compensatory eye and head responses was computed using waveform correlation of head-in-space and eye-in-head or body-in-space and head-on-body velocity respectively (Cullen et al. 1996). Typically, this measurement was taken during the ~100 msec interval following the onset of a transient perturbation. Data segments that included anti-compensatory rapid eye movements were excluded.

2.1.3 Active Head Movements

During experimentation, the animals were able to move their heads at-will and frequently generated bursts of spontaneous head movements. Although some of these movements appeared to be irritative, i.e., rapid shaking of the head, most were exploratory movements during which the animal smelled, chewed or oriented toward some feature of the experimental environment. All analyses of active movements included in this report occurred in the absence of any passive rotational stimulus and all occurred in the dark unless described otherwise.

Two broad strategies were employed to analyze active head movements. First, segments of active head movements (> 5 msec in duration) were selected by a software algorithm using two criteria: that no passive stimulus was present and head speed exceeded 5 deg/sec. Each segment was analyzed to compute the gain of the compensatory response by regression of eye-in-head against head-in-space velocity. The latency of the compensatory eye response was determined by cross-correlating these variables over the same data segments. The latency was assumed to be the lag associated with the maximum correlation coefficient. Alternatively, some data segments were selected during which discrete head and eye movements occurred and which approximated the speeds and accelerations of the passive whole body transient perturbations discussed in the previous section. Each of these segments was analyzed for gain and latency using the same approach described above for the automated analysis. For those segments, brief intervals containing rapid eye movements were excluded from the analysis. About 150 minutes total of experimental time in the intact animals was included, from which 31 minutes were selected as being long enough with sufficient head movement activity to warrant

analysis; in lesioned animals the corresponding totals were 163 (control) and 250 minutes (post lesion). All of these segments were selected using a computer algorithm and were typical and representative of the animals' behavior.

2.2 Lesion Studies

Six guinea pigs were prepared for eye and head movement recording as described above.

2.2.1 Surgical Procedures

After collection of control data, a post-auricular approach was used to access the mastoid bulla. The malleus-incus complex was drilled until the oval window could be visualized, taking care not to perforate the tympanic membrane. Using a 30-gauge needle, a cochleostomy was created at the oval window, followed by 100 μl of streptomycin injection (400 mg/ml). The middle ear cavity was also filled with the streptomycin solution. The skin was closed with 3-0 Ethicon nylon-interrupted sutures and the procedure was repeated in the opposite ear to create bilateral lesions. Extent of the lesions was confirmed by histological examination at the completion of the behavioral experiments (Figure 2.2).

2.2.2 Data Collection and Analysis

Post-lesion data were collected at one week and at additional intervals up to ~4 months post lesion. In each animal, the extent of the lesion was confirmed by light and electron microscopy of whole mount and thin-sectioned tissue from the semicircular canals and otolith organs.

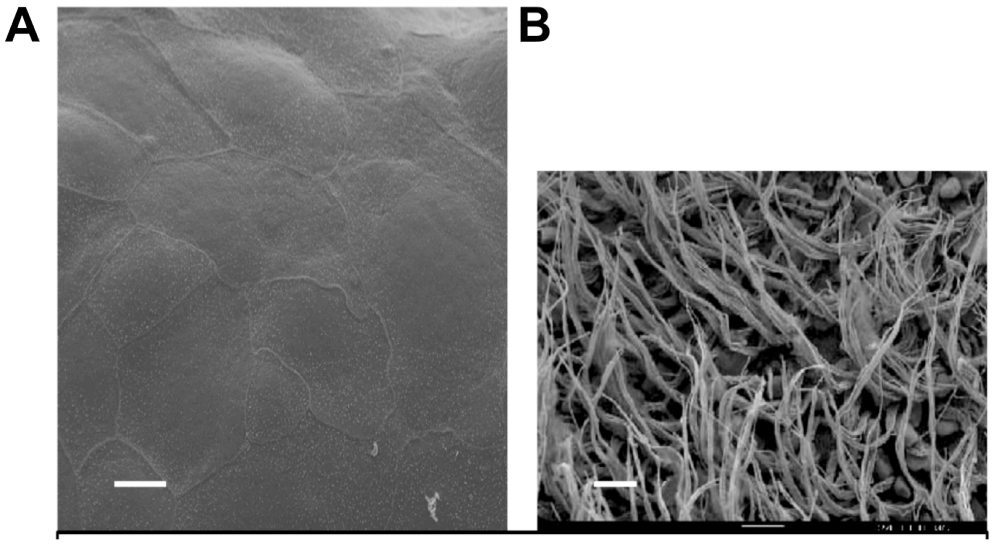

Figure 2.2 Example scanning electron micrographs of lesioned and control guinea pig vestibular epithelium.

Both scale bars correspond to 10 microns. **A** 2 weeks after intratympanic streptomycin injection a complete loss of hair cells is seen in the crysta ampularis. **B** Crysta ampularis of a normal control animal; an intact population of hair cells can be seen. (Images courtesy of Y. Raphael).

Animals' behavior was analyzed for both passively stimulated and actively-generated head movements as described above. Passive vestibular stimulation was performed at 2-4 week intervals for up to six months after lesion. These data were compared among test dates as well as to control data collected prior to the lesion. Active head movements were extracted automatically, as described above, at multiple test dates throughout the period of recovery and compared among post-lesion and control test dates.

2.3 Galvanic Vestibular Stimulation

Three normal guinea pigs were prepared for bilateral galvanic vestibular stimulation to be performed concurrently with behavioral recordings/vestibular stimulation described above.

2.3.1 Surgical Preparation

Stimulating electrodes were implanted bilaterally in the middle ear. The electrodes and leads were assembled prior to the surgery and gas sterilized. Each electrode consisted of two Teflon-coated, 32-gauge, platinum-iridium wires (A-M Systems, Sequim, WA) soldered to a set of stainless steel connectors. Each electrode had a ball, 0.05 mm in diameter, on the implanted end. A retro-auricular incision was made and the dorsal bulla exposed. The bulla was drilled to provide access to the middle ear. Using a surgical microscope, the ossicles, cochlea and round window were observed through the opening as landmarks for placement of the stimulating electrodes. One electrode was placed near the round window, wedged in place and fixed with Vetbond (3M, St. Paul, MN). The second electrode, serving as a ground, was implanted in the bulla distal to the first electrode and fixed in place with Vetbond. Metabond was used to seal the bulla opening. The leads from both electrodes were led subcutaneously to the skull and attached to a previously constructed acrylic pad.

2.3.2 Experimental Design

2.3.2.1 Bilateral Galvanic Vestibular Stimulation

Guinea pigs were placed in the apparatus as described above for behavioral vestibular stimulation. Same stimuli were employed – sinusoidal rotations around the earth vertical axis at frequencies between 0.1 and 8 Hz and transient velocity rotations, both using velocities between 20 and 90 deg/sec. Currents ranging between 20 and 80 μA were applied either cathodally or anodally (to achieve either excitation or inhibition of the vestibular periphery, respectively) and were timed to occur in relation to the vestibular

stimulus. Currents were applied bilaterally and in temporal synchrony using separate constant current isolated pulse stimulators (Model 2100, A-M Systems) for each ear. Currents were balanced between the two ears so that no eye movements were invoked in the absence of rotational stimuli with either cathodal or anodal stimulation.

2.3.2.2 Head Restraint Experiments

To control for a possible effect of head restraint on GVS responses, animals were placed in a modified body restraint with an attached mold of the head. To create the custom mold, a guinea pig was anesthetized and tightly wrapped in protective covering. The area around its head was then filled with dental impression material (AlgiNot, Kerr Corp., Sybron Dental Specialties, Washington, D.C.). Once hardened, the mold could be attached to the body restraint and tightened comfortably around the animal's head. The animal was then placed in the recording set up and vestibular and galvanic stimulation were applied as described above.

2.3.3 Data Analysis

2.3.3.1 Sinusoidal Stimulation

For sinusoidal rotations, we fit each data trace (eye-in-space, eye-in-head, head-in-space, and head-on-body velocities) with a sinusoidal curve at the same frequency as applied turntable rotation. The data fit was used to compute the amplitude and phase of the response relative to the stimulus. To quantify VOR gain, we computed the ratio of the eye-in-head velocity and head-in-space velocity amplitudes; for compensatory head movement, we divided the head-on-body velocity by that of the body-in-space. VOR and

compensatory head movement phase shifts were computed by subtracting phase values from the same pairs of fits as the gains.

Sinusoidal stimuli were typically applied in blocks of 10 (low frequencies) or 20 (high frequencies) cycles of the same amplitude and frequency. Galvanic stimulation was applied during the first half (5 or 10 cycles) of a block; the remaining cycles were used as control data (Figure 5.1A, top trace). Cycles with and without GVS were fit separately; the cycle immediately preceding the onset and the cycle following the offset of stimulation were omitted to avoid possible GVS onset/offset transients. Since galvanic stimulation was applied for half of the sinusoidal stimulus block, each set of cycles with GVS was paired with a subsequent set of control cycles within the same block. This procedure yielded a pair of amplitude and phase values for both compensatory eye and head movements. The value of each parameter during GVS was subtracted from the matching control value, *e.g.*,

$$\delta \dot{EIH} = \dot{EIH}_{control} - \dot{EIH}_{GVS} \qquad \textbf{Equation 1}$$

$$\delta \dot{HIS} = \dot{HIS}_{control} - \dot{HIS}_{GVS} \qquad \textbf{Equation 2}$$

These δ values were used to represent the change in compensatory eye-in-head (Equation 1) or head-in-space (Equation 2) movements related to GVS. δ values were separated into two groups according to the frequency of sinusoidal rotation: a "low frequency group" (0.1, 0.2, 0.5, 1, 2, 5 Hz) and a "high frequency group (8, 10 Hz); the distinction was made based on our previous findings that inertial forces appear to dominate head rotations above 5 Hz (Chapter 3). Differences in phase shift between GVS and control cycles were also pair wise computed to determine possible influences of galvanic stimulation.

2.3.3.2 Transient Stimulation

For transient step trials, our approach differed in several key aspects. Unlike sinusoidal trials where GVS was applied over consecutive cycles of motion, each step was treated as a single trial. On GVS trials, constant current was applied for 800 msec, beginning 400 msec before and persisting 400 msec after the onset of body acceleration. Thus, any onset transients of GVS occurred well before the onset of body acceleration. GVS was applied for approximately 50% of all transient trials, selected randomly by the computer. The values of eye and head velocity were again measured at 90% of the peak body velocity; control and GVS step values were grouped separately. We also examined δ values at the time of peak body acceleration and during the steady state portion of each step and found that the results were qualitatively similar regardless of the time at which the measurement was made. To quantify eye movements, the eye-in-head velocity value was plotted against the head-in-space velocity value for each step, and an overall slope was computed for all control points using robust regression (rightward and leftward steps were analyzed separately). Slopes were similarly computed for all GVS points for each animal's test date. These slopes were used as an estimate of the gain of the VOR for each animal, for trials with and without GVS. This analysis yielded a set of VOR gain value pairs whose differences (Equation 1) represented the effects of GVS. Head movement responses were analyzed in a similar fashion.

2.3.3.3 Active Head Movements

To evaluate effects of GVS on compensatory eye movement responses to active, self-generated head movement, animals were placed in the experimental setup and allowed to move their heads freely without external vestibular stimulation. Eye and head movements

were recorded as described above. GVS was applied randomly during epochs of active head movement. Control and intervening GVS blocks of active head movement were analyzed to calculate compensatory eye movement gain and eye movement latency with respect to an active head movement. The gains were computed using data samples taken over the entirety of the control or GVS block and performing a robust regression of eye-in-head versus head-in-space velocities. Time points where both head-in-space and eye-in-space velocities were in the same direction were excluded, as they represented anti-compensatory behavior. To calculate latency we first divided each control or GVS block into 25 msec segments. For each segment, a cross-correlation of head-in-space and eye-in-head velocities was then performed, and only latencies with negative correlation coefficients were considered as per Cullen et al. 1996. For the GVS versus control comparison, the gains and latencies of consecutive control and GVS blocks were subtracted (Equation 1), yielding a δ for each pair of control and GVS blocks.

2.3.3.4 Statistical Analysis

To ascertain the significance of changes in each of the variables, a nonparametric permutation test was performed. Following the general procedure described by Nichols and Holmes 2002, we examined each distribution of pair differences (*e.g.*, control VOR minus GVS VOR gains, for all dates). From each distribution, we calculated the mean of the differences as the observed statistic. Using this mean, we compared it against a null distribution representing the null hypothesis that the test condition (GVS or control) has no effect on behavior. The null distribution was generated by relabeling the test condition of the values in each pair difference and calculating the mean of the differences after relabeling. If computationally feasible, the values of the null distribution were composed

of the means from a complete permutation of all label orders. To make the required computational state feasible, if the number of permutations exceeded 2^14, a randomized permutation test was performed instead with 10,000 permutations, each with randomly chosen labels. The *p-value* was calculated as the proportion of statistic values in the null distribution equal to, or more extreme than the observed mean (Nichols and Holmes 2002).

Chapter 3

Guinea Pig Responses to Passive Whole Body Rotations

The goal of this initial study was two-fold. First, the study served to characterize vestibular responses in the guinea pig. Although some previous work had been done looking at the vestibular responses of this animal to rotational stimuli (Gresty 1975; Escudero et al. 1993), we expanded the study to a broader range of rotational frequencies and velocities and perhaps most importantly performed all of the work in a head-unrestrained preparation. This approach allowed us to better characterize how the animal uses its eye and head together in a coordinated manner to achieve vestibular compensation.

Looking at this coordination was, in fact, the second goal of the study. Although much work has been done to look at the VOR in a number of different species, until recently relatively few studies have been performed to look at eye and head movements as they occur naturally, in concert. For example, in the guinea pig, the one study that has attempted to characterize both eye and head movements examined them in separate experiments – one examining the eyes and the other the head (Escudero et al. 1993). Understanding the synergy that occurs between the head and eye was an important motivating factor behind the experiments described in this chapter.

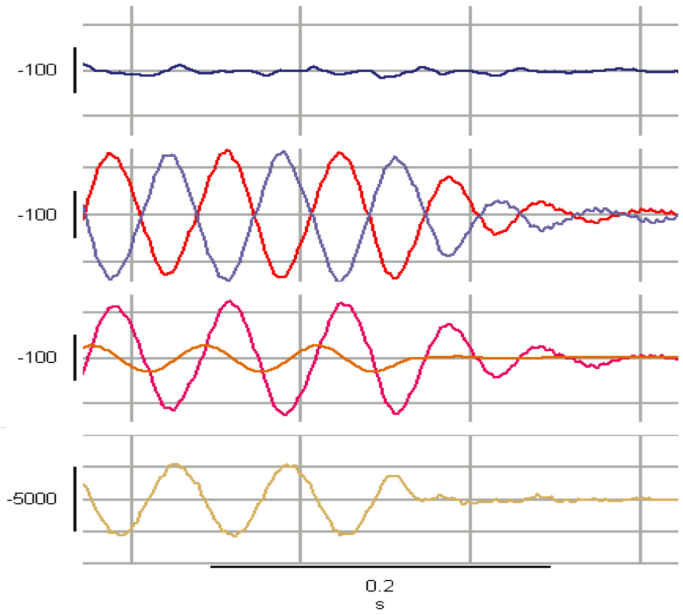

Figure 3.1: Eye and head movement response to a 15 Hz sinusoidal rotation in the dark.

Head-on-body velocity (*magenta*) is much greater than body velocity (*orange*), phase leads body velocity by nearly 90° and is aligned with acceleration (*yellow*). Despite the large head-in-space velocity (*red*) and phase lead, the eye movement response is ~180° out of phase (*light blue*) and nearly perfectly compensatory as shown by the eye-in-space ("gaze") trace (*dark blue*).

3.1 Sinusoidal Rotation

To compare our findings with previous reports (*e.g.*, Escudero et al. 1993, Gresty 1975), we first tested animals using sinusoidal stimulation. Our results were in agreement with those studies at the relatively low frequencies previously employed (≤ 2Hz). At higher frequencies, we saw evidence that the vestibular system was no longer able to control head movements due to inertia.

3.1.1 Inertial Effects on Head Movements at High Frequencies of Rotation (> 5Hz)

At frequencies above 5 Hz we found an enhancement of the VOR and a significant loss of head stability that had not previously been reported. Figure 3.1 shows representative eye and head responses from a guinea pig rotated in the dark at 15 Hz. At

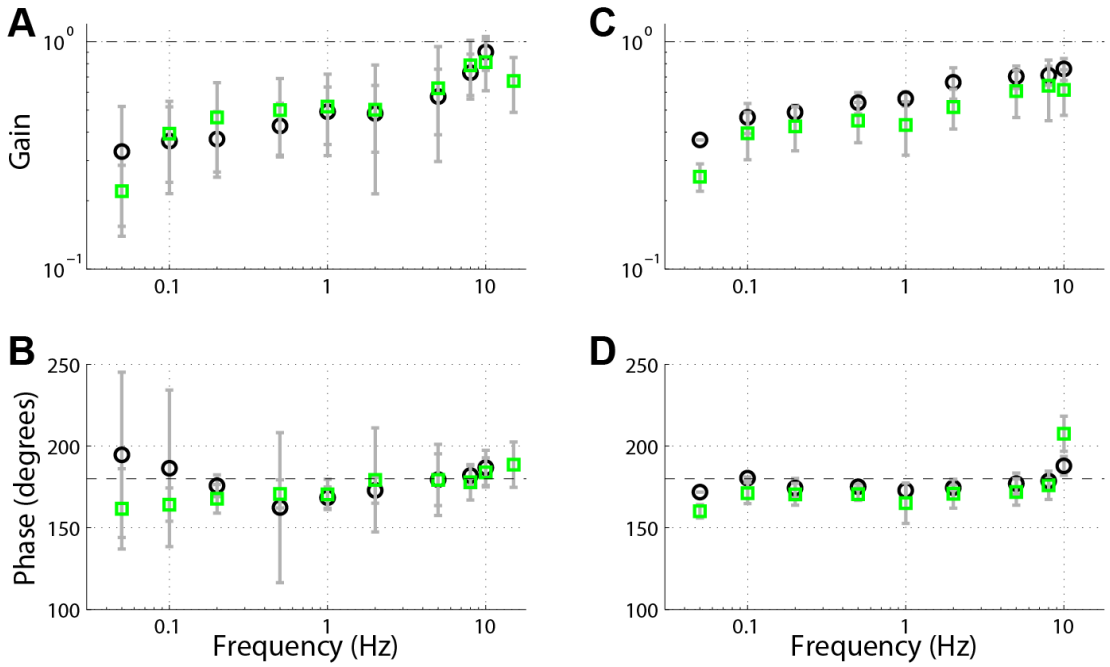

Figure 3.2: VOR frequency responses at 30 & 90 deg/s.

Average frequency responses for the VOR of the 8 guinea pigs. *Green squares*: dark, *black circles*: light. Dashed, black horizontal lines at gain of 1 and phase of 180 degrees represent perfect compensatory response. **A** & **B** frequency responses at 30 deg/s; **C** & **D** frequency responses at 90 deg/s. Gains increase across the frequency range in light and dark; no statistical difference can be seen between the two (**A** & **C**). At the lowest frequencies (0.05 & 0.1 Hz) there is a visible phase lag in the dark that is eliminated in the light at 30 deg/s (**B**). This relationship disappears at the higher stimulus velocity (**D**). Overall VOR performance (gain & phase) improves with increase in frequency.

this frequency, head-on-body velocity exceeded body velocity and was phase shifted nearly 90 degrees. Although head movements were clearly not compensatory, the upper and second from the top panels (Figure 3.1) show that the VOR continued to be effective in stabilizing gaze, since eye-in-head velocity was opposite in direction and nearly equal in magnitude to head-in-space velocity.

3.1.2 VOR Responses

We compared data at three peak velocities (30, 60 and 90 deg/s) in order to establish the linearity of the VOR response to periodic stimuli. Figure 3.2 shows the averaged frequency VOR response data as Bode plots for eight guinea pigs tested in these

experiments (only 30 and 90 deg/s shown). In agreement with previous findings, our data showed little difference in the frequency response to 30, 60 or 90 deg/s rotations, suggesting that the VOR is linear over the range of head velocities and accelerations provided by our rotational stimuli (compare Figure 3.2A & B and Figure 3.2C & D). Figure 3.2A & B shows a gradual increase in average VOR gain from 0.3 at frequencies less than 0.1 Hz to nearly compensatory (> 0.8) at frequencies greater than 8 Hz. Phase shifts were compensatory (~180 degrees) at all frequencies, although there was a small phase lead (*e.g.*, 12.3 degree lead at 0.2 Hz, 30 deg/s) at frequencies less than 0.2 Hz in the dark. This phase lead was reduced in the light for the 30 deg/s stimulus (Figure 3.2C).

Our data also demonstrated reduced phase leads in the light at frequencies below 0.2 Hz (dark: phase lead=18.4 deg/s; light: phase lag 14.6 deg/s, at 0.05 Hz and 30 deg/s). Overall, VOR gain in the light was not significantly greater at any tested frequency using the Tukey-Kramer significance criterion ($\alpha = 0.05$). This observation may reflect the relatively homogenous nature of the guinea pig's retina and the lack of a smooth pursuit response (Marlinksy and Kröller 2000). Nevertheless, the reduced phase lead for the 30 deg/s rotations (< 0.2 Hz) are consistent with the idea that vision does contribute to retinal stability, perhaps through pathways related to the optokinetic reflex (*e.g.*, Andrews et al. 1997).

3.1.3 Head Movement Responses

Figure 3.3 shows the averaged head movement frequency response data for the same animals, collected during the same sessions simultaneously with eye movement data. Similar to the VOR data shown in Figure 3.2, there was little difference between the frequency response to 30 deg/s and 90 deg/s rotations suggesting that the head movement

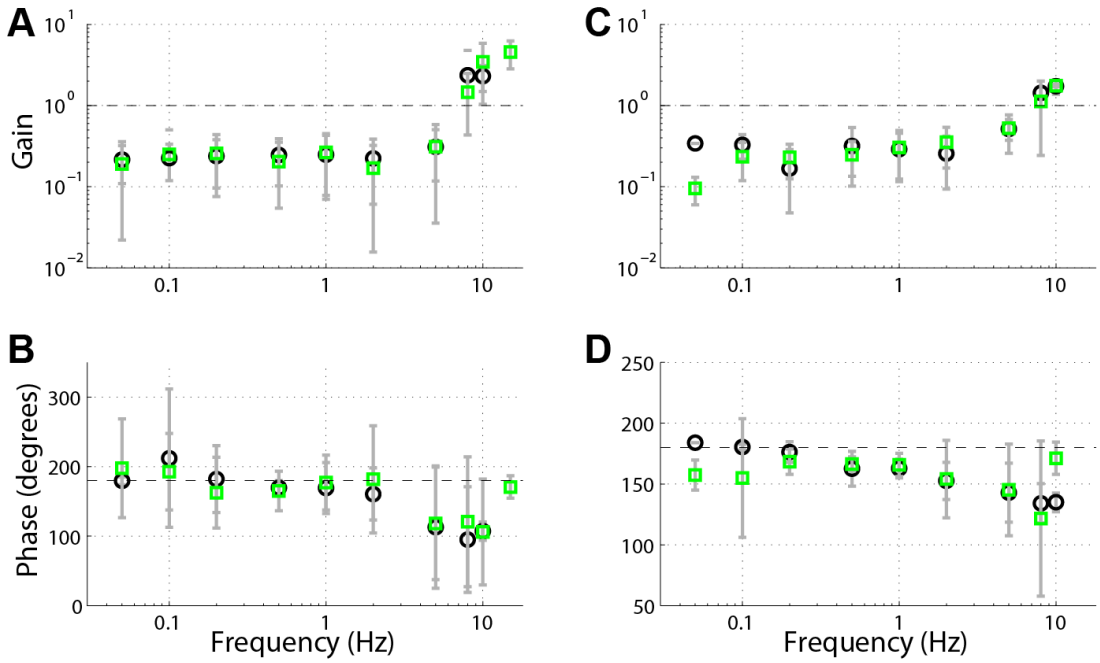

Figure 3.3: Head-movement frequency response plots for 30 & 90 deg/s.

Green squares: dark, *black circles*: light. Dashed, black horizontal lines at gain of 1 and phase of 180 degrees represent perfect compensatory response. **A** & **B** average head movement frequency responses for stimulus oscillations of 30 deg/s; **C** & **D** average head movement frequency response of 90 deg/s stimuli. For frequencies below 5 Hz, the gains at both velocities are very low (around 0.2) and phase values are ~180 degrees. At higher frequencies, gains increase to values close to or even greater than 1 and phases shifts to values near 90 degrees.

responses are also linear over the range of body velocities and accelerations provided by the rotational stimulus. At frequencies less than 5 Hz, the animals' head movements were compensatory in direction with modest phase leads (*e.g.*, 17 degree phase lead at 0.2 Hz and 1.8 degree phase lag at 2 Hz, Figure 3.3C & D). However, head movement amplitudes were low (*e.g.*, a gain of 0.25 at 0.2 Hz, and 0.31 at 5 Hz, at 30 deg/s). These findings are similar to those reported by Escudero (< 2 Hz, Escudero et al. 1993). A novel finding is the large increase in head velocity that occurred for frequencies greater than 2 Hz, coupled with phase shifts that approached 90 degrees (*e.g.*, a gain of 3.5 and a 74 degree phase lead at 10 Hz, 30 deg/s). This behavior is suggestive of a resonance phenomenon in guinea pigs similar to that reported for cat (Goldberg and Peterson 1986)

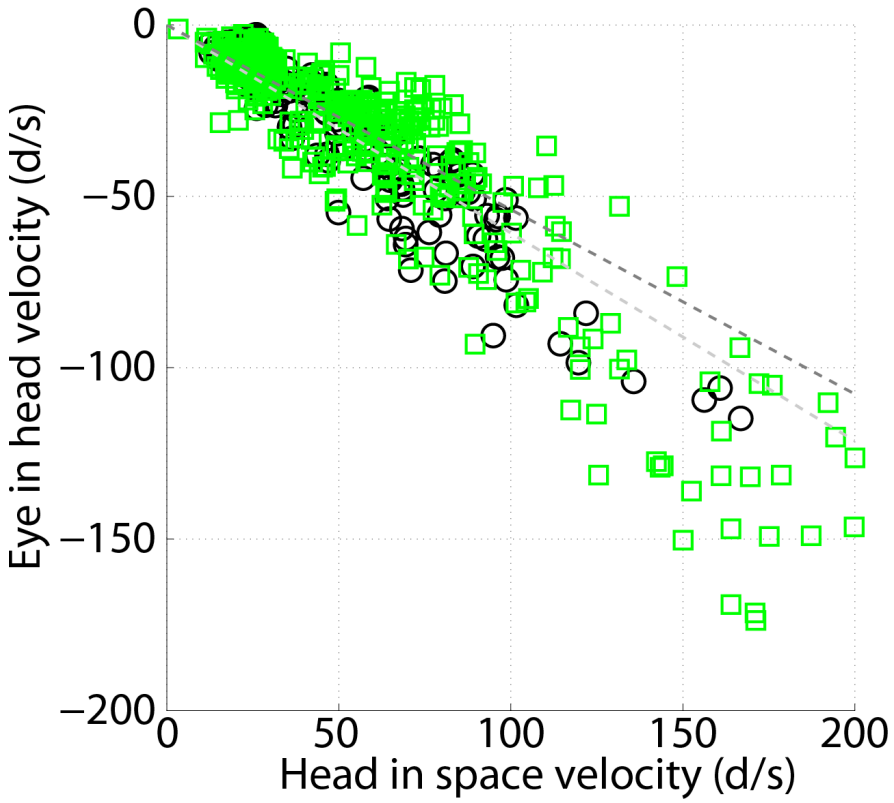

Figure 3.4: Eye-in-Head versus Body-in-Space velocity for sinusoidal stimulus.

Average velocity of the eye relative to the head was plotted against the corresponding average head-in-space velocity for each set of cycles analyzed, at all frequencies. Green squares, black line: dark; black circles, gray line: light. The slope represents the average VOR response across all head velocity values (*green*: dark, slope = -0.54; *black*: light, slope = -0.61). Note the possible increase in slope at velocities above 100 deg/s, which closely corresponds to the increase in gains at higher frequencies, which frequently leads to high head velocities.

and human head movements (Keshner et al. 1995; Keshner and Peterson 1995) and implies that inertial forces dominated neck reflexes at the higher frequencies of body rotation. Although head movements were clearly not compensatory, the gain of the VOR was enhanced at these high frequencies. Thus the vestibular system is able to respond to high acceleration head movements and actually improves its performance when compensatory head movements no longer contribute to gaze stability (Figure 3.1).

3.1.4 Interaction Between Eye and Head Movements

Figure 3.2 and Figure 3.3 illustrate the VOR and VCR in traditional Bode plot format. For the VOR, this presentation may obscure the precise relationship between head and eye speed since the speed of the unrestrained head can vary across frequency and body velocity. To clarify this relationship, Figure 3.4 directly shows the relationship between eye-in-head and head-in-space speed.

The head-free guinea pig yields a broad and continuous set of head velocities since they are controlled not only by the stimulus but also by the head movements produced by the animal. Consistent with the sinusoidal gain data, the slope of the eye-in-head versus the head-in-space velocity in the dark was constant at 0.54 (s.e. = 0.006) for velocities between -100 deg/s and 100 deg/s at any frequency. The relationship was more variable at head speeds above 80 deg/s, consistent with the increase in gain observed in Figure 3.2. Higher frequencies (> 5 Hz) correlated with higher velocities (~100 deg/s) and at these values VOR gain was greatest. The phase of the VOR response remained constant and nearly compensatory at even the highest velocity values.

3.2 Transient Velocity Steps

In addition to sinusoidal rotation we also used transient velocity stimuli to emulate a more natural motion. The same peak velocity values as during sinusoidal rotation were employed.

3.2.1 Eye and Head Position Comparison

To determine whether the animals tended to maintain the position of their eye and head closely coupled during both types of stimulation, we examined changes in the

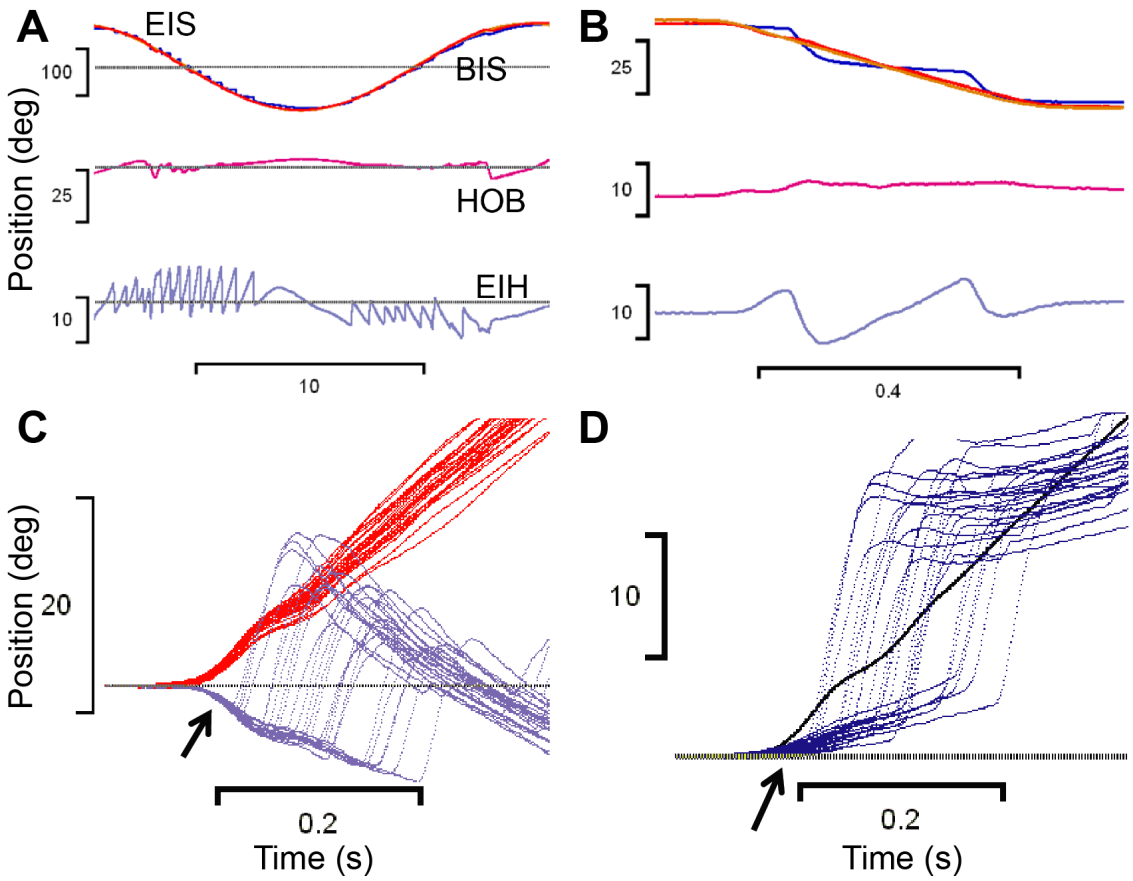

Figure 3.5: Eye and head positions during sinusoidal and transient velocity stimulation.

A During low frequency (0.05 Hz) sinusoidal PWBR, the animal's body is rotated through a ±180 deg angle; the head remains aligned with the body (BIS) throughout the cycle. The position of the eye-in-space (EIS) also tracks the head with a series of rapid eye movements and stable fixation intervals. A small counter-rotation of the head relative to the body can be seen (~5 degrees, HOB) and eye position within the orbit deviates less than ±12 degrees (EIH). **B** The animal's response to a transient perturbation exhibits a similar pattern with body and head in space aligned and orbital eye position maintained within a narrow range. Traces are ordered top to bottom as labeled in **A**. **C** & **D** Superimposed trials of transient perturbations showing a stereotypical pattern of head in space and eye movements. **C** *Red traces*: head-in-space position, *blue traces*: EIH position. **D** *Blue traces*: EIS position, *black trace*: average head-in-space position.

animals' eye and head position in response to vestibular stimulation. During rotation in the dark (or light, not shown), the guinea pig's head is so closely aligned with the body's longitudinal axis that the two traces cannot be distinguished from one another (body in space, BIS, and head in space, overlap; Figure 3.5A & B, uppermost traces, orange and

red). Head rotation relative to the body (HOB, Figure 3.5A & B, middle traces) is minimal, even during the large amplitude (> 100 deg) body rotation of the low frequency (0.05 Hz) sinusoidal stimulus shown in Figure 3.5A. Rapid anti-compensatory and slow compensatory eye movements are evident in the traces representing eye position within the orbit (EIH, Figure 3.5A & B, lowermost traces). The rapid and slow eye movements have similar amplitudes but opposite directions; thus, eye position within the orbit remains within ~12 degrees of center. Eye position in space (often referred to as gaze, the sum of eye-in-head plus head-in-space) tracks the movement of the head-in-space although it is offset by ~75 degrees in the lateral-eyed guinea pig.

Figure 3.5C & D show eye and head positions during onsets of a number of superimposed transient velocity trials. Each trial represents a single transient velocity stimulus rotation at a given velocity. Overall, the initial movement of the eye is in the opposite direction of the head (Figure 3.5C, arrow) at a speed that nearly matches head speed; thus the eye in space position is initially constant (Figure 3.5D, arrow). The initial compensatory eye movement is interrupted by an anti-compensatory rapid eye movement in the opposite direction; subsequent compensatory movements rotate the eye back toward its initial position in the orbit.

3.2.2 Velocity Analysis

Analogous to Figure 3.5A & B, Figure 3.6A & B illustrates the time course of body, head and eye velocity during a single transient velocity trial in response to an abrupt clockwise rotation. The initial head-in-space trajectory is a rightward ramp whilst the eye-in-orbit, after a brief delay, rotates in the opposite (compensatory) direction (Figure 3.6A, long arrow). Figure 3.5B shows that the rapid eye movements tend to “look ahead”

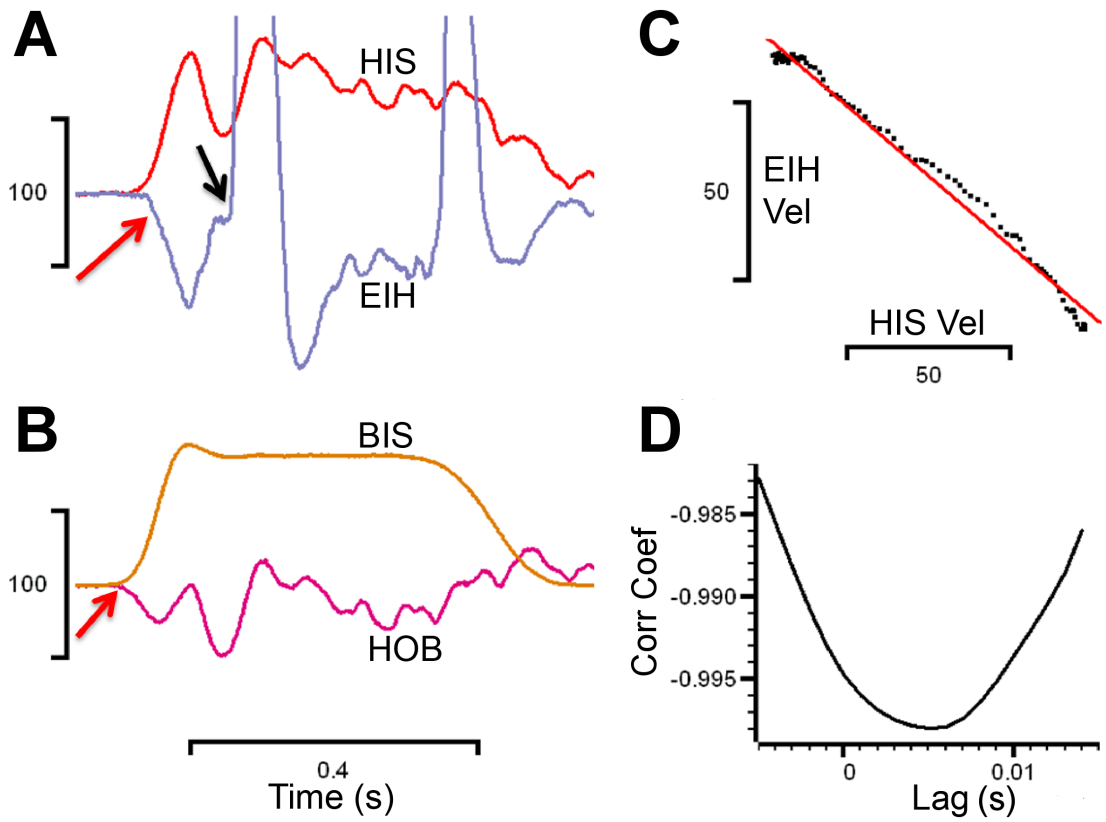

Figure 3.6: Representative head and eye velocity responses in response to transient step PWBR.

A & **B** Velocity records for the transient perturbation shown in Figure 3.5B. In **A**, the initial eye movement (*long arrow*, EIH trace) is compensatory but interrupted by an anti-compensatory rapid eye movement (*short arrow*). In **B**, the initial head movement (*arrow*) is compensatory and followed by decreasing amplitude oscillations. **C** Initial eye velocity is proportional to head velocity (linear regression slope = -0.79). **D** The initial eye movement lags the head movement by ~5.5 msec.

in the sense that they overshoot head position (dark trace) at the time of their occurrence. The initial head-on-body movement occurs with zero latency (Figure 3.6B, arrow) and reflects the inertial tendency of the head-in-space to remain stationary. Figure 3.7D shows the mean latency of the onset of head-in-space movement as ~23 msec with respect to the onset of body motion. The distribution of latencies is skewed in the positive direction as would be expected for an inertial lag (Figure 3.7D). The subsequent response of the head is an oscillation that typically decays to zero velocity during a trial. The initial

compensatory movement of the eye-in-head velocity (Figure 3.6A, long arrow) is delayed with respect to head velocity and is interrupted by an anti-compensatory rapid eye movement (short arrow), which in turn is followed by an alternating sequence of fast anti- and slow compensatory eye movements (Figure 3.6A).

3.2.2.1 VOR Responses

To further quantify these responses, we measured eye velocity relative to the head and compared it to head velocity in space for each passive body rotation. Figure 3.6C shows the regression of eye-in-head versus head-in-space velocity during the initial ~100 msec of the trial shown in Figure 3.6A. Over this interval, eye and head velocity are linearly related with a slope of -0.6 ($r^2 = 0.96$). Figure 3.7A shows a similar relationship between eye and head velocity measured for all guinea pigs at the 90% point of body velocity (see Methods). Compensatory eye velocity is well correlated with head velocity regardless of trial or animal ($r^2 = 0.92$ for light, and 0.87 dark). The slope of the linear relationship is 0.54 (se = 0.007) for light trials and 0.46 (se = 0.006) for dark trials and represents the average gain of the VOR. Compensatory eye movements in the dark and light were similar. For a statistical confirmation, an ANOVA with a Tukey-Kramer adjustment for multiple comparisons was performed for a pair wise comparison of each guinea pig's performance in the light versus dark. No significant difference was found ($\alpha = 0.05$), with one outlier (one animal on a single test date).

Figure 3.6D illustrates a waveform correlation of eye-in-head and head-in-space velocity during the initial ~100 msec of the trial shown in Figure 3.6A. Prior to the first anti-compensatory eye movement, the waveforms are highly correlated with a lag of ~6 msec. This lag is an estimate of the latency of the VOR for this trial. Figure 3.7B shows

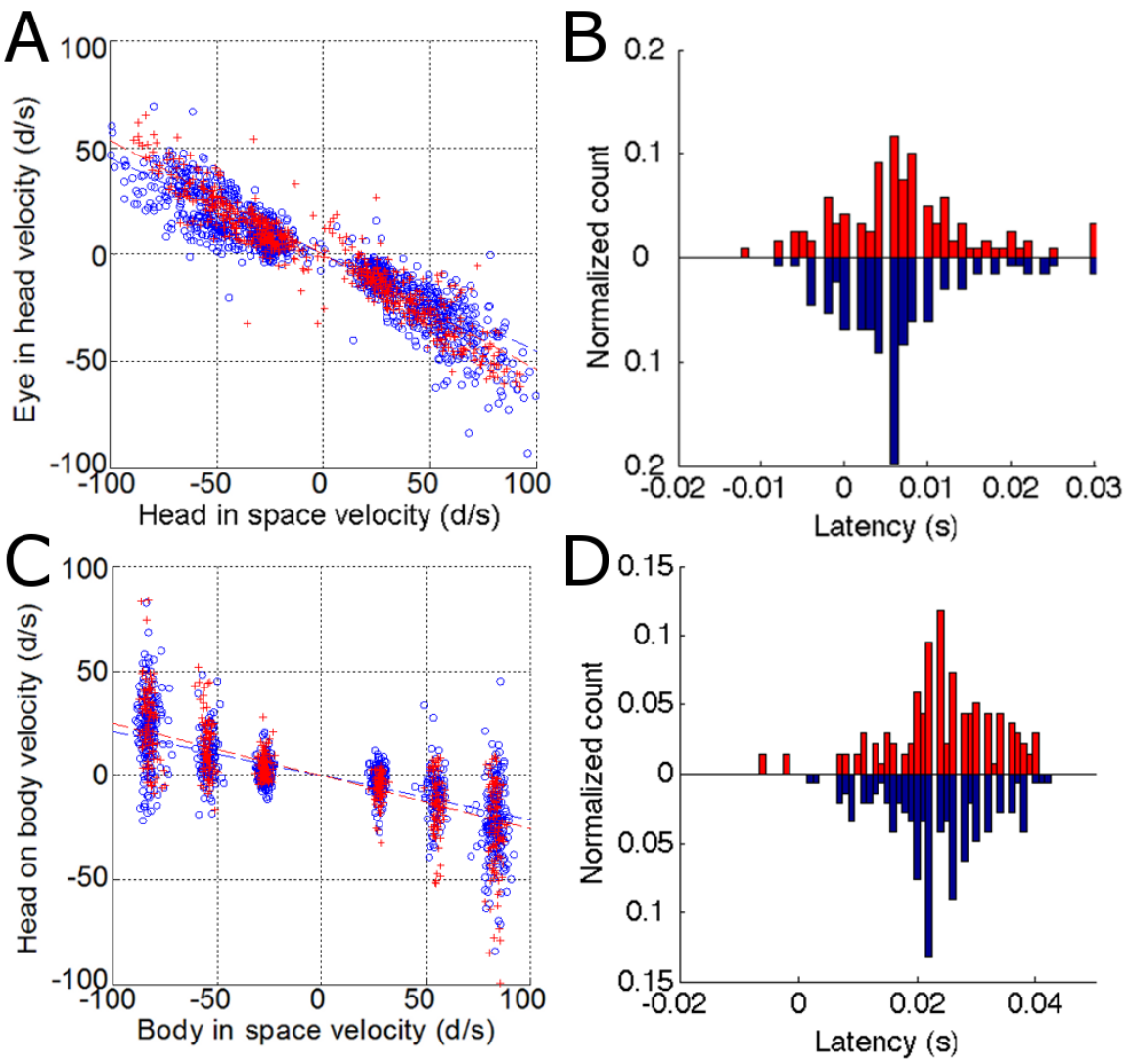

Figure 3.7: In response to brief, PWBR (velocity steps), evoked eye and head movements are compensatory.

A Eye-in-head velocity is proportional to head-in-space velocity (*blue*: dark, linear regression slope = -0.45; *red*: light, linear regression slope = -0.54). **B** Distribution of the initial eye movement latency for 90 deg/s velocity steps. The mean latency of the compensatory ocular responses is 7 ± 9 msec. *Red bars*: clockwise steps; *blue bars*: counterclockwise steps. **C** Head-on-body velocity is proportional to body-in-space velocity (*blue*: dark, linear regression slope = -0.21; *red*: light, linear regression slope = -0.25) for the same velocity steps shown in **A**. The 6 groupings represent the three perturbation amplitudes (30, 60, 90 deg/s) and two directions. **D** Distribution of the initial head movement latency for 90 deg/s velocity steps. The mean latency of the compensatory head responses is 24 ± 9 msec (clockwise) and 23 ± 8 ms (counterclockwise direction).

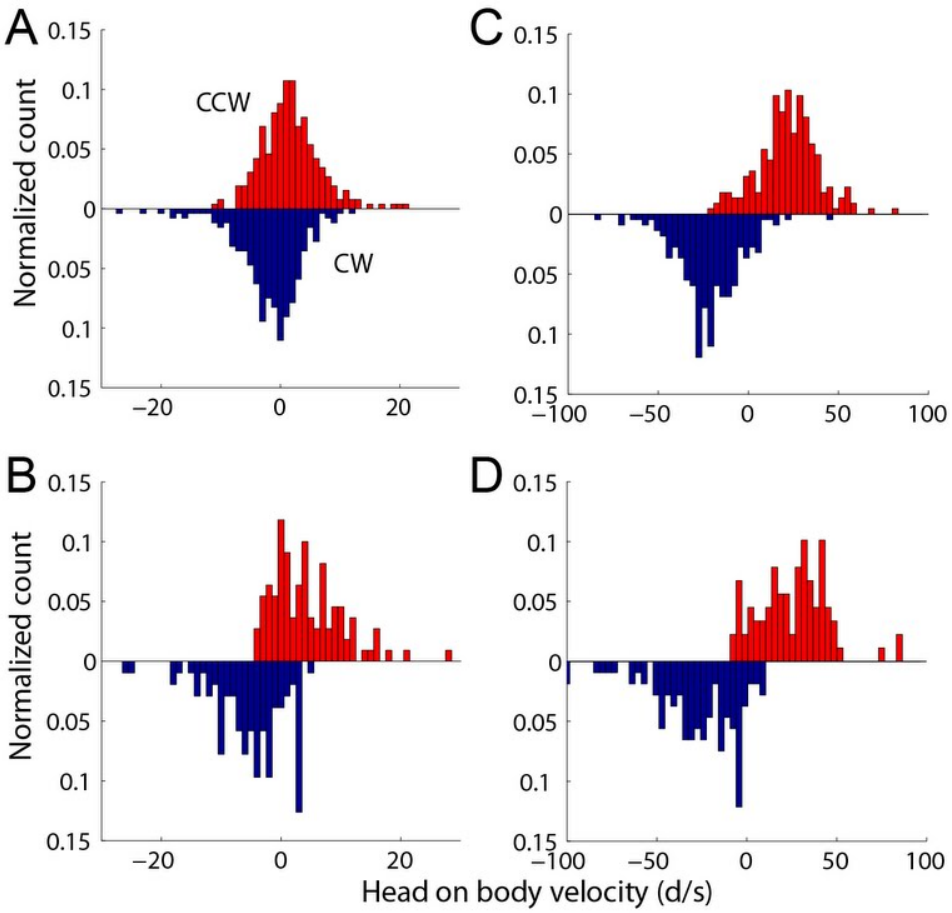

Figure 3.8: Comparison of compensatory head movement responses to velocity steps in light and darkness.

Histograms of evoked head-on- body velocities for 30 deg/s (**A**, **B**) and 90 deg/s (**C**, **D**) velocity steps in dark and light, respectively. *Red*: counterclockwise steps, *blue*: clockwise steps. In darkness **A**, the majority of responses are centered about zero, although skewed in a compensatory direction (opposite stimulus velocity). In the light **B**, the compensatory bias is more apparent. At higher velocities, the distributions are shifted toward the compensatory direction for both dark and light conditions (**C**, **D**).

the distribution of latencies across 251 90 deg/s trials for multiple animals; latencies were skewed in the positive direction with means of 6.2 and 7.4 msec for clockwise and counter-clockwise rotations respectively. These two distributions were shown to be the same following a t-test ($\alpha = 0.05$).

3.2.2.2 Head Movement Responses

The initial head velocity provoked by passive whole body rotation shows considerable variability from trial to trial. Figure 3.7C illustrates initial head speed relative to the body as a function of body-in-space speed. Since the body movement was strictly controlled, body-in-space velocities are distributed tightly around the 30, 60 and 90 deg/s values. Unlike the relationship of eye-in-head with head-in-space (Figure 3.6C, 3.7A), head-on-body velocity was not well correlated with body-in-space velocity ($r^2 = 0.62$ for light and dark). Mean head velocity did, however, increase with body velocity (slope = 0.21 for dark and 0.25 for light). For passive body turns of a particular speed, a broad range of possible head movement velocities occurred. This observation is further illustrated by the distribution of head velocities shown in Figure 3.8. Panels A & C show histograms of head speed for 30 and 90 deg/s body velocity transients delivered in darkness. Head speeds are randomly distributed about mean values that are compensatory for body velocity: for counterclockwise 30 deg/s body velocity, mean head-on-body velocity was +1.7 deg/s; for clockwise 30 deg/s body velocity mean head-on-body velocity was -1.8 deg/s. At higher speeds the mean values shifted more strongly in the compensatory direction, -21.8 deg/s and +21.9 deg/s for ±90 deg/s transients respectively. For each distribution, the mean values are significantly different from zero (t-test, $\alpha = 0.05$) and consistent with the mean slope in Figure 3.7C. Although mean values of head speed are compensatory, the distribution of head speeds is random and normally distributed. To ensure that these distributions were not a result of differences among animals, the same analysis was done for each animal independently. In all cases, we confirmed the

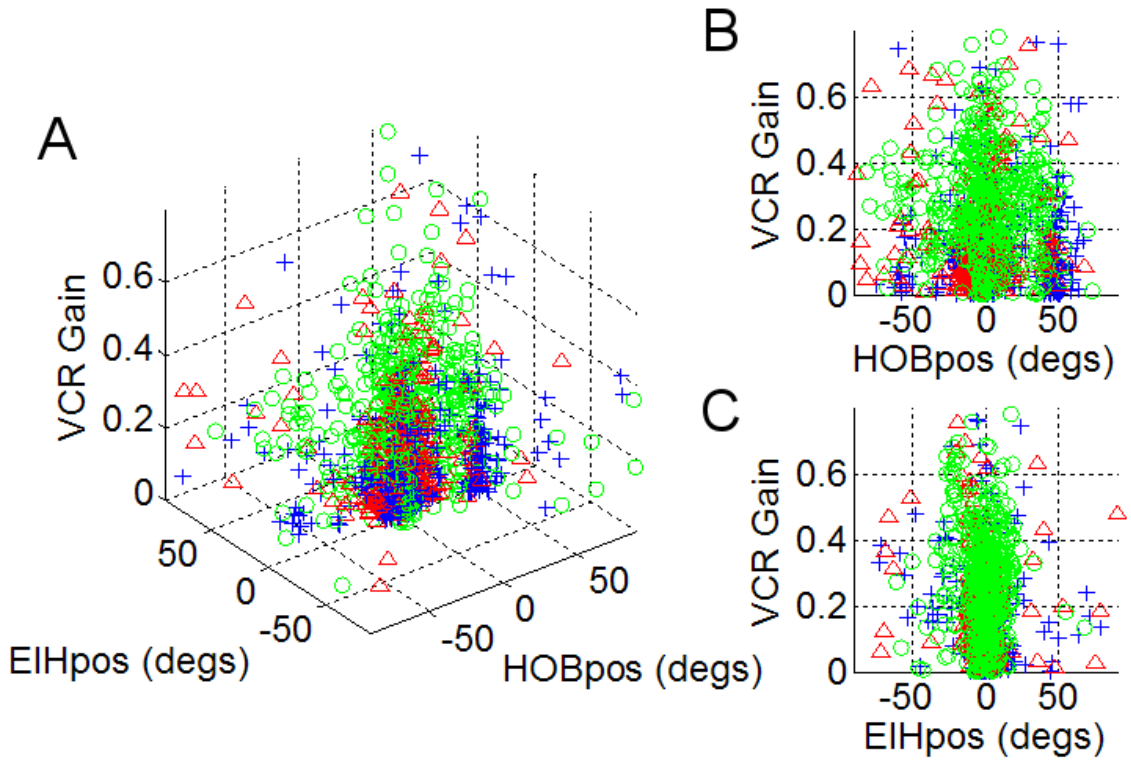

Figure 3.9: Head movement gain as function of initial head and eye position.

Position of the animals' head and eye was recorded immediately prior to the onset of the step and plotted against the compensatory head response gain (Head-on-body velocity/Body-in-space velocity) at the 90% of the velocity increase for the corresponding step. *Blue* +: 30 deg/s; *red triangles*: 60 deg/s; *green circles*: 90 deg/s step velocities. For steps at all three velocities there is no relationship between initial position of eye or head and the gain compensatory head movement.

existence of comparable response variability for each guinea pig, as was shown for the entire population.

The variability of the compensatory head responses to transient steps was unexpected. At each body velocity, head movements ranged from almost completely compensatory, where little or no VOR was required to stabilize gaze, to anti-compensatory. For example, Figure 3.8C shows that the 5 to 95% spread values for counterclockwise rotations ranged from -49 deg/s to 4.6 deg/s whilst the mean VOR gain was 0.47 across all stimulus velocities.

Head movement response variability might be dependent on the initial position of the head relative to the trunk or to eye position relative to the head. For example, if the head were initially turned to the right, then a compensatory head movement to a leftward body rotation might be less than if the head were initially turned to the left. To investigate this possibility, initial eye-in-head or head-on-body position was recorded at the onset of the transient stimulus. Plots of head-on-body velocity versus initial head and/or eye position showed that the compensatory head movements were not systematically related to eye or head position (Figure 3.9).

There was no significant difference between light and dark trials when the three stimulus velocities were combined. Performing a pair wise comparison, as for the VOR, showed variability in each animal's performance between and within light and dark conditions but no discernable trend. However, an effect of testing in the light on head movements was evident at the lowest (30 deg/s) stimulus speed as illustrated in Figure 3.8. In the dark (Figure 3.8A) at 30 deg/s, mean head-on-body velocity was +1.7 deg/s for counterclockwise and -1.8 deg/s for clockwise stimulus directions. In the light, however, the means were +4.2 deg/s and -5.3 deg/s respectively (Figure 3.8B). Statistical comparison of the corresponding means (*e.g.*, dark leftward vs. light leftward) at 30 deg/s shows that they are significantly different for both directions (ANOVA, $\alpha = 0.05$). However, when the same comparison is performed for the 90 deg/s data, the means are not significantly different (light: +25.7 and -26.4; dark: +22.0 and -21.8, Figure 3.8C & D).

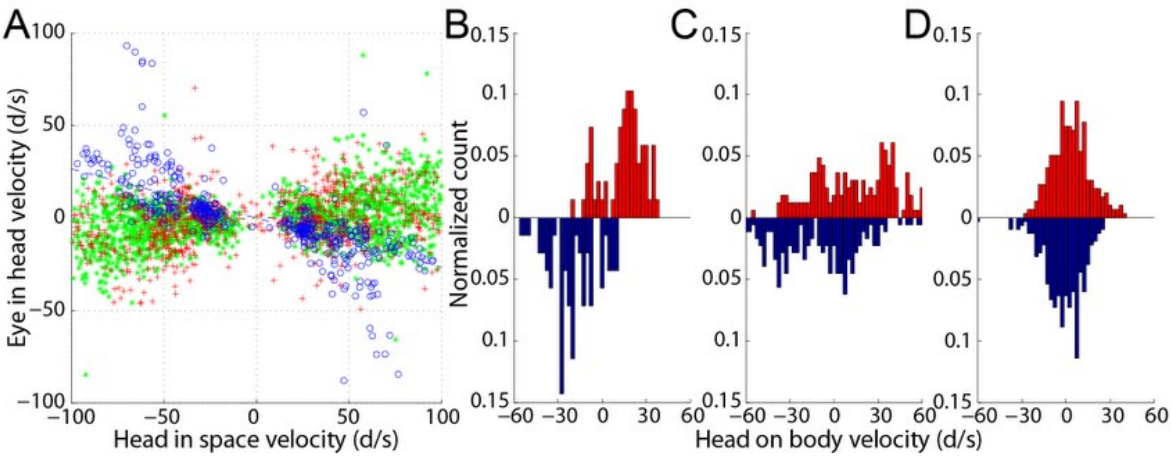

Figure 3.10: Bilateral chemical lesions of the peripheral vestibular system eliminate head and eye responses to passive velocity steps.

A Eye-in-head versus head-in-space velocity control responses prior to lesion (slope = -0.27, *blue circles*); 1 week post-lesion (*red* +); and 4 weeks post-lesion (*green* *). Post-lesion slopes were ≈ + 0.03 and +0.07, respectively. **B–D** Histograms of evoked head-on-body velocities for 90 deg/s steps **B** Control. Note compensatory shift; mean = 14.64 (CW) & -18.84 (CCW) deg/s; SD = 13.83 & 15.99 deg/s, respectively. **C** One week post-lesion, compensatory bias is less: mean = 13.47 (CW) & -9.92 (CCW) deg/s; SD = 26.52 & 28.41 deg/s. **D** Four weeks post-lesion. No significant compensatory bias remains: mean = 3.53 (CW) & -1.46 (CCW) deg/s; SD = 12.91 & 14.16 deg/s.

3.3 Responses to Passive Vestibular Stimulation in Animals with Complete, Bilateral Lesions of the Vestibular Periphery

Animals with complete bilateral vestibular lesions were tested to confirm that the eye and head responses reported above were dependent on afferent vestibular activity.

3.3.1 Sinusoidal Rotation

For sinusoidal stimuli, at one and four weeks after the lesion, compensatory responses to low frequency stimulation (< 2 Hz) were nearly zero. At higher frequencies, the VOR was not compensatory but instead, eye movements had significant phase leads relative to head movement. There was no recovery of the VOR measured up to four weeks post-lesion.

3.3.2 Transient Velocity Steps

Pre- and post-lesion responses were also measured using transient velocity steps. Figure 3.10A shows the relationship between compensatory eye velocity and head-in-space velocity. Prior to the lesion (blue circles), there is a robust VOR response similar to that shown in Figure 3.7A (slope ~ -0.3). However, after the lesion (red + & green *), the compensatory responses are completely abolished; eye speeds are randomly distributed about zero.

Figure 3.10B-D shows results of a similar analysis of head movements. Figure 3.10B shows a distribution of head movement amplitudes before the lesion: comparable to Figure 3.8B there is a shift in mean head speed that is compensatory (mean = 14.6 & -18.8 deg/s; std = 13.8 & 16.0 deg/s). One week post-lesion (Figure 3.10C), there is no evident compensatory bias in the head speed responses. Four weeks post-lesion, (Figure 3.10D) responses are more stereotyped but there is no recovery of the normal compensatory bias (means = 3.5 & -1.4 deg/s; std = 12.9 & 14.2 deg/s). The absence of post-lesion compensatory eye and head responses demonstrates that the responses measured in intact animals depended, at least in part, on vestibular afference.

3.4 Discussion

The goals of this study were to quantitatively characterize the VOR in a head unrestrained preparation and determine the roles played by vestibular and neck reflexes in stabilizing the head and eyes in space during passive whole body rotations. With the exception of Gresty's study in 1975 (data recorded from a single animal) and Escudero's study in 1993 (eye and head not recorded at the same time), these issues have not been

addressed systematically in the guinea pig. The relative lack of basic studies is surprising since translational studies of vestibular evoked myogenic potentials (VEMP, Yang and Young 2005; Lue et al. 2008), mechanisms of ototoxicity (Song et al. 1997; Sha and Schacht 2000) and investigations of sensory cell regeneration within the labyrinth (Forge et al. 1993; Walsh et al. 2000; Kim et al. 2007) often rely on quantitative characterization of the guinea pig's VOR.

Typically, the head and eye movements that occurred in response to passive perturbations of body position in space acted to maintain a relatively constant relationship of eye to head and head to body. For example, the results (Figure 3.5A & B) clearly show that the guinea pig maintained its head aligned to the body axis during periodic or transient passive rotations in space. This finding was somewhat surprising since the vestibulo-collic reflex (VCR) is expected to act synergistically with the VOR to stabilize gaze direction (eye plus head position) in space. However, in our experimental conditions, the VCR was minimally responsive during passive perturbations and the head moved with the body.

The eye also maintained a fixed position with respect to the head as eye position within the orbit rarely deviated more than ±15 deg (Figure 3.5A & B). Despite the occurrence of VOR-driven compensatory movements, positional stability of the eye in the orbit was achieved by anti-compensatory rapid eye movements that re-centered orbital eye position. The net result of these coordinated head and eye responses was to shift gaze in the same direction as the body movement. This behavior is consistent with the panoramic vision of an afoveate, lateral-eyed animal, as there is no need to aim the eye at a specific location in space. Interestingly, this interpretation is at odds with the

afoveate rabbit, which is described as maintaining gaze stability in space, similar to descriptions of primate behavior (Fuller 1981; Collewijn 1977). Fuller did describe instances when the rabbit's head and eyes moved with the body, but he characterized these as periods of "visual inattentiveness". We would not regard the guinea pigs in our study as "inattentive" because their "en bloc" behavior (where their head and eye aligned with the body) was typical of every animal, in both, light or darkness. However, the animals may have been "visually" inattentive some of the time in the sense that there was little of interest in the test environment to which they might seek to orient. In the next chapter, however, we describe active head movements made by the same guinea pigs during the same experimental sessions that did provoke a coordinated pattern of head and eye movements similar to that of primates and rabbits.

The gain of the VOR in response to passive rotation in the dark was less than perfect (Figure 3.7). Not surprisingly, the VOR was enhanced in the light when the perturbation was low frequency (< 0.2 Hz) or low velocity (< 30 deg/s). More interestingly, the VOR was also enhanced significantly during high acceleration stimuli that provoked large head velocity responses (Figure 3.1). Minor et al. (1999), in head-restrained squirrel monkeys, and Hoshowsky et al. (1994), in humans, have also reported enhanced VOR gains using high frequency and high acceleration stimuli. The enhanced response might be related to attentional mechanisms, but video recordings of the animals and comparison of their responses across conditions do not support this explanation. Gain variations across animals remained constant and those that exhibited higher gains maintained those across all test dates. This observation suggests that the responses to passive rotation reflect a default gain state idiosyncratic to each animal. The default state might represent a

behavioral compromise between the opposing goals of stabilizing the retinal image and the maintenance of eye position within the orbit. Consistent with this idea, the gain of the VOR is greater during self-generated head movements suggesting that its state is determined by the behavioral context (for further discussion, see Chapter 4).

A final point that will be discussed in more detail in the next chapter is the finding that the latency of the compensatory VOR responses was 6-7 msec, consistent with data from primates (Snyder and King 1992; Crane and Demer 1998; Minor et al. 1999; Huterer and Cullen 2002) and the relatively short neural pathway from sensor to eye movement. The ~7 msec latency to passive perturbations is in contrast to the near zero latency compensatory responses that occur during self-generated (active) head movements (Chapter 4).

In their natural environment, guinea pigs often move their heads rapidly. To determine how guinea pigs achieve retinal stability over a broad, natural range of head velocities and accelerations, we examined the VOR at stimulus frequencies up to 15 Hz (acceleration ~5000 deg/s/s) and during transient head movements with accelerations up to 2500 deg/s/s. The initial response of the head to an abrupt body rotation is to remain stationary in space. As a result, head relative to body velocity initially mirrors the body's speed. We approximated the guinea pig's head/neck biomechanics by a second order model similar to that suggested for cats and humans (Goldberg and Peterson 1986; Peng, et al. 1996) and simulated responses similar to those shown in Figure 3.6B. In the model (Simulink, MATLAB), the initial movement of the head is determined by the interplay of three passive biomechanical parameters: head inertia, neck stiffness and viscosity. The model effectively captured the initial head response to body rotation and confirmed the

inertial character of the response with the caveat that neck muscle stiffness and viscosity at the onset are partially determined by tonic innervation. However, the model failed to capture the variability that occurred at the end of the imposed acceleration (Figure 3.7C & Figure 3.8). In fact, significant variability in head-on-body speed can first be detected soon after peak body acceleration (40-50 msec after the onset of motion). Because of its delayed onset, we hypothesize that the variability reflects a central interaction of biomechanics with changes in activity within descending pathways that convey voluntary control strategies (vestibulo- and/or reticulo-spinal tracts) and/or intrinsic spinal mechanisms (*e.g.*, cervico-collic or stretch reflex modulation of neck stiffness or viscosity). We suggest the latter to be more likely, as the stretch reflex would tend to restore the position of the head with respect to the body axis. The second order model fails to capture the variability since its parameters are fixed at the onset of the perturbation. Additional experiments would be required to determine the relative influence of tonic innervation, descending modulation (*e.g.*, the VCR), intra-spinal mechanisms and "passive" biomechanics.

In the intact guinea pig, the distributions of initial head velocity are skewed in a compensatory direction so as to temporarily stabilize head position in space and not relative to the body. Functionally, this response would augment the VOR and improve gaze (eye + head) and retinal image stability in space. However, subsequent head and rapid eye movements restore the alignment of head and eye with the body axis. It is tempting to assume that the initial response of the head is inertial as suggested by the model simulations and that the VCR plays at most a minor role in the behavior. However, Figure 3.10B-D shows that after chemical lesions, which completely destroy the

vestibular sensory receptors, head velocity is evenly distributed around zero – i.e., with no preference for the compensatory direction. This result is surprising since one would expect the inertial lag alone to shift the distributions in a compensatory direction. Additional analyses of animals with compensated vestibular lesions suggest that neck stiffness and viscosity increase with time following a bilateral lesion (as illustrated by changes in head-on-body velocities from 1 to 4 weeks post-lesion, Figure 3.10C & D). The mechanism that accounts for this change is not known. However, the "stiffened" neck would effectively reduce the inertial lag of the head and any "compensatory" head relative to body peak velocity (Figure 3.6B, arrow). Thus, changes in neck stiffness may account for the lack of significant bias in the post lesion velocity distributions. The proposed mechanism is reminiscent of an "en bloc" strategy to stabilize the head on the body that is employed by many human patients with bilateral vestibular loss (Horak 2010). Although intact guinea pigs occasionally use an en bloc strategy to rapidly reorient themselves (a hop), we believe the hypothesized changes in neck stiffness represent a compensatory mechanism that helps the animal to maintain alignment of head and body in response to passive perturbations.

Head alignment was not uniformly maintained across all stimuli in the intact guinea pig. Although the head is relatively stable for moderate perturbations (*e.g.*, Figure 3.6), animals had difficulty stabilizing their heads during high frequency and/or high acceleration periodic stimuli (Figure 3.1). Two effects were striking: first there was a rapid increase of head velocity that could exceed body velocity by a factor of 4 or more; second, head velocity phase lead increased to 90 deg or in-phase with body acceleration (Figure 3.1). The phase shift implies that head velocity was determined by acceleration,

which implies that inertial forces dominated vestibular and neck reflexes; the animal was unable to stabilize his head in space. This failure was not one of vestibular sensation since VOR gain was actually enhanced during these episodes. The VOR data are similar to those reported for head-restrained primates rotated at high frequencies (Minor et al. 1999; Huterer and Cullen 2002).

Chapter 4

Guinea Pig Responses to Self-Generated (Voluntary) Head Movements

An interesting aspect of eye-head coordination is the interplay between the two during self-generated head movements. There are a number of differences between voluntary motion and passive whole body rotation. First, since the animals initiate the head movement, additional information is available to the nervous system regarding the head movement prior to any vestibular feedback. Second, depending on the animal's intention for the head movement, the necessary eye movement behavior may vary. For example, if a subject is attempting to switch his direction of regard (or gaze) then the VOR must be suppressed. Otherwise, the VOR would be counterproductive to the head movement, as the goal of the VOR is to maintain constant gaze. In contrast, if the head movement is initiated without the intent to shift gaze, for example in the case of chewing or irritative head shaking, a compensatory eye movement may be required to maintain a still image of the world on the animal's retina. This study was conducted to better understand eye-head coordination during self-generated head movements and quantify the characteristics of the eye movement responses during normal active head movements of the guinea pig.

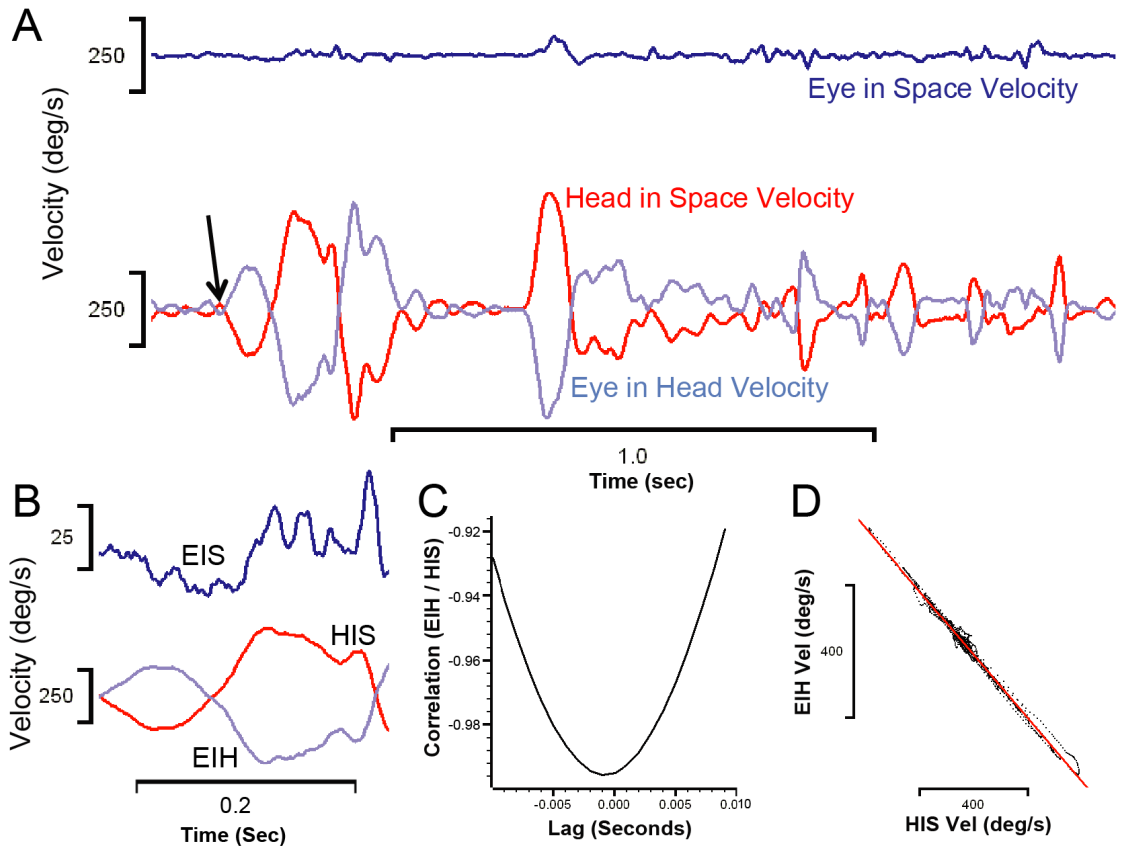

Figure 4.1: Anticipatory eye movements that preserve retinal image stability occur in temporal synchrony with head movements.

A Example of self-generated head movements and eye movement responses. *Upper, blue trace* eye-in-space (gaze) velocity*; lower blue trace* eye-in-head velocity; *lower red trace* head-in-space velocity. **B** Portion of record shown in **A** (*arrow*) with expanded gaze velocity scale. **C** Waveform cross-correlation of the data segment shown in **A**. The anticipatory response latency is the lag (-1 msec) at the maximum correlation. **D** Linear regression analysis of eye-in-head and head-in-space velocities for the data segment shown in **A**. The regression slope is -0.95.

4.1 Active Head Movements in Normal Animals

Figure 4.1A illustrates a 2 second-long sequence of self-generated head movements in the dark. During this sequence, the guinea pig made large head turns with speeds that exceeded 400 deg/s (lower red trace). Eye-in-head velocity (lower blue trace) mirrored the head-in-space, and changes in head speed and direction were matched by changes in eye speed and direction. The upper blue trace shows that gaze velocity, the sum of eye-

in-head and head-in-space, was nearly zero throughout the sequence. The stability of gaze is more clearly seen in Figure 4.1B where the gaze velocity scale is expanded by a factor of ten for the data segment beginning at the arrow. The apparent temporal synchrony of the eye and head data suggests that the eye movement anticipates the head movement. This idea is confirmed by the waveform correlation (Figure 4.1C) which demonstrates that the eye-in-head movements led head-in-space movements by ~1 msec. The negative (anticipatory) latency of this response is in contrast to the VOR-initiated compensatory eye movements, for which the latency was ~7 msec (Chapter 3).

Figure 4.1D shows that the anticipatory eye movement accurately mirrored head velocity; eye-in-head velocity was inversely proportional to head-in space velocity (regression slope = -0.95). The near perfect ocular compensation of the voluntary head turn contrasts with the less than perfect VOR compensation of passive perturbations (regression slope ~-0.5, Chapter 3).

Figure 4.2 shows two more samples of gaze shifts initiated by head turns that represent the typical pattern of these movements in guinea pigs. Both examples are from the same animal: the records in Figure 4.2A were obtained in darkness, those in Figure 4.2B in the light. Across all animals, we found no substantive differences between responses that occurred in the light and those that occurred in darkness. Both examples show that a head movement initiates the gaze shift (upper panels, gray traces) and is followed by a rapid eye movement (upper panels, black traces) that orients the eye in the new gaze direction (Mirenowicz and Hardy 1992). With the exception of the reversed order of head and eye movement, the pattern resembles that of primates. However, the guinea pig does not have a fovea. Thus, although the rapid eye movement is saccadic in its kinematics, it is

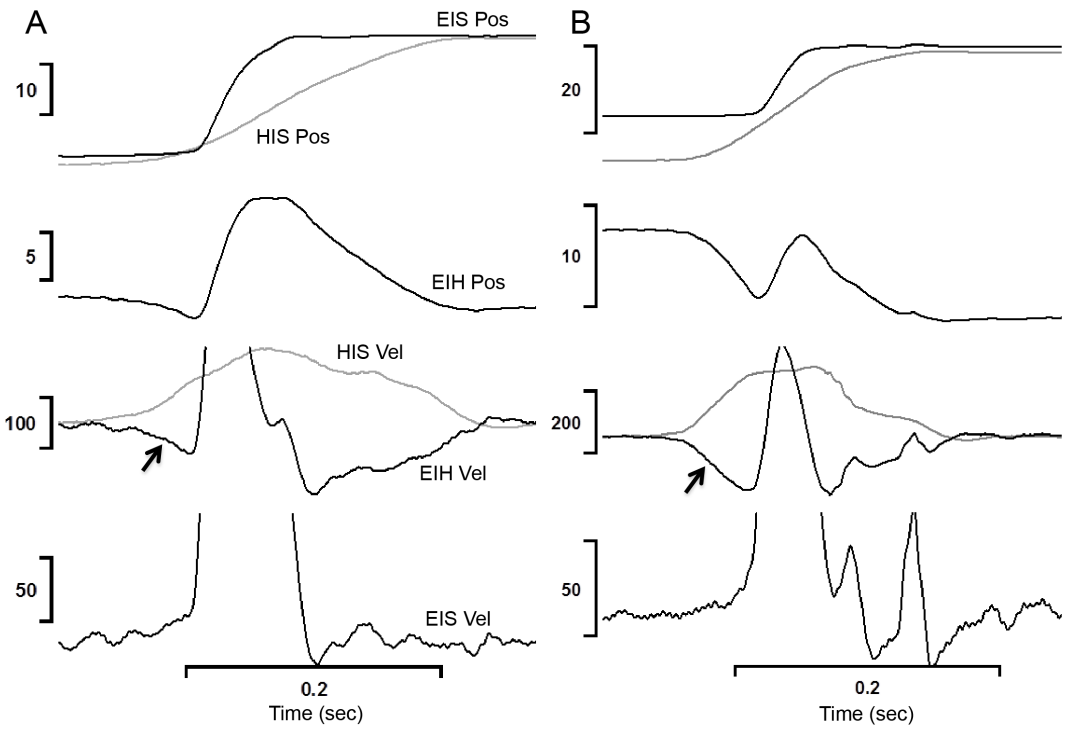

Figure 4.2: Two examples of anticipatory eye movements during self-generated rightward head movements.

In both panels, the uppermost traces are head (*gray*) and eye (*black*) position in space; 2nd panel from top shows eye-in-head position (*black*); 3rd panel from top shows head (*gray*) velocity in space and eye (*black*) velocity in relative to the head; 4th panel from top shows eye velocity in space (*black*). The arrows indicate the anticipatory eye movement that precedes the rapid eye movement.

analogous to a vestibular quick phase in that it predictively corrects for the change in eye position produced by the image stabilizing slow phase rotation of the eye.

The eye-in-head position records (second panel from the top) illustrate the complementary interaction of the slow and rapid components of the ocular response. In Figure 4.2A, the final position of the eye-in-head is centered after the ~25 degree head turn; in Figure 4.2B, the eye is initially off-center and is returned to a central position at the end of the head turn. The VOR is usually assumed to produce the compensatory ocular rotation; in these examples, however, the latency of the response is too short for it to be produced by the VOR. Figure 4.3A & C show waveform correlations for the initial

compensatory eye movements (arrows, Figure 4.2A & B respectively) that confirm latencies less than zero and thus demonstrate the anticipatory nature of these responses. Although the initial compensatory response shown in Figure 4.2A (arrow) was anticipatory, the compensatory response following the rapid eye movement had a longer latency (Figure 4.3B, 8.2 msec) consistent with this segment having been produced by the VOR. The corresponding data segment in Figure 4.2B was interrupted by two small anti-compensatory rapid eye movements that caused the intervening compensatory intervals to be too short for the computation of latency using waveform correlation. The lower panels, Figure 4.3 D-F, show regression analyses of eye-in-head relative to head-in-space for the corresponding data segments whose correlations are illustrated in Figure 4.3 A-C; for each data segment, the relationship was linear (Figure 4.3D, slope=-0.75; Figure 4.3E, slope=-0.89; Figure 4.3F, slope=-0.94).

Figure 4.4A shows the distribution of latencies for 74 segments of anticipatory responses to self-generated head movements in 3 animals. To compute lag (or response latency), cross correlations of the eye-in-head and head-in-space velocities were performed. The mean anticipatory latency was 0.0001 ± 0.0025 sec (standard deviation). To ensure that segment lengths were not confounding the results, a regression of segment length to lag was performed and no relationship was found ($r^2 = 0.029$). Additionally, the 74 segments were broken up into 25 msec long intervals, analyzed for latency and binned. The procedure confirmed the results shown in Figure 4.4 (mean -0.2 ± 0.27 msec, n = 47,827). Most of the computed latencies were less than zero verifying the anticipatory nature of the responses illustrated in Figure 4.1 & Figure 4.2.

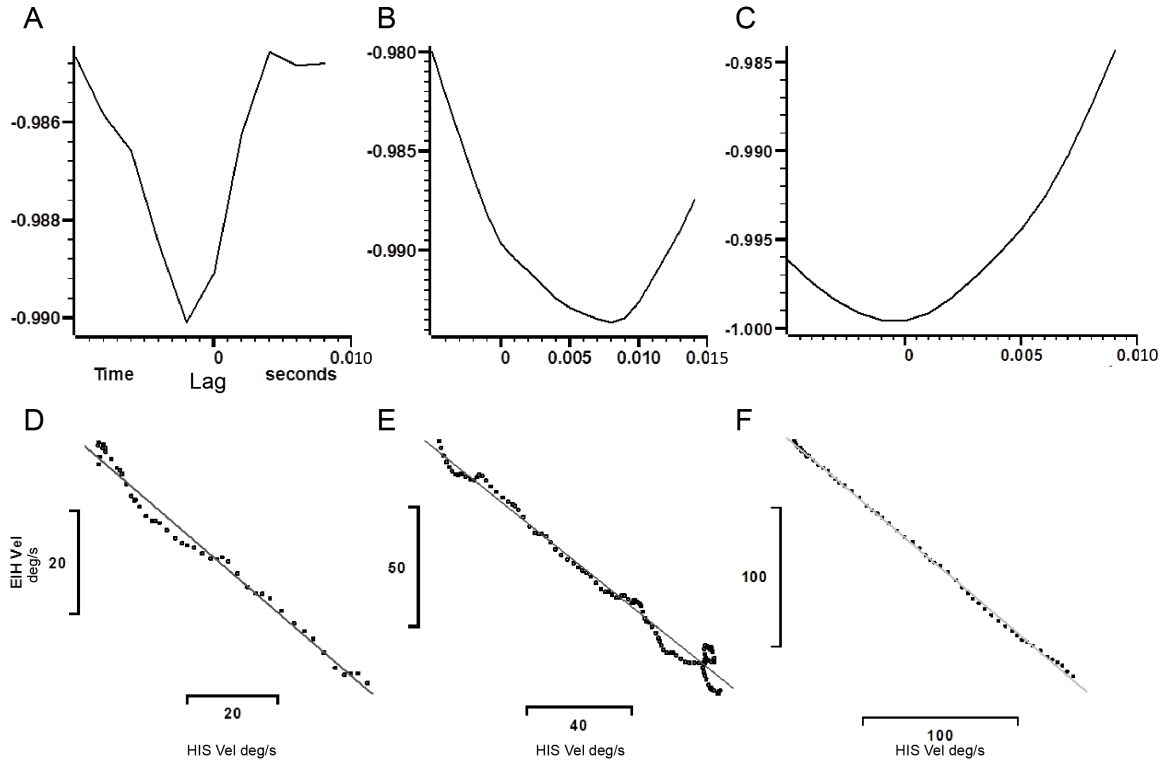

Figure 4.3: Cross correlation and regression analyses of the data segments illustrated in Figure 4.2.

A Waveform correlation for the segment indicated by the arrow in Figure 4.2A. The latency is -2 msec. **B** Waveform correlation for the data segment that follows the rapid eye movement in Figure 4.2A. The latency is 8 msec. **C** Waveform correlation for the segment indicated by the arrow in Figure 4.2B. The latency is -1 msec. **D** Regression analysis for the segment indicated by the arrow in Figure 4.2A. The regression slope is -0.75. **E** Linear regression analysis for the segment that follows the rapid eye movement in Figure 4.2A. The regression slope is -0.89. **F** Regression analysis for the segment indicated by the arrow in Figure 4.2B. The regression slope is -0.94.

We analyzed the 74 data segments further to establish the relationship of head and eye velocity. Eye and head velocity were recorded for multiple segments of active head movement that occurred in the absence of passive rotation. Linear regressions of eye-in-head versus head-in-space velocity were done for consecutive points of each active head movement segment. Segments for which no valid cross correlation value could be found were excluded from the analysis. Figure 4.4B shows the resultant distribution of slope values. Statistically, the slopes were normally distributed with a mean of -0.80, i.e., the

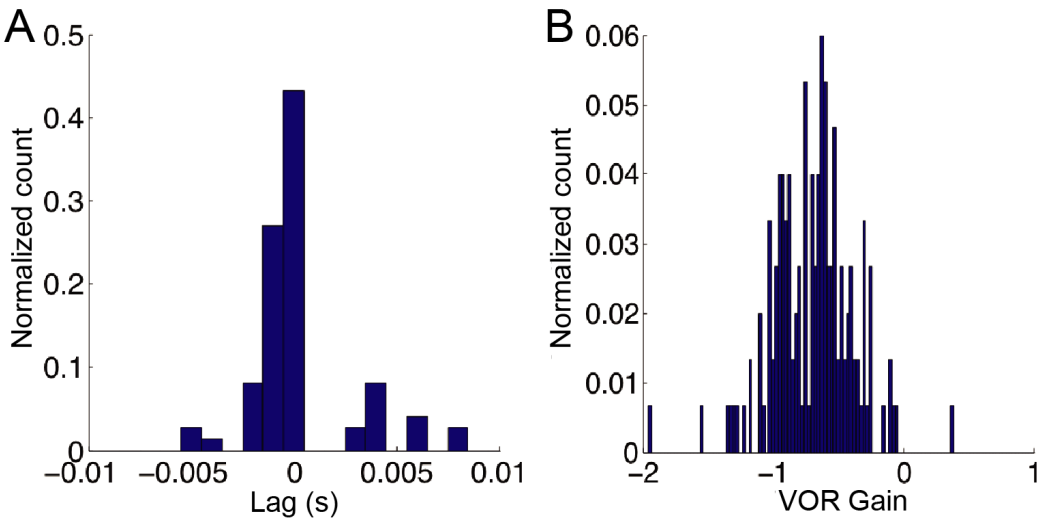

Figure 4.4: Latencies and gains of compensatory eye movements.

A Distribution of eye movement latencies (lags) associated with self-generated head movements. **B** Distribution of regression slopes (compensatory gain) of eye versus head velocity associated with self-generated head movements. The ‘‘normalized count’’ is the count of items in each bin divided by the total number of counts.

anticipatory movements compensated on average for 80% of head velocity. To determine if there was a relationship between response gain and latency, the gain values were re-plotted subject to certain conditions. The data set was divided into two distributions – one with only gain values associated with lags less than 2 msec (n = 61) and another with values associated with lags greater than or equal to 2 msec. The mean of the smaller lag subset was slightly higher (-0.81± 0.23) than that of the other subset (mean = -0.74 ± 0.19).

4.2 Active Head Movements in Animals with Bilateral Vestibular Lesions

None of the guinea pigs with bilateral peripheral vestibular lesions were able to produce eye movements that could compensate for unpredictable, passively-induced head movements; this deficit persisted for the entire post-lesion survival time of 4 months

(Chapter 3). However, within one week post-lesion, the same animals were able to effectively compensate for self-generated head movements. Figure 4.5 shows two representative samples of anticipatory responses in one animal 4 months post-lesion. Figure 4.5A illustrates the animal's initial lack of response to a passive perturbation followed by a robust anticipatory response to a self-generated head turn. Initial eye-in-head velocity (black trace, 3rd panel from top) was persistently zero immediately after the abrupt onset of head and body rotation (arrow). As a result, initial eye-in-space (gaze) velocity (arrow, lower panel) tracked head velocity. In this data segment, ~150 msec after the passive perturbation, the animal actively counter-rotated its head (gray traces, upper & 3rd panel). The head turn was accompanied by an anticipatory ocular counter-rotation (3rd panel, black trace, latency=-2 msec) that produced a gaze velocity of close to zero deg/s during the head movement (regression slope = -0.86, eye relative to head).

Figure 4.5B illustrates another example of a post-lesion anticipatory response. In this record, the guinea pig actively rotated its head counterclockwise through an angle of nearly 50 degrees (gray trace, upper panel). In temporal synchrony with the voluntary head turn, the eye-in-head counter-rotated (2nd panel from top) and gaze velocity (bottom panel) was zero prior to the occurrence of the rapid eye movement. One noteworthy feature of the post-lesion data can be seen in this Figure: deviations of eye-in-head position were frequently greater than in the intact animal because rapid anti-compensatory eye movements either failed to occur (Figure 4.5A) or compensated inaccurately for the ocular counter-rotation (Figure 4.5B).

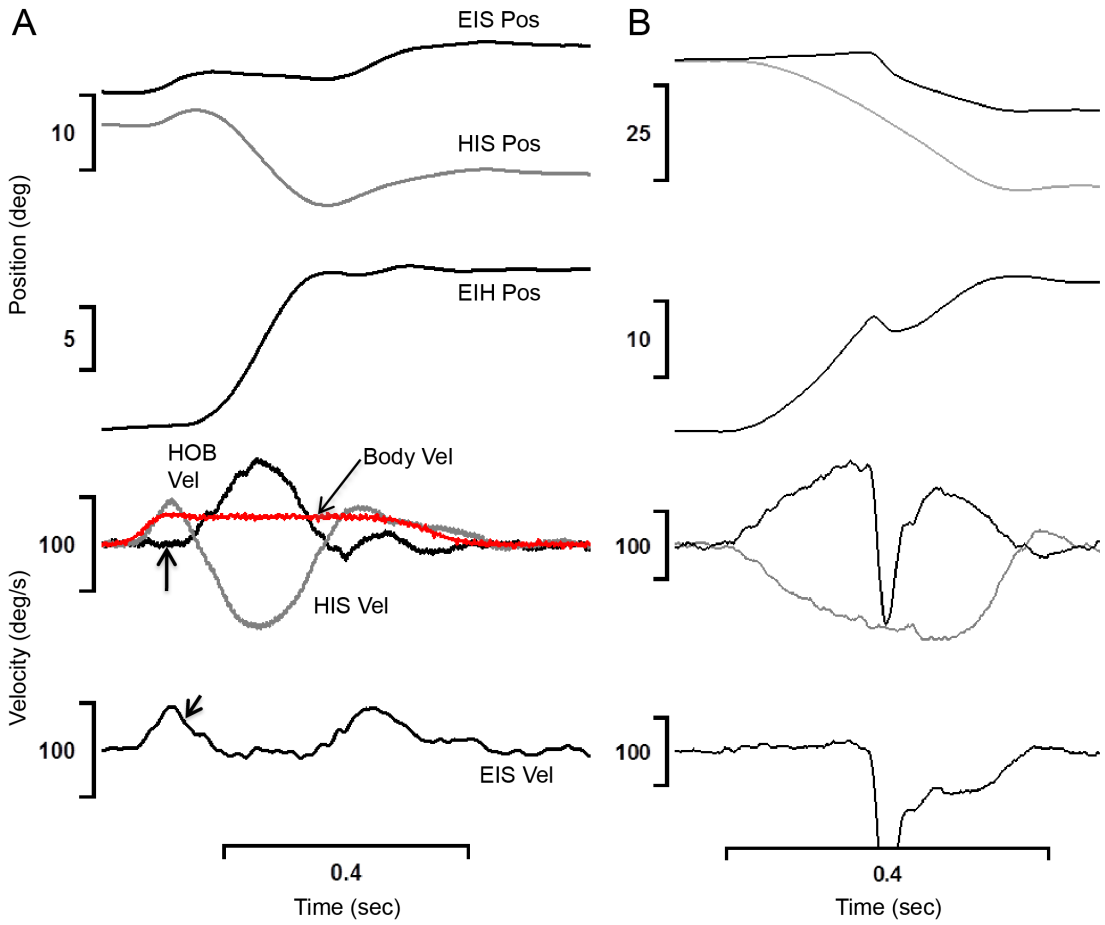

Figure 4.5: Two examples of anticipatory eye movements in an animal 4 months after a complete bilateral vestibular lesion.

A Passive perturbation followed by an active head movement. **B** Active head movement. Traces are ordered as in Figure 4.2

To compare anticipatory eye movements in lesioned animals to those in intact animals, voluntary head movement segments were selected from control and 2 week post-lesion recordings. Figure 4.6 shows distributions of latency (A) and gain (B) for anticipatory responses in control and 2-week post lesion animals. Two weeks post-lesion the mean lag of the anticipatory eye movements in responses to head movements was -0.001 ± 0.001 sec (n = 371), and was indistinguishable (t-test, α=0.05) from the control responses of the same animals (-0.001 ± 0.001 sec, n = 242). Figure 4.6B shows the distributions of

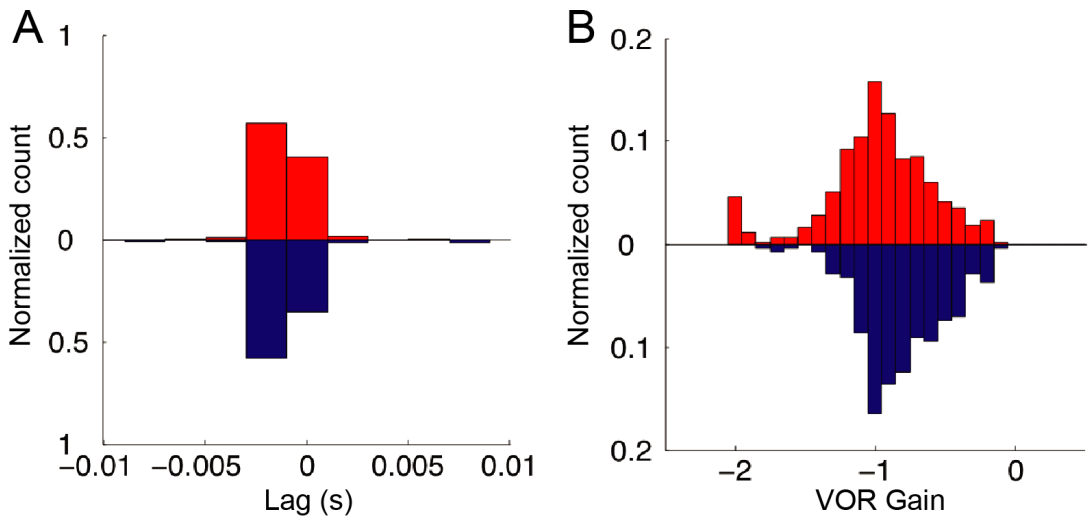

Figure 4.6: Latencies and gains of compensatory eye movements 2 weeks after complete bilateral lesion of the vestibular periphery.

Distribution of anticipatory eye movement latencies (**A**) and regression slopes (gain, **B**) in five animals recorded 2 weeks after bilateral vestibular lesions. Upper half (*red)* of each panel shows data from lesioned animals; lower half (*blue*) is control data.

response gains. Two weeks post lesion the mean response gain was -1.11 ± 1.22, greater than that measured in the control condition (-0.80 ± 0.30). The pre- and post-lesion distributions were statistically different (t-test, α=0.05).

4.3 Discussion

We describe, for the first time, a novel compensatory and *anticipatory* ocular response that occurs in conjunction with self-generated head movements. Gresty's (1975) pioneering study of the unrestrained guinea pig described the pattern of head and eye movements associated with voluntary gaze shifts, but because of technical limitations, he could not measure the latency of compensatory eye movements. However, he concluded that the "vestibular-ocular reflex is utilized in a frequency range in which it produces perfect compensation for fast programmed head movements". In this study, we find that the anticipatory eye movements are unlikely to be dependent solely on vestibular sensory

inflow since they occur in animals with complete bilateral vestibular lesions (Figure 4.5) and are synchronous with head motion. Although the extravestibular origin of these responses could not be determined with certainty by our experiments, we believe that the timing of these anticipatory responses renders neck muscle proprioception or other sensory afferents unlikely origins for these responses that precede any detectable movement of the head (Figure 4.3). However, we cannot exclude the possibility of EMG activity in neck musculature that precedes actual movement of the head and modulates the discharge of secondary vestibular neurons (Vibert et al. 1999). Alternatively, the anticipatory responses may be dependent on motor efference ("efference copy", von Holst and Mittelstaedt 1950; Mittelstaedt 1971). Dichgans et al. (1973) described compensatory eye movements in non-human primates that were associated with self-generated head movements and were not dependent on vestibular sensation since they were observed to occur in animals with bilateral labyrinthectomies. Furthermore, the compensatory responses were still present after surgical lesions interrupted proprioceptive input from the cervical spinal cord. Newlands et al. (1999, 2001) reported similar findings in monkeys with bilateral canal plugs or unilateral vestibular lesions. Although these authors believed the compensatory eye movements developed as an adaptive response to vestibular lesions, Zhou et al. (2010) recently reported zero latency compensatory eye movements in intact monkeys during voluntary head turns, suggesting that anticipatory responses are part of an animal's normal behavioral repertoire. This behavior may be more common in more species than previously suspected. In swimming Xenopus tadpoles (Combes et al. 2008) and in lamprey during fictive swimming (Grillner 2008), similar patterns of anticipatory ocular responses have been described.

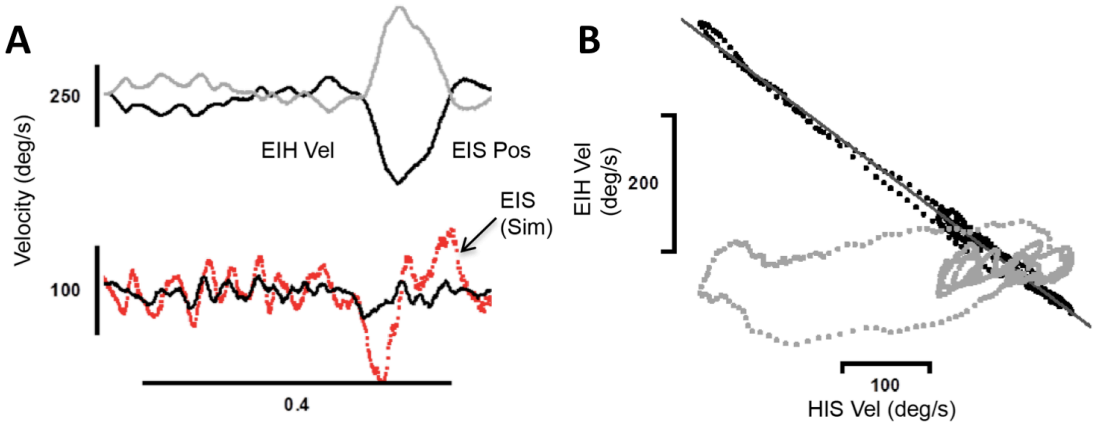

Figure 4.7: Temporal synchrony of anticipatory eye movements with head movement improves retinal stability.

A Upper panel head-in- space velocity (*gray*); eye-in-head velocity (*black*). Lower panel eye- in-space (gaze) velocity (*black*) and simulated eye-in-space if the anticipatory response were delayed 7 msec (*red*). **B** Regression analysis of eye and head velocity. *Black dots*, actual data; *gray dots*, delayed data.

We found the anticipatory responses to differ from those produced by the VOR alone in two significant ways. First, anticipatory responses are characterized by temporal synchrony with voluntary head movements (~1 msec versus ~7 msec for the VOR). Second, the anticipatory responses have higher gains (0.80 versus 0.46 for the VOR, Chapter 3). Although it is obvious that higher gain should produce better compensation, it is less obvious that temporal synchrony might be behaviorally significant. Figure 4.7 illustrates the effect of simulating a delay of eye-in-head velocity equal to the average latency of the VOR. The upper panel of Figure 4.7A shows a representative segment of a compensated active head movement. The anticipatory eye movement (black trace) was temporally synchronized with the head movement (gray trace, latency = 0 msec) and compensatory eye velocity was linearly related to head velocity (regression slope = -0.97, Figure 4.7B). The black trace in the lower panel of Figure 4.7A shows that the guinea pig's eye-in-space (gaze) velocity was near zero during most of the active head movement segment. However, if the anticipatory eye movement were delayed by the

latency of the VOR (~7 msec), then retinal image stability would be significantly worsened as indicated by the larger and more variable gaze velocity (red trace, Figure 4.7A). During the active head movement, the short delay associated with the VOR is sufficient to disrupt the correlation between eye and head velocities (Figure 4.7B, gray data points). Although it is unlikely that the VOR would produce errors of this magnitude, this example suggests that even a small temporal lag during a self-generated rapid head movement has the potential to produce large image slip velocities across the retina. Although a lack of temporal synchrony may only briefly degrade the guinea pig's already poor visual acuity, more significantly, it may hinder the animal's ability to detect movement of an object in its environment (Land 1999). Self-generated movements are purposeful – for example, the guinea pig may shift its gaze toward a sound or odor that might signal the presence of a predator. A heightened ability to detect movement in the environment would be critical at such times.

Anticipatory movements are typically associated with voluntary head movements; however, a similar mechanism could assist the VOR when the head is passively perturbed so as to produce repetitive and predictable head movements. For example, the guinea pig's head oscillates in response to abrupt acceleration transients with a natural frequency of 12-14 Hz (Chapter 3). In this frequency range a 7 msec time lag would be expected to produce up to 36 degrees of phase lag, enough to disrupt image stability unless otherwise compensated. Previous studies of the monkey's VOR (Huterer and Cullen 2002; Minor et al. 1999; Ramachandran and Lisberger 2005) have shown that VOR responses to periodic stimuli up to 50 Hz exhibit less phase shift than would be predicted by the 7 msec latency of the reflex. In order to account for their results, Ramachandran and Lisberger proposed

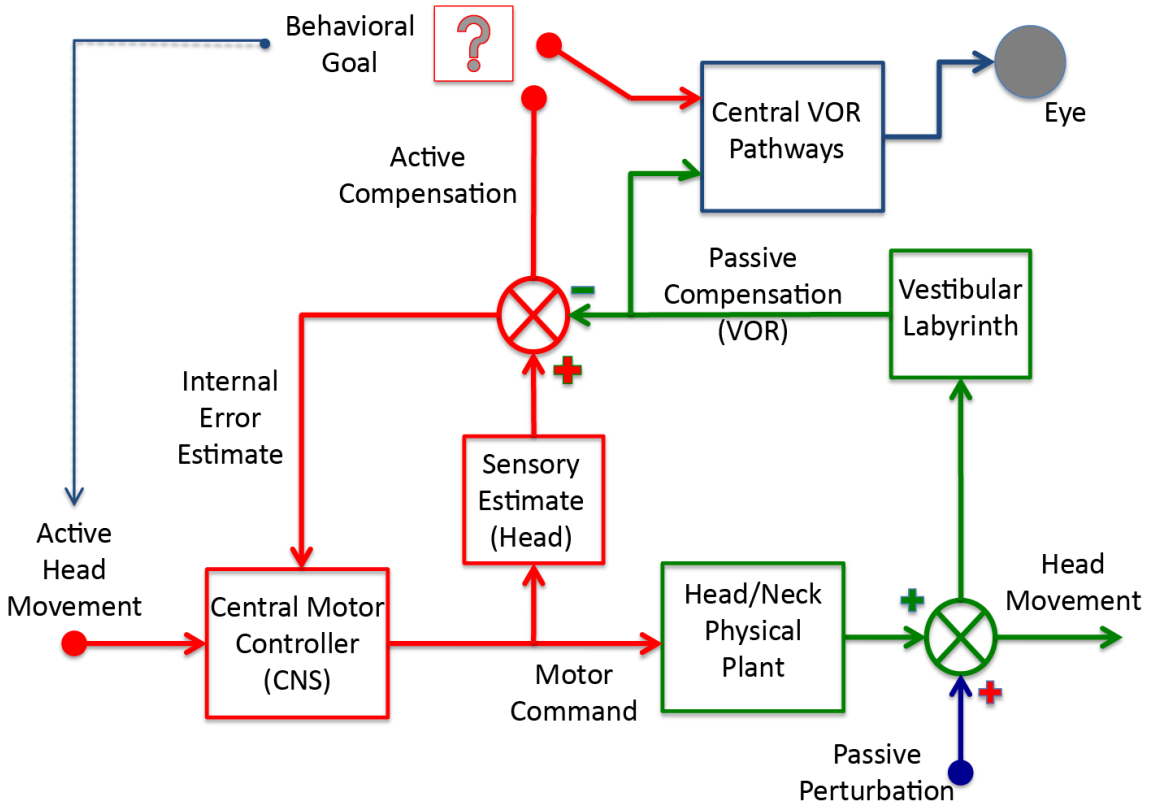

Figure 4.8: Conceptual feed-forward model of proposed anticipatory eye movement mechanism.

Details in text.

a model with negative latencies. The negative latency pathway was suggested as a computational "placeholder" for primary afferent fibers (*e.g.*, Hullar et al. 2005) with sufficient phase leads to account for the required "negative" latency at high frequencies.

Alternatively, we propose that the VOR may be assisted by a feed-forward predictive mechanism (see below) that senses vestibular and proprioceptive feedback during periodic (predictable) head motion. Consistent with this idea, we observed synchronous compensatory responses during many data segments with high frequency head oscillations induced by passive perturbations. Such a mechanism would be useful since the head plant has been shown to exhibit instability in many species (humans, Keshner et al. 1995; Peng et al. 1999; cats, Peterson et al. 1981; for guinea pig see Chapter 3). We

suggest that the proposed anticipatory mechanism that stabilizes retinal images during self-generated head movements might also play a role in stabilizing gaze during passive head perturbations that induce head oscillations.

Figure 4.8 presents a conceptual model of how this proposed mechanism and the VOR might interact. The model hypothesizes feed-forward control of the head for voluntary movements (Frens and Donchin 2009; McNeilage et al. 2008; Shadmehr et al. 2010; Wolpert and Miall 1996). If an animal is passively perturbed so as to produce an unexpected head movement, the vestibular system will sense that movement and produce compensatory eye movements via the VOR pathways. For self-generated (voluntary) head movements, the feed-forward controller produces the command to move the head. In a feed-forward model, neural circuits that implement an internal model of head/neck plant dynamics and the vestibular sensory apparatus are presumed to exist. These neural circuits generate both a motor command that is appropriate to move the head (but inappropriate to move the eyes) and an estimate of the sensory response. The anticipated sensory estimate of the impending head movement must be correctly scaled and transformed into a vestibular coordinate frame so as to be directly comparable to an actual vestibular sensory signal. One purpose of this signal is to enable the brain to distinguish vestibular sensation resultant from an active head movement from that caused by external perturbations that also produce head movement. In the model this task is accomplished by comparison (at the summing junction) of the sensory estimate to the actual sensory signal. We propose that the difference of these two signals (H_{est}-H_{ves}) is used to produce the anticipatory response. If it were added to the vestibular signal in the central VOR pathways, the net input to the VOR would be H_{est}. If the motor controller

were accurate and there were no external perturbations, then this estimate would faithfully mimic the actual vestibular sensory inflow and thus would produce an accurate anticipatory eye movement. If either the estimate were in error, or if there were unexpected perturbations of head movement, then this signal could be used centrally to modify the ongoing head movement and the ocular response (Lehnen et al. 2009). The switch labeled "behavioral goal" allows for this possibility; if H_{est}-H_{ves} is not zero, then the best strategy might be to rely more heavily on the actual vestibular inflow rather than the estimate. In a bilaterally lesioned animal, the pathways associated with the "vestibular labyrinth" are destroyed and there is no passive VOR. However, the sensory estimate may be used directly to drive compensatory eye movements such as those shown in Figure 4.5A. This model is consistent with our findings and previous studies (*e.g.*, Bizzi et al. 1971; Newlands et al. 2001; Lehnen et al. 2009) but remains hypothetical until tested by future experiments. One aspect of the model merits attention: if the sensory estimate is encoded in vestibular coordinates, then it may be difficult to distinguish it using single unit recordings from an actual sensory response unless the head trajectory is perturbed in an unpredictable manner.

Chapter 5

Galvanic Vestibular Stimulation in Head Unrestrained Guinea Pig

To further probe eye and head movement responses during voluntary head movements and PWBR, we performed galvanic stimulation in head-unrestrained guinea pigs. The current study presents novel evidence for effects of GVS on vestibularly induced, compensatory head movements. Surprisingly, GVS also has a significant effect on the VOR of head-unrestrained guinea pigs during both sinusoidal and transient head motion over a broad range of movement frequencies and velocities. In contrast, there is no effect of GVS on the anticipatory compensatory eye movements that occur during self-generated (active) head movements of these animals (Chapter 4).

The approach of the current study differs in two critical ways from previous work. First, our experiments were done in the guinea pig, unlike the seminal studies of Minor and Goldberg (1991); second, both eye and head responses to horizontal vestibular stimulation were measured in animals whose heads were unrestrained, a more natural paradigm. Previously published reports from another laboratory have described the effects of GVS on vestibular nerve activity in the guinea pig. Kim and Curthoys (2004) replicated, in anesthetized guinea pigs, the preferential effect of GVS on irregular afferents found in chinchilla (*e.g.* Baird et al. 1988) and monkey (*e.g.* Goldberg et al. 1984). However, the effect of GVS on primary afferent activity does not necessarily

predict its effect on the VOR. For example, there may be species-specific differences in central processing of afferent vestibular signals. Guinea pigs are lateral-eyed, afoveate, terrestrial animals and, unlike primates, they do not produce smooth pursuit eye movements (Marlinsky and Kröller 2000), which share central pathways that process signals related to the VOR in monkeys (Roy and Cullen 2002). Furthermore, in the mouse, another afoveate species, Beraneck and Cullen (2007) showed that putative secondary vestibular neurons with eye position sensitivity ("ES neurons") encode a signal correlated with eye velocity during optokinetic nystagmus (OKN). However, in contrast to previous findings in primates, mouse vestibular-only cells ("VO neurons") do not exhibit activity related to either the visual motion stimulus or eye movement during OKN.

The presence or absence of head restraint is another potentially significant factor, since any head movement (active or passive) that is concurrent with the rotational stimulus will directly modify the afferent vestibular signal itself as well as produce proprioceptive inflow to vestibular neurons (Roy and Cullen 2002). Moreover, head movement has been shown to alter experimental outcomes in a number of behaviors, for example sound localization (Populin 2006). In the case of the vestibular system, specifically, electrophysiological recordings from the primate vestibular nucleus have shown that the activity of VO neurons changes when the head is allowed to move (for review, see Cullen and Roy 2004). Chapter 3 describes VOR and head movement responses in head-unrestrained guinea pigs over a wide range of passive whole body rotational speeds and frequencies. These results were comparable to responses previously shown in guinea pigs tested with restrained heads (Escudero et al. 1993). In the two

studies, VOR performance (gain) was measured to be quite similar despite a difference in head restraint. That finding indicates that signal processing in the VOR pathways is not substantially modified when the head is free to move as compared to head restrained experiments. In agreement with those findings, we confirm in this report that the presence or absence of head restraint does not influence the effect of GVS on the guinea pig's VOR.

5.1 Sinusoidal Rotation

5.1.1 Inhibitory GVS

Inhibitory galvanic vestibular stimulation (anodal GVS) had a significant effect on compensatory head and eye movements as shown in Figure 5.1. Figure 5.1A shows an animal's responses to 5 Hz sinusoidal rotations at 20 deg/sec with bilateral anodal galvanic stimulation (cycles 2-4) and without GVS (cycles 7-11). The effect of GVS is clearly visible in the eye-in-space, or gaze velocity trace (dark blue trace) and in the head-on-body velocity trace (maroon trace). During stimulation eye-in-space velocity (gaze velocity) is greater, indicating smaller compensatory eye movements in response to the externally driven rotation. The stimulus-evoked suppression of compensatory eye velocity is clearly shown in the higher temporal resolution panel Figure 5.1B and the plot of eye versus head velocity (Figure 5.1C, regression slope with GVS = -0.57) as compared to cycles without GVS (Figure 5.1D, regression slope = -0.76). Similarly, Figure 5.1E-G shows that compensatory head relative to body velocity is reduced during anodal GVS stimulation. Figure 5.1F shows head plotted versus body velocity with GVS (regression slope = -0.06) and Figure 5.1G without GVS (regression slope = -0.18).

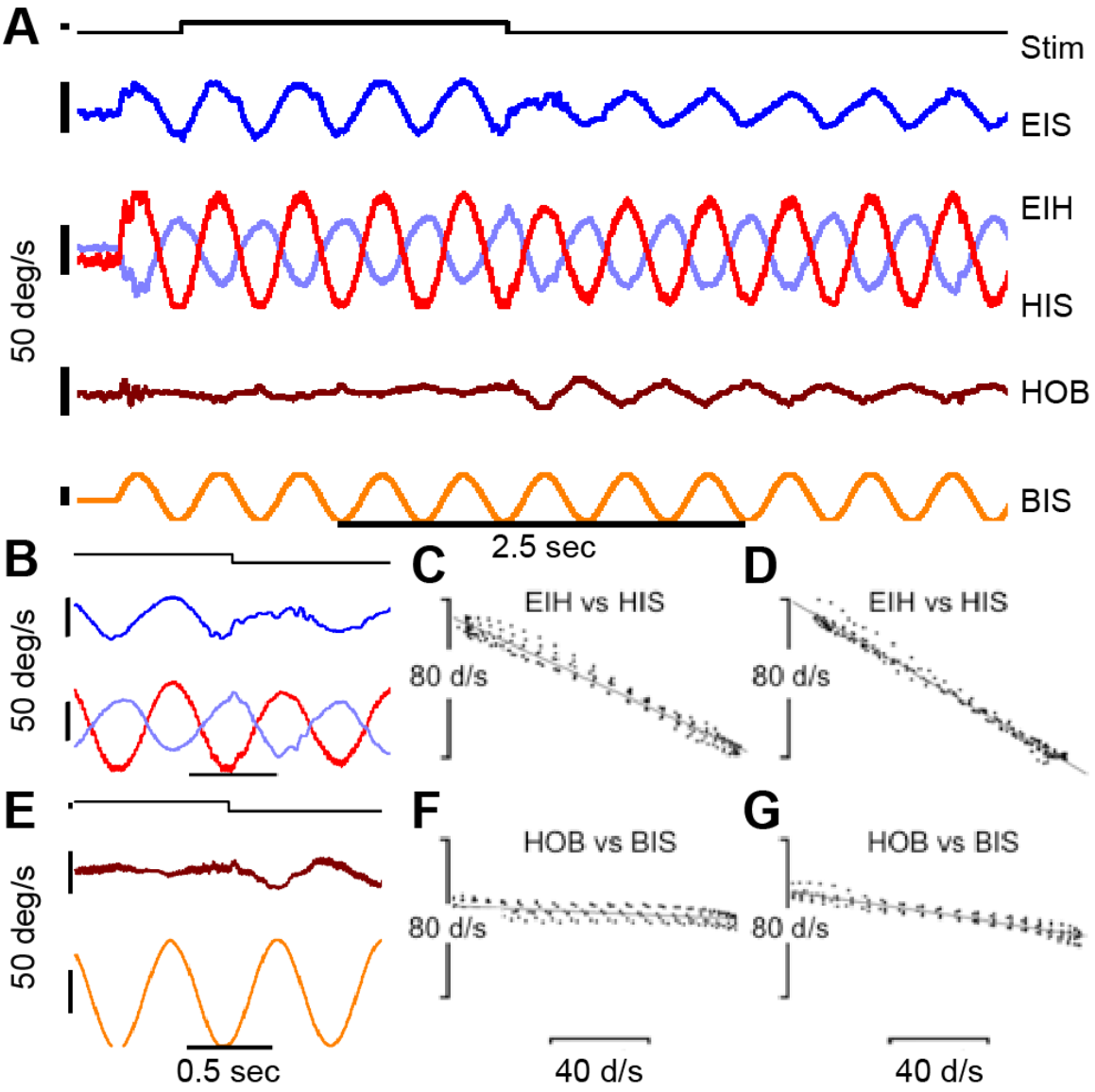

Figure 5.1: Anodal GVS suppression of compensatory eye and head movements.

A 5 Hz sinusoidal rotation at 20 deg/sec with bilateral anodal galvanic stimulation (cycles 2-5) and without GVS (cycles 6-11). During stimulation (Stim, elevated upper trace) eye-in-head velocity (EIH, *light blue*) and head-on-body velocity (HOB, *maroon*) are decreased. **B** Higher temporal resolution panel of cycles 5 & 6 showing eye-in-space (EIS), eye-in-head (EIH) and head-in-space (HIS) velocity. **C** Eye versus head velocity for cycles 2-5, regression slope (VOR gain) = -0.57. **D** Eye versus head velocity for cycles 6-11 without GVS, regression slope (VOR gain) = -0.76. **E** Higher temporal resolution panel of cycles 5 & 6 showing head-in space (HIS, upper trace), head-on-body (HOB, *maroon*), and body-in-space (BIS, *orange*). **F** Head versus body velocity for cycles 2-5, with GVS, regression slope (VCR gain) = -0.06). **G** Head versus body velocity for cycles 6-11, without GVS, regression slope (VCR gain) = -0.18.

5.1.1.1 GVS Effects on Compensatory Eye Movements

To illustrate the effects of anodal GVS on the VOR across all tested frequencies, Figure 5.2 shows a representative frequency response of a single animal on a single test date. The animal shows a clear decrement in the VOR gain across all frequencies between 0.2 and 5 Hz (*e.g.*, control: 0.61 ± 0.05, GVS: 0.49 ± 0.11 at 0.2 Hz; control: 0.75 ± 0.03, GVS: 0.57 ± 0.03 at 2 Hz). At 8 Hz, however, the discrepancy is no longer evident (control: 0.82 ± 0.09, GVS: 0.85 ± 0.03). GVS appears to have had no effect on

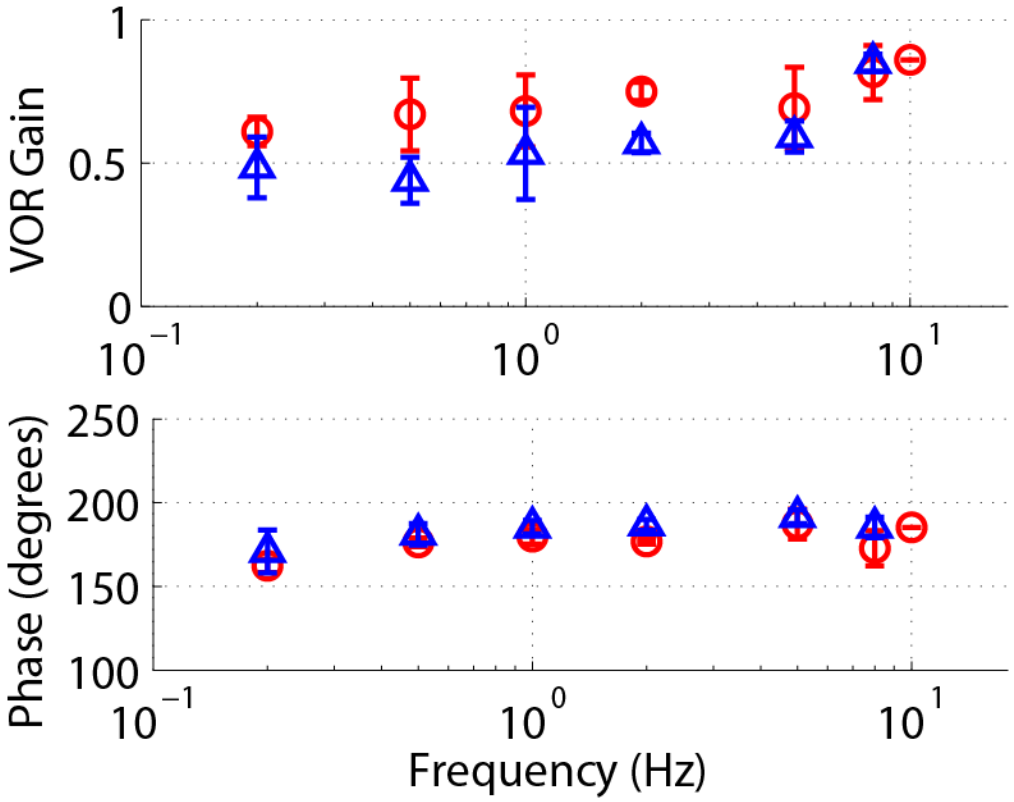

Figure 5.2: Frequency response plot for VOR responses with and without anodal GVS.

VOR gain (top panel) and phase (bottom panel) values are plotted at each tested frequency of rotation for a single animal, across all 20 deg/s rotations on a single test date. Responses without GVS (*red*) have significantly higher gains than those during anodal GVS (*blue*). Phase values, however, remain unchanged across the two conditions.

the phases of the VOR response at any of the tested frequencies (control phase values ranged from 162.33 to 187.18 degrees and GVS phase values ranged from 170.85 to 191.66 degrees).

To further quantify these changes across all instances of sinusoidal rotations, differences between GVS and control responses were computed for each of the velocities as described in Chapter 2.3.3 (each difference yielded a corresponding δ value). For compensatory eye movements, $\delta H\dot{I}S$ is plotted against $\delta E\dot{I}H$ for each paired sinusoidal cycle (Figure 5.3A & C, inhibitory & excitatory respectively). Accordingly, each plotted point represents a change in eye and head performance for an instance of sinusoidal stimulation with and without GVS. If GVS does not have a consistent effect on gain, then the δ values should be distributed randomly about the origin. If compensatory eye movements are suppressed by inhibitory GVS, then $\delta E\dot{I}H$ values will be positive (see Equation 1). Similarly, if compensatory head movements are suppressed by GVS, then the amount of head movement relative to the world will be greater during stimulation than during control, yielding negative $\delta H\dot{I}S$ values. Therefore the majority of the points would be located in the second quadrant of Figure 5.3A (negative $\delta H\dot{I}S$ and positive $\delta E\dot{I}H$). As described in Chapter 2.3.3, sinusoidal stimuli are categorized as either "low frequency" (blue, Figure 5.3) or "high frequency" (red, Figure 5.3). Figure 5.3B & D represents the number of data points in each of the quadrants for inhibitory or excitatory GVS, respectively; and quantify the distributions of δ values plotted in Figure 5.3A & C. For the inhibitory trials, Figure 5.3B shows the distributions of δ pairs plotted in Figure 5.3A. The distributions are consistent with the hypothesis that the greatest number of δ values for low frequency sinusoidal rotations should be located in the second quadrant.

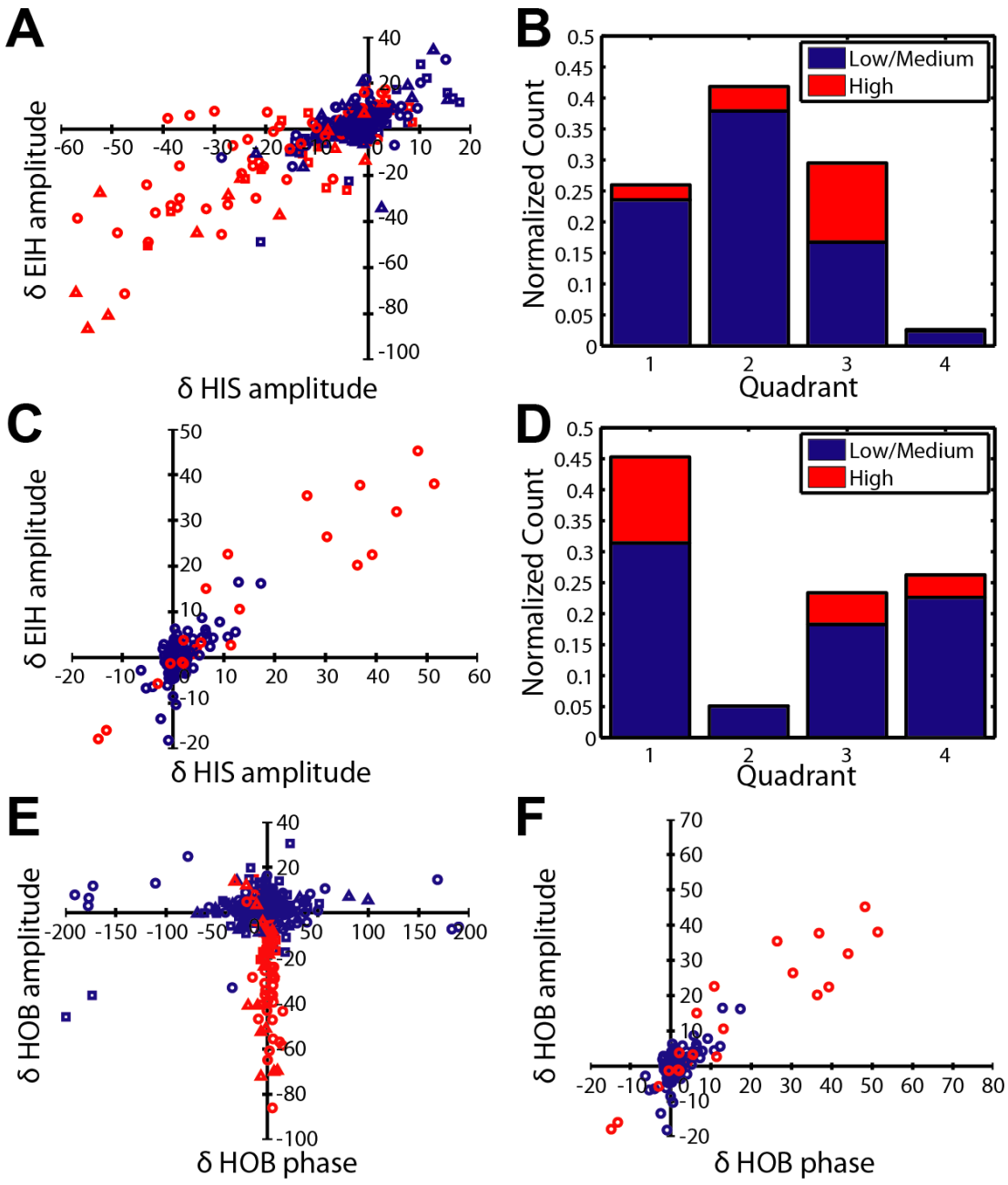

Figure 5.3: Changes in EIH, HIS and HOB velocity during sinusoidal rotation were induced by anodal and cathodal GVS.

A Computed δ values (Equations 1 & 2) of EIH and HIS velocity with anodal GVS. **B** Number of points that fall in each quadrant in **A**. **C** Computed δ values (Equations 1 & 2) of EIH and HIS velocity with cathodal GVS. **D** Number of points that fall in each quadrant in **C**. **E** Changes in HOB velocity amplitude and phase with anodal GVS. **F** Changes in HOB velocity amplitude and phase with cathodal GVS. *Blue:* Low frequency sinusoidal rotation (≤5Hz), *red:* high frequency sinusoidal rotation (> 5Hz).

5.1.1.2 GVS Effects Depend on Rotational Frequency

An interesting result of this analysis is the difference in the distributions for low and high frequency stimulation. Eye and head responses to stimulations below 8Hz are clearly suppressed by anodal GVS, indicating reduced gaze stability (Figure 5.3A & B, quadrant II, blue). However, responses above 8Hz are different for the compensatory eye movements as compared to the compensatory head movements. The majority of the ($\delta H\dot{I}S$, $\delta E\dot{I}H$) value pairs associated with high frequency stimuli fall in quadrant III in Figure 5.3A & B (red), indicating that with anodal GVS there was an increase in head velocity relative to space. Paradoxically, the gain of the VOR increased despite anodal GVS, resulting in an unexpected (compared to the low frequency stimulus results) improvement in ocular compensation. We showed earlier that for frequencies above 5 Hz, compensatory eye movements are enhanced and the animals' head movements appear to be inertially driven (Chapter 3).

To determine if an inertial component is the basis for this behavior, amplitudes and phases of the responses were examined. For the high frequency data, phase shifts between stimulus and head-on-body velocity approached 90 degrees (phase lead, control: 78.49 ± 37.32 deg; anodal GVS: 77.22 ± 37.42 deg). Thus, the motion of the head on body was nearly in phase with body acceleration consistent with the inertial hypothesis (Chapter 3). Furthermore, inhibitory GVS had little effect on the phase shift of the head re body at high frequencies (Figure 5.3E, red).

During high frequency rotation, head velocity increased and could even exceed stimulus velocity. Head velocity was further increased with inhibitory GVS current, suggesting that vestibular inputs to the neck continued to exert an effect on head stability

during inertially driven head oscillations. For low frequency oscillations, the differences between control and anodal GVS head-on-body velocities were positive (Figure 5.3E, blue), indicating a decrement in compensatory head-on-body velocity with inhibitory GVS (phase shifts approximated 180 degrees; control: 159.84 ± 61.87 deg, anodal GVS: 156.27 ± 59.32 deg). Thus anodal GVS caused significant decreases in ocular stability (Table 5.1), which were reflected by parallel increases in eye and head movement relative to the world.

5.1.2 Effects of Excitatory GVS

Excitatory GVS (cathodal GVS) caused an overall improvement in ocular stability (as shown by changes in eye-in-space velocity, Table 5.1) that was, however, much smaller than the loss of stability associated with anodal GVS. During cathodal GVS, animals showed a decrease in head velocity relative to the world and an increase in compensatory eye velocity (as is indicated by the presence of δ values in quadrant IV, Figure 5.3C & D). However, these effects were not significant for low frequency rotations. Interestingly, cathodal GVS affected the phase shifts more than the gains of the compensatory responses. Although no significant change in head-in-space phase values was found (*e.g.* low frequencies, control: 169.34 ± 20.61 deg; cathodal GVS: 171.30 ± 8.72 deg), eye-in-head phase values showed a significant difference (p = 0.01, Table 5.1). Though the changes in eye-in-head and head-on-body velocities were not significant at low frequencies, they did additively contribute to an overall decrease in eye-in-space velocity, and therefore a significant improvement in gaze stabilization (p = 0.03, Table 5.1). The improvement in gaze velocity was also evident at high frequencies (EIS, Table 5.1).

Frequency		*p-value* Control – anodal GVS	Control – cathodal GVS	Control - Control
Low	HIS Amp	**0.00***	0.19	0.39
	HIS Phase	0.37	0.20	0.42
	EIS Amp	**0.00***	**0.03‡**	0.05
	EIS Phase	0.05	0.07	0.38
	EIH Amp	**0.00‡**	0.17	0.38
	EIH Phase	0.23	**0.01‡**	0.32
	HOB Amp	**0.00‡**	0.30	0.31
	HOB Phase	**0.00***	**0.00‡**	0.50
High	HIS Amp	**0.00***	**0.01‡**	0.08
	HIS Phase	0.22	0.48	0.10
	EIS Amp	**0.00***	**0.02‡**	0.22
	EIS Phase	**0.01***	**0.00‡**	0.42
	EIH Amp	**0.00***	**0.03‡**	0.09
	EIH Phase	0.14	**0.00***	0.16
	HOB Amp	**0.00***	**0.01‡**	**0.02***
	HOB Phase	0.09	0.32	0.11

Table 5.1: Results of nonparametric tests for inhibitory and excitatory GVS conditions (sinusoidal rotations).
Bold * indicates means significantly smaller than null distribution, *bold ǂ* indicates means significantly larger than null distribution at $p < 0.05$.

Eye and head movements during high frequency stimulation, when paired with cathodal GVS, displayed a decrease (functionally an improvement) in head-in-space velocity but also a decrease in VOR performance (quadrant I, Figure 5.3C & D, red). This result mirrors the increased velocity of the head relative to space and the increase in VOR performance shown for inhibitory GVS. A comparison of Figure 5.3E & F illustrates the persistent influence of vestibular control on the neck at high frequencies. Whereas anodal GVS caused an increase in the velocity of the head relative to body (Figure 5.3E, red), cathodal GVS caused a decrease of the head-on-body velocity (Figure 5.3F, red). The phase shifts of the head movements were unaffected ($p = 0.32$ Table 5.1; control: 106.76 ± 31.90 deg; cathodal GVS: 106.57 ± 27.00 deg). The results are consistent with our previous conclusion that the large increase in head velocity at high

frequencies, associated with a 90 degree phase shift, is driven by a decrease in the vestibular system's control of head stability (Chapter 3).

5.1.3 Statistical Validation

To ensure that the differences in the distributions of δ values between control and GVS conditions were real and not an artifact of sampling, we looked at similar pairs of control sinusoids and computed the difference of those. Comparing the distribution of these differences to the null distribution (see Methods) generated from the set yielded no significant difference between the two (Table 5.1, Control-Control).

5.1.4 Stimulation in the Light

For some test dates we performed the experiments in light as well as darkness to determine whether the response to stimulation was dependent on vision. For both anodal GVS and control conditions, we found effects of light on eye-in-space and head-in-space amplitudes (Kolmogorov-Smirnov two-sample test, $p < 0.05$) consistent with earlier findings (Chapter 3). We examined whether the influence of vision was quantitatively different in control versus GVS conditions and found no effect for either eye or head responses (all *p-values* > 0.05, unbalanced Two-Way ANOVA).

5.2 Transient Velocity Steps

5.2.1 Stimulation in the Dark

During transient rotations GVS had a significant effect on the magnitude of compensatory eye movements (Table 5.2), but a smaller effect on compensatory head movements. Figure 5.4 shows averaged transient responses of one animal at 30 deg/s

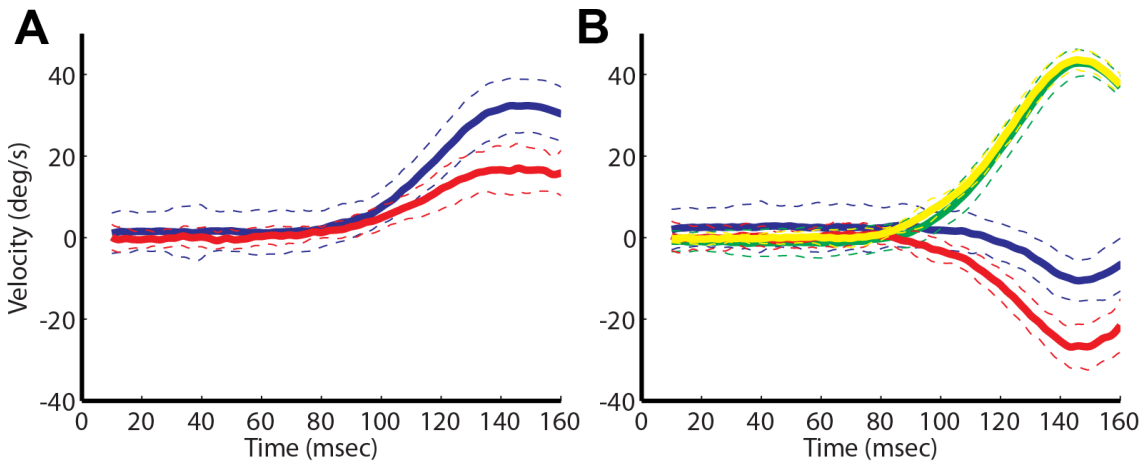

Figure 5.4: Anodal GVS suppression of compensatory eye movements.

Each panel shows averaged responses to 30 deg/s velocity steps during a representative experiment. **A** Averaged eye-in-space velocity: *red*, control; *blue*, anodal GVS. **B** Averaged head-in-space velocity: *yellow*, anodal GVS; *green*, control. Averaged eye-in-head velocity: *blue*, anodal GVS; *red*, control. Dashed lines represent one standard deviation.

(W3, Table 5.2). Figure 5.4A shows reduced gaze stability on trials with anodal GVS compared to control trials (compare eye-in-space with GVS, blue trace, to eye-in-space without GVS, red trace). An increase in eye velocity would be expected if head-in-space velocity also increased as a result of GVS suppression of compensatory head movement. However, as shown in Figure 5.4B, for this data set head in space velocity is nearly identical on GVS trials (green trace) as on control trials (yellow trace). Figure 5.4B illustrates the suppression of eye-in-head velocity on GVS trials (blue trace) compared to the control trials (red trace, same data set as Figure 5.4A).

Anodal GVS reduced the mean VOR gain (HIS vs EIH slope, Figure 5.5A) for all animals and test dates. The effect is most evident for rightward steps, although it is significant in both directions (right: $p < 0.001$; left: $p < 0.05$). The apparent asymmetry is related to an asymmetry in the animals' control responses since control gains were higher for rightward steps (during GVS and control experiments). No significant effect on

Animal/ Date	Left Control – anodal GVS	Right Control – anodal GVS
D1	-0.19	-0.02
D2	0.03	-0.08
V1	-0.07	-0.26
V2	-0.02	-0.26
V3	-0.02	-0.11
V4	0.12	-0.24
W1	-0.21	-0.11
W2	-0.12	-0.29
W3	0.01	-0.30
W4	-0.32	-0.19
HF1	-0.01	-0.23

Table 5.2: VOR gain differences between control and anodal GVS (transient velocity steps).

compensatory head movements was detected in either direction (mean δ of BIS vs. HOB slopes: -0.01 & 0.01, *p-values*: 0.33 & 0.22 left and rightward respectively). Cathodal GVS effects were similar to those of anodal GVS. VOR gains were enhanced by excitatory electrical stimulation (*e.g.* Figure 5.5B, W2 Table 5.2), but cathodal GVS did not produce a systematic change in head movements.

Weak galvanic currents suppress irregular afferents. Based on their responses to angular acceleration, one might expect to detect a change in VOR latency if these afferents were selectively ablated by GVS. However, there were no significant latency changes for either eye or head responses with either cathodal or anodal stimulation (*e.g.* anodal GVS: head $p = 0.48$ & $p = 0.23$; eye $p = 0.06$ & $p = 0.68$, right and left rotations, respectively).

5.2.2 Stimulation in the Light

As with periodic rotation, experiments were performed in light and darkness during some test dates. To determine if there was an effect of light on an animal's performance

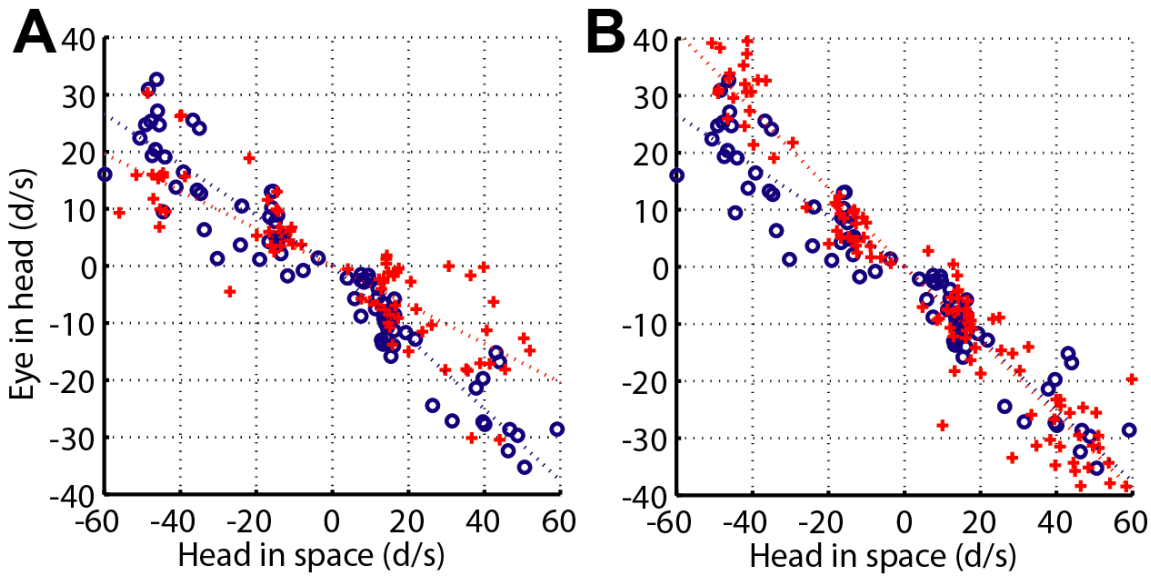

Figure 5.5: Eye-in-Head vs. Head-in-Space data from a representative experiment.
A VOR suppression with anodal GVS. **B** VOR enhancement with cathodal GVS. *Blue circles* represent control trials and *red + symbols* represent GVS trials. (W2, Table 2)

we compared values obtained in the light with those obtained in the dark on the same test date. We found no effect of light in the control condition on eye-in-space or head-in-space movement (Kolmogorov-Smirnov two-sample test, $p < 0.05$, Chapter 3). In agreement with the results of periodic stimulation, there was no interaction between light and galvanic stimulation for eye or head responses (all *p-values* > 0.05, unbalanced Two-Way ANOVA).

5.3 Effects of GVS on VOR in Head-Fixed Guinea Pig

Minor and Goldberg (1991), among others, reported no suppression of the VOR during anodal GVS. Their experiments were performed in head-restrained primates. To test whether the differences in our results were due to the ability of the animals' heads to move freely, we repeated the experiments (both sines and transient steps) in an animal with a restrained head. For both sinusoidal and transient rotations we found anodal GVS

to suppress the VOR. For sinusoidal rotations, eye-in-head velocity decreased across all frequencies, causing a significant decrement in the animal's ability to maintain gaze ($p < 0.001$). For transient steps, the decrease in performance closely mimicked those reported for head-unrestrained animals (see Table 5.2, HF1). The overall decrease in VOR gain was significant (ANOVA, $p < 0.05$).

5.4 Active Head Movements

GVS did not influence compensatory eye movements associated with voluntary head movements. As reported in Chapter 4, for compensatory eye movement, mean gain (eye/head) across all animals during active head movements was higher than during passive stimulation (active: gain = 0.91 ± 0.11; passive periodic rotation: low frequency VOR gain = 0.59 ± 0.18, high frequency VOR gain = 0.81 ± 0.10; passive transient steps: right VOR gain = 0.45 ± 0.21, left VOR gain = 0.31 ± 0.16). Furthermore, the mean latency of compensatory ocular responses in relation to active head movement was effectively zero (mean = -0.06 ± 0.18 msec) consistent with the anticipatory nature of the movement (Chapter 4). In our previous study, we reported that anticipatory responses occurred even in the absence of a functional vestibular system suggesting that GVS should have no effect on anticipatory responses. Consistent with this hypothesis, there was no measurable difference between compensatory eye movements that occurred with and those that occurred without GVS during self-generated head movements (anodal GVS: $p = 0.08$; cathodal GVS: $p = 0.19$).

To determine if GVS influenced self-generated head velocity we compared distributions of head-in-space velocity during active movement epochs that occurred in

the absence of GVS with those that occurred during GVS. No statistical difference was found (anodal GVS: $p = 0.17$; cathodal GVS: $p = 0.26$) suggesting that changes in vestibular afference, as modulated by GVS, do not significantly alter the trajectories of planned voluntary head movements.

During epochs of active head movement the animals occasionally generated eye and head movements in the same direction, presumably to shift their line of sight. To compare if there was relatively more gaze change occurring under either of the GVS or control conditions, we examined data samples where eye-in-head and head-in-space velocities had the same sign (because the eye and head are moving in the same direction, the instances are anti-compensatory). We analyzed eye velocity amplitude during these epochs of anti-compensatory movement to determine if there was any influence of GVS on the generated quick phases. During cathodal GVS, the proportion of eye movements in the anti-compensatory direction was greater than control ($p = 0.02$), although these epochs corresponded to significantly lower mean eye-in-space velocities than control (Kolmogorov-Smirnov two-sample test, $p < 0.05$; Means: Right: control = 38.98 deg/s, cathodal GVS = 26.00 deg/s; Left: control = -41.45 deg/s, cathodal GVS = -34.36 deg/s). For anodal GVS, there was a trend for a higher number of anti-compensatory eye movements than control ($p = 0.05$). The distribution of eye-in-space velocities corresponding to these movements was also different between control and GVS (Kolmogorov-Smirnov two-sample test, $p < 0.05$), although no clear trend in mean velocity changes could be ascertained (Right: control = 60.39 deg/s, anodal GVS = -57.47 deg/s; Left: control = -56.46 deg/s, anodal GVS = -72.19 deg/s).

5.5 Discussion

5.5.1 GVS Effects on the VCR

Irregular vestibular afferents exhibit more phasic responses to angular rotation and innervate central vestibular neurons that project to the cervical spinal cord (Boyle et al. 1992). It has been hypothesized that their dynamic characteristics could help compensate for the inertial and biomechanical properties of the head and neck; thus the phase advanced signal encoded by these afferents might significantly influence the VCR and vestibular control of head stability (Bilotto et al. 1982; Boyle et al. 1992; Fernandez and Goldberg 1971; Peterson et al. 1988; Schor et al. 1998). The goal of this study was to directly test this hypothesis. Previous studies have established that weak anodal currents selectively suppress the discharge of irregular afferents in primates and guinea pigs (Minor and Goldberg 1991; Kim and Curthoys 2004). If irregular afferent activity is functionally significant for the VCR, then anodal galvanic stimulation should reduce VCR gain and potentially destabilize the head-in-space during passive whole body rotation in animals whose heads are unrestrained. Our experimental results are evidence in support of this hypothesis because systematic decreases in compensatory head velocity relative to body velocity occurred when anodal GVS was applied (Figure 5.1 and 5.2 and Table 5.1). In some instances, the suppression of the VCR was substantial. For example, nearly a 70% reduction in gain is illustrated in Figure 5.1, panels F and G during 2 Hz sinusoidal rotations.

Additionally, during high frequency sinusoidal oscillations (8 Hz), when head speeds exceeded imposed stimulus speeds and were likely driven by inertial forces, anodal GVS further destabilized the head in space resulting in greater head-in-space velocity

compared to control cycles. Thus, despite the presence of large inertial forces, irregular inputs to central vestibular neurons with descending axons exerted a significant influence on head stability at high rotational frequencies consistent with their dynamic properties. An interesting aspect of the high frequency sinusoidal data was that anodal GVS had no significant influence on phase shift (Table 5.1, Figure 5.3E & F), despite the phase advanced dynamics of the irregular afferents. Instead, phase shifts clustered near 90 degrees reflecting the inertial character of the head response.

5.5.2 GVS Effects on the VOR

In contrast to vestibular control of the head, it is widely believed that signals encoded by regular vestibular afferents dominate activity in the direct VOR pathway. In what is now a classical study, Minor and Goldberg (1991) showed that anodal GVS had no effect on the VOR of monkeys during passive sinusoidal or transient rotations, and this finding was subsequently confirmed by two other laboratories (Angelaki and Perachio 1993; Chen-Huang et al. 1997). The result is somewhat surprising since irregular afferents provide synaptic inputs to central neurons in VOR pathways as well as to neurons in VCR pathways (Highstein et al. 1987). Unexpectedly, we found significant effects of GVS on the guinea pig's VOR (Figure 5.1 – Figure 5.5 and Table 5.1 & Table 5.2). This result is not due to suppression (or activation) of regular as well as irregular afferents. Kim and Curthoys (2004) showed that weak galvanic currents, similar to those employed in this study, selectively suppress irregular afferents in guinea pigs just as they do in non-human primates. Thus, we must assume that the GVS suppression of the VOR is a species-based difference and irregular vestibular afferent signals play a more direct role in producing the guinea pig's VOR than they do in the primate.

Although detailed evidence for the central connectivity of afferent classes is not available in the guinea pig, it is unlikely that the basic vestibular circuits in the brainstem are fundamentally different (Babalian et al. 1997; Burian et al. 1990; Ris et al. 1995). Thus, it is likely that regular and irregular afferents provide synaptic inputs to central neurons in the VOR pathways as they do in primates, although the relative synaptic weights may be different. To account for our experimental findings, we hypothesize that polysynaptic, possibly extra-vestibular, inputs sculpt vestibular afference in accord with species-specific behavior. In particular, there are fundamental differences in the relative importance of vestibular and visual sensation in afoveate guinea pigs as compared to primates with well-developed visual systems. Guinea pigs live in burrows and feed at dawn and dusk in their natural habitat (Finlay 1981). They do not have foveas; their retinas are relatively homogeneous and have a low density of photoreceptors, primarily rods (Choudhury 1978; Hughes 1977) and they have poor visual acuity (estimated maximally at 2.7 c/deg, Buttery et al. 1991). Consistent with this morphology and lifestyle, guinea pigs make few spontaneous rapid eye movements and do not produce smooth pursuit (Escudero et al. 1993).

5.5.3 Ocular Compensation During Self-Generated Head Movements

During self-generated head movements we were unable to detect any influence of GVS on head or eye speed. This result extends our previous finding that anticipatory compensatory eye movements associated with active head movements occur independently of vestibular afference (Chapter 4). Whatever the source of the anticipatory motor command (proprioceptive or efference copy), the anticipatory response is more effective than the passive VOR in stabilizing the eye in space (Chapter

4). Retinal stability during rapid self-generated head turns may be important for the animal to distinguish object motion in the environment from self-motion (Land 1999). Thus, an anticipatory movement in association with a self-generated head or body movement may not only reduce retinal slip, but also provide perceptual suppression of any remaining slip associated with the animal's voluntary movement.

This result is evidence that during active head turns in the intact guinea pig, the anticipatory response produces all or most of the ocular compensation. If there were a significant and synergistic passive VOR component, then that component would have been suppressed by GVS and we should have detected a difference in the compensatory eye movements that occurred during stimulated and control epochs of self-generated head movement. Thus, this result supports the idea that an efference copy of the motor command to move the head is used to cancel the sensory consequence (reafference) of the planned head movement (Cullen et al. 2011; Sadeghi et al. 2010; von Holst and Mittelstaedt 1950, Chapter 4). If the active head movement occurs as planned, then the vestibular nerve signal is effectively nullified centrally by the efference copy and would produce no VOR-related eye movement. Our GVS data are consistent with this model of vestibular processing and the principle of reafference (von Holst and Mittelstaedt 1950).

Chapter 6

Conclusions and Significance

This thesis work represents a comprehensive description of eye and head movements associated with vestibular compensation in the guinea pig. The findings are important both as a description of guinea pig behavior and in a broader context of use of afoveate animals in vestibular research. The guinea pig has been an important participant in biomedical research since the 17th century, having even visited space as part of the Soviet Space Program in 1961 (Burgess and Dubbs 2007). During its tenure as a medical aid and research subject, the species has allowed scientists to make a variety of advances benefitting a number of animal species, including humans. Despite its usefulness, the guinea pig model has some shortcomings for translational research as it differs from primates in a variety of ways. In the visual system, for example, the guinea pig lacks a fovea and retinal information is transmitted at a much lower rate than human (Koch et al. 2006) to a simplified set of central visual pathways (Lui and Aldon 1997).

Conversely, however, it is the guinea pig's relative simplicity that has made it invaluable to advances in neuroscience research. In evolutionary terms, the animal provides a simpler set of neuronal pathways that can be understood before looking at the complexity superimposed onto them in the primate. Additionally, sharing a number of similarities with other afoveate species, such as mice (for examples in the vestibular

system see Beraneck and Cullen 2007), it provides researchers with a bridge between different scientific approaches (*e.g., in vivo* electrophysiology and behavior in primates, and genetic manipulations in mice are not easily interchanged between the two species). In some instances, the guinea pig is not only a link because of its physical characteristics (size, ease of training and behavioral testing, etc.) but because of intermediate aspects of its neurological development. For example, in Lui et al. (1994), by showing similarities between the guinea pig's OKN and cortical projections to the primate (which differed from that of a mouse and rabbit), researchers were able to determine the contribution of the nucleus of the optic tract to OKN.

6.1 VOR and Differences Between Guinea Pig and Primate

This thesis provides a number of unexpected differences between previous findings in the primate and the guinea pig. These differences are both interesting and important, highlighting points of contrast between primate and guinea pig that could, when further explored, lead to an improved understanding of the vestibular system. The vestibular system is evolutionarily highly conserved (Mo et al. 2010) and so any differences among species could represent additional layers resulting from evolution of other related systems, *e.g.*, vision. Study of a simplified model allows for a teasing out of the more complex set of components in more highly developed species.

One possible instance of this layering is highlighted in this thesis – the functional importance of retinal stability between foveate and afoveate species. The VOR stabilizes retinal images during head movements. In primates, retinal image stability maximizes visual acuity. However, guinea pigs have poor acuity so reduction of blur due to image motion is unlikely to be a primary function of the guinea pig's VOR. Land (1999) and

Walls (1962) argue that detection of motion in the external environment is also dependent on retinal stability; if the eye moves in space then the brain cannot determine if the motion of a retinal image is induced by the animal's own movement or by external movement in the environment. For example, if one spins and then abruptly stops, the world is perceived as spinning for a brief time. The misperception occurs because vestibular sensation causes eye movements that persist after one stops spinning. Additionally, detection of relative motion between an object and its environment is enhanced by retinal image stability (Nakayama 1981). In accord with Land, we suggest the function of the guinea pig's VOR is to stabilize retinal images so the animal may better distinguish self motion from movements of other animals or objects (*e.g.*, a predator) in the environment. This functional distinction is likely to represent a fundamental difference between foveate and afoveate species in how vestibular nuclei neurons integrate vestibular inputs with inputs related to visual sensation.

In the primate, a powerful set of visual mechanisms assist or even supplant vestibular control of compensatory eye movements. First, foveal-based smooth pursuit eye movements stabilize retinal images on the fovea regardless of the source of image motion (self-generated or external). During passive rotation, the gain of the VOR is significantly improved during visual fixation of a target (Baloh and Halmagyi 1996; Schweigart et al. 1999) because smooth pursuit and the VOR act synergistically. In the clinic, vestibular nystagmus is readily suppressed by real or even imagined fixation targets (reviewed in Baloh and Halmagyi 1996), a clear demonstration that visual inputs or cognition can dominate vestibular signals in the VOR pathway. Second, fusional vergence - necessary

for binocular vision - also stabilizes retinal images because it maintains binocular correspondence of foveated targets (reviewed in Baloh and Halmagyi 1996).

In the guinea pig, foveal visual mechanisms are not available to assist the VOR in stabilizing the retinal image. Instead, we hypothesize that irregular vestibular afferents, because of their greater sensitivity to angular acceleration (Goldberg 2000), enhance VOR responses to movement and thus play a greater role in image stabilization in the guinea pig than they do in the primate. The influence of irregular afferents on secondary vestibular neurons in primates may be regulated or suppressed to allow for the more powerful visual mechanisms to dominate or modulate vestibular signals. This idea is consistent with previous findings, which showed that polysynaptic inhibitory pathways could be responsible for the central cancellation of irregular afferent inputs in primates (Chen-Huang et al. 1997). Although anodal GVS had no effect on the averaged activity of secondary vestibular neurons (Type I PVP), the activity of individual cells was modulated by GVS (Chen-Huang et al. 1997) confirming the presence of irregular inputs on those cells. This result and our data suggest that recordings from central vestibular neurons in guinea pigs will be needed to determine the influence of irregular inputs to specific classes of vestibular neurons, *e.g.*, those likely to project into the VCR-related pathways (VO neurons) and those that project into VOR pathways (cells with eye movement sensitivity). For example, Beraneck and Cullen (2007) recorded from identified secondary vestibular neurons in the afoveate mouse. They found that the firing rates of only a subset of neurons with eye movement sensitivity, "ES" neurons, were modulated by optokinetic signals. In primates, eye movement and vestibular-only (VO

cells) exhibit firing rate modulation correlated with optokinetic stimulation (Waespe and Henn 1977).

Although the VOR effectively stabilizes retinal images for rapid movements, it performs poorly for low frequency stimuli (Baird et al. 1988; Highstein et al. 2005; for review, Goldberg, 2000). However, in the light, full field, slowly moving images induce an optokinetic reflex in guinea pigs (and other species) that reduces retinal slip over a frequency range where the VOR is deficient (Azzena et al. 1974; Lui et al. 1999; Marlinsky and Kröller 2000). Consistent with these reports, we found, in the light, that the guinea pig's compensatory eye movements were enhanced during low velocity periodic motion (Chapter 3). However, anodal GVS had no effect on low frequency visual enhancement of the VOR; visual enhancement and anodal GVS suppression both occurred, but there was no interaction (Chapter 5) suggesting either linear addition of signals in a common circuit, or that optokinetic and GVS influences were exerted in parallel pathways. This result is also consistent with the finding of Angelaki et al. (1992) that anodal GVS had no effect on OKN or OKAN in squirrel monkeys.

6.2 Active Head Movements and Synergy Between Guinea Pig and Primate Research

Although, as discussed above, some of the findings in this thesis point to a possible divergence between primate and guinea pig, there are others that serve to confirm and further explain phenomena previously reported in the primate. Specifically, Chapter 4 describes anticipatory, compensatory eye movements that occur during voluntary head movement in the guinea pig. Although the result is novel, it extends previous findings of

Dichgans et al. (1973), who reported a similar type of compensatory eye movements in rhesus macaques with bilateral labyrinthectomies.

In this study, Dichgans et al. concluded that the compensatory eye movement behavior they saw after the labyrinthectomy was the result of "a complex process" that involved significant rewiring that would have to occur after the insult as an adaptive mechanism. This conclusion was partially motivated by the authors' findings that the gain of the compensatory eye movements was unchanged and nearly perfectly compensatory in the intact primate during both active and passive head movements. Additionally, the compensatory eye movement response was not affected by vision and was abolished when only neck proprioceptive input was available (during rotations of the body under a stationary head). In sum, these results led the scientists to conclude that prior to a vestibular lesion compensatory eye movements to active head motion are solely vestibular in nature – the VOR. Interestingly, the authors saw some compensatory eye movements during active head motion within the first week after the labyrinthectomy and reported a marked improvement in these in the following weeks (90% compensatory one month post-lesion). Ultimately, the overall results of the experiment led the authors to conclude that there is an adaptive mechanism that becomes responsible for preprogramming compensatory eye movements (only during active head motion) in animals with a complete vestibular lesion.

This thesis is in agreement with the insight provided by Dichgans et al. (1973) regarding the presence of preprogrammed compensatory eye movements during voluntary head motion. However, in contrast to the previous work, work summarized in Chapter 4 clearly shows that these preprogrammed movements are present not only in

animals with a vestibular insult but also in subjects with an entirely intact vestibular system. This result was facilitated by the animal model chosen in this thesis and its differences from the primate. Since, unlike the primate, the guinea pig does not have a VOR that is perfectly compensatory during passive rotations, the improved gain of compensatory eye movements during active head movements provided an important clue to its differences from the VOR. The lack of saccades in the guinea pig's behavioral repertoire further simplified the analysis of the compensatory eye movement behavior. This instance is a good example of how the guinea pig can provide a simpler model for studying a behavior or system that has evolved in complexity but is ubiquitous to all species. Since the findings reported in Chapter 4, Zhou et al. (2010, personal communication) have reported similar zero-latency compensatory eye movements in the primate, indicating that the behavior is not unique to the guinea pig but can be translated to foveate species.

Chapter 7

Future Directions

This work has opened up several additional research paths related to each of the findings. For completeness, experiments need to be done in both guinea pig and primate to better understand the parallels between the two species as they are illuminated by the behavioral differences reported here.

7.1 Probing Head Movement Variability

The observed variability of compensatory head movement responses (Chapter 3) to transient velocity vestibular stimulation could not be accounted for by variations in orbital eye position, head position relative to the body or state of alertness. To determine the muscular and thus cervical motoneuron activity related to the variability found in head movement, electromyographic (EMG) recordings should be made in muscles related to horizontal VCR (*e.g.,* obliquus capitis inferior) and correlated with head movements during PWBR. Descending vestibular inputs play a key role in producing the compensatory head movement response. In conjunction with EMG recordings, vestibular inputs should be manipulated using GVS to selectively activate or suppress the irregular subset of vestibular afferents in order to assess their contribution to cervical motoneuron activity.

7.2 Anticipatory Compensatory Eye Movements During Voluntary Head Motion

Our finding that compensatory eye movements during active head movements are not vestibular in nature (or have only a limited vestibular component) bears further investigation (Chapter 4). Electrophysiological recordings in primate and guinea pig vestibular nucleus during active head motion would provide initial validation of our results. Previous studies have shown suppression in vestibular activity during active head movement (Roy and Cullen 2004) that has been attributed to the need to suppress vestibular reflexes during active gaze shifts. However, further investigation is needed in subjects where active head movements are not done for purposes of gaze reorientation.

Much work has been done to understand the role of efference copy in allowing the vestibular system (and by extension the subject) to distinguish between active head movements, when reflexes may need to be suppressed, and passive perturbation (reviewed in Cullen et al. 2011). As part of our own work, we have suggested a model of how efferent information may lead to compensatory eye movement responses that are more efficient than the VOR. This model should be tested behaviorally and electrophysiologically in both the guinea pig and the primate. Experiments need to be performed in animals with both intact and compromised vestibular systems in order to determine how the network regulating these eye movements may be modified without a properly functioning vestibular system.

EMG recordings during active head movements should be conducted in order to further rule out the role of proprioception in the anticipatory eye movement responses. Based on the experiments presented in Chapter 4, EMG activity in neck musculature that

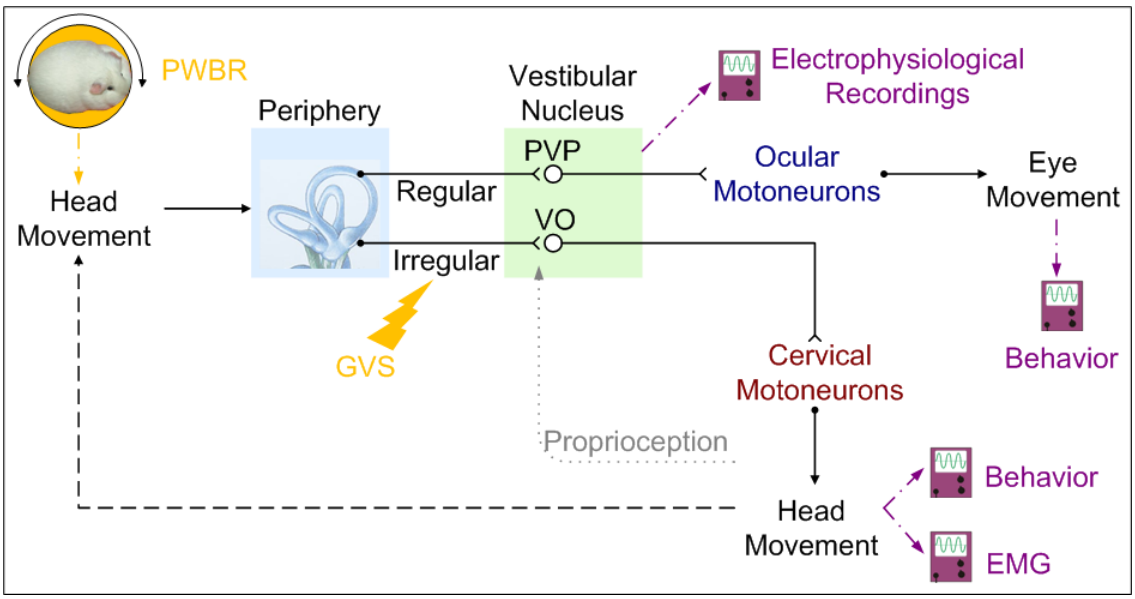

Figure 7.1: Potential approach to probing the guinea pig's vestibular system. Simplified circuit with suggested combination of experimental manipulations. Yellow indicates experimental inputs, purple indicates experimentally measured outputs.

precedes actual movement of the head and modulates the discharge of secondary vestibular neurons (Vibert et al. 1999) could not be ruled out as the putative signal responsible for the compensatory eye movements. However, Dichgans et al. (1973) described similar compensatory eye movements in non-human primates that were still present after surgical lesions that interrupted proprioceptive input from the cervical spinal cord. Confirmation of these findings in the guinea pig using EMG recordings is needed.

7.3 Understanding Differences in Effects of GVS Between Guinea Pig and Primate

An exciting result of this work has been the effect of GVS on guinea pig VOR (Chapter 5). Although we have worked to rule out the effects of head restraint on the discrepancy between our and previous results, head restraint must be further ruled out as a factor by testing the effects of GVS on the VOR of a head-unrestrained primate.

Although we do not anticipate changes in the outcome, the species differences we have illuminated indicate that the most parsimonious explanation may not be applicable when drawing conclusions based on experiments done in a foveate versus afoveate species.

In the guinea pig, electrophysiological recordings from the vestibular nucleus akin to those presented in Chen-Huang et al. (1997) would provide neurophysiological information that can be correlated with the behavior reported in Chapter 5. For completeness experiments should include both vestibular and optokinetic stimulation in conjunction with recordings and GVS. This parallel investigation of the effects of GVS in the guinea pig to ones done in primate is particularly important in light of current studies that are underway to develop a vestibular prosthesis using electrical stimulation of the vestibular afferent fibers in the chinchilla (Della Santina et al. 2007). The chinchilla is similar to the guinea pig in that it is an afoveate and lateral-eyed animal, with VOR gains between 0.4 and 0.6 (Migliaccio et al. 2010). These similarities indicate that the chinchilla's response to GVS must be examined. If the effects are similar to those in the guinea pig, additional investigation will be required in order to understand the prosthetic's efficacy in the primate and its eventual translation to human patient populations.

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