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Eye - head coordination in the guinea pig I. Responses to passive whole body rotations

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Abstract

Vestibular reflexes act to stabilize the head and eyes in space during locomotion. Head stability is essential for postural control whereas retinal image stability enhances visual acuity and may be essential for an animal to distinguish self-motion from that of an object in the environment. Guinea pig eye and head movements were measured during passive whole body rotation in order to assess the efficacy of vestibular reflexes. The vestibulo-ocular reflex (VOR) produced compensatory eye movements with a latency of ~7 msec that compensated for 46% of head movement in the dark and only slightly more in the light (54%). Head movements, in response to abrupt body rotations, also contributed to retinal stability (21% in the dark; 25% in the light) but exhibited significant variability. Although compensatory eye velocity produced by the VOR was well correlated with head-in-space velocity, compensatory head-on-body speed and direction were variable and poorly correlated with body speed. The compensatory head movements appeared to be determined by passive biomechanical (e.g., inertial effects, initial tonus) and active mechanisms (the vestibulo-collic reflex or VCR). Chemically induced, bilateral lesions of the peripheral vestibular system abolished both compensatory head and eye movement responses.

Keywords

vestibulo-ocular reflex; vestibulo-collic reflex; eye movements; aminoglycoside vestibular lesions

Introduction

Vestibular control is a multi-component process that involves coordination between eye, head and body movements to allow for the stabilization of the trunk in space during locomotion, and for stabilization of the eyes in space to maintain a steady image on the retina. A traditional, head-restrained approach has been instrumental in a number of experiments to elucidate the nature of vestibular reflexes and compensatory eye movements. In some studies, scientists have also employed an experimental approach that allows one to simultaneously examine coordinated eye and head movements (Bizzi et al. 1971; Gresty 1975; Gdowski & McCrea 1999; Roy & Cullen 1998, 2002). Such an approach allows for the study of eye-head coordination using more natural behavior. In this paper we present data from guinea pigs that were allowed to move their heads in a completely natural manner during passive stimulation,

allowing for simultaneous analysis of their eye and head movements. In a companion paper (Shanidze et al. 2010), we will describe an active response to self-generated head movements.

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 & McCrea 1999; Roy & Cullen 2001, 2002; Cullen & 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 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, Li & Nevill 1998; Kopke et al. 2001; Walsh et al. 2000; Yamane et al. 1995); and to evaluate aminoglycoside ototoxicity (Forge & Li 2000; Pettorossi et al. 1986; Bamonte et al. 1986; Jones 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 anticomensatory 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 1971) except that the rapid eye movement follows the head movement. With the exception of Gresty's report (1975), this study is the first systematic effort to determine how eye movements are coordinated with passive head and body movements in the guinea pig.

In this paper we show that during passive whole body rotation, the VOR partially reduces retinal image motion. However, in the head unrestrained animal, compensatory head movements also contribute to this behavioral goal in synergy with the VOR. Although the VOR is tightly related to the speed of the head in space, the speed and direction of evoked head movements is variable suggesting an interaction of descending control signals, such as the vestibulo-colic reflex, with intra-spinal mechanisms (e.g., stretch reflexes) and the intrinsic biomechanics of the head, neck and trunk.

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.

Nine male guinea pigs (5 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 & Bui 2001, Bui & Vingrys 1999) would be correlated to differences in vestibular behavior. Since there were no differences in their eye and head movements in response to whole body angular rotations, all of their data were combined for this report.

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 comfortably adjusted to 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 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. Robinson 1963 in human; Fuchs & Robinson 1966 in monkey; Stahl et al 2000 (eye), Baker 2005 and Takemura & King 2005 (head) 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. A Primelec search coil system (D. Florin, Ostring, Sw; model CS681) generated three orthogonal electromagnetic fields about the guinea pig. The Primelec 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.

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 velocity of rotations was 30, 60 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 30, 60 or 90 deg/s. These accelerations were comparable to those produced by the animals themselves during active head movements made in the same experimental sessions. The animals were randomly rotated to the right or left on successive trials. Up to 120 randomly interleaved trials in each direction were obtained in each experimental session.

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 Spike 2 (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 sec. time constant was applied to all acquired position channels and eye and head velocity were computed by differentiating the position data.

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. An experimenter verified each set of removed eye movements. Desaccaded cycles were averaged and fit with a sinusoid using a least squares fit criterion. Head-on-body and eye-in-head velocities were computed by subtracting body velocity from the directly measured head-in-space velocity, and head-in-space velocity from the directly measured 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, Zhou et al 2003) and typically before the occurrence of any quick phases. Values for the body-, head- and eye-in-space velocities were measured and 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.

Six additional guinea pigs were prepared for eye and head movement recording as described above. 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-g needle, a cochleostomy was created at the oval window, followed by 100 μ l of streptomycin injection (400 mg/ml). The middle ear cavity was also filled with the streptomycin solution. The skin was closed with 3-0 nylon interrupted sutures and the procedure was repeated in the opposite ear to create bilateral lesions. 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.

Results

Passive whole body rotation

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, HIS, overlap; Figs 1a&b, uppermost traces). Head rotation relative to the body (HOB, Figs 1a&b, middle traces) is minimal, even during the large amplitude (>100 deg) body rotation shown in Fig. 1a. Rapid anti-compensatory and slow compensatory eye movements are evident in the traces representing eye position within the orbit (EIH, Figs 1a&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. Figs 1c&d illustrate the time course of body, head and eye velocity during a single 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 (Fig. 1c, long arrow). A rapid, anti-compensatory eye movement that is saccadic in nature (Fig. 1c, short arrow) interrupts the compensatory eye movement. Fig. 1b shows that the rapid eye movements tend to "look ahead" 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 (Fig. 1d, arrow) and reflects the inertial tendency of the head-in-space to remain stationary. Fig. 2d 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. 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 (Fig. 1c, 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 (Fig. 1c).

To quantify these responses, we measured eye velocity relative to the head and compared it to head velocity in space for each passive body rotation. Fig. 1e shows the regression of eye-in-head versus head-in-space velocity during the initial ~100 msec of the trial shown in Fig. 1c. Over this interval, eye and head velocity are linearly related with a slope of -0.6 ($r^2 = 0.96$). Fig. 2a 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 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).

Fig. 1f illustrates a waveform correlation of eye-in-head and head-in-space velocity during the initial ~100 msec of the trial shown in Fig. 1c. 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. Fig. 2b shows 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 ($\alpha = 0.05$).

The initial head velocity provoked by passive whole body rotation shows considerable variability from trial to trial. Fig. 2c 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 (Figs 1e, 2a), 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 Fig. 3. 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 Fig. 2c. 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 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, Fig. 3c 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.

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 Fig. 3. In the dark (Fig. 3a) 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 (Fig. 3b). 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, Fig. 3c & d).

To compare our findings with previous reports (e.g., Escudero et al. 1993, Gresty 1975), we also tested animals using sinusoidal stimulation. Our results were in agreement with those studies at the relatively low frequencies previously employed (≤ 2 Hz). At frequencies above 5 Hz, however, we found an enhancement of the VOR and a significant loss of head stability that had not previously been reported. Fig. 4 shows representative eye and head responses from a guinea pig rotated in the dark at 10 Hz. At this frequency, head-on-body velocity exceeded body velocity and was phase shifted nearly 90 degrees (bottom panel, lighter trace). Although head movements were clearly not compensatory, the upper and middle panels 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.

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. 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. However, there was 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.

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 & Kröllner 2000). Nevertheless, the reduced phase lead for the 30 deg/s rotations (< 0.2 Hz) and the enhanced compensatory head movement responses shown in Fig. 3b 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).

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). 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 & Peterson 1986) and human head movements (Keshner, et al. 1995; Keshner & 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 (Fig. 4). The phase of the VOR remained constant and nearly compensatory for even the highest head velocities.

Vestibular Responses in Lesioned Animals

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

Pre- and post-lesion responses were also measured using transient velocity steps. Fig. 5a shows the relationship between compensatory eye velocity and head-in-space velocity. Prior to the lesion (black circles), there is a robust VOR response similar to that shown in Fig. 2a (slope ~ -0.3). However, after the lesion (+ & *), the compensatory responses are completely abolished; eye speeds are randomly distributed about zero.

Figs 5b–d show results of a similar analysis of head movements. Fig. 5b shows head movements before the lesion: comparable to Fig. 3b 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 (Fig. 5c), there is no evident compensatory bias in the head speed responses. Four weeks post-lesion, (Fig. 5d) 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.

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 & Young 2005; Lue et al. 2008), mechanisms of ototoxicity (Song et al. 1997; Sha & 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 during passive stimulation. For example, the results (Figs 1a&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 (Figs 1a&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; Collewyn 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 in the sense that there was little of interest in the test environment to which they might seek to orient. In a companion paper, however, we describe active head movements made by the same guinea pigs during the same experimental sessions that did provoke coordinated head and eye movements similar to those of primates and rabbits.

The gain of the VOR in response to passive rotation in the dark was less than perfect (Fig. 2). 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 (Fig. 4). 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 (Shanidze et al. 2010).

A final point that will be discussed in more detail in the second paper is our finding that the latency of the compensatory VOR responses was 6–7 msec, consistent with data from primates (Snyder & King 1992; Crane & Demer 1998; Minor et al. 1999; Huterer & 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 (Shanidze et al. 2010).

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 & Peterson 1986; Peng, et al. 1996) and simulated responses similar to those shown in Fig. 1d. 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 (Figs 2c & 3). 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, Figs 5b–d show 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, Figs 5c&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 (Fig. 1d, 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 to 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., Fig. 1), animals had difficulty stabilizing their heads during high frequency and/or high acceleration periodic stimuli (Fig. 4). 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 (Fig. 4). 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 & Cullen 2002).

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Fig. 1. Representative head and eye movements in response to passive whole body rotation (PWBR)
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 (arrow). A small counter-rotation of the head relative to the body can be seen (~ 5 deg, 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 panel A. **C & D.** Velocity records for the transient perturbation shown in panel B. In **C**, the initial eye movement (long arrow, EIH trace) is compensatory but interrupted by an anti-compensatory rapid eye movement (short arrow). In **D**, the initial head movement (arrow) is compensatory and followed by decreasing amplitude oscillations. **E.** Initial eye velocity is proportional to head velocity (linear regression slope = -0.79). **F.** The initial eye movement lags the head movement by ~ 5.5 msec

Fig. 2. In response to brief, passive whole body perturbations (velocity steps), evoked eye and head movements are compensatory

A. Eye-in-head velocity is proportional to head-in-space velocity (linear regression slope = -0.45). **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. Top bars: clockwise steps; bottom bars: counterclockwise steps. **C.** Head-on-body velocity is proportional to body-in-space velocity (linear regression slope = -0.21) for the same velocity steps shown in panel **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 msec (counterclockwise direction)

Fig. 3. Comparison of compensatory head movement responses to velocity steps in light and darkness

Histograms of evoked head-on-body velocities for 30 deg/s (panels **A** & **B**) and 90 deg/s (panels **C** & **D**) velocity steps in dark and light, respectively. Black: counterclockwise steps, gray: 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 towards the compensatory direction for both dark and light conditions (**C** & **D**)

Fig. 4. Eye and head movement response to 10Hz sinusoidal rotation in the dark

Head-on-body velocity is much greater than body velocity and phase leads body velocity by nearly 90 degrees (dashed line). Despite the large head velocity and phase lead, the eye movement response is ~180 deg out of phase (dashed line, middle traces) and nearly perfectly compensatory as shown by the eye-in-space (“gaze”) trace

Fig. 5. Bilateral chemical lesions of the peripheral vestibular system eliminate head and eye responses to passive velocity steps

A. Eye-in-head vs. head-in-space velocity control responses prior to lesion (slope = -0.27 , black circles); one week post lesion (dark gray); and four weeks post lesion (light gray). Post lesion slopes were $\approx +0.03$ and $+0.07$ respectively. **B–D.** Histograms of evoked head-on-body velocities for $90^\circ/\text{s}$ steps **B.** Control. Note compensatory shift (mean = 14.64 (CW) & -18.84 (CCW) $^\circ/\text{s}$; std = 13.83 & 15.99 $^\circ/\text{s}$ respectively). **C.** One week post lesion, compensatory bias is less (means = 13.47 (CW) & -9.92 (CCW) $^\circ/\text{s}$; std = 26.52 & 28.41 $^\circ/\text{s}$). **D.** Four weeks post lesion. No significant compensatory bias remains (means = 3.53 (CW) & -1.46 (CCW) $^\circ/\text{s}$; std = 12.91 & 14.16 $^\circ/\text{s}$)