The Contributions of Residual Function and Cross Coupling to the Horizontal Angular Vestibulo-ocular Reflex after Bilateral Horizontal Semicircular Canal Plugs

by

Gavin Eugene Guy Jones

A thesis submitted in conformity with the requirements for the degree of Master of Science Graduate Department of Physiology University of Toronto

© Copyright by Gavin Eugene Guy Jones (1999)
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author’s permission.

L’auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L’auteur conserve la propriété du droit d’auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-45562-9
Abstract:
The Contributions of Residual Function and Cross Coupling to the Horizontal Angular Vestibulo-ocular Reflex after Bilateral Horizontal Semicircular Canal Plugs

For completion of MSc. degree (1999), Dept. of Physiology, University of Toronto. Gavin Eugene Guy Jones

The vestibulo-ocular reflex (VOR) functions to stabilize most of the retina during head movement. The eye movement produced by the lateral and medial recti, which is primarily a response to activation of the horizontal semicircular canals, will be called the horizontal angular VOR (hVOR).

Several factors may contribute to the hVOR produced by rotation after plugging both lateral canals. One is residual function. This arises from the remaining transducing properties of the plugged canal. Another, cross-coupling, is the term given to the neural connections that produce a horizontal eye movement as a result of stimulation of the four intact vertical canals. By studying the eye velocity produced as a result of sinusoidal oscillation about an Earth-vertical axis in the dark, one can attempt to separate these factors.

After plugging both horizontal canals, a response was observed that is consistent with a physical model for residual function. That is, the response occurred predominantly at high frequencies and consistently showed a phase lead. In both cats, we believe that cross coupling was also observed. In one animal, the input of cross coupling increased its efficacy after plugging.
Contents

1. Summary .......................................................... 7

2. Introduction ....................................................... 8
   2.1 Representation of rotations ................................ 9
   2.2 Transduction of angular rotation .......................... 9
   2.3 Horizontal angular VOR ................................. 9
   2.4 hVOR Frequency profile ................................. 10
   2.5 Cross coupling ........................................... 11
   2.6 Residual function ........................................ 14
   2.7 Contribution of the otolith organs ..................... 15
   2.8 Cervico-ocular reflex .................................... 16
   2.9 Eye position effects on the hVOR ....................... 16
   2.10 Hypotheses ............................................... 18

3. Methods ............................................................ 19
   3.1 Rationale .................................................. 19
   3.2 Rotation stimulus ......................................... 21
   3.3 Apparatus .................................................. 22
   3.4 Data acquisition .......................................... 23
   3.5 Controls .................................................. 24
      3.5.1 Cervico-ocular reflex .............................. 24
      3.5.2 Effect of anaesthesia .............................. 24
      3.5.3 Apparatus-related controls ....................... 25
   3.6 Surgical Procedures ....................................... 25
      3.6.1 Head holder surgery ................................ 26
      3.6.2 Eye coil surgery ................................... 26
3.6.3 Canal plug surgery ........................................... 27
3.7 Training ......................................................... 28
3.8 Alertness strategies ........................................... 28
3.9 Paradigms ....................................................... 29
3.10 Data analysis .................................................. 30

4. Results ............................................................ 32

4.1 Controls ......................................................... 32
  4.1.1 The effect of the cervico-ocular reflex ................. 32
  4.1.2 Recordings before plugging ............................ 33
  4.1.3 The effect of anaesthesia ............................. 35

4.2 Time course of recovery ..................................... 36

4.3 Gain and phase properties after bilateral hSCC plugs ... 37
  4.3.1 Gain and phase at different frequencies ............... 37
  4.3.2 Gain and phase across different pitch angles ...... 39
  4.3.3 Cross coupling in cat T ................................ 41

5. Discussion ....................................................... 43

5.1 Cross coupling ............................................... 43

5.2 Residual function ............................................ 45

5.3 Cervico-ocular reflex (COR) ................................ 45

5.4 Conclusions .................................................. 46

Bibliography ...................................................... 47

Figures .............................................................. 52

Tables ............................................................... 91
List of Figures

1. Picture of vertebrate labyrinth .................................. 52
2. Representation of rotations ........................................ 53
3. Canal Orientations .................................................. 54
4. Canal and recti misalignments ..................................... 55
5. Bode plots for canal model ........................................ 56
6. Otolith movement during rotation ................................ 57
7. Head holder arch ..................................................... 58
8. Cat box, head post, recording conditions ...................... 59
9. Rotation axis stimulation .......................................... 60
10. Sinusoid example ................................................... 61
11. Velocity pulse example ............................................ 62
12. Plugged canal ........................................................ 63
13. Post-plug COR data, cat G ........................................ 64
14. Pre- and post-plug COR data, cat T ............................. 65
15. Pre-plug frequency vs. phase and gain, cat T ................ 66
16. Pre-plug frequency vs. phase, cat G ............................ 67
17. Pre-plug frequency vs. gain, cat G .............................. 68
18. Pre-plug pitch angle vs. gain and phase, cat G ............... 69
19. Pre-plug pitch angle vs. gain and phase, cat T ............... 70
20. Anaesthesia time course control, cat T ......................... 71
21. Post plug 8 Hz time course (gain), cat G, angles 0 to 15 degrees down ........ 72
22. Post plug 8 Hz time course (gain), cat G, angles 20 to 35 degrees down .... 73
23. Post plug 2 Hz time course (gain), cat G ....................... 74
24. Post plug frequency vs. gain, cat G ........................................... 75
25. Post plug frequency vs. phase, cat G ........................................... 76
26. Post plug frequency vs. gain and phase, cat T, 3 weeks after plugging .... 77
27. Post plug frequency vs. gain and phase, cat T, 3 months after plugging ... 78
28. Post plug frequency vs. gain and phase, cat T, 6 months after plugging ... 79
29. Post plug pitch angle vs. gain, cat G ............................................. 80
30. Post plug pitch angle vs. phase, cat G ......................................... 81
31. Post plug pitch angle vs. gain, cat T, 3 weeks after plugging ............... 82
32. Post plug pitch angle vs. gain, cat T, 3 months after plugging ............... 83
33. Post plug pitch angle vs. gain, cat T, 6 months after plugging ............... 84
34. Pre-plug cross coupling estimation, cat T, example 1 Hz ....................... 85
35. Area comparison, cat T, 1 Hz example .......................................... 86
36. Pre-plug cross coupling estimation, cat T, 0.1 to 2 Hz ........................ 87
37. Post-plug area comparison, cat T, 0.1 to 2 Hz, 3 weeks after plugging .... 88
38. Post-plug area comparison, cat T, 0.1 to 2 Hz, 3 months after plugging .... 89
39. Post-plug area comparison, cat T, 0.1 to 2 Hz, 6 months after plugging .... 90

List of Tables

1. Description of stimuli used in both cats ........................................ 91
2. Results of area comparison for cat T after plugging ........................ 92
1. Summary

The vestibulo ocular reflex (VOR) is a short latency eye response whose primary goal is to stabilize images on the retina during head movement. The response to angular rotation has been separated into three components, each defined by the eye movement response, generated by conjugate pairs of recti. The component that is produced by activation and inactivation of the horizontal recti, the horizontal component (hVOR), is the subject of this project. In particular, two aspects of the hVOR were studied after plugging the horizontal semicircular canals (hSCC) in two cats. These aspects will be referred to as residual function and cross coupling. Plugging entails drilling a hole in the bony labyrinth and inserting periosteum, thereby theoretically removing transduction in that canal. Three hSCCs provide the majority of the drive for hVOR activation in the normal condition.

One contribution to the hVOR after bilateral hSCC injury is termed residual function. This refers to the remaining transducing properties of the plugged canals. Plugging prevents normal transduction within the plugged canal. However, fluid can still move in the plugged canal. Before this thesis, the influence of residual function had not been studied in the VOR. Residual function is based on a model put forth by Rabbitt (1996). Non-zero gains observed only at high frequencies, with phase leads are characteristics of residual function. Another factor that this study attempts to observe is cross coupling. Cross coupling describes activation of the hVOR by the other four (intact) canals. The contribution of cross coupling is necessary for an accurate VOR. Finally, the cervico-ocular reflex (COR) was studied.

The residual function and cross coupling factors were separated after plugging by recording the gain and phase of the hVOR while the subject was rotated using different Earth-vertical axes of rotation with respect to the head. For rotations in the best plane of the horizontal canals, the cross coupling contribution should be minimal, while the contribution of residual function should be maximal. Also different frequencies of rotation were used, from 0.1 to 8 Hz. At low frequencies, Rabbitt (1996) predicts that residual function does not exist. At high frequencies, residual function should be present. The cross coupling contribution is hypothesized to occur throughout the frequency spectrum, for the frequencies between 0.1 and 8 Hz. Therefore, there is no way of observing residual function alone, but we believe that its presence has been shown in this thesis.
The COR was measured by rotating the cat's body while the head was stationary. The COR response before and after plugging was minimal.

For residual function, phase leads of 30 degrees were commonly observed. For both cats, all averaged phase values acquired after plugging were positive (indicating a phase lead). Many were in the range 20 to 40 degrees. For cat G, gain values at 8 Hz were approximately 20% of pre-plug values. Gain values at lower frequencies declined in a fashion that approximated the response from a plugged canal, as hypothesized by Rabbitt (1996).

The influence of cross coupling was measured in one cat by estimating the input of cross coupling, and comparing that estimate to the measured response after plugging, using frequencies where cross coupling is the primary contribution to VOR gain. At all times after plugging (three weeks post-plug, 3 months post plug, and 6 months post plug), the cross coupling input was larger than pre-plug estimations. From 3 months to 6 months after plugging, the increase was larger than 70% for frequencies 0.25 Hz and 1 Hz. Because of these results, we say that cross coupling has increased in efficacy. However, total gain values for stimuli that should activate primarily cross coupling did not exceed 0.2. Many of the gain averages for both cats were 0.1.

Together, our experiments show that after injury, the hVOR response gets contributions from residual function, cross coupling. The COR does not seem to contribute to the response, nor does it increase any time after plugging. The cross coupling influence has been shown to increase long after plugging.

2. Introduction

The vestibular system senses and responds to head movement in space. The vestibular end organ is commonly referred to as the inner ear or labyrinth. The labyrinth consists of components that sense linear and angular motion. The utricle and saccule (or otolith organs) respond to linear motion. Angular acceleration is sensed by three more or less mutually orthogonal torus-like structures called semicircular canals. For a picture of a generalized labyrinth, see figure 1.

The vestibulo-ocular reflex (VOR) is a response that depends on our ability to sense head motion. It allows images to remain fixed on most of the retina during head movement by attempting to produce an eye
movement in the opposite direction of the head movement with an amplitude being approximately equal to the head velocity.

2.1 Representation of rotations

Rotations can be represented in a way analogous to translational movement or position. Both rotations and translations must specify amplitude and direction. Also, rotations must be described with respect to some reference position. Rotations can be described by either planes or vectors (see figure 2). The rotation axis lies along the vector. Vectors are useful in describing vector projections onto a surface or a plane. Planes are useful in correlating rotations with anatomical constructs, such as the canals. This thesis will use both planes and vectors to describe the head velocity stimulus and the eye velocity response.

2.2 Transduction of angular rotation

Angular rotation is sensed by the canals, which modify and transmit their signals. The canals respond to angular acceleration. However, the signal the canals convey is angular velocity. Hence, it is said that the canal performs an integration procedure (Wilson and Jones, 1979). When the head is rotated, this rotation will cause movement of endolymph fluid within the canal lumen. The fluid movement is due to inertial forces. As a result of this fluid movement, pressure is exerted on an compliant structure called the cupula. This pressure will cause the cupula to bend, which in turn will cause hairs originating from hair cells to bend. The hairs (cilia) are embedded in the cupula. The bending of the cilia result in an increase of the potential across the hair cells’ membranes. This is the causal relationship between angular acceleration and receptor potential in the sensory cell. Next, the signal is conveyed to neurons (vestibular afferents) which transmit their signals to the CNS. Each afferent fires with a basal frequency or resting discharge. For rotation in the canal plane in one direction, the afferents’ firing rate will increase. For rotation in the opposite direction, the firing rate will decrease. By this method, rotation is sensed, integrated, and sent to central structures.

2.3 Horizontal angular VOR

The vestibular end-organ can sense rotation in any plane, but because of anatomical factors, accurate interpretation of angular velocity in three dimensions is made difficult. For rotation in one direction, it is said that we have a conjugate pair of canals (Wilson and Jones, 1979). Each member of the conjugate pair lies in
approximately the same plane as its counterpart. For rotation in the optimal direction, the afferent signal from one of the canals is increased, while the other canal is decreased (Goldberg and Fernandez, ed. 1976). For rotation in the horizontal plane, the conjugate canals are the right and left horizontal (or lateral) canals. The other sets of conjugate pairs are: right anterior-left posterior (RALP) and left anterior-right posterior (LARP) canals. As can be seen from figure 3, the conjugate pairs do not lie in one plane. Hence, there is no one rotation plane that can activate two canals to the exclusion of the others. This is because at least four canals must be activated for a particular plane of rotation. For most rotations, all six canals sense the rotation. There are other imperfections in the semicircular canal layout. The canal planes are not exactly orthogonal to each other. Interpretation of net angular velocity is difficult, but not impossible when the vector components are not orthogonal. The canal is not a perfect torus; it is curved slightly. As a result, transduction of angular velocity is not in a single plane. Because of the three facts mentioned above, interpretation of angular velocity based solely on the vector addition of the signals from the end-organ would result in consistent misalignments between the VOR's input and output. The CNS must interpret this varied set of information appropriately to interpret the correct head velocity, and hence the appropriate direction for the VOR.

The VOR is organized in such a way that it can essentially be described by three sub-reflexes, with one being in roughly the horizontal direction. Three pairs of extraocular muscles move each eye. The optimal pulling direction of each set of muscles can be described by a plane (figure 4). The horizontal component is tilted nose up from horizontal stereotaxic coordinates by approximately 23 degrees (see figure 3 and 4). This component of the angular VOR is called the horizontal angular VOR, or hVOR. The primary input to the hVOR comes from the two horizontal semicircular canals. For the purposes of this study, the hVOR will be defined by its output: movement of the eyes in the plane of the horizontal canals, i.e. in the plane that is horizontal when the animal's head is tilted approximately 20 degrees down from stereotaxic position. The eye movement produced by the hVOR is achieved primarily by a combination of excitation and inhibition of the medial and lateral recti of both eyes. The hVOR can be activated by any of the three canals. By definition, then, any factors producing an eye movement in the specified plane as a result of rotation can be said to contribute to the hVOR.

2.4 hVOR frequency profile
Depending on the stimulus, the hVOR exhibits varying responses, as shown by two properties: gain and phase. Gain refers to the ratio of output to input. For the hVOR, this paper will define gain as \( ([\text{horizontal eye velocity}]^{-1})/\text{[head speed]} \). For both eye and head velocity/speed measurements, the first derivative of position is used. For passive whole body rotation in the dark, the eyes move with a gain under 1. If a sinusoidal rotation is applied, the eye movement is approximately 180 degrees out of phase with the head velocity. Phase will refer to the temporal separation of the peaks of eye and head velocity, measured in degrees. For this thesis, if the peaks of the eye and head velocities will occur at the same moment in time, but with opposite directions, then a phase value of zero will be given. A negative phase value will indicate that the eye velocity peaks at a later time than the head velocity. This is also called a phase lag. A positive phase value occurs when the peak in eye velocity occurs before the peak head velocity. This is also called a phase lead. For frequencies of 3 to 8 Hz, there is little data in cats. However, by inference, conclusions can be drawn about the nature of gain and phase properties of the hVOR in cats in that range of frequencies. A model of a canal with a simplified cupula predicts the gain and phase properties as seen in figure 5 (Wilson and Jones, 1979). Generally, the same trend in gain and phase was observed on the canal afferent signal (Fernandez and Goldberg, 1971). However, the afferent and model data come to the same qualitative conclusions. At low frequencies, the hVOR appears to have a gain of under unity with a slight phase lead. In middle-range of frequencies, the gain is close to unity and the phase is close to zero. Paige (1983a, in the squirrel monkey), Baarsma and Collewijn (1974, in the rabbit) and Skavenski and Robinson (1973, in rhesus monkeys) have shown that the hVOR in darkness has a gain just below 1 and a phase of approximately zero in the middle frequency range. At high frequencies, the gain is approximately unity, and the phase is lagged. There is at least one study which looked at the gain and phase properties in cats in the high frequency range 5 to 8 Hz. Broussard and Bhatia (1996), observed that the reflex exhibits a phase lag at high frequencies, and a phase lead at low frequencies as long as the end-organ is undamaged. For the purposes of this study, the low frequency range is below 1 Hz, the high frequency range is 5 Hz and above, and the middle range is the frequency range in between.

2.5 Cross coupling
For the purposes of this thesis, cross coupling refers to the input of the 4 vertical (2 posterior and 2 anterior) canals to the hVOR. For the VOR to be accurate, the three sub-reflexes must interact with one another. The degree of interaction depends on the geometric misalignments of the sense organs and the extraocular muscles. The pulling directions of the horizontal recti lie very close to the best planes of the horizontal canals. There are similar close alignments of the vertical and oblique recti and RALP (Right Anterior-Left Posterior coordinate) and LARP (Left Anterior-Right Posterior coordinate) canals (Highstein, 1988). There are misalignments between the best plane of the horizontal canals and the plane of the horizontal recti. Figure 4 shows how the two planes are tilted up in the same direction, but differ in the magnitude of the tilt. It should be noted that in cats, as in other species, the magnitude of misalignment between the horizontal canals and horizontal recti is small compared to the misalignment in the RALP and LARP sub-reflexes (Blanks et. al., 1972, Carpenter et. al., 1988, Graf et. al., Ezure and Graf 1984, Highstein, 1988). I estimate the misalignment to be 10 degrees in the hVOR, and approximately 15 degrees in the RALP and LARP coordinates (Blanks et. al., 1972, Bohmer and Hess, 1985, Carpenter, 1988, Estes et. al., 1975, Highstein, 1988, Yakushin et. al., 1995). However, differences may exist depending on the species, individual, and whether or not the VOR coordinates are set up bilaterally. Theoretically, it is possible to have three separate sub-reflexes, with conjugate canals affecting only four extraocular muscles, and not giving input to the other eight. This would result in consistent misalignments in the angular VOR. It has been shown (Crawford and Vilis, 1991, for example) that the observed misalignment of input-output is very small, too small given the misalignments between the canals and extraocular muscles (Carpenter, ed. 1988, Blanks et. al., 1972.) Since the input and output coordinate systems are slightly different, and the VOR is accurate, three segregated, separate sub-reflexes do not exist. For an accurate VOR, there must be crossed input for each coordinate system (the horizontal system, the “LARP” system, and the “RALP” system). To summarize, the need for cross coupling arises from the fact that the orientations of the inputs and outputs of the three components of the angular VOR are slightly different.

The capacity for large gain changes in the hVOR based on the crossed input is large, even though the hVOR itself does not appear to show a large contribution from cross coupling. Past experiments (Yakushin et al., 1995, Baker, 1982) have shown that there is little change in the cross coupled response after plugging both horizontal canals, as measured by hVOR gain. However, an increase over time of the cross coupled
response has been observed elsewhere (Angelaki et al., 1996). Secondary neurons that carry a predominantly horizontal signal have been found to respond to rotations in directions other than those described by the best plane of the conjugate horizontal canals (Baker et al., 1984, Graf et al., 1993). Maximal activation vectors for individual cells did not appear to correspond to the spatial orientation of one or both horizontal canals for some cells. It has been shown that the misalignment on the secondary neurons does not originate from the primary afferent input from horizontal canals (Ezure et. al., 1978, Estes et al., 1975, Myers and Lewis, 1991). Although Estes et al. (1975) concluded that the optimal sensitivity of the primary afferents did not coincide with the physical layout of the horizontal canals, the optimal sensitivities were only slightly different. The above facts would lead one to conclude that there is canal convergence on a portion of horizontal secondary cells. However, the cross-coupled influence on the hVOR itself is slight (Tusa et al., 1996). One possible way of reconciling these conflicting observations is to point out that the cross coupled input likely comes from all four vertical canals. If the subject's head was rotated about an axis that was tilted nasally in the pitch plane, away from the best axis of the horizontal canals, then a counter-clockwise component would be needed as well as a rightward component for the eye movement response. If this rotation was applied, then it would affect all six canals. The left horizontal, right posterior, and right anterior canals would be activated while the right lateral, left posterior and left anterior canals would be inhibited. Similarly, for rotation in the RALP plane, there is excitatory modulation from one canal and inhibitory influence from the other. For any rotation, there are both excitatory and inhibitory influences from one set of conjugate canals. If we assume that the cross coupled inputs of each of the four vertical canals are approximately equal, then the net input from cross coupling would be very close to zero. This is because the hVOR is being activated by afferent input from one canal in the conjugate set, and inhibited from the other conjugate canal. If equal input from all four vertical canals exist in the population of horizontal interneurons, but individual secondary neurons carry differential input from one or two canals, then it would be possible to observe a large cross coupled response on the secondary neurons (Graf et. al., 1993), while the hVOR would show no such response. This idea that there is population wide cancellation but individual signal on neurons was also put forth by Chen and McCrea (1996). However, the population wide cancellation that they studied was the input from regularly discharging afferents. If the idea of cancellation on a population basis existed, the capacity for adaptation of cross coupled input would be large. We could not see the raw material for adaptation of the
cross coupled input by simply looking at the VOR. It would appear that cross coupling did not exist. But on individual neurons, the extent of the cross coupled input could be seen. By changing synaptic efficacies, the cancellation effect could be reduced. There could be several sites that could affect an increase in the input from cross coupling. One is the synapse between the horizontal interneuron and the abducens nucleus. Another is the synapse between the neuron carrying the vertical canal signal and the horizontal secondary neuron. By changing both, the up-regulation of the input from cross coupling can be magnified. In short, the vertical canals will be activated for horizontal rotation (first paragraph of section 2.3). That signal likely exists on secondary neurons (this paragraph). In other words, some horizontal secondary neurons get input from more than one canal, however, the hVOR itself aligns closely with the horizontal canals. It is likely that the secondary neurons' input from the 4 vertical canals cancel out downstream, hence the hVOR does not the same signal. Hence, the capacity for up-regulation of the crossed input is large because of the nature of the system.

2.6 Residual function

Residual function refers to the remaining transducing properties of a canal which is occluded by a plugging procedure. Canal plugging refers to a form of damage that blocks movement of endolymphatic fluid, while the cupula is intact (for a full description, see the first paragraph of section 3.1). A theoretical model for residual function has been developed (Rabbitt, 1996), and predicts a response that increases with the frequency of rotation, and is significantly phase-led. This differs from the normal high-frequency response, which is phase-lagged. Rabbitt's model is based on a canal which is distensible. In this canal, cupular movement is due to two factors. One is based on the pressure gradient on either side of the cupula, AP. This will result in bending of the cupula in the transverse direction. The other is based on the uniform pressure within the ampulla lumen, P(o). This pressure will cause the ampulla to expand outward, deforming the cupula. During low frequencies of rotation, in the intact canal, AP is the primary influence on cupular distortion. At high frequencies, in the intact canal, P(o) plays a role. This is because as fluid cannot move quickly from an area of large surface area to an area of small surface area, eg. from the ampulla to the canal arm. This will result in buildup of pressure. Hence P(o) will be large for high frequencies. The distortion of the cupula due to P(o) is phase lagged.
When the canal is plugged, the largest pressure gradient is no longer on either side of the cupula, but is on either side of the plug. Hence $\Delta P$ in the ampulla will be reduced. There is still a pressure gradient across the cupula, but it is no longer the largest in the canal. The positive pressure that contributed to $\Delta P$ in the normal canal also contributed to the large value for $P(o)$. In the plugged canal, the large positive pressure in the largest pressure gradient is not in the ampulla, hence the expansionary influence on the ampulla is not as large. In other words, the influence of $P(o)$ is reduced. Since $P(o)$ is lower, then the phase lag is not as large a factor. $\Delta P$ has a phase lead, which results in the phase led signal. For lower frequencies of rotation, the pressure gradient across the cupula is not large enough to bend the cupula enough for transduction. Only at higher frequencies is the pressure gradient across the cupula large enough for transduction. Needless to say, residual function can only exist in a plugged canal which has an intact cupula. If the cupula is destroyed, residual function is theoretically impossible. The model predicts the output of the plugged canal, not any reflex, per se. A plugged canal may still sense angular acceleration, but the properties of the velocity signal on the afferents may be very different from the normal sense organ. The high frequency, phase led transduction is a trademark of residual function, as proposed by Rabbitt.

2.7 Contribution of the otolith organs

The otolith organs could conceivably transduce angular rotation. In theory, this is possible because each set of otolith organs will generate differing signals during rotation. If the subject is rotated about a dorso-ventral axis that is centered in the head, then each individual set of otolith organs would experience linear acceleration, as seen in figure 6. If the rotation axis was located midway between the two sets of labyrinths, then one set of otolith organs would experience linear acceleration in one direction, while the other set of otoliths would sense linear acceleration in the opposite direction. The only correct way to interpret this information is to conclude that the head is rotating. It is uncertain whether the brain combines this information properly in order to allow transduction of this form to contribute to the hVOR. It remains to be qualitatively observed. However, there is evidence that suggests that transduction from otolith organs may not be able to interpret yaw rotation at low angular velocities (low radial accelerations). Cohen et. al. (1983) have shown that after plugging 6 canals, the VOR response was negligible. It is possible that if higher velocities were used, the otoliths may have been activated. Goldberg and Fernandez (1976) have shown that
the utricular afferents will not increase their firing rate above threshold for rotations of angular velocities of at least 10 deg./s. Therefore, if the otolith organs cannot sense the radial acceleration from the angular velocity observed, then they should not contribute to the hVOR. The CNS cannot interpret a signal that does not exist.

For rotations whose maximal angular velocity is 10 degrees per second or less, otolith transduction of angular velocity does not exist.

2.8 Cervico-ocular reflex

The Cervico-ocular reflex (COR) is another eye stabilization mechanism which has unique properties, and serves to stabilize retinal images. Like the VOR, the COR also initiates an eye movement in response to head movement. However, here, sensory stimulation for the reflex is proprioceptive input from neck muscles (Bronstein et. al., 1995, Gioanni et. al., 1997). Therefore, the stimulus for this reflex is head on body movement, or bending of the neck. If the head rotates to the right on the body, the cervico-ocular reflex (COR) will initiate a leftward eye rotation. It has been determined that passive flexion of a cat's neck can allow for movement of the head on the neck of over 100 degrees, with minimal forces applied at a point 2 cm away from the C1 area, the approximate centre of rotation (Graf et al., 1997). This reflex is not as exact as the VOR. This means that an oblique head on body rotation will produce at least a horizontal eye movement. The misalignment between the input and output is much greater than the VOR (Bronstein and Hood, 1986). This would imply that the yaw component of the COR should not show consistent deviations related to static pitch position of the head with respect to the body. The magnitude of the COR is much lower, especially in subjects with no damage to their vestibular organs. However, after injury, an up-regulation of the COR magnitude was observed that later subsided to pre-injury values. The initial post-injury increase occurred seven weeks after the injury, and the return to normal gain values was observed 6 months after the injury (Bronstein et. al., 1995). The COR as an eye stabilization mechanism is not completely compensatory, but it appears that it can increase its efficacy in the absence of other eye stabilization mechanisms.

2.9 Eye position effects on the hVOR

It has been shown that for a given head velocity, different eye velocity responses are possible, and may be influenced by current eye position (Misslisch et. al., 1994; Misslisch et. al., 1996). For the VOR to be effective, it must be a consistent response to head movement. For a head rotation in a particular direction,
the eye velocity response should be in the opposite direction. There should not be any limitations on the eye movement response of the VOR. It has been observed that the VOR may be at least partially subjected to a constraint on eye velocities in three dimensions, called Listing's Law (Misslisch et al., 1994, Misslisch et al., 1996).

Listing's Law states that the eye will only be in certain positions. Specifically, the eye may take up any vertical and horizontal position, but the torsional position will remain constant, as compared to a reference position called primary position (Tweed et al., 1992). One definition of primary position is a normal to Listing's plane (Crawford and Vilis, 1995, Heap, 1990, Tweed and Vilis, 1990, Suzuki et al., ed. 1994, Straumann et al., 1991). Listing's plane describes the position the eye may take when obeying Listing's Law. When position vectors are plotted with respect to primary position, all the vectors fall into this plane. The plane is approximately aligned with the coronal plane, although it is tilted up slightly in the pitch plane (Tweed et al., 1990). There is one corollary to Listing's Law. The velocity vectors that the eye may assume also fit in a plane. When the gaze is in primary position, Listing's plane, and this velocity plane are in the same orientation. However, when the eye deviates away from primary position, the velocity plane is also tilted (Tweed et al., 1990). If the eye is in a position 20 degrees upward, the velocity plane is tilted up by 10 degrees. In general, if the eye is tilted x degrees, the velocity plane is also tilted, in the same direction, but with magnitude x/2.

It has been determined that the VOR obeys Listing's law partially. In other words, the torsional velocities the VOR can produce are limited. If the eye is positioned upward from primary gaze in pitch, then the velocity plane is tilted upward by half the magnitude of the eye's tilt. The VOR response vector is tilted as well, but by less than the velocity plane's tilt. Specifically, the VOR vector is tilted away from the ideal compensatory response by half, towards the velocity plane. The effective result on the VOR response is that it will have a have a slight torsional component and the horizontal component will be slightly reduced. This restriction has a large influence on the torsional VOR but only a slight effect on the horizontal VOR (Misslisch et al., 1994). For example there was approximately a 8 degree rightward tilt of eye velocity axes for 20 degree leftward deviation in eye position for the torsional VOR. For the horizontal VOR, a 20 degree deviation of the static eye position upward, a tilt of approximately 3 degrees up in the eye velocity was the result (Misslisch et al., 1994). For the shift in the horizontal VOR the reduction in the horizontal component
is virtually negligible, from 100% to 99.76% of non-tilted values. Misslisch et. al. (1994) determined that the VOR is partially compensatory and partially obeys Listing’s Law. Hence, the dependence on eye position will attenuate the hVOR, but the effect is minimal for oblique positions of 20 degrees or less. The extraocular muscles change their pulling direction as the eye deviates. For example, if the eye is deviated upward, the pulling direction of the lateral rectus will now be torsional and downward, as well as horizontal. Miller and Robinson (1984, 1987), and Miller (1989) have quantified the possible change in pulling directions. This shift is too little to account for the change observed in the VOR (based on the torsional VOR, as observed by Misslisch et. al., in 1994 and 1996). Hence, the cause of the shift is yet to be determined, but if there are consistent oblique eye positions during the VOR, the hVOR may be affected.

2.10 Hypotheses

The study of residual function is important in understanding whether such a phenomenon exists, and whether it can influence a motor system, such as the hVOR. Future canal plug studies may have to include this transduction in analyzing high frequency plugged hVOR responses if residual function can influence the hVOR. Understanding the extent of the adaptation undergone by cross coupling would set a precedent for the malleability of coordinate systems, and how the brain interprets the external world.

This project will attempt to determine the contributions of residual function and cross coupling to the hVOR after bilateral horizontal semicircular canal plugs.

Generally, the project will attempt to test three predictions:

1. Residual function of the plugged horizontal canals can generate a noticeable response in the hVOR in the best plane of the horizontal canals or in other planes.

2. Cross coupling will increase its contribution to the hVOR after plugging.

3. The cervico-ocular reflex (COR) can increase its gain after the plugs.

There are several ways that we can attempt to test whether the statements are valid. A description of the rationale is provided in the first section of the methods.
3 Methods

3.1 Rationale

We will attempt to determine whether cross coupling, residual function, and the cervico-ocular reflex contribute to the VOR gain recorded after plugging. Our approach is to occlude both horizontal canals (hSCCs) by plugging. Plugging is a common approach to studying hVOR adaptation (Baker et al., 1982, Paige, 1983, Yakushin et al., 1995). The surgical procedure was originated by Ewald (1892), and modified by Money and Scott (1962). Canal plugging theoretically eliminates transduction of angular velocity by the plugged canal (Bohmer et al., 1985, Correia and Money, 1970, Raphan et al., 1983), while the resting rate of the afferents that innervate the damaged canal remains relatively normal (Barmack and Pettorossi, 1988, Goldberg and Fernandez, 1975, Paige, 1983). We will rotate the cats about an Earth-vertical axis. The head rotation will be composed of velocity sinusoids. The resultant eye velocity should approximate a sinusoid. The hVOR shows different gain and phase properties when different frequencies of head rotation are used. These differences, primarily based on phase characteristics, will allow the experimenter to determine which factor or factors are contributing to the eye movement during rotation.

First I will describe how we will attempt to quantify the cross coupled input. We will determine the VOR gain and phase at different frequencies of the hVOR in the intact animal. We will assume that cross coupling will have the same phase properties across the various frequencies both before and after the plugs. At low frequencies, phase characteristics do not appear to differ from data in normal animals (Baker et. al., 1982, Baker et. al., 1984, Tusa, et al., 1996, and Yakushin et. al., 1995). Next, we will assume that the low frequency hVOR is not contaminated with a response originating from the damaged canals. Rabbitt's model has proposed that transduction in the plugged canal is most prevalent at high frequencies, and is minimal at low frequencies. At frequencies below 2 Hz, the transduction of angular velocity by the plugged canal is negligible, according to the model. Hence, cross coupling should be the major input to the low frequency VOR. Also, as the rotation axis is tilted in the pitch plane, away from the best plane of the horizontal canals, the vertical canals should be activated to a larger degree without cross coupling. There should be a cosine relationship when the tilt in the rotation axis (away from the best plane of the horizontal canals) is plotted vs.
VOR gain (defined earlier). If the gain is larger than that predicted by a cosine relationship, then we can conclude that there is significant cross coupling, i.e., the cross coupled input has increased. Yakushin et al. (1995) has shown that there is a cosine relationship, as described above. In practice, it is difficult to determine the exact nature of the cosine relationship. For example, if the misalignment between the horizontal canals and the horizontal recti in one individual is large initially, then the cross coupled input should be large, even before plugging the horizontal canals. This could lead to the conclusion that adaptation had occurred after plugging, when the truth may be that the cross coupled input was simply large initially, and didn't change after plugging. Fitting a cosine curve assumes that this is the standard for cross coupling in any animal, while there may be differences in individuals. For this reason (inter-individual differences), determining cross coupling by fitting a cosine curve may lead to inaccurate conclusions about adaptation of cross coupling after a plug. In cat G, that is the only comparison that is possible. However, conclusions cannot be drawn from such analysis. In the cat T, we have attempted to quantify the adaptation of the cross coupled input in a different way, described in section 2.10. In short, cross coupling can be quantified by examining gain at different pitch angles.

We can also attempt to determine the contribution of residual function to the post plug response. Rabbitt's model predicts that residual function is noticeable at high frequencies, above 5 Hz, and drops off substantially when low frequency stimulation is applied. The model also predicts that the angular velocity sensed by the plugged canal will show a phase lead. This suggests that if a phase-led response is observed, whose gain is most substantial at high frequencies, and gradually drops off as lower frequency stimuli are applied, residual function of the plugged canal has produced that response.

Residual function and cross coupling can be separated by the plane of stimulus rotation. Some rotation axes will have greater torsional components. The axis of rotation will be tilted in the pitch plane, either forward or backward. If the head rotation axis is tilted in the pitch plane, the vertical canals should be stimulated to a larger degree, resulting in an increase in the contribution of cross coupling in the eye velocity response. Conversely, the contribution of residual function should be less for rotation about axes that have both yaw and roll components. The opposite should be true for rotation in a plane that is close to the best plane of both lateral canals. The contribution of residual function should be at its highest at pitch angles close
to the horizontal canals (if a high enough frequency is used), and the contribution of cross coupling should be small, since the vertical canals will be almost perpendicular to the plane of rotation.

We also attempted to quantify the contribution of the COR. Measurement of the COR is important because the COR may contribute to the measured eye response to whole body rotations in the dark. If the COR contributes to the response observed during head rotation, the eye movement recordings in response to body rotation (referred to as 'hVOR', in quotes, for short) after plugging may be due to neither residual function nor cross coupling. At the very least, the COR response could contaminate the recorded eye velocity responses, and it may be difficult to fit a sinusoid to the low-frequency gain. Another problem is that if the COR contributed a small percentage to the 'hVOR' recordings, misinterpretation of the relative contributions of residual function and cross coupling is possible under some stimulus conditions. Therefore, it is imperative to quantify some characteristics of the COR, such as its frequency characteristics, phase, and gain magnitude. It should be noted that the level of possible neck-bending that may occur in the whole body rotations is small, and cannot be measured accurately with our apparatus. Hence it is impossible to mimic the neck-bending artifact which may occur during head rotation trials. We will produce bending of the neck whose magnitude is probably much greater (by at least one order of magnitude) than the possible artifact. If we can show that a very large neck-bending will produce a minuscule gain, then we can reasonably conclude that neck-bending of small magnitude will not contribute to the hVOR response. In this way, the COR contribution will be analyzed.

3.2 Rotation Stimulus

Several different forms of the same stimulus were utilized. One elderly cat (cat G) and one young cat (cat T) were subjected to Earth-vertical, whole-body rotation in darkness. Sinusoidal oscillations and velocity pulses were applied. Sinusoids ranging from 0.1 Hz to 8 Hz were used. For one trial, only one frequency was applied. For all sinusoidal rotations of the head, the maximum velocity applied was 10 degrees per second. For COR experiments, peak velocities were 20 deg./s. and 10 deg./s. COR recordings were also made in the dark and in the light. Any COR averages contained trials acquired in both darkness and with the lights on, and all peak velocities. It was determined in two controls that these factors did not affect the recorded COR gain or phase (data not shown). Velocity pulses were used solely for calibration
purposes and estimating alertness in this project. For velocity pulse rotation, the plateau velocity was 20 degrees per second, with a rise time of 10 milliseconds, and the plateau duration was 300 milliseconds. Eye movements were recorded in response to these types of passive head rotation.

3.3 Apparatus

Eye position recordings were as follows. Measurements were made using the Helmholtz field coil-scleral search coil technique. For eye movement recordings, dual magnetic fields were generated by 2 perpendicular field coils (Remmel Labs). Current induced in the implanted scleral search coil was separated into vertical and horizontal components. This separation was possible because the horizontal and vertical magnetic fields oscillated at different frequencies. The horizontal and vertical components were low-pass filtered electronically, with a roll-off of 500 Hz. Data acquisition, interpretation, and analysis was done by pentium and pentium II based computers, using Labview software (National Instruments). Both eye and head position signals were also filtered, using Butterworth filters (30 Hz low frequency cutoff) within the Labview program. For stimulus frequencies below 0.65, the sampling rate was 130 Hz. Above 0.65, the sampling rate was 200 *[the frequency used].

The rotation and head fixation apparatus will now be described. Rotation was applied to a table which contained the animal, field coils, and recording apparatus. Whole body rotations were carried out, except for experiments which tested the COR. The animal's head was rotated with the same velocity as the table. Rotations occurred in the dark, with curtains and black walls to ensure that light did not leak into the recording room. Only the animal's head was rotated with exactly the same velocity as the table. The head was rigidly connected to the table. This was accomplished by implanting a cylindrical metal rim to the animal's head. The metal rim (called a head holder) was fixed to screws implanted in the cats' cranium by dental cement. The cylindrical rim was screwed to a round head post which was attached to the table via a rigid arch (figures 7 and 8). Hence, the table velocity that was used was applied directly to the cat's head.

The axis of rotation had several different orientations when viewed with respect to the cat's head. The animal's head was rotated about an Earth vertical axis for all trials. It was as if the rotation axis tilted in the pitch plane (in head coordinates) for some stimuli. This was accomplished by rotating the animal's head in the pitch plane and fixing it in the new pitch position (see figure 9). Earth vertical rotation was then
applied. The effect was that a rotation with different torsional components could be administered (figure 9 b). This effect was achieved by installing a swiveling head holder arch on the turn table in place of the fixed pitch position arch, shown in figure 7. Hence the swiveling arch allowed different pitch positions of the cat's head.

A different apparatus was used for the neck-bending required for the COR recordings. During rotations, the cat's body was packed in styrofoam to ensure that the body did not slip within the cat box while the body-on-head rotation was applied. The animal sat in the same cat box as the VOR recordings, but its head was attached to a wooden superstructure via the head holder cylinder implantation and a long head post. The wooden superstructure sat on the ground and didn't move during recordings. This prevented head rotation in space. The field coils were supported by the wooden superstructure as well, so that any eye velocity which was observed corresponded to an eye movement produced by the COR. The table (and hence body) rotated, while the head was held in place. The result was bending of the neck.

3.4 Data Acquisition

The recording apparatus was calibrated daily at one pitch angle, and weekly at several pitch angles. Calibration was done by attaching a scleral coil to a pointer which could swivel back and forth horizontally on a protractor. The current was then correlated to eye position. To ensure that there were no changes in position recordings from day to day, at the beginning of each day, the system was tested. For this, the protractor was used. The position recording was noted while the protractor was in different horizontal positions (ten degrees left, zero and ten degrees right). Once a week, the calibration procedure was used at six different pitch angles. Pitch angles in this range, in 5 or 10 degree increments, were used for VOR recordings on the cats.

Recordings each day took approximately 30 minutes. This was the length of time we determined that accurate recordings could be made. With longer recording times, the cat did not remain alert, and the results were affected. During each day's session, the cat's head was fixed in a certain pitch position (via the swiveling arch), and recordings were made at different frequencies, as shown in table 1. Figure 10 shows the average head and eye traces for one day; that is, one day's averaged recording at one frequency and one pitch angle. Each trace consisted of at least 15 sinusoids, and many were averages of over 25 sinusoids.
Approximately 24 of these averages were acquired during a daily session. Sometimes velocity pulses were used (figure 11), to give the experimenter an indication of the cat's alertness. Once this was completed, the head was fixed in a new pitch position, and the recordings at different frequencies occurred again. This went on until approximately thirty minutes expired.

3.5 Controls

There were several controls, some of which will only be mentioned, not shown in the results section.

3.5.1 Cervico-ocular Reflex

The COR was recorded because it may have contributed to the recorded VOR response. The COR was measured after plugging both lateral canals in cat G, starting 7 months after his plugs. In cat T, the COR was measured before plugging, 3 weeks after plugging, and 15 weeks after plugging. In both animals, recordings were made in darkness and in the light. Recordings were made with peak body velocities of 10 and 20 degrees per second. Initially, the recordings were made in the light, to ensure that the cat was not injured. A person sat in the recording room and observed the experiment, trying to determine whether the cat felt discomfort. Once it was determined that the recordings could not injure or result in undue stress, subsequent recordings were made in darkness.

3.5.2 Effect of Anesthesia

We attempted to determine when the effect of anesthetic wore off after surgery. The anesthetic used was isoflurane, usually given in doses from 2-3%. After surgery, it took approximately 30 minutes for the cats to regain consciousness. The effect of anesthetic on the hVOR has not been fully quantified. Keller (1976) has shown that anesthetics may influence the eye velocity. Anesthetics may modify a centrally originating efferent effect (Wilson and Jones, 1977). It should be noted that in our experiments, no recordings were made while the cats were under anesthetic. The effect anesthetic may have on this project is probably solely on the cat's alertness. If we recorded too soon after the bilateral plugs, the animal may have been sleepy, and the gain values could have been depressed because of the anesthetic. Regardless of the cause, we tried to determine the time course of the effect. VOR recordings were made in cat T after it had been under anesthetic for 6 hours. Recordings were made at frequencies from 0.1 to 8 Hz, the frequencies
that were to be used after the plugs. Recordings were made every six hours after surgery. Alertness was determined by comparing the gain values after anesthetic administration with the gain values on days when the animal was alert. Also, a subjective estimation of drowsiness was made. This was done by noting the animal's horizontal and vertical eye position during the recordings in the dark. If the eye position drifted by a noticeable amount (at least 4-5 degrees in a 10 or 15 second window), then the animal was said to be drowsy. If the eye position remained relatively stable, we estimated that the animal was alert. The anesthetic control was done for cat T only.

3.5.3 Apparatus-related controls

Several controls were used. The primary one consisted of placing a block of wood in the apparatus and recording the gain. This block of wood has approximately the same mass and volume as that of a cat's head (331g and 26.25 cubic cm). The block of wood is fixed to a cylindrical head holder by dental acrylic. The cat's and block's head holders are identical. The block of wood also has a 20 mm diameter eye coil fixed to its frontal surface, and an eye coil connector, identical to cats' eye coil connectors, was fixed to its top surface by dental acrylic. Velocity sinusoids were then applied to the table, and gain and phase were recorded. If the gain values for the block of wood were above 0.01, the reason for the increased 'gain' was discovered and removed before subjecting the cat to rotations. Generally, before new apparatus (for example, the COR apparatus and the swiveling head holder arch) were installed, recordings were made from the block of wood to ensure that artifacts were not introduced because of the new hardware. Further, for approximately a week after installing the new apparatus, recordings from the block of wood were made immediately before and/or after animal recordings for that day. Recordings from the block of wood also were made periodically, when a large amount of variability appeared in the animal recordings. Recordings from the block of wood was the primary method to point out and remove any artifacts before recording sessions involving the cats.

3.6 Surgical Procedures

There were several common practices before every surgery. Surgeries were performed after the cat's training had been completed. For the head holder surgery, the cats' heads were fastened to a stereotaxic apparatus. For subsequent surgeries, the animals' head was held still by a clamp which was attached to a rod which has an attachment for the head holder implant. Pre-medication and post-surgery drug administration
was essentially the same for all surgeries. Before surgery, the cat was injected intramuscularly with 0.5 ml premix (containing the substances Demerol, Acepromazine, and atropine). i.v. saline was also utilized during and before the surgical procedures. During the surgeries, the animal was given 2-3% isoflurane gas, via endotracheal intubation. For eye coil surgeries, 1-3 drops of Diphenyl T (active substance: phenylephrine hydrochloride) were given to ensure retraction of the cat's nictitating membrane. After surgery, the cats were given 0.5 ml penlong (penicillin) and 0.1 ml Buprenorphine (an analgesic). For seven days post-surgery, the animals received 2 pills per day of 62.5 mg clavamox, an antibiotic given as a prophylactic. After the plugging procedure, cat G was given i.v. fluids for 2.5 days post-op, because of possible dehydration. At various times after their first recordings were made, cats G and T were given chloramphenicol ophthalmic ointment and eye drops, to treat infections from whichever eye had the coil. In addition, cat G was given gentamycin ophthalmic ointment periodically. Gain values were compared before and after the initiation of gentamycin administration, and the gains were virtually identical (data not shown). This suggests that local administration of the ototoxic drug did not affect the labyrinth of cat G. These were the procedures before and after surgeries, as well as procedures that were common for all surgeries.

3.6.1 Head holder surgery

The orientation of the head holder and hardware used will now be described. The head holder was implanted, along with the eye coil, during a 4-5 hour surgery. For the surgery, three bent steel plates were used. The plates were attached to the animal's skull by 6 mm screws. Two screws were used for each plate. The head holder was then fixed to the plates by dental acrylic. For both Cat T and G, the head holder was positioned such that the cat was oriented 23 degrees down when the reading on the arch was 0 degrees. Hence, to position the head 10 degrees up from the stereotaxic position, the value on the arch would have to be 33 degrees up (+33 degrees), and for the head to be positioned 40 degrees down, the arch would have to have a position of 17 degrees down (-17 degrees). This is how the head holders were oriented.

3.6.2 Eye coil surgery

The eye coil surgery will now be explained. Eye coils were prepared from teflon-insulated steel wire. Coils had three turns, 2 around the diameter of the coil, and the final turn wrapped around the other 2 coils. Before eye surgery, Diphenyl-T eye ointment was administered, to retract the nictitating membrane and
reduce swelling. During surgery, the sclera was exposed, and the eye coil was attached to the sclera by 4 sutures. The sutures were either 5-0 silk or 5-0 polyester. The coil was placed posterior to the cornea, but anterior to the extraocular muscles. The coil was soldered to a two pronged, male connector (Winchester socket) which was fixed to the cat’s supraorbital bone. This was done by attaching dental cement to the connector and 2 mm screws which were implanted in the supraorbital bone. In this way, the eye coil surgery was carried out.

For both animals, more than one eye coil surgery was carried out. For cat G, there were two eye coil surgeries. The first was before the plugs in his right eye, and the second was after plugging, in the left eye. For cat T, eight eye coils were used. Because the coils were prone to detaching from the sclera in this animal, they had to be removed and replaced several times.

After each surgery, eye coils were calibrated. Calibration consisted of rotating the animal using velocity pulses in the light with an experimenter in front of the animals to ensure the best eye stabilization. Under these conditions, we assumed that the steady-state gain (while pulse velocity was constant) was 1. I then adjusted a multiplication factor until it should give a gain value of 1 under the conditions described above. Velocity pulse experiments were then carried out in the light with an experimenter in front of the cat with the new multiplication factor. If the experiment did not yield gain values that averaged 1, the whole calibration process was repeated, until the velocity pulse in the light yielded an average gain of 1. This way, we could ensure that all the data from one animal were equivalent, in spite of the use of different eye coils.

3.6.3 Canal plug surgery

The canal was plugged by the method described below. The procedure entails carefully opening the bony canal for approximately 3 mm with a diamond burr. Next, a piece of periosteum is inserted into the opening. The canal was exposed by first exposing the temporal bone. Then, the external auditory pathway was followed, and the incus was observed. A portion of the incus is parallel to the horizontal canal. This is how the horizontal canal was recognized. Bone was drilled away, and the canal was exposed. The bony arm of the canal was drilled open until endolymphatic fluid leaked out of the opening. The canal was then plugged with periosteum. This procedure was done on both horizontal canals. For a pictorial description of a plugged and unplugged canal, see figure 12.
3.7 Training

The performance required of the cats during the VOR recordings was minimal. The primary requirement was that the animal sit passively in the box while recordings were made in the dark, and in some cases in the light. It was beneficial if the animals did not attempt to move their heads during the experiment, so as not to put undue pressure on the surgically attached head holder. The training required was not extensive.

The training procedure occurred in increments. The animal was first trained to walk into the bag where it would remain during the recordings. The cat’s body would be in the bag while its head was exposed. The next step was to allow the animal to sit in the box for a period of time. The time was gradually lengthened until the subject could sit in the box for an extended time period, usually 30 minutes. Finally, after the eye coil and head holder were surgically attached, the cat was trained to sit comfortably while in the box, and its head was held still via the arch.

3.8 Alertness Strategies

We attempted to keep the cat awake and alert by several methods. One of the largest estimated contributors to day-to-day variability was sleepiness. We attempted to minimize this in several ways. First, we kept the duration of the recording sessions to a minimum. We recorded once a day, at most, except immediately after the plugs. We also monitored alertness by the eye position (described above), velocity pulse gain and overshoot, and other subjective measures, such as the cat’s behavior. Figure 11 shows one example of an average from a velocity pulse trial. When the animal is sleepy, the overshoot becomes much smaller. We determined the optimal length for recording. Secondly, we sometimes used D-amphetamine sulfate to prevent sleepiness. Use of this drug allowed longer recording sessions, typically 45 minutes. This drug was only given on up to 5 consecutive days, and in increasing dosage to ensure that the drug was effective each day. Third, we changed the light cycle in the room in which the animals were housed. It has been shown that ambient light affects sleep cycles (Czeisler et. al., 1982). Also, nocturnal animals such as cats are more active in the dark. Hence, since most recordings were normally made during daylight hours, the light and dark setting in the cat housing room were changed, so that during daylight hours, the lights were out. The transition was made from normal lighting times to the reversed times by moving the light cycle forward.
by three hour increments until the light cycle was completely reversed. It has been shown that by moving the cycle forward, and not backward, the greatest effect on sleep cycles can occur (Moore-Ede and Richardson, 1985, and Turek, 1986). By these three methods, we attempted to minimize the effect of alertness on the variability in our recordings, both before and after plugging.

3.9 Paradigms

Averages were made to account for day-to-day variability. Recordings were made on several different days to ensure that alertness, noise, and other unforeseen factors would not add or subtract from the VOR gain and phase values. Under each circumstance, at least five recordings were made, and then averaged. For instance, before plugging, there were 5 recordings made at 8 Hz, and with the cat’s head fixed at pitch angle 50 degrees down from the stereotaxic position. Comparisons were made of gain before and after plugging, under the same circumstances. Comparisons were also made of two or more gain values, each at different pitch angles, and each at the same frequency. Averages of pre and post-plug gains and phase were acquired and compared to one another.

Data were acquired for time courses (time after plug vs. gain plots) after plugging in slightly different ways. Cat G was the first cat we experimented on. From the information acquired from this animal, we changed some of the recording paradigms for cat T. In cat G after plugging, recordings were made at 8 Hz and 2 Hz at all pitch angles that were available, in 5 degree increments, from 0 degrees to 35 degrees down, and also recordings at 23 degrees down. We concluded from the data in cat G that 5 degree increments in pitch angle is excessive, and that the time course data could be spaced out further (longer time between analogous recordings). So, in cat T, recordings were made at less pitch angles and more frequencies, particularly the low frequencies 0.1, 0.25, and 0.5 Hz. Most of the same procedures were used for the two cats after plugging. However, before plugging, some of the procedures differ. Table 1 shows the sinusoidal stimuli used for the two animals.

COR recordings were made as well. For these, pre and post plug recordings were made for cat T only. COR recordings from cat G were made only after the plugs. Frequencies used were 0.5, 1, 2, 2.5, 5, 7, and 8 Hz. For the 2-8 Hz recordings, the peak maximum head velocity was 20 deg/s. For the other two frequencies, the peak maximum head velocity was 10 deg/s.
3.10 Data Analysis

This section will describe how we attempted to quantify the contribution of cross coupling in cat T after plugging. There are several assumptions in this line of reasoning, but I think that they are valid. In any case, they cancel each other out when comparisons are made. First, we assume that the pre-plug gain recordings are due to horizontal canal activation and vertical canal activation (cross coupling). If we remove the input from the horizontal canals, we will be left with the vertical canal activation. This was accomplished by fitting the pre-plug data to a second order regression curve at a particular frequency (figure 34). The horizontal canal contribution was approximated to a cosine curve, whose peak matched the peak of the regression function. When the cosine curve was subtracted from the fitted curve, the difference was assumed to be cross coupling. By using this, it is possible to underestimate cross coupling slightly. When rotating in the best plane of the horizontal canals, there should be slight vertical canal activation. By adjusting the cosine curve so that its maximum has the same gain value as the fitted curve, we implicitly assume that the contribution of cross coupling when rotating in the best plane of the horizontal canals is zero. From Blanks et. al., 1972, I assume that the angle between the rotation plane and a plane of one vertical canal is 89 degrees, not 90. If the canal is activated by projection onto the canal plane, then the cross coupling input should be \( \cos(89 \text{ degrees}) \) or 1.6% of the optimal response. If we assume that the optimal input of cross coupling is 0.8 (the approximate value for hVOR gain across pitch angles), then 1.6% of 0.8 is 0.012. Therefore, we are underestimating the cross coupled input by 0.012. This amount is only slightly above noise level, as measured with the block of wood (described in an earlier section). Hence, my underestimation is approximately the same magnitude as the noise level. The assumption, then, seems valid, with this recording apparatus. This is a better estimation of cross coupling than simply assuming the cross coupled input fits a sine curve because there may be other factors that influence gain (section 2.9, for example). These factors would be difficult to estimate, and cannot be accounted for. By the subtraction method, the cross coupling input is inferred from existing data, and the other mitigating factors are removed, assuming they are additive. After determining the contribution of cross coupling, we fit a fourth order best fit to the post plug data at various times after plugging (early, late and very late data). The adaptation is the area in between the curves (figure 37, 38 and 39). By comparing these areas at the three different times after plugging, we can see
whether the input from cross coupling has been increasing with time. We can make the same comparison at several different frequencies, and see whether the same trends occur (whether the area stays the same, or is increasing). In determining which frequencies to use, I chose those that likely were not contaminated with residual function (2 Hz or below). Also, I only used frequencies whose maxima for regression fits were between 20 and 30 degrees. This is because if it were not the case, I would not know where to move the cosine curve to.

There are several values in using the area between curves approach used in cat T. The first is that when determining the cross coupling from pre-plug data, many of the unforeseen factors that may contribute to the response cancel each other out when the curves are subtracted from one another. Another benefit is that there is an internal test for validity. I assumed that the primary contribution to the pre-plug hVOR is from the horizontal canals. If so, it would be reasonable to conclude that the maximum of the second order fit should be close to the best plane of the horizontal canals, approximately 23 degrees down. Since the largest gain contribution is from the horizontal canals, the gain itself should also show a maximum where the canals are. I only used frequencies that yielded a second order fit with a maximum between 20 and 30 degrees down from the stereotaxic longitudinal plane. The frequencies 0.1, 0.25, 1 and 2 Hz fit that criterion. Only 0.5 Hz data did not. Another benefit from using this approach is that there are four independent ways of looking at cross coupling: the four different frequencies. By applying the same analysis to the four chosen frequencies, there are effectively four different ways of looking at the same phenomenon. If they show the same trends, then it would make any conclusions we draw that much more valid. For example, if there were increases in area for 0.1 Hz base on comparing the three sets of post plug data, and if that were also true for the other three frequencies used (when making the comparison), then it would be valid to conclude that the cross coupled response underwent up-regulation. Further, if the magnitudes of the area changes were similar, then that would further our conclusion. If there were differences in how the areas changed and in which direction, then we could either conclude that the test contains too many imperfections, and that any trends are lost, or we could conclude that there is no adaptation in the cross coupling contribution.
4. Results

Averages were made, which were separated and plotted, and organized in several ways. Generally, data can be plotted in two ways: gain (or phase) vs. frequency at different pitch angles, or gain (or phase) vs. pitch angle at different frequencies. The former should give the reader insight into the output of the residual function, while the latter should give a rough indication of the contribution of cross coupling at the frequency tested. Data were collected from two cats, and each set of data will be shown separately. For the frequencies and pitch angles used before and after plugging, see Table 1. In cat G, the averages can be separated into two sets: before plugging and after plugging. After the plugs, the averages are composed of trials encompassing the first 5 months. In cat T, data can be separated into four sets: before plugging, immediately after plugging (early), 3 months after plugging (late data), and 6 months after plugging (very late data). There are many plots, but they are grouped in two levels: the higher structure is pre, post, late post, and very late post, while within that structure gain and phase are plotted vs. frequency and also vs. pitch angle.

COR data were plotted only after plugging for cat G, since the apparatus for COR recordings was not completed early enough to get recordings before plugging that cat. For cat T, recordings were made before plugging, 3 weeks, and 3.25 to 4 months after plugging. This was to make recordings analogous to Bronstein et al. (1995) who observed a noticeable increase in the COR 3 weeks after vestibular damage that was significantly lower 7 months later. For COR data in both animals, phase data is not shown. The values for phase varied considerably, as evidenced by the fact that standard deviation values for some phase averages were over 300 degrees.

4.1 Controls

4.1.1 The effect of the Cervico-ocular reflex

The COR was studied in cat G and T. During recordings, the amount of neck-bending was at least one order of magnitude greater than the estimated neck flexion artefact which may have occurred during VOR recordings. The value in this control lies in the fact that it could rule out the COR as a major contributing factor if the gain values of the COR are very different from the ‘VOR’ recordings made after plugging. For example; if there were a large spike in ‘VOR’ gain recording after plugging (in the plot time after plugs vs.
gain) and there was a corresponding spike in COR recording at the same time. the VOR spike could be attributed to the influence of the COR. However, if we were to have a 0.3 gain measurement for the 'VOR' and a gain of .03 was observed for COR, we could not conclude that the COR contributed 10% of the 'VOR' response. This is because the stimulus for the COR recordings does not resemble the possible artefact in 'VOR' recordings we were trying to observe. Therefore, we will not attempt to draw quantitative conclusions about the contribution of the COR to our 'VOR' data.

Figure 13 and 14 show COR data acquired in both cats. Figure 13 shows the COR gain 5 months after plugging in cat G. It is obvious that the gain was high at low and high frequencies, and the phase showed no consistent trends (data not shown). The magnitude of the COR was only above the noise level for the highest and lowest frequencies. From the block of wood control (section 2.5.3), we determined that the noise level for this apparatus resulted in gain values of not more than 0.01. However, we could rule out the possibility of noise in the animal recordings. It may have been that for animal recordings, there was noise that could not be simulated by the block of wood control. In cat T, the averaged results for all recordings are shown in figure 14. The three sets of data were taken at various times before and after plugging. Each set of averages took 10 days to acquire and consisted of five individual experiments. For cat T (figure 14), note the lack of significant gain changes in each set of data. The actual magnitude of gain values was quite low. Since a stimulus which was much larger than the COR artefact has resulted in a minuscule gain, then the COR artefact itself must also have had a much smaller gain than that observed in the COR recordings. These sets of data indicate that the COR was not a contributing factor in our VOR recordings.

4.1.2 Recordings before plugging

Recordings were made before plugging to determine the gain and phase properties of the hVOR under normal conditions in each of the two animals. Pre-plug gain and phase data can be seen in figures 15-19 for both animals.

Figure 15 shows the pre-plug VOR data for cat T. Phase data for cat T is shown in figure 15 'A'. Phase was approximately zero for 10 degrees down, 0.1 Hz. For the averages at 8 Hz, a phase lag of approximately 30 degrees was observed. For the frequencies in between, generally, the phase was close to zero until 1 Hz, and as higher frequencies were used, a phase lag became noticeable, which increased as the
frequency used was higher. This trend is evident at all the pitch angles tested. The observed phase profile was consistent with previous observations (Broussard and Bhatia, 1996), and such a trend has been generally observed in other vestibular-based responses (Estes and Sasaki, 1978). Although only standard deviation values are only shown for one pitch angle, the standard deviations were never greater than 16 degrees, and most were smaller than 10 degrees. For a particular frequency, averages at each pitch angle fit within two standard deviations of any other average, for a different pitch angle. In other words, the results at a frequency were not very different at each pitch angle. This is obvious from the fact that many of the 8 lines are within one standard deviation of the 40 degree down average.

Gain averages can be seen in figure 15 'B'. Here, too, the standard deviations for the 40 degrees down average are shown as an example. The gain values appeared to be less than unity for the low frequencies, but steadily increased for frequencies above 5 Hz. The low frequency (0.1 Hz to 2.5 Hz) gain averages did not appear to show a consistent trend with pitch angle. This trend appeared to be evident at all the pitch angles used. The trend of larger standard deviations for high frequencies was true for all the pitch angles. We estimate that the largest contribution to day to day variations in gain for an analogous stimulus was lack of cat alertness. It also appears that the high frequencies are more prominently effected by lack of alertness. Hence, larger standard deviations for high frequencies would be expected.

Figures 16 and 17 show Bode plots acquired in cat G before plugging. In general, the same observations shown in cat T were seen in the phase data for cat G. The phase was approximately zero, and if frequencies above 1 were used, a phase lag became evident. Unfortunately, for cat G, the lowest frequency used was 0.5 Hz, so the pre-plug trends observed in the low frequency data observed in cat T cannot all be substantiated. For cat G, more pitch angles were used. Recordings were made with pitch angle values of 5 degree increments, not 10. In cat G, therefore, there are twice as many plots for the frequency vs. phase averages. As in cat T, figure 16 showed a phase lag at high frequencies. The lag at the highest frequency was 31.1 degrees. The values for each plot never differed by more than two standard deviations, except for frequencies 5, 7, and 8 Hz in the 10 degrees up plot. Eleven of the twelve plots agreed with one another quite well. The phase characteristics observed in other studies (such as Broussard and Bhatia, 1996) and in cat T
were substantiated in cat G. It would seem that the zero phase for low frequencies, and increasing lag with increasing frequency, was a common phenomenon in the hVOR of normal cats.

Figure 17 shows frequency vs. gain data for cat G before plugging. In general, standard deviation values were higher for gains in cat G than T. This is probably because strategies for keeping the cat awake and alert during recordings were determined and refined on the cat G, and were optimally put to use on cat T. As in cat T, the gain appears to be level at approximately 0.75 for frequencies below 5 Hz. This trend seems evident for all 12 pitch angles used, except, perhaps for the 0 degrees plot. For the frequencies 5, 7, and 8 Hz, the average gain values appear higher than at 0.5, 1, and 2 Hz. Again, this was observed in both cats. Generally, in two cats and 20 plots (pitch angles), frequency vs. gain and phase plots gave rise to the same observations, at least qualitatively.

Figure 18 shows pitch angle vs. gain and phase for cat G. In 'A', it appears that gain was unrelated to pitch angle. However, a relative maximum in gain appeared between 20 and 30 degrees down from the stereotaxic horizontal plane. From figure 18 'B', there appeared to be no relationship between pitch angle and phase that was consistent with each of the six plots (frequencies). Several characteristics appeared, which, as we shall see, were confirmed by data from cat T.

Figure 19 shows pitch angle vs. average phase and gain for cat T before plugging. 'A' shows that there was no trend for pitch angle vs. phase that was consistently shown in all nine plots (frequencies). 'B' shows that there appeared to be a gain maximum at 23 degrees for 8 of the 9 frequencies used. It also seems that the gain was lower for extreme pitch angles. Standard deviations for gain were shown for 0.5 Hz data in both animals, in figure 18 'A' and 19 'B'. Standard deviations were lower in cat T than in cat G for all frequencies. This allowed us to make quantitative, as well as qualitative observations in cat T that would be difficult to make in cat G.

4.1.3 The effect of anaesthesia

In cat T, we recorded gain and phase of the VOR in response to sinusoidal and velocity pulse stimuli at several times after plugging. Gain was recorded across all frequencies, but only three high frequencies (5, 7 and 8 Hz) and three low frequencies (0.1, 0.25 and 0.5 Hz) are shown. This cat's high frequency gain decreased much more when fatigued. However, from this control, gain values reached pre-anaesthesia values
at the same time for all the frequencies used. As figure 20 illustrates, gain values steadily increased after the anaesthesia was discontinued. Note that after 18 hours, all of the gain values had reached values that were close to the pre-anaesthesia averages. (i.e. gain values began to fall within the range of the standard deviations from the pre-anaesthesia values). The only exception was 8 Hz, but for this frequency, gain values never fell well within the range predicted by the pre-anaesthesia average and standard deviation. The pre-anaesthesia averages are the same shown in graphs 15 ‘B’ and 19 ‘B’. We determined that 18 hours after the cat was removed from anaesthesia, accurate recordings could be made. At this time, most gain values returned to values that were within the limits set by the standard deviation and average gain under normal circumstances. Therefore, in cat T, we recorded gain and phase 18 hours after the bilateral plug surgery, no sooner.

In cat G, the first recordings used for this thesis were taken 3 days after plugging. That animal only recovered from the surgery completely at that time. Cat G had to be fed I.V. fluids, did not eat for 3 days, and appeared ill until 3 days post-surgery. For that reason, the first data point included in averages were taken at that time.

4.2 Time course of recovery

Data from at least 40 hours after bilateral plugging in cat G are shown. In cat G, the relationship between time after plugging and gain can be seen in figures 21 to 23. For 8 Hz data (figures 21 and 22), it appeared that there was little correlation between gain and time after the plugs except for pitch angles that are closest to the estimated plane of the best canals (20 to 30 degrees down from the stereotaxic longitudinal plane, in figure 22). Note the low correlation coefficients. This showed that there wasn’t a consistent increase with time, or levelling off. Figure 23 shows a sample of 2 Hz time course data. At 2 Hz, the gain was relatively constant after plugging. Note that the gain scale is the same as in figures 21 and 22. The slight increases seen in figures 21 and 22 were completely absent in the 2 Hz time courses (figure 23). For other pitch angles at 2 Hz, the results looked similar. Hence, it appears that at the lowest frequency tested in cat G, 2 Hz, there was no gain increase in the first 2 months after plugging. In cat T, the recordings were made differently. Three sets of post-plug recordings were made, not one continuous set of data. Hence, no time
course plots are shown. From data shown from cat G, it is not obvious that learning has occurred in the first 5 months after plugging.

4.3 Gain and phase properties after bilateral hSCC plugs

4.3.1 Gain and phase at different frequencies

For the four sets of averages at three different times in two different cats, gain and phase showed characteristics that were consistent with a response that was at least in part due to residual function of plugged canals. In cat G, after plugging, the frequency vs. gain plots are shown in figure 24. Note the high gain for the high frequency averages. This phenomenon occurred at all of the 10 pitch angles used. Gain values from 0.5 to 8 Hz closely resembled the output of residual function, qualitatively. That is, the gain was negligible at low frequencies, and incrementally increased as higher frequencies were tested. Standard deviations are shown only for data at 40 degrees down. Standard deviations for other pitch angles were similar. It can be seen that many of the high frequency gain values were more than two standard deviations from noise level (gain value of 0.02). This trend was evident at all pitch angles used. For 40 and 50 degrees down, the average gain values at 0.1 Hz were also high. For 0.1 and 0.25 Hz data, the points that contributed to the average were only acquired many months after plugging. For both 40 and 50 degrees down, there were two points that contributed high gains. It is possible that learning occurred in the cross coupling response, and recordings were made before and after learning. This could also explain the large standard deviations. Bode plots for phase data can be seen in figure 25. From 'A' and 'B', there did not appear to be a consistent trend for frequency vs. phase data when looking at only the low frequencies. In fact, the range for the average phase data appeared quite large. In 'A' at 1 Hz, the range in the averages was just under 100 degrees. Although the standard deviations are only shown for 40 degrees down, the values for standard deviation were large for all phase data, especially low frequency data. In this sense, the 40 degrees down data was an adequate representation for the trend observed in standard deviations. Magnitudes were generally large for frequencies below 5 Hz. Below this frequency, phase was unreliable. At high frequencies, phase can be used for predictions, at least qualitatively. From figure 25 'A' and 'B', the range in 8 Hz average phase values was just over 30 degrees, while at 0.5 degrees, the range was well over 100 degrees. Gain averages for 8 Hz were
all above zero (figure 25 ‘B’). It seems clear that for cat G, low frequency phase data was unreliable, while high frequency phase data showed a persistent lead.

In figures 26 to 28 frequency vs. gain and phase are shown for cat T at three different times after plugging. The early data can be seen in figure 26. For these data, which were collected between 18 hours and 3 weeks after plugging, the gain values for all plots were low, regardless of frequency (figure 26 ‘A’). Only 0 degrees and 30 degrees down showed larger values for high frequency gain. Almost all gain values were within 2 standard deviations from the noise level. With such low gains, phase data has become unreliable, as can be seen in figure 26 ‘B’. The range for many averages was large, and the standard deviations were large as well, even at high frequencies, as can be seen from 50 degrees down data. Figure 27 shows frequency vs. average gain and phase 3 months after plugging in cat T. From figure 27 ‘A’, the increased high frequency gain evident in cat G can be seen in cat T as well. The low frequency gains remained slightly above noise level, except for one extreme pitch angle (10 degrees up from the stereotaxic horizontal plane). In figure 27 ‘B’, we can see the average phase data. Large amounts of variability in the averages and large standard deviations shows that phase has remained unreliable. For the two lowest frequencies, the standard deviations were smaller. This was true not only for the 50 degrees down data, but for the other pitch angles as well (not shown). Figure 28 shows frequency vs. average gain and phase for data acquired 6 months after plugging in cat T. From figure 28 ‘A’, many of the trends observed in the data from cat G asserted themselves in pitch angle vs. average gain plots in cat T. The high frequency gain was much larger, and was greater than two standard deviations away from noise level for 7 of the 8 pitch angles used. In fact comparing averages from 3 weeks post-plugs to 6 months post-plugs, of the 24 averages that were compared (8 averages at each of the frequencies 5, 7 and 8 Hz), 18 were significantly different, P=0.05. The gain values have increased significantly for the high frequency data. There were also increased gain values for gain at the lowest frequencies and most extreme pitch angles (10 degrees up, 0, and 50 degrees down from the stereotaxic horizontal plane). This may have been due to the contribution of cross coupling. Here, generally, for the three plots mentioned previously, the standard deviations were much smaller than those observed in cat G. It was possible that recordings were made after learning had manifested itself, in cat G. In figure 28 ‘B’, we can see that low frequency phase remained unreliable, due to large standard deviations and
a large range for average values at a particular frequency. For 7 and 8 Hz, however, the standard deviations were much smaller, and the ranges were just under 20 degrees for both frequencies. It seems that, as in cat G, we can assume that the high frequency phase lead was a characteristic of cat T's post-plug hVOR.

Qualitatively, the same trends were observed in both cats, but for data in cat T, these trends showed themselves most prominently 6 months after plugging.

4.3.2 Gain and phase across different pitch angles

The relationship between pitch angle and gain, is shown at different frequencies. From figures 29 to 33, the data from cats G and T can be seen.

Figure 29 shows pitch angle vs. average gain for cat G after plugging. In figure 29 'A' we can see that the gain was larger for the higher frequencies, but the gain did not appear to change with pitch angle. Figure 29 'B' shows low frequency average gain taken from cat G. For comparison, a cosine curve is shown whose minimum is at 23 degrees down from the stereotaxic horizontal plane, which we estimate to be the best plane of the horizontal canals. The gain value for the cosine's minimum was zero. It is likely that cross coupling provided the primary input to the hVOR after plugging at these frequencies. It is possible that the 2 Hz points were an exception, since at this frequency, residual function's contribution may have been non-zero. It is also possible that cross coupling did not provide the largest contribution for 2 Hz averages. Thirteen of the average values had gain values that were lower than the cosine curve. Fifty two of the average gain values had gain values that were above the cosine curve. Also, many of the average values did not fit the cosine curve well. Therefore, we did not estimate cross coupling by fitting cosine curves, or adjusted cosine curves. It should be pointed out that of the 65 averages, only 19 were not within one standard deviation of the cosine curve. Therefore, if comparisons with cosine curves were an appropriate way of estimating cross coupling, then it appeared that cross coupling was present in cat G after plugging.

Figure 30 shows pitch angle vs. average phase for cat G after plugging. For all the frequencies used, it appeared that the phase showed no trend across pitch angles. For reasons described earlier, low frequency gain was unreliable. At high frequencies, the phase lead was persistent across all pitch angles, and did not appear to modulate with pitch angle. This would have been observed if input from at least one plugged canal existed.
Figure 31 shows pitch angle vs. gain and phase data acquired from cat T between 18 hours and 3 weeks after plugging. From 'A', for all the frequencies except one, gain values all showed a maximum at 23 degrees down from the stereotaxic horizontal plane. Gain values also were generally higher for extreme pitch angles. Although no cosine curve is shown for comparison, the plots did not resemble a cosine curve in the least, partially due to the maximum, not minimum, at the estimated best plane of the horizontal canals. In figure 31 'B', as in cat G, it can be seen that low frequency gain could not allow us to make any inferences. High frequency gain was unreliable as well. The magnitudes for standard deviations were high for 5 to 8 Hz (not shown) as well as 0.5 Hz.

Figures 32 and 33 shows pitch angle vs. gain and phase data acquired 3 and 6 months, respectively, after plugging in cat T. From figure 32 'A', it is obvious that the gains at high frequencies were higher than lower frequencies. It is not clear whether the gain values were higher for extreme pitch angles. 'B' shows that phase data remains unreliable for the low frequencies, and generally, there were higher phase values for high frequencies. Figure 33 'A' shows that there appeared to be two distinct sets of average gain data, high frequencies and low frequencies. The gains were much higher for high frequencies, and there was a minimum at 23 degrees down, an unexpected observation. This would be difficult to explain. It is possible that the contribution of residual function was constant across pitch angles (as was shown in cat G), and the added cross coupled response could have provided the observed minima. Low frequency data also showed minima, and for 4 of the 6 frequencies, the minimum was at 23 degrees down from the stereotaxic horizontal plane. 'B' showed that again, low frequency phase was unreliable, and that the phase lead at high frequencies did not show a trend with pitch angle.

Generally, pitch angle vs. gain data should have given an indication of cross coupling. Fitting cosine curves may have been inappropriate for this study. However, comparing gain values to cosine curves, as in figure 29 'B', if cross coupling fits a cosine curve, then since the gain values were close to the curve, cross coupling has contributed to the post plug response in cat G. For cat T, estimation of cross coupling was done in a different way.
4.3.3 Cross coupling in cat T

For this cat, we attempted to quantify the adaptation of cross coupling by estimating the cross coupled input based on pre-plug data and then noticing how post plug values compared to the pre-plug values at different times after plugging. Figure 34 shows how pre-plug cross coupling is estimated. The top graph shows the averages, standard deviations, and the second order regression curve for 1 Hz pre-plug data. For this regression line, the maximum was at 27 degrees down. Second order curves were fit similarly for frequencies between 0.1 and 2 Hz. If the maximum of the fitted curve was not between 20 degrees and 30 degrees down, then data from that frequency was not used for cross coupling estimation. This criterion assumes that before plugging, input from the horizontal canals provided the major input, and there was some input from the other 4 canals (there is some cross coupling). If this were true, then a maximum would be expected, and it should have manifested itself at a pitch angle close to the best plane of the horizontal canals. If the maximum was at 40 degrees down or some other extreme angle, this may have meant that the data could not have been fit with a curve appropriately, and estimation of cross coupling at this frequency may have led to false conclusions. The bottom graph shows that regression line and a cosine curve (dotted line) whose maximum matches that of the regression line. This is the estimated contribution from the horizontal canals. If the curves are subtracted, the pre-plug cross coupled input can be estimated. This is what the dashed line represents.

Figure 35 shows how comparisons were made at various times after plugging, using the same frequency. The dashed line is the estimated pre-plug cross coupled input at that frequency. The solid lines in 'A', 'B', and 'C' show fourth order regression lines for pitch angle vs. gain data at the times indicated at 1 Hz. A fourth order fit was used because it closely fit the data. These fits were made at three different times and for four different frequencies (figures 37 to 39). Hence, twelve fourth order fits were made. The correlation coefficients for each of the fits was above 0.9, and for 9 of the 12 fits, 'r' was above 0.97. It is also possible to use second order fits. Regardless of the learning, a parabola or stretched cosine curve would show the contribution of cross coupling. The fit in 'b' resembled a second order fit. However, we wanted the regression curves to fit the average gain data closely. The amount of learning that cross coupling has undergone since before plugging was estimated by the area between the curves. For each time, the area
values are shown to the right of the graphs. The area has increased from pre-plug values, so that all areas are positive. For each different interval, the areas have increased. For the last interval (between 3 and 6 months after plugging), the area increase was quite large.

Figure 36 shows the estimations of pre-plug cross coupling for the four frequencies used. 0.5 Hz data was not included because the second-order fit of pre-plug data did not yield a maximum between 20 and 30 degrees down from the stereotaxic horizontal plane. 2 Hz data was used because it was estimated to be the lower limit for residual function. However, this frequency may have been contaminated with a response from residual function. Figure 37 shows the comparison between pre-plug cross coupling and the gain recorded between 18 hours and 3 weeks after plugging at the four frequencies. Although there are some places where the pre-plug curve crossed the post-plug curve, all areas, in figures 37, 38 and 39 were positive. Table 2 shows the results from the analysis. The areas increased for every time interval except one. Also, for two frequencies (0.25 and 1 Hz), the increase in gain was large during the time from 3 to 6 months after plugging. The four frequencies were independent measures of cross coupling. It was likely that the 2 Hz data included contributions from residual function as well as cross coupling. If this were the case, and if the learning in residual function occurred at different times than cross coupling, then the 2 Hz data would not agree with the others. If this method was not measuring any true phenomenon or phenomena, the results would be random. If so, then the changes in area could have been negative as well as positive. The agreement between frequencies shows that this method can show the learning in the cross coupled input.

This form of estimating cross coupling was not used on cat G, since the lowest frequency used before plugging was 0.5 Hz. For the three frequencies at 2 Hz or lower, used before plugging, the maxima for the regression curve was not between 20 and 30 degrees down. Also, the standard deviation for gain values in cat G were higher than in cat T. Hence, estimation of cross coupling could have been obscured by day to day variability.
5. Discussion

5.1 Cross coupling

Data from cats T and G have shown that cross coupling exists in the animals after plugging. In cat T, it was shown that the cross coupled input had increased with time. With the lateral canals plugged, only the vertical canals should produce the hVOR at low frequencies. Many of the data points for gain vs. pitch angle curves for cat G at frequencies 0.1 and 0.5 are close to the cosine curve shown in the figure 29. Since we did not record low frequencies before plugging, we cannot make statements about the adaptation of cross coupling in that animal. The vertical canal input appears to have been amplified by the hVOR. Whether this larger than expected response existed in the non-plugged cat, we cannot say.

The results from cat T allowed a different way of estimating the input of cross coupling. The data that resulted from this analysis can be seen in table 2. There appears to be a large increase in the cross coupled input between 3 and 6 months. There is an increase at all but one frequency, and for two, the increase is quite noticeable. It's possible that the 2 Hz responses have been contaminated by contribution from residual function. These results show that cross coupling has undergone learning.

This conclusion is consistent with other findings, at least qualitatively. Yakushin et al., 1995 has shown that up-regulation of the cross coupled response did not occur. However, Angelaki and Hess (1996) have shown that up-regulation does occur if the VOR is observed for a longer time after plugging. Our data from cat T bear that out. Up-regulation does occur, but takes at least 6 months to assert itself. Quantitatively, gain values at pitch angles at 40 degrees down and lower differ from those observed in cats in Baker et al., 1982. Baker only used frequencies between 0.1 and 2.5 Hz, and was able to use more extreme pitch angles than this project. However there was much overlap in both frequency and pitch angle. For the most extreme pitch angles used in this study, the gain values we observed were approximately 40% of those observed in Baker et. al., 1982. It is possible that inter-individual differences may give rise to the discrepancy. The cross coupling component of the hVOR does appear to be slightly larger than expected for a particular animal, and the cross coupled input does show a slight increase in efficacy, but this is only evident long after plugging.
Cross coupling may or may not play a role in coordinate transformation in the alert, post-plug animal. The recorded gain is much too small to prevent retinal image slip under natural conditions after plugging. The general behaviour of the cats does suggest that both sensation and eye movements are compensatory for angular motion in the horizontal direction. It would be important to note that our experimental conditions do not reflect natural conditions. In the alert cat, there may be active and passive horizontal head movements, but they are not always about an Earth-vertical axis. It would be possible to use otolith modulation to add to the horizontal eye movements. Angelaki and Hess (1995) have shown that in animals with both horizontal canals plugged, there are larger magnitude eye movements for an equivalent horizontal head rotation when the axis is not Earth-vertical, compared to rotation where the axis is Earth vertical. One factor that likely does not contribute to eye stabilization is the presence of efference copies during active head movements (Khalsa et al., 1987). A possible contributor to eye stabilization in the alert animal is the optokinetic response (OKR). The OKR undoubtedly plays a role in compensating for low frequency horizontal head movements. It has been shown that optical input can affect change in VOR efficiency (Gonshor and Melvill-Jones, 1976, Miles and Egihmy, 1980 and others). Therefore, the VOR gets input from the optical system. It may be possible that the OKR can be modified after vestibular damage. It should be noted that changes in the horizontal OKR could not be elicited when VOR changes were produced (Lisberger et. al., 1981). However, in that study, the VOR learning was induced by magnifying spectacles, not vestibular damage. It seems clear that the VOR gets input from the OKR, but the OKR doesn't appear to get input from the VOR. OKR gain may or may not increase after vestibular damage. Finally, the effects of residual function may contribute as well, but primarily to head movements with high frequency components. For all these reasons, cross coupling does not need to contribute a large component to post-plug eye movement behaviour during the normal everyday activities of the alert animal with both horizontal canals plugged. Perhaps because there are other eye stabilization mechanisms during normal behaviour, increases in the cross coupled input do not need to manifest themselves until much several months have passed after plugging. The observation that the cross coupled contribution does not compensate for the passive head motion does not mean that compensation for head motion does not occur.
5.2 Residual function

Residual function does appear to play a role in the hVOR after plugging. Based on our results, this appears evident. The high-frequency phase led results shown in two animals would seem to point to that. The phase lead is consistent with the output of residual function as described by Rabbitt’s model. Normally, a phase lag is evident. Further evidence for the presence of residual function comes from the bode plots from cat G after plugging (figure 24). They look very similar to Bode plots of the predicted transduction in Rabbitt’s model. The roll-off with decreasing frequency is consistent with transduction predicted by the model. From cat T, the result appears less pronounced. We suspect that one cupula was destroyed in the plugging procedure. If this were so, the increased gain at high frequencies would still be evident, but the increase would not be as great. In most of our data, there appears to be some influence of cross coupling, even for rotations in the best plane of both horizontal canals. This is not unreasonable, since stimulating one set of canals while excluding transduction from the four others is impossible (see introduction). Therefore, we could not study residual function alone. The response due to residual function does not appear to increase for 5 months after plugging. Quantifying the adaptation of residual function is difficult. If I were to compare the areas under the regression line at various times after plugging, then I could not conclusively say that residual function has undergone learning. Since 8 Hz data likely has both residual function and cross coupling as contributing factors, it would be possible to argue that cross coupling, not residual function has undergone the adaptation. That argument would be strengthened by the fact that it has already been shown that adaptation of the cross coupled response does occur 6 months after plugging, using the low frequencies as an indicator. Since there appears no quantitative proof that we can activate the hVOR by residual function alone, it is difficult to draw conclusions except to say that it does exist. For this reason, comparisons were not made after plugging. At this point, there appears to be no way to explain these results except to say that residual function has contributed to the measured hVOR.

5.3 Cervico-ocular reflex (COR)

It appeared from our results that the COR activation was minimal. Our COR experiments have shown that the COR probably did not contribute to the measured gain in hVOR experiments. No increase in COR gain during weeks after plugging was observed in our experiments. This may be due to species
differences. We can say that there is no increase in COR gain with time, and that for small amplitude movements, the COR is not significant.

In natural situations, the COR may be more effective, though. Under normal conditions, COR gain may be higher than that observed in our experiments. It has been shown (Thurtell et al., 1998) that there may be a difference between the compensatory eye movements as a result of active and passive head on body movement. This may affect several eye stabilization mechanisms, which may also include the COR. Our experiments were by no means an exhaustive survey of COR function, therefore I can only speculate about its role in the alert cat after plugging.

5.4 Conclusions

These data suggest that cross coupling increases several months after plugging. Second, the data also support the existence of residual function in a plugged canal. The fact that residual function has been observed will affect interpretation of high-frequency gain data after plugging in future studies.


Chen-Huang, C., R. A. McCrea, et al. (1996). "Contributions of regularly and irregularly discharging vestibular-nerve inputs to the discharge of central vestibular neurons in the alert squirrel monkey." 2


Hong, J.A. Modification of the disynaptic vestibulo-ocular reflex pathway after a unilateral canal plug in the cat. Msc. Thesis University of Toronto, pg. 27.


Figure 1. Generalized representation of a vertebrate labyrinth and cochlea, including saccular, utricular and cochlear nerves.
Figure 2. Representation of rotations. A) Rotational vectors. View is from behind the subject. The right-hand rule is used. Therefore, a rightward rotation is described by a downward pointing vector, a clockwise rotation is described by a nasally pointing vector, and an upward rotation is described by a rightward (from the subject’s perspective) vector. B) Rotations, as shown by planes. Horizontal (yaw) rotation is rotation in the yaw (longitudinal) plane. Rotation in the pitch (sagittal) plane is vertical rotation, and torsional rotation is rotation in the roll (coronal) plane.
Figure 3. Representation of feline canal orientations. Black, yellow, and purple denote anterior, posterior and horizontal canals, respectively.
Figure 4. Unilateral representation of canal and muscle planes, using humans as the basis for tilt values. The same qualitative differences exist in all animals (values taken from Highstein, 1988 and Wilson, ed. 1979).
Figure 5. A general description of cupular frequency response, based on a 'simplified cupula canal model', put forth by Jones and Milsum, 1965. Taken from Wilson and Jones, ed. 1979.
Figure 6. For rightward head rotation, there is radial acceleration in opposite directions, as shown. If the otoliths can sense and integrate that information, the appropriate interpretation would be that the subject is rotating.
Figure 7. Swivelling head holder arch. The upper portion can be rotated in the pitch plane and fixed in the new position, allowing different rotational planes as the stimulus head velocity. In this case the upper portion of the arch is being rotated forward, or pitched down.
Figure 8. Representations of the field coil, cat box, and head post in 'A'. 'B' shows the cat in the recording apparatus. Taken from Hong, 1998.
Figure 9. A) Shows that the axis of head rotation is constant in space, but the animal's head can be moved and fixed in the new pitch position. The rotation axis will then change with respect to the head. B) Effective result of changes in pitch position. Different stimulus axes are made possible.
Fig. 10. Data from one trial in cat G before plugging. 32 rotation cycles were averaged for this example. Rotations were acquired at 5 Hz at pitch angle 30 degrees down. Head and eye velocities were fitted with sinusiods (not shown). Gain is the ratio of maximum eye velocity to maximum head velocity of the peaks of each fitted curve. Phase is temporal separation of the peaks of the fitted curves, in degrees.
steady state

Figure 11. Plot of time vs. velocity for one velocity pulse average in cat G. Data are from 23 degrees down, and peak head velocity is 30 degrees/second before plugging. Note lack of initial overshoot in eye velocity trace and low steady state gain. These are indicators of a lack of alertness.
Figure 12. Representations of A) a canal under normal conditions and B) a plugged canal. A part of the canal arm is drilled away, across the whole cross-section, and periosteum is placed on either side of the arm. The surgeon tried not to damage the cupula, since it is necessary for residual function.
Figure 13. COR data from cat G after plugging. Frequencies used were 0.5, 1, 2, 2.5, 5, 7, and 8 Hz. Averages were composed of trials acquired 6 to 9 months after plugging. Rotations were body-on-head. For all points, n=5. Error bars denote standard deviations.
Figure 14. Averaged data for COR in cat T before plugging, 3 weeks after plugging, and 13 weeks after plugging. Rotations were body-on-head. Error bars are standard deviations. For all points, n=5.
Figure 15. Plots in cat T before plugging at 8 pitch angles, shown in the legends. Negative values for pitch angle denote pitch nose down. 'A' shows frequency vs. average phase. Negative values for phase indicate phase lag. 'B' shows frequency vs. average gain. Data are averages of 5 points. Standard deviations are shown only for data acquired at 40 degrees down from the stereotaxic horizontal plane (40 degrees).
Figure 16. Plots of frequency vs. average phase for cat G before plugging. A) shows plots taken at pitch angles from 40 degrees down from the stereotaxic horizontal plane to 20 degrees down from the stereotaxic horizontal plane. B) shows plots from 15 degrees down from the stereotaxic horizontal plane to 10 degrees up. Averages are of 8 points. Standard deviations are shown only for pitch angle -40 degrees.
Figure 17. Plots of frequency vs. average gain for cat G before plugging. A) shows plots taken at pitch angles from 40 degrees down from the stereotaxic horizontal plane to 20 degrees down from the stereotaxic horizontal plane. B) shows plots from 15 degrees down from the stereotaxic horizontal plane to 10 degrees up. Averages are of 8 points. Standard deviations are shown only for pitch angle -40 degrees (40 degrees down from the stereotaxic horizontal plane).
Figure 18. Plots of Pitch angle vs. average gain and phase for cat G before plugging. In 'A', graph shows pitch angle vs. gain data for plots taken at frequencies between 0.5 Hz to 8 Hz. Negative values for pitch angle denote pitch nose down. 'B' shows pitch angle vs. phase data. Negative values for phase denote phase lag (eye with respect to head). Averages consist of 8 individual experiments. Standard deviations are shown only for 0.5 Hz data.
Figure 19. Plots in cat T before plugging at 9 frequencies, shown in the legends. Negative values for pitch angle denote pitch nose down. 'A' shows pitch angle vs. average phase. Negative values for phase indicate phase lag. 'B' shows pitch angle vs. average gain. Data are averages of 5 points. Standard deviations are shown only for 0.5 Hz data.
Figure 20. Plots of time after removal of anesthesia vs. gain for cat T. Data were acquired at the frequency indicated and at pitch angle 23 degrees down from the stereotaxic longitudinal plane. The three highest and lowest frequencies tested are plotted. The points before time of zero hours are averages taken at 23 degrees before plugging.
Figure 21. Time course data for the gain of cat G. Data are from trials taken after the plugs at 8 Hz. Regression lines are first or second order best fits. Correlation coefficient values for regression lines are shown in upper left corner.
Figure 22. Time course data for the gain of cat G. Data are from trials taken after the plugs at 8 Hz. Regression lines are first or second order best fits. Correlation coefficient values for regression lines are shown in upper left corner.
Figure 23. Time course for cat G after bilateral horizontal semicircular plugs. Time vs. gain graphs for 2 Hz data. Note different time scale, but the same gain scale as 8 Hz time course plots.
Figure 24. Plots of frequency vs. average gain for cat G after plugging. A) shows plots taken at pitch angles from 50 degrees down from the stereotaxic horizontal plane to 25 degrees down from the stereotaxic horizontal plane. B) shows plots from 23 degrees down from the stereotaxic horizontal plane to 10 degrees up. Averages are of at least 5 points. Standard deviations are shown only for pitch angle -40 degrees. Data were acquired between 2 days to 5 months after plugging.
Figure 25. Plots of frequency vs. average phase for cat G after plugging. A) shows plots taken at pitch angles from 50 degrees down from the stereotaxic horizontal plane to 23 degrees down from the stereotaxic horizontal plane. B) shows plots from 20 degrees down from the stereotaxic horizontal plane to 10 degrees up. Averages are of at least 5 points. Standard deviations are shown only for pitch angle -40 degrees.
Figure 26. Post plug average data from cat T. Data were acquired 18 hours to 3 weeks after plugging. 'A' shows frequency vs. gain. 'B' shows frequency vs. phase. Positive values for phase indicate a phase lead. Averages consist of 5 individual points. Standard deviations are not shown except for 40 degrees down, in both 'A' and 'B', to indicate the amount of variability found in phase data.
Figure 27. Post plug average data from cat T. Data were acquired 3 months to 3.5 months after plugging. 'A' shows frequency vs. gain. 'B' shows frequency vs. phase. Positive values for phase indicate a phase lead. Averages consist of 5 individual points. Standard deviations are not shown except for 40 degrees down, in both 'A' and 'B', to indicate the amount of variability found in phase data.
Figure 28. Post plug average data from cat T. Data were acquired 6 months to 6.4 months after plugging. 'A' shows frequency vs. gain. 'B' shows frequency vs. phase. Positive values for phase indicate a phase lead. Averages consist of 5 individual points. Standard deviations are not shown except for 40 degrees down, in both 'A' and 'B', to indicate the amount of variability found in phase data.
Figure 29. Plots of pitch angle vs. gain for cat G after plugging. 'A' shows pitch angle vs. gain for frequencies between 2.5 and 8 Hz. Negative values for pitch angle indicate pitch nose down. 'B' shows pitch angle vs. gain for frequencies 0.1 to 2 Hz. Large solid line is a cosine curve, for comparison whose minimum is 0 gain at 23 degrees down from the stereotaxic horizontal plane. Averages consist of at least 5 individual trials. Data were acquired from 2 days to 5 months after plugging.
Figure 30. Plots of pitch angle vs. phase for cat G after plugging. Negative values for pitch angle indicate pitch nose down from the stereotaxic horizontal plane. Negative values for phase indicate lag. Averages consist of at least 5 individual trials. Data were acquired from 2 days to 5 months after plugging.
Figure 31. Post plug average data from cat T after plugging. Data were acquired 18 hours to 3 weeks after plugging. 'A' shows pitch angle vs. gain. Negative values for pitch angle denote pitch nose down. 'B' shows pitch angle vs. phase. Positive values for phase indicate a phase lead. Averages consist of 5 individual points. Standard deviations are not shown except for 0.5 Hz, in both 'A' and 'B', to indicate the large amount of variability found in phase data.
Figure 32. Post plug average data from cat T. Data were acquired 3 months to 3.5 months after plugging. 'A' shows pitch angle vs. gain. Negative values for pitch angle denote pitch nose down. 'B' shows pitch angle vs. phase. Positive values for phase indicate a phase lead. Averages consist of 5 individual points. Standard deviations are shown for 0.5 Hz data in both 'A' and 'B'. For other frequencies, standard deviations are not shown.
Figure 33. Post plug average data from cat T. Data were acquired 6 months to 6.4 months after plugging. 'A' shows pitch angle vs. gain. 'B' shows pitch angle vs. phase. Negative values for pitch angle denote pitch nose down from the stereotaxic horizontal plane. Positive values for phase indicate a phase lead. Averages consist of 5 individual points. Standard deviations are not shown except for 40 degrees down, in both 'A' and 'B', to indicate the amount of variability found in phase data.
Figure 34. The contribution due to cross coupling was determined (1 Hz). The solid lines in both cases are second order regression lines based on pre-plug data from cat T (raw data shown in top curve). The dotted line is a cosine curve shifted so that its maximum is the same as the regression line's maximum. This represents the contribution from the horizontal canals. The dashed line is the contribution of cross coupling. It is the difference between the solid line and the dotted line.
Figure 35. Plots to determine the influence of cross coupling. Solid lines are fourth order best fit regression lines. Dashed lines represent pre-plug cross coupling at each frequency shown. The adaptation in cross coupling is the area between the two curves. Areas are shown to the right of each graph. Regression lines are from after plug (as indicated by graph title). Note the increase in area over time. Data are from 14z, cat T.
Figure 36. Plot to determine the influence of cross coupling. The solid line represents a second order regression line from cat T before plugging at frequencies 0.1 to 2 Hz (as shown in figure 17). The dashed line is a cosine function whose maximum has the same x and y values as the maximum of the solid line. The dotted line represents the influence of cross coupling, and is the difference between the dashed line and the solid line.
Figure 37. Plots to determine the influence of cross coupling. Solid line is a fourth order fit of data taken from cat T after plugging (early data). Dashed line represents cross coupling at the frequency indicated by the graph titles, as calculated in figure 68. The area in between the two graphs represents the adaptation in the cross coupled response.
Figure 38. Plots to determine the influence of cross coupling. Solid lines are fourth order best fit regression lines. Dashed lines represent pre-plug cross coupling at each frequency shown. The adaptation in cross coupling is the area between the two curves. Regression lines are from data taken 3 months after plugging.
Figure 39. Plots to determine the influence of cross coupling. Solid lines are fourth order best fit regression lines. Dashed lines represent pre-plug cross coupling at each frequency shown. The adaptation in cross coupling is the area between the two curves. Regression lines are from data taken 6 months after plugging.
<table>
<thead>
<tr>
<th>Cat</th>
<th>Pitch angles used before plugging</th>
<th>after plugging</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>10,5,0,-5,-10,-15,-20,-23</td>
<td>10,5,0,-5,-10,-15,-20,-23,-25,-30,-35,-40</td>
</tr>
<tr>
<td>T</td>
<td>10,0,-10,-20,-23,-30,-40,-50</td>
<td>10,0,-10,-20,-23,-30,-40,-50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cat</th>
<th>Frequencies (Hz) used before plugging</th>
<th>after plugging</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>0.5, 1, 2, 5, 7, 8</td>
<td>0.1, 0.25, 0.5, 1, 2, 2.5, 5, 7, 8</td>
</tr>
<tr>
<td>T</td>
<td>0.1, 0.25, 0.5, 1, 2, 2.5, 5, 7, 8</td>
<td>0.1, 0.25, 0.5, 1, 2, 2.5, 5, 7, 8</td>
</tr>
</tbody>
</table>

Table 1 Sinusoidal frequencies and pitch angles used for
<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Time after Plugging (months)</th>
<th>Area between curves</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 Hz</td>
<td>0.75</td>
<td>2.786</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>5.051</td>
</tr>
<tr>
<td>0.1 Hz</td>
<td>6.00</td>
<td>5.669</td>
</tr>
<tr>
<td>0.25 Hz</td>
<td>0.75</td>
<td>5.475</td>
</tr>
<tr>
<td>0.25 Hz</td>
<td>3.00</td>
<td>5.623</td>
</tr>
<tr>
<td>0.25 Hz</td>
<td>6.00</td>
<td>8.792</td>
</tr>
<tr>
<td>1 Hz</td>
<td>0.75</td>
<td>4.438</td>
</tr>
<tr>
<td>1 Hz</td>
<td>3.00</td>
<td>4.799</td>
</tr>
<tr>
<td>1 Hz</td>
<td>6.00</td>
<td>7.198</td>
</tr>
<tr>
<td>2 Hz</td>
<td>0.75</td>
<td>2.491</td>
</tr>
<tr>
<td>2 Hz</td>
<td>3.00</td>
<td>4.467</td>
</tr>
<tr>
<td>2 Hz</td>
<td>6.00</td>
<td>4.072</td>
</tr>
</tbody>
</table>

Table 2. Area totals for cat T after plugging.