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MODIFICATION OF THE DISYNAPTIC VESTIBULO-OCULAR REFLEX PATHWAY AFTER A UNILATERAL CANAL PLUG IN THE CAT

by

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

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Abstract

The vestibulo-ocular reflex (VOR) stabilizes gaze by making smooth compensatory eye movements during head rotations. The VOR can recalibrate itself. This occurs when the visual input is magnified or miniturized, or after peripheral damage such as a blocking the fluid flow in the semicircular canals. I hypothesized the disynaptic VOR pathway was modifiable after plugging a canal and the pathway transmitted high frequency signals.

Horizontal eye movements were recorded when the cats were rotated about a vertical axis or by electrically activating the primary vestibular afferents through electrodes. One cat had one horizontal semicircular canal plugged and two cats wore either x2.0 and/or x0.25 spectacles continuously for 4-day periods.

After the canal plug surgery and while the cats wore spectacles, consistent changes in the earliest portion of the evoked eye movement occurred as the VOR gain (eye velocity/head velocity) changed. The earliest portion of the eye movement was probably caused by the activation of the disynaptic pathway. Larger changes were seen after the canal plug than during adaptation to spectacles. The disynaptic pathway was modifiable at all frequencies and did not show a preference for high frequencies after the canal plug surgery. The disynaptic pathway may contain a site of learning that is revealed after an injury.
## Contents

1 INTRODUCTION .............................. 1
   1.1 Neural pathways .......................... 2
   1.2 Potential sites of learning ............... 5
      1.2.1 First suggested site of learning ....... 7
      1.2.2 Second suggested site of learning .... 9
      1.2.3 Third suggested site of learning ..... 11
   1.3 VOR frequency response ................... 13
   1.4 My questions ............................ 15

2 METHODS ..................................... 17
   2.1 Equipment ................................ 17
   2.2 Summary of cat operations and protocol .. 18
   2.3 Stimuli .................................. 20
      2.3.1 Velocity pulses ....................... 20
      2.3.2 Sinusoids .............................. 22
      2.3.3 Sum-of-sines .......................... 24
      2.3.4 Current pulses ......................... 25
   2.4 Training ................................ 27
   2.5 Surgery ................................ 28
      2.5.1 Surgery preparation .................... 28
      2.5.2 Head holder surgery ................... 29
      2.5.3 Eye coil surgery ....................... 29
      2.5.4 Ear electrode surgery .................. 31
      2.5.5 Canal plug ............................. 33
   2.6 Protocols ................................. 34
      2.6.1 Calibration ............................ 34
      2.6.2 Optically induced motor learning ...... 35
2.7 Controls ........................................... 37
  2.7.1 Sham surgery ............................. 37
  2.7.2 Anesthesia control and frequency of stimulation ........ 38
2.8 Summary of calculations ......................... 38

3 RESULTS ............................................. 44
  3.1 Controls ......................................... 44
    3.1.1 Effects of anesthesia and frequency of electrical stimulation of the labyrinth .......................... 44
    3.1.2 Sham surgery ................................ 46
  3.2 Eye movements after a canal plug and during optically induced motor learning ....................... 48
    3.2.1 VOR response to velocity pulses .......... 48
    3.2.2 VOR response to sinusoids ................. 52
    3.2.3 VOR response to sum-of-sines ............... 55
    3.2.4 Evoked eye movement caused by current pulses .................. 59
  3.3 Modification index ............................. 60

4 DISCUSSION ........................................ 69

Glossary ............................................. 75

Bibliography ....................................... 77
List of Tables

1  Summary of the cat operations and protocol .......................... 19
2  Stimulus used to rotate the cat with spectacles ........................ 36
3  Latency of the evoked eye response after the current pulse .......... 59

List of Figures

1  Some of the neural pathways of the horizontal VOR .................. 3
2  Disynaptic VOR pathway .............................................. 6
3  Measuring equipment .................................................. 17
4  Four stimuli ............................................................ 21
5  Spectral analysis ........................................................ 24
6  Current series .......................................................... 25
7  Apparatus ............................................................... 27
8  Eye coil with 3 turns .................................................... 30
9  Electrode placement in the inner ear .................................. 32
10  Plugged Canal ........................................................... 33
11  Spectacles worn by the cat .............................................. 36
12  Flow chart of the data .................................................. 39
13  Calculation of the modification index ................................ 41
14  Anesthesia control ...................................................... 45
15  Sham surgery ........................................................... 47
16  VOR response to velocity pulses ...................................... 49
17  VOR gain during velocity pulses ...................................... 50
18  Dynamic index .......................................................... 51
19  Normal frequency response of the VOR .............................. 52
20  The time course of the VOR gain and phase during sinusoidal osci- 53
    llations after a canal plug surgery ..................................
<table>
<thead>
<tr>
<th>Page</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>VOR frequency response after a canal plug surgery</td>
</tr>
<tr>
<td>22</td>
<td>The VOR response to sinusoidal rotations after wearing spectacles for two days</td>
</tr>
<tr>
<td>23</td>
<td>The VOR response to sum-of-sines stimulation, after a canal plug surgery</td>
</tr>
<tr>
<td>24</td>
<td>Eye movement evoked by current pulses</td>
</tr>
<tr>
<td>25</td>
<td>Calculation of the modification index: Time slices</td>
</tr>
<tr>
<td>26</td>
<td>The modification index using VOR gain from velocity pulses</td>
</tr>
<tr>
<td>27</td>
<td>The modification index using VOR gain from sinusoidal rotations</td>
</tr>
<tr>
<td>28</td>
<td>The modification index using VOR gain from sum-of-sines oscillations</td>
</tr>
<tr>
<td>29</td>
<td>The modification index at the peak eye velocity</td>
</tr>
<tr>
<td>30</td>
<td>Correlation coefficients</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

The vestibular apparatus in the inner ear detects angular and linear acceleration of the head. The inner ear is composed of two parts: the bony and membranous labyrinth. The membraneous labyrinth contains the angular and linear head acceleration sensory mechanisms along with the cochlea. Three roughly orthogonal semicircular canals, in each of the inner ears, detect the rotational motion of the head [3]. This study is concerned with the eye movements resulting from the activation of the horizontal semicircular canals - the horizontal angular vestibulo-ocular reflex. From here on, the horizontal angular vestibulo-ocular reflex will be referred to as the VOR. The VOR stabilizes gaze by making smooth compensatory eye movements during head rotations.

Given persistent retinal image motion during head turns, the reflex must adjust its gain to eliminate the image motion. This process is called motor learning because the amplitude of evoked eye response from vestibular stimulation is different from normal and the changes are retained if the factors causing the changes, are eliminated. Telescopic spectacles can induce motor learning. The VOR response increases or decreases if the subject wears magnifying or miniaturizing spectacles, respectively [44]. The learned changes are retained if the subject is in the dark or does not receive vestibular stimulation [44]. After peripheral damage to the vestibular system, the VOR is no longer able to produce eye movements equal and opposite to head movements. Peripheral damage can be caused by mechanically blocking one canal (canal plug) or removing one labyrinth (labyrinthectomy). The eye movements are half the needed velocity. The VOR goes through a process of vestibular compensation to counter balance the effects of the lesion. The VOR increases its response to about 80% of its normal value. Vestibular compensation is assumed to be due to changes in synaptic strength, plasticity of the central nervous system.

Generally, the mechanisms involved in learning are unknown, but some progress
has been made determining where learning occurs. Recently, Lisberger (1994) [33] combined two theories suggesting the vestibular input to the Purkinje cells in the paraflocculus and flocculus of the cerebellum [29] and the vestibular input to the flocculus target neurons (FTNs) in the medial vestibular nuclei [48] are sites of learning in the VOR. These ideas stem from experiments using telescopic spectacles. Experiments causing vestibular compensation to occur after a labyrinthectomy or canal plug has lead Galiana to suggest the vestibular signals from the commissure to the FTNs may be a site of learning [21]. This study attempts to contribute to possible sites of learning in the VOR. I ask whether the disynaptic VOR pathway contains a site of learning after a canal plug. The disynaptic pathway has been studied extensively and if a site of learning occurs there, then further studies can be performed to understand the cellular mechanisms driving plasticity.

1.1 Neural pathways

Figure 1 shows some of the horizontal VOR pathways. The VOR parallel pathways include the disynaptic pathway [16], the commissural pathway [61] and a path through the cerebellum [29]. The disynaptic pathway consists of three sets of neurons: the primary vestibular afferents, the secondary vestibular neurons (PVPs and FTNs) and the extraocular motoneuron. The commissural pathway consists of a network of type I and type II vestibular neurons communicating across the midline of the brain. The cerebellum receives vestibular and visual signals. The visual signals come from the accessory optic tract via the inferior olive. The cerebellum transmit signals to the vestibular nucleus.

The disynaptic VOR pathway contains three sets of neurons: primary afferents, secondary vestibular neurons and extraocular motoneurons [16]. Each semicircular canal contains an enlarged area, the ampulla that in turn contains the sensory epithelium. The primary afferents receive input from the sensory hair cells embedded in the cupula of the semicircular canal [25]. The cupula acts as a diaphragm
Figure 1: Some of the neural pathways of the horizontal VOR.

The flocculus target neurons (FTN), cerebellum, and nucleus prepositus hypoglossi are only shown on one side of the brain. The other structures are shown on both sides of the midline. The vertical dotted line represents the midline of the brain. All synaptic connections are excitatory unless otherwise indicated with a "-". The "--/-" represents polysynaptic connections.
stretched across the cross section of the ampulla. The movement of the cupula translates to the sensory hair cells. During head rotations, the endolymph in the canal experiences inertial forces. As the head begins to rotate, the endolymph lags behind and causes the cupula to lean in the direction opposite to the head. The stereocilia then bend towards or away from the kinocilium, and cause depolarization or hyperpolarization of the sensory hair cells, respectively. If the head rotation is sustained, the endolymph will catch up to the head rotation and the stereocilia and kinocilium straighten up. When the head slows and stops the reverse happens. In an overly simplified approximation, the canal acts as an integrator receiving a head acceleration signal and transmitting a head velocity signal to the primary afferents. For sustained signals with high frequencies, the primary afferents show a phase lead and a gain enhancement [17]. The more irregular the spontaneous firing patterns of the afferent the greater the phase lead and gain enhancement.

Flocculus target neurons (FTNs) and position-vestibular-pause neurons (PVPs) are secondary vestibular neurons in the medial vestibular nucleus [60][59][9]. Figure 1 shows the location of the FTNs and PVPs in the VOR pathways. Most of the secondary neurons in the disynaptic pathway are PVPs [60]. PVPs receive monosynaptic excitatory input from ipsilateral primary afferents. They fire in relation to eye position, vestibular rotations and pause during saccades. FTNs receive monosynaptic, excitatory input from the ipsilateral primary afferents, longer latency excitatory input from the contralateral primary afferents, and monosynaptic, inhibitory input from the Purkinje cells in the flocculus cerebellum [9][36]. FTNs and PVPs monosynaptically project to the abducens nucleus. Most PVPs project to the contralateral abducens nucleus. The possible third neuron in the medial vestibular nucleus are the eye and head velocity neurons (EHVs). EHV s discharge in relation to eye and head velocity in the same direction. Scudder and Fuchs [60] suggests EHV s contribute to the disynaptic pathway and consist of FTNs. Other vestibular interneurons exist, but will not be discussed.
Extraocular motoneurons from the abducens nucleus project to the ipsilateral lateral rectus muscle and to the contralateral oculomotor nucleus. The motoneurons from the oculomotor nucleus project to the ipsilateral medial rectus muscle. The co-activation of the lateral and rectus muscles produces horizontal eye movements.

Figure 2 shows the changes in neural activity when the head turns to the left and the VOR causes the eyes to move to the right. The direction of the arrows beside the neurons show an increase or decrease firing rate from normal, and all shown synaptic connections are excitatory. Briefly during a left horizontal head rotation, the vestibular hair cells in the right horizontal semicircular canal hyperpolarize. This causes the primary vestibular afferents and secondary vestibular neurons to decrease their firing activity. Concurrently the sensory hair cells in the left horizontal semicircular canal depolarizes and increases the activity of the primary and secondary vestibular neurons. Axons of the secondary vestibular neurons cross the midline and excite or inhibit the motoneurons. The right lateral and left medial rectus muscle increase activity, while the left lateral and right medial rectus muscle decrease activity. This causes the eyeballs to turn to the right.

1.2 Potential sites of learning

The VOR’s ability to recalibrate allows it to be a good model to study neural plasticity in the central nervous system. Recently some studies have focused on the cellular changes during VOR compensation. More studies can be focused on the neural mechanisms of plasticity if the sites of learning can be found. The following sections will discuss some of suggested sites of learning. The first two suggested sites of learning stem mainly from telescopic spectacle experiments, while the third site comes from experiments when one labyrinth has been removed.
Figure 2: Disynaptic VOR pathway

During a leftward head turn, the eyes turn to the right. The increase, ↑, and decrease, ↓, activity of the neurons are shown beside each neuron in the disynaptic pathway (primary afferents → secondary vestibular neurons → motoneuron). All the shown synaptic connections are excitatory.
1.2.1 First suggested site of learning

Lisberger et al. (1986) [36] suggested the VOR is composed of modifiable and unmodifiable pathways. During motor learning the modifiable pathway of the VOR changes to stabilize gaze. The separation of the VOR pathways into modifiable and unmodifiable components can be seen during a rapid acceleration, for example a head velocity pulse stimulation (30°/s in 50ms) when the monkey has adapted to telescopic spectacles [32] [31]. Magnifying or miniaturizing spectacles cause the VOR gain to exponentially approach an asymptote, larger or smaller from normal respectively in 2-4 days [44] [12] [32]. During a rapid head acceleration stimulus, the first 5ms of the eye movement resulting from the normal and adapted VOR is the same in the primate. The eye movement resulting from the unmodifiable pathway occur earlier than the modifiable pathway. The early portion of the eye movement was caused by the activation of disynaptic pathway. Since it takes longer for the FTNs to respond to the head turn than PVPs, Lisberger suggested the modifiable pathway included the FTNs while the unmodifiable pathway included the PVPs.

The latency calculated from the onset of the rapid head acceleration depends on the mechanics of the turntable and the filters used to process the signals. The primary afferents are activated with a latency after the onset of the head acceleration ranging from 4-18ms [36]. A stimulating electrode in contact with the perilymph surrounding the semicircular canals bypasses the mechanical encoding of the canals and almost simultaneously activates the primary afferents and evokes an eye movement [26]. Electrical stimulation of the labyrinth causes the primary afferents to respond 0.2-0.5ms later [5]. The latency difference between the modifiable and unmodifiable pathways is zero when the labyrinth is stimulated using current pulses [7]. Although no latency difference is present, the dynamics of the modifiable and unmodifiable pathways are different. The latency from stimulating the labyrinth using current pulses to the motoneuron is 1.3-2.0ms in the monkey.
The latency from stimulating the abducens nucleus or the oculomotor nucleus to the onset of the eye motion is 3ms in the cat [43]. The total latency from electrically stimulating the labyrinth and the onset of an eye movement is about 5ms. It takes another 5.5ms (3.9-8.8ms) and 6.5ms (4.1-9.8ms) for the medial rectus and lateral rectus eye muscles to reach their peak, respectively [43]. The latencies measured after electrically stimulating the neurons depend on the filters used to process the recorded signals. Electrical stimulation of the labyrinth is a better method to study the modifiable and unmodifiable components of the VOR.

During motor learning, the evoked eye movement to current pulses changes in relation to changes in VOR gain measured by rotation. Activation of the disynaptic pathway shows small changes during optically induced motor learning [7]. Although this method cannot distinguish which of the secondary vestibular neurons (the FTNs and/or PVPs) cause the modifiability of the pathway, Broussard et al. [9] suggest the FTNs are responsible for the small change during VOR adaptation [7]. FTNs have exhibited firing behaviors that change according to the increases and decreases in VOR gain.

The FTNs adapt to telescopic spectacles by exhibiting a change in directional sensitivity, while the PVPs do not [38] [37]. During normal to high VOR gains, FTNs increase firing during ipsilateral head rotations and decrease firing during contralateral head rotations. This firing behavior is classified as type I. The reverse is observed during low VOR gains. FTNs decrease firing during ipsilateral head rotations and increase firing during contralateral head rotations. This firing behavior is classified as type II. The FTNs receive excitatory vestibular input from the ipsi- and contralateral labyrinth [9]. The directional sensitivity change in FTNs is thought to be caused by a dominating effect either from the ipsilateral or contralateral vestibular input. It is thought that during normal to high VOR gains the dominant input to the FTNs are the ipsilateral vestibular input, while during low VOR gains, the dominant input comes from the contralateral vestibular input [9].
The changes in the FTNs are in the correct direction to account for the VOR gain changes. In contrast to the FTNs, the PVPs show very little to no change during VOR gain changes. PVPs are usually type I neurons, with a few exceptions of type II.

Long-term potentiation (LTP) [4] may be the cellular mechanism involved in learning. It is thought that long-term potentiation may be used to store long term memory. Long term potentiation occurs in the medial vestibular nucleus. High frequency stimulation of synaptic input can increase the synaptic strength or efficiency of synaptic transmission for hours or days. The synapse between two neurons can be strengthened if the neurons act synchronously. High frequency (200Hz) current stimulations of the primary afferents result in long-lasting potentiation in the ipsilateral medial vestibular nuclei slices of rat brains [10].

1.2.2 Second suggested site of learning

In addition to the FTNs, the Purkinje cells in the cerebellar flocculus also change during learning, implying both are involved in learning. Figure 1 shows the input signals the Purkinje cells receive. The Purkinje cells receive vestibular input via the mossy fibers from the primary afferents and visual input via the climbing fibers from the inferior olive [29]. The Purkinje cells increase firing activity when the VOR gain is below normal. They are highly unlikely to cause the earliest modifiable component of the VOR, because of their long latency after the onset of a vestibular stimulation. However Purkinje cells may be responsible for longer latency modifiable components of the VOR. Broussard et al. (1992) found a component of the VOR that was modifiable, 30ms after the current pulse.

Previously it was thought the VOR had one site of learning and it was either in the brainstem where the FTNs were located [48] or in the cerebellum where the Purkinje cells were located [29]. Miles et al. (1981) [48] suggested the VOR had one site of learning and that was in the brainstem. He recorded the firing activity
of the Purkinje cells in the cerebellum and found the following surprising result. The Purkinje cells showed appropriate changes to cause the VOR gain to change when recorded during the VOR, but changed in the wrong direction when recorded during VOR cancellation (the eyes do not move during a head rotation). Miles continued to develop his theory and thought the signals from the cerebellum acted as a teacher, guiding learning in the brainstem.

Ito (1972) [29] thought the vestibular input to the Purkinje cells through the mossy fibers were modifiable. The modification was thought to occur when the parallel fibers and climbing fibers were activated at the same time. Results supporting the ideas of Ito are the cerebellar lesion studies. Removal of the cerebellum and/or flocculus removes the adaptive changes to the VOR caused by subjects wearing reversing or telescopic spectacles. In addition, it prevents the ability of the VOR to learn, but does not affect the normal VOR [58] [35]. Evidence has been presented suggesting long-term depression may occur in the cerebellum [30]. The long-term depression decreases the efficacy of a synapse that occurs when the parallel fiber synapses on a Purkinje cell and are activated in synchrony with climbing fibers.

Since then Lisberger has suggested that there are more than one site of learning [33]. The second suggested site of learning is the vestibular input to the Purkinje cells. The Purkinje cells exhibit a peculiar behavior. The firing activity of the Purkinje cells changed appropriately to cause VOR adaptation if the cells were recorded during the VOR but not during VOR cancellation [46]. The behavior of the Purkinje cells could by resolved if motor learning in the VOR occurred at more than one site. The model suggested a learning site at the vestibular input to the FTNs and Purkinje cells [33]. The model includes the positive feedback between the brainstem and the cerebellum suggested by Miles et al. [47], since the Purkinje cells receive head and eye velocity signals. The vestibular input to the Purkinje cells included a gain control and a dynamic element. The dynamic element in a recurrent
positive feedback loop allowed small subtle changes to be converted to a gradual increase or decay of potential [39]. The visual input, vestibular inputs and the eye movements could vary as a function of time. Subtle changes at the cellular level were able to cause changes at the behavioral level. This could be accomplished by changes in the cellular mechanisms, such as long-term depression.

1.2.3 Third suggested site of learning

Lisberger [33] has suggested one of the sites of learning in the brainstem is the vestibular input to the FTNs. His model does not predict the exact site. The vestibular input may be directly from the ipsilateral primary afferents and/or from the contralateral primary afferents. Unlike Lisberger’s model [33], Galiana did not collapse the two sides of the brain into one side. Galiana has suggested the commissural input to the FTNs is a site of learning in the VOR [21]. Broussard and Lisberger [9] found the FTNs receive excitatory vestibular input from the contralateral labyrinth. The signal path from the contralateral labyrinth to the FTNs is unknown.

The bilateral model of the VOR suggests that the vestibular commissure connecting the vestibular nuclei may be a site of learning, after a unilateral labyrinthectomy or unilateral canal plug [21]. In the model, two interdependent, closed, positive feedback loops across the commissure between the vestibular nuclei show that a gain change in one feedback loop will affect the gain of the other feedback loop. The commissural feedback loop includes the FTNs. Small changes in the closed loops can result in noticeable changes in the overall system. The restoration of the central system following peripheral injury could be attributed to the VOR gain changes in the closed commissural loops. Broussard et al [7] suggested after accidentally plugging the horizontal canal and learning continued, the site of learning probably occurred where vestibular signals from both sides of the brainstem converge. The neurons in the vestibular commissure have been shown to
change following vestibular damage.

Two methods of causing vestibular damage are by performing a labyrinthectomy or by plugging a canal. One difference between a unilateral labyrinthectomy and canal plug is that the former disrupts the natural resting rate of the primary afferents causing a spontaneous rhythmic beating of the eyes, nystagmus. The unilateral labyrinthectomy and unilateral canal plug both reduce the normal vestibular input by about 50%. The reduction of vestibular input reduces the VOR gain to about 50% of normal [52] [18] [40] [11] [54]. The VOR goes through a process of compensation to readjust itself to stabilize gaze.

The frequency of occurrence and resting firing rate of ipsilesional type I neurons after a lesion drops to close to nothing. A network of type I and type II neurons occur across the vestibular commissure. Type I neurons receive monosynaptic inhibitory input from type II neurons, (see figure 1). Type II neurons receive excitatory input from contralateral type I neurons, across the vestibular commissure [61]. The drastically reduced firing rate of ipsilesional type I neurons after a peripheral injury is thought to be caused by the absence of excitatory ipsilesional vestibular input and an enhanced commissural inhibition from the contralateral type II neurons, which have lost the excitatory input from the lesioned side [54]. The frequency of occurrence and firing rate of the ipsilesional type II neurons, and contralesional type I and type II neurons are the same or very close to normal. The recovery of the resting firing rate of ipsilesion type I neurons in 2-3 days correlates with the recovery of posture imbalances seen immediately after the lesion [22] [54] [64] [65] [57]. The VOR partially recovers to a gain of about 80% of normal [53][18]. The resting activity of type I neurons on both sides of the midline is equal, but lower than normal. The type II neurons are equally common across the midline. The VOR gain is thought to recover when bilateral symmetry is regained in the time varying responses of the vestibular nucleus [22] [54].

Exposure to light affects the recovery of VOR during compensation. The VOR
does not compensate if a monkey is kept in the dark after a unilateral labyrinthectomy. Once the monkey is exposed to light, the VOR will compensate[19].

1.3 VOR frequency response

The VOR exhibits signs that suggest high and low frequencies signals are processed differently. The function of the VOR to make compensatory eye movements during head rotations suggests a 180° phase difference is present between the head and eye velocity signals. As previously mentioned the mechanical encoding performed by the semicircular canals provide roughly about half of the required phase difference. The contribution of the extraocular muscles and the orbital mechanics have been found by Skavenski and Robinson (1973) [62]. The contribution is greatest at the high frequencies. Skavenski and Robinson (1973) [62], summarizes the phase contributions of the canals, extraocular muscles and the orbital mechanics and the predicted contribution from the brainstem. The contribution from the brainstem is least at the high frequencies. The mechanics of the brainstem can be modeled using two parallel pathways to process high and low frequencies differently. The first pathway consist of an integrator for low frequency signals before entering the extraocular motoneurons; the second pathway directly projects to the extraocular motoneurons and leave the high frequencies to be integrated by the orbital mechanics [62]. The oculomotor plant acts as a low pass filter. The gain is constant at frequencies below 0.1Hz and above this shows decreasing gain and increasing phase lag.

The normal frequency response of the VOR is not uniform across all frequencies. The VOR gain is less than unity at low frequencies, approaches and maintains a gain of one at frequencies below 4Hz. At frequencies above 4Hz, the VOR gain is greater than unity [50] [66]. It is thought the wide dynamic range of primary afferents [17] is responsible for the different frequency response of the VOR. At low frequencies, the gain of the primary afferents is below one. The primary affer-
ent gain is constant at one between 1Hz and about 5Hz. At high frequencies, the primary afferent gain is greater than one, and this gain increases as the frequency increases. The primary afferents with different dynamics project to different parts of the vestibular nucleus. This separation may be preserved at the motoneuron level [45] [49].

At low frequencies, signals in the VOR pathway pass through the nucleus prepositus hypoglossi. The nucleus prepositus hypoglossi acts as a neural integrator [20]. Figure 1 shows the neural connections to the nucleus prepositus hypoglossi. The nucleus prepositus hypoglossi receives input from the ipsi- and contralateral vestibular nerve at disynaptic latencies, projects to the abducens nucleus, cerebellum cortex and receives information from the flocculus [2].

The neurons in the medial vestibular nucleus exhibit frequency dependencies. The spike generator in the medial vestibular nucleus neurons is linear and has a unity gain and zero phase lead during low frequencies. The lower the resting firing rate of the neuron, the lower the frequency when the gain starts to fall from unity and when the phase lead increases. Signals entering the medial vestibular nucleus are linearly related to the output of the MVN neurons. There is a little phase lead decrease at high frequencies [15].

During motor learning, the VOR gain is not uniform across all frequencies. More VOR gain changes are seen at low frequencies than at high frequencies. Robinson [58] found larger gain changes at low frequencies than at high frequencies. If telescopic spectacles are worn, then the VOR shows a broad and large VOR gain change centered at the frequency used to rotate the subject during adaptation [34]. The large VOR gain change decreases as the frequency used to rotate the subject during adaptation increases. Raymond and Lisberger (1996) [56] found the VOR gain changed very little at frequencies greater than 5Hz. A phase lead is seen at the frequencies above the adapting frequency and a phase lag is seen at frequencies below the adapting frequency [34]. After unilateral vestibular dam-
age, an asymmetric VOR response occurs during high frequency rotations [66]. It has been suggested that parallel frequency selective channels exist in the VOR and each channel has its own modifiable gain element.

1.4 My questions

The main goal of this study was to show whether the disynaptic pathway is modifiable and is the disynaptic pathway modifiable more at high frequencies than at low frequencies. This requires the VOR to undergo adaptation using telescopic spectacles or through compensation for a canal plug.

Is the disynaptic pathway modifiable after a canal plug? To answer the question I stimulated the labyrinth using single current pulses and measured the VOR response to rotations about a vertical axis. Electrical stimulation of the labyrinth allows us to estimate the neurons responsible for the eye response caused by the current pulse. Since small changes appeared in the earliest portion (0-14ms after the current pulse) of the evoked eye movement, the disynaptic pathway may contain a site of learning.

Another question I asked was whether or not the disynaptic pathway transmits high frequency signals. It is known that during VOR adaptation, the VOR gain is greatest at the frequency used during adaptation [34]. This suggests there are selective frequency pathways [34]. During adaptation to telescopic spectacles, smaller gain changes are seen at frequencies greater than 5Hz than at lower frequencies [56]. This suggests the neural pathway associated with VOR adaptation is also associated with the change occurring at the low frequencies. Humans stimulated by a high acceleration head impulse after a unilateral vestibular neurectomy had a greatly reduced VOR gain during contralesional rotations [27]. Rotations towards the intact side had a VOR gain just below normal. To answer the previously mentioned question the cat was oscillated about a vertical axis through a range of frequencies (0.5-8.0Hz) after a canal plug surgery. I determine if the changes in
the disynaptic pathway occurred at the same time as the VOR gain change during high frequency rotation.

I subjected the cats to telescopic spectacles and/or a unilateral horizontal canal plug. To obtain a clearer picture of the VOR gain time course, I recorded soon after the canal plug surgery. I tested the VOR using velocity pulses, sinusoidal rotations (0.5-8Hz), and sum-of-sines oscillations and activated the VOR pathways by electrically stimulating the labyrinth. The results showed the disynaptic pathway was clearly more modifiable during vestibular compensation than during vestibular adaptation. The disynaptic pathway modification was affected at all frequencies.
2 METHODS

Two female cats (Cat R, Cat Z) and one male (Cat O) cat were used. The male cat was neutered. The age of the cats ranged from 5 to 6 months at the beginning of the study and 10 to 16 months at the end of the study. I used kittens because their VOR compensates better after peripheral damage than in adult cats [40]. Each cat slept in its own cage during the night and interacted with other cats during the day. I recorded the response of the horizontal VOR to various stimuli rotations about a vertical axis and the evoked eye movement to electrical stimulation of the labyrinth. The data recorded during each stimulus was stored in its own file in the computer and later analyzed. Horizontal eye movements were recorded.

2.1 Equipment

Figure 3 shows a diagram of the equipment. LabVIEW, a graphical programming package, and the data acquisition card were from National Instruments. Neurokinetics produced the table to turn the cat. Signals from the computer were also sent to the current isolator and probe, to deliver constant current pulses to the labyrinth.
The stimulator was made by Frederick Haer & Co. Inside the experimental room the lights were turned off and music or noise was played over the speakers and/or came from someone making noise, the "noise maker", during the experimental recordings. This was done to keep the cat alert.

Eye movements were recorded using a magnetic search coil. The eye movement monitor model EM3 was made by Remmel Labs. Two Helmholtz coil pairs were placed orthogonally to each other along the skeleton of a cube, the field coils. Current in the field coils produced an oscillating horizontal and vertical magnetic field at 50kHz and 75kHz, respectively. This allowed the horizontal and vertical eye movements to be separated. A uniform magnetic field was produced in the center. The eye coil voltage was linear within 5% for angles less than 30° from the center. At the center of the magnetic field the eye coil monitor was accurate to 4 arc minutes. The magnetic field induced a voltage in the eye coil proportional to the cosine of the angle between the eye-coil axis and the field coil. The signal leaving the eye coil monitor was low-pass filtered. All channels during the velocity pulse stimulation and current pulse stimulation were sampled at 1000 samples per second. Channels recorded during sinusoidal oscillations less than 0.65Hz were sampled at 130 samples per second. At stimulating frequencies higher than 0.65Hz, the sampling frequency was at two hundred times the stimulating frequency. The signals were digitally low-pass filtered using Butterworth filters with a stopband of 55-65Hz, to remove power-line interference. The horizontal and vertical eye positions were computed and differentiated. The eye velocity signal was filtered using a low pass filter with a cutoff frequency at 175Hz.

2.2 Summary of cat operations and protocol

Each cat had 1 or 2 eye coils implanted in the right (R) and/or left (L) eye to record the horizontal eye position. This is summarized in table 1. The cats were
restrained from making any head movements. The VOR pathways were activated either by whole body rotations about a vertical axis or by electrically activating the primary afferents. Biphasic current pulses were sent through an electrode in contact with the perilymph of the inner ear to activate the primary afferents. The cats either wore spectacles or had a canal plug or both to induce motor learning in the VOR. Cat R and Cat Z wore factor of 2 (x2) and/or factor of 0.25 (x0.25) magnifying spectacles continuously for 3 days. The flow of endolymph in the right horizontal canal plug of Cat Z was blocked (canal plug). Immediately following the canal plug, I recorded from Cat Z every few hours. Cat Z wore spectacles 5 months after the canal plug surgery. Cat Z has two entries in the table 1, because she had two eye coils. A series of control studies were performed. Cat O was used in three control studies: sham surgery, anesthesia and frequent recordings. The sham surgery attempted to determine if the cutting and drilling during surgery affected the VOR. The anesthesia control attempted to determine how long the anesthesia affected the VOR after surgery. The last control study determined if the current pulses scheduled approximately every 3 hours may increase the activation of the VOR pathway. Unforeseen health problems with the cats prevented all the

Table 1: Summary of the cat operations and protocol

(R/L = right/left side of the cat's head)

<table>
<thead>
<tr>
<th>Cat</th>
<th>Eye Coil</th>
<th>Electrode</th>
<th>Spectacle</th>
<th>Canal Plug</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>L</td>
<td>L</td>
<td>x2, x0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z</td>
<td>R</td>
<td>L</td>
<td>x0.25</td>
<td>R</td>
<td>Sham Surgery</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
<td>Anesthesia</td>
</tr>
<tr>
<td>Z</td>
<td>L</td>
<td>R</td>
<td></td>
<td></td>
<td>Frequent Recording</td>
</tr>
<tr>
<td>O</td>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
individual experiments (spectacles, canal plug and controls) from being performed on the same cat.

2.3 Stimuli

The cat was restrained on a table, and whole body rotations activated the VOR by rotating the table. The cat was rotated using velocity pulses, sinusoids and sum-of-sines stimulus to activate the VOR. I also activated the VOR pathway by applying current pulses to the labyrinth. These can be seen in figure 4 along with the averaged typical horizontal eye velocity response. The eye velocity traces are shown as a dotted line. Ideally, the eye velocity should be a mirror image of the head velocity. Individual VOR responses were averaged together with the assumption an ideal VOR response exist and the averaged response will converge to it.

Calibration factors were calculated before normal data was collected. After the head holder pedestal was implanted on the cat, I calculated the calibration factor of the horizontal head velocity to obtain a peak head velocity of 10°/s during sinusoidal oscillations. Once the eye coil was implanted in the cat, the cat was rotated in the light using a velocity pulse stimulus. The VOR gain was measured and the calibration factor was computed to obtain an idealized VOR gain of unity in the light. I calculated the VOR gain from each of the vestibular rotational stimulations.

2.3.1 Velocity pulses

Figure 4A shows the velocity pulse stimulus used to activate the VOR. The velocity pulse consists of a 10ms rise time, steady state duration of 300ms and steady state velocity of 20°/s. The mechanics of the table converted the 10ms rise time to a 90ms rise time with an overshoot. The table moved 6° during one rotation. These parameters were chosen because the number of quick phases made by the cat was small. The table moved leftward or rightward in a pseudo random sequence separated by 700ms. I analyzed the files by averaging 5-15 responses to right and left
Figure 4: Four stimuli
The average stimuli used during this study and the average VOR response are shown. The solid line is the stimulus, and the dotted line is the horizontal eye velocity. The number of responses averaged is represented by n. (A) Velocity Pulse (n=5). The 'ss' shows the steady state portion of the pulse used to calculate the VOR gain. (B) Sinusoidal oscillations (n=21) (C) Sum-of-sines oscillations (n=20) (D) Current Pulse (n=170).
head turns, separately. Responses with quick phases were not used to calculate the average. Once the responses were averaged the VOR gain was calculated. The “steady state” portion was used to calculate the gain. The steady state portion is shown in figure 4A as ’ss’. The VOR gain was calculated as:

$$Gain_{vp} = \frac{Average\ Steady\ State\ EyeVelocity}{Average\ Steady\ State\ HeadVelocity}$$

The subscript $vp$ refers to velocity pulse. The maxima and minima gradually decrease as time increases. The calculated VOR gain may be higher or lower than the actual VOR gain if fluctuations are biased by the maxima or minima, respectively. The amount of bias could be minimized by choosing a steady state portion that a maximum was counterbalanced by a minimum. The dynamic index (DI) was also calculated as:

$$DI = \frac{Peak\ EyeVelocity}{Average\ Steady\ State\ EyeVelocity}$$

to quantify the overshoot seen in the eye response. The dynamic index calculation includes the mechanics of the table and is only a rough estimate of the VOR response to high and low frequencies.

2.3.2 Sinusoids

Figure 4B illustrates one cycle of a sinusoidal oscillation with the corresponding eye velocity response. I recorded the VOR frequency response when the table rotated at one frequency in a sinusoidal pattern at 0.5Hz, 1Hz, 2Hz, 5Hz, 6Hz, 7Hz, and 8Hz. The peak table velocity was 10°/s. At these frequencies, the table rotated 3.18°, 1.59°, 0.79°, 0.31°, 0.27°, 0.22°, and 0.20° peak, at 0.5Hz, 1Hz, 2Hz, 5Hz, 6Hz, 7Hz, and 8Hz, respectively. At frequencies higher than 8Hz, the flexion of the apparatus introduced artifacts in the signal. This was seen by a non-zero VOR "gain" from a wooden block model (300g) of a cat head attached to the apparatus. Normally during sinusoidal recordings between 0.5Hz and 8.0Hz, the block of wood had a "gain" ranging from 0.01 to 0.03. In the worst case, when
the measured VOR gain of the cat was about 0.40, the artifact contributed about 1-2%. Recordings from a freshly euthanized cat (30 minutes) had a VOR "gain" not different from the block of wood taken immediately after the euthanized cat. Therefore the amount of artifact in the system was insignificant.

Responses to sinusoidal rotations were analyzed by averaging 20-30 eye velocity traces without saccades. Sinewaves were fitted to the average head and eye velocities using the Levenberg-Marquardt algorithm. This algorithm found the least square set of coefficients that best fit the data at ±80° around the positive or negative peak eye velocity. Just as the VOR gain was calculated from the velocity pulse stimulation, the VOR gain was calculated from the sinusoidal rotations at each of the various frequencies. In this case, the VOR gain was calculated using the amplitude of the fitted sinewave as follows:

\[ Gain_s = \frac{\text{PeakEyeVelocity}}{\text{PeakHeadVelocity}} \]

The subscript \(s\) refers to the sinusoid. The phase was calculated as the angle by which the eye velocity led the head velocity. A phase lead was positive.

Although a nystagmus was small or nonexistent after a plug, any such nystagmus would enter the data as a DC offset in the VOR response to sinusoids. The DC offset is also the baseline that divides the cycle into two half cycles of the same duration [40]. To detect the asymmetry in the VOR response, the two half cycles need to be fitted separately. The baseline was subtracted from the average eye velocity, forcing the break point to be at zero velocity. The eye velocity was broken at the midpoint between the rightward and leftward peaks. The midpoint was determined visually and verified by fitting a sinewave to the average. If the fitted sinewave was centered about 0°/s then the midpoint was found. Each half cycle was duplicated, inverted, combined with the original to make a complete cycle and fitted with a sinewave. The VOR gain and phase was calculated when the head turned to the right and when the head turned to the left.
The frequency decomposition of the head and eye velocity during sum-of-sines rotations for one averaged file is shown. The VOR gain is calculated as the eye velocity/head velocity.

2.3.3 Sum-of-sines

Figure 4C shows the sum-of-sines stimulus. I was interested in the VOR recovery within the first 48 hours after the canal plug surgery and thought the cat might not be awake enough to respond to all the sinusoidal rotations mentioned above. I therefore decided to use a sum-of-sines stimulus, which was much quicker than the series of sinusoidal rotations. The sum-of-sines stimulus was composed of 0.5Hz, 1Hz, 2Hz, 4Hz and 8Hz, with individual peak amplitudes of 5°/s. The frequencies were chosen to have the same frequency range as the testing frequencies of the sinusoids and to be harmonics of each other. The stimulus curve had a period of two hundred milliseconds. I analyzed the files by averaging 15-25 responses during 200ms intervals. A spectral analysis was performed using a Fast Fourier Transform and the VOR gain at each frequency was calculated. Figure 5 shows the spectral analysis of the head and eye velocity during the sum-of-sines rotations. The VOR
Figure 6: Current series
The peak eye velocity evoked by electrically stimulating the labyrinth versus the stimulating current pulse is shown. Standard deviation bars are shown (n=3). The lowest current to induce an eye movement is at 200μA. A biphasic current pulse is also shown. The threshold current is 200μA.

Gain was calculated as eye velocity/head velocity. All the frequencies used to rotate the table were present in the spectral analysis. However the eye velocity did have components at frequencies not used to rotate the table. These components were small.

2.3.4 Current pulses
Figure 4D shows a current pulse and an eye response caused by it. The table did not move during current pulse stimulation. A stimulating electrode in contact with the perilymph of the inner ear delivered 200-300 0.2ms bipolar, rectangular current pulses. Since the perilymph surrounds the semicircular canals it was necessary
for the electrode to come in contact with the perilymph to activate the primary vestibular afferents. A current pulse sent through the electrode caused the almost synchronous activation of the primary afferents. 2.4s separated each current pulse for Cat R and 600ms for Cat Z and Cat O. Cat R had a longer interpulse time because the eye velocity evoked from a series of current pulses decreased when the interpulse time was short. The current sent through the electrode stimulated the VOR pathways. The current pulse caused the eye to move away from the side of stimulation, before returning back to the side of stimulation. In addition to activating the primary afferents, high enough currents could activate the nearby facial nerve, resulting in a facial twitch. Facial twitches did not enter the eye trace, because I recorded the contralateral eye movements or used low stimulating currents.

Each cat was stimulated at a standard current, calculated as double the lowest stimulating current needed to produce an eye movement (the threshold current). The threshold current was found by the recording the cat’s eye movement to a series of currents. Figure 6 shows the average peak eye velocity evoked by current pulses of Cat O from 3 different days, 1, 11 and 14 days before the control experiments. The threshold current of Cat O was 200μA. Cat O and Cat Z was stimulated at 400μA, and Cat R was stimulated at 300μA. Other currents were used and the results did not depend on the current.

The eye velocity evoked by the current pulse was found to depend on the eye position. Vertical and horizontal eye position responses within ±20° and ±10° of center, respectively were included. Between 100-200 eye responses were averaged. Most averages contained 150-200 eye responses. The standard deviation of the averaged response was about 3°/s.

Implanted metal electrodes bring potential problems to the experiment. In Cat O, the peak eye velocity evoked by current pulses was variable before the sham surgery, and this probably continued after the sham surgery (see figure 15B). High intensity current stimulation can cause heating or electrochemical effects, resulting
in the break down of body fluids producing gas bubbles, toxic products and a harmful pH. Polarization can occur when a current flows through the silver electrode. If the charge build up is large enough, the potential difference can cause hydrolysis to occur [14]. The change in pH and pressure can cause damage to the surrounding tissue.

2.4 Training

Each day, the cats were not fed until after the training session. This was continued during the experimental stage. Training usually took five consecutive days to complete. Each training session lasted 15-30 minutes. Each cat was rewarded with soft cat food or baby food when he/she performed the desired task. Each cat learned to enter a canvas bag, and sit with torso inside and head outside, as the bag was tied. The cat in the bag was placed in the recording box and placed so as to lie in the prone position. Figure 7A shows a perspective view of the apparatus. The
surrounding coils provided the magnetic field needed to record the eye movements. The cylinders were used to attach the cat’s head to the apparatus. Figure 7B shows the cat in the box. Metal bars along the top of the box, and a wooden board at the back of the box restrained the cat from making large movements. The cat was trained to rest comfortably in the box. Once training and surgery was completed, the cat’s head was attached to the apparatus using the cylinders. The small cylinder was attached to the hollow head holder pedestal implanted on the cat’s head. The large, hollow cylinder connected the small cylinder to the apparatus, joined by screws. The connectors on the cat’s head connect the eye coil and the ear electrode to the computer and current stimulator, respectively. The apparatus shown in figure 7A was mounted to a table that turns by a servomotor.

2.5 Surgery

When training was completed, the cats were ready for surgery. Surgery occurred under sterile conditions. We implanted a head holder pedestal (to immobilize the head), 1 or 2 coils around the eye (to measure the eye position) and, 1 or 2 electrodes in the middle ear to stimulate the labyrinth. During a subsequent surgery, we plugged the horizontal canal. Stainless steel screws and dental acrylic anchored the hardware in place. Before surgery, the cat was injected intramuscularly (I.M.) with 0.5cc of a mixture of Demerol, acepromazine and atropine. During surgery, the cat was given 1-3% of isoflurane. Immediately after surgery, 0.1cc I.M. of Buprenorphine, a pain killer, and 0.5cc I.M. of Penlong, an antibiotic, were injected in the cat. Twice a day for 7 days after the surgery, 62.5mg of Clavamox was given to the cat, as an antibiotic.

2.5.1 Surgery preparation

Before each surgery, the cat fasted at least 14 hours. I injected the cat with the premedication as mentioned above to reduce pain and alertness. The cat was given
anesthesia and connected to an I.V. (90% saline). Throughout surgery, the heart rate was monitored using a stethoscope and the percentage of isoflurane adjusted to maintain a steady heart rate.

2.5.2 Head holder surgery

We implanted the head holder pedestal, and eye coil during a 3–4 hour session. The head holder pedestal was a hollow, stainless steel cylinder, 1 cm in height and had a 1.5 cm diameter. Stainless steel screws fastened bent steel rectangular plates, fixation plates to the skull. Three to four fixation plates were used, involving 6–8 screws. The head holder pedestal was attached to the converging point of the fixation plates, using dental acrylic. Cat Z’s head holder was oriented so when she was restrained in the apparatus her head was pitched 21° nose down. Cat O’s head was pitched 23° nose down in the apparatus. This increased the activation of the horizontal canals during rotations by positioning the horizontal canals to be more parallel to the plane of rotation, while decreasing the contributions of the vertical canals. Cat R’s head had a 0° pitch in the apparatus. This did not make a difference. The results of Cat R and Cat Z during optically induced motor learning were the same.

2.5.3 Eye coil surgery

An eye coil was made from stainless steel wire insulated with Teflon, before the surgery. The coil either had 3 (Cat R, Cat O) or 4 (Cat Z) turns. If the coil had 3 turns, the first 2 turns had the diameter of the eye ball, while the third turn consisted of the wire spiralling around the first two turns. An illustration of an eye coil is seen in figure 8. During implantation of the eye coil, an ophthalmic lubricating ointment was given to maintain moisture in the eye not receiving the eye coil. In the eye receiving the coil, 3–4 drops of 1% Neo-Synephrine retracted the nictitating membrane. This simplified the procedure of implanting the eye coil. In addition,
The eye coil was attached to the sclera. The cat was placed in the magnetic field and the induce current in the coil was recorded. This allowed the eye movements to be recorded.
we irrigated the surface of the eye with sterile saline to prevent dehydration. We dissected the conjunctiva away from the sclera along the perimeter of the eye. Four sutures sewn in the sclera, somewhat evenly spaced around the eye, guided and held the eye coil in place. This allowed the eye coil to rotate with the eye. The eye coil could not be seen when looking at the cat. 5-0 polyester sutures were used in the first two cats, and 5-0 silk sutures were used in the subsequent cat.

An oval shaped piece of skin (1cm x 0.5cm) was removed from the supraorbital bone. Two 2mm diameter holes were drilled in the bone for two stainless steel screws. The leads of the eye coil ran underneath the skin to the surface of the head where the oval shaped skin was removed. The insulation on the ends of the eye coil leads was stripped, and the wire was soldered to a connector, Winchester socket. Dental acrylic cemented the socket to the screws. Cat R had an 18mm diameter eye coil in the right eye. Cat Z had a 17mm diameter eye coil in the right eye during the canal plug experiments, followed by an 18mm diameter eye coil in the left eye during the spectacle experiments. Cat O had an 18mm diameter eye coil in the left eye.

2.5.4 Ear electrode surgery

Implanting the stimulating electrodes in the ear took about 4 hours. A teflon, insulated, silver stimulating and a ground electrode were implanted in the middle ear. An incision exposed the temporal bone. The middle ear was exposed after following the external auditory canal. A small hole, using a hand drill (0.009")", the size of the electrode and deep enough to meet the perilymph in the inner ear, was made in the bony promontory. The perilymph surrounds the semicircular canals. The 1mm length exposed tip of the stimulating electrode was placed in the small hole. The ground wire with a 1mm diameter spherical tip was placed on the floor of the middle ear. Figure 9 shows the location of the implanted electrodes in the middle ear. Similar to the eye coil leads, the electrode leads were connected to a separate
Figure 9: Electrode placement in the inner ear

The figure of the human ear shows the analogous place of the stimulating 'S' and ground 'G' electrode in the cats, respectively.
Winchester socket on the supraorbital bone.

Cat O’s labyrinth was accidentally damaged while implanting his right stimulating electrode. The injury resulted in an asymmetric eye response during the rightward and leftward head rotations. The control experiments did not begin until 30 days after the electrode surgery, to give the VOR time to stabilize.

2.5.5 Canal plug

Cat Z had a unilateral horizontal canal plug. The canal plug surgery mechanically blocks fluid flow in the semicircular canal. The pressure caused by the plug is dispersed because of the shared endolymphatic tract among the other semicircular canals and the otoliths. The plugged canal reduces the amount of vestibular input into the VOR neural pathways. Similar to the electrode implantation surgery, the canal plug surgery involved an incision exposing the temporal bone. The external auditory pathway was followed and the incus was exposed. The long portion of the incus lies parallel to the horizontal canal. The labyrinthine bone was carefully drilled away, avoiding the nearby facial nerve. We drilled a small opening, 2-3mm in the lateralmost portion of the horizontal canal, causing the endolymph
to seep out. The opening in the canal was quickly plugged up with pieces of periosteum. Figure 10 shows a canal that was plugged. Plugging was complete when endolymph no longer seeped out. Once the canal was plugged another 30 minutes passed while the cat’s ear was being closed up and the anesthesia was shut off.

Cat Z’s left horizontal canal was plugged. After four hours of surgery, Cat Z was in the dark until the first recording session. Data was collected roughly every 3 hours for 20 hours, then every 6 hours for 12 hours, then every 12 hours for 7 days, then once a day for 4 days, then once every 3-4 days until 3.5 months after the plug. Approximately a month passed before daily recordings were resumed in preparation to put spectacles in Cat Z. During the first 17 hours after the surgery, Cat Z freely roamed around in the lab once she was allowed in the light. Afterwards, Cat Z returned to the animal facility. She resumed her normal routine of being in her cage during the night, playing with other cats in a room during the day and in the lab during the recording period.

2.6 Protocols

2.6.1 Calibration

Each day the eye coil monitor was calibrated before recording. A duplicate of the eye coil implanted in the cat was attached to a protractor and placed in the magnetic field. The coil was rotated about a vertical axis between $\pm 10^\circ$ and $\pm 20^\circ$ from center ($0^\circ$ was straight forward) and monitored to ensure the correct coil position was acquired by the computer. During recordings with the cat, all measurements were taken in the dark to eliminate any visual input, thus leaving only vestibular inputs to be responsible for the eye movements produced. The room was painted black to absorb any light that may be in the room, and considered dark according to human eyes. Human eyes take about 15 minutes to dark-adapt.

Each stimulus was collected in a data file. Several data files were collected during one recording session for each stimulus. The longest file covered about 8 min-
utes of data. However, most data files contained about 2 minutes. The lights were on between recording data files to prevent the cat from becoming dark-adapted in case of an undetectable light leak. If the recording session went smoothly, recording time lasted for about 35 minutes during the canal plug experiments, but lasted about 2 hours during the spectacle experiments. The 2 hours include 1 hour of rotating the cat in the light while wearing spectacles and 1 hour of recording.

When the cat was sleepy, the loss of the initial overshoot in response to velocity pulses or a drifting eye position was seen. The cat was kept alert by playing sounds over the speakers, and/or by someone making noise in the experimental room. Sometimes, I wiped the cat’s face with a wet cloth or offered baby food in front of the cat to maintain alertness. If these methods did not keep the cat awake, the experiment was stopped. The cat would then be given a holiday or injected with amphetamine the next day. If amphetamine (0.5-0.6 mg/kg I.M.) [52] [40] was given to the cat (Cat R, Cat Z) data was recorded 90 minutes later. If the administration of amphetamine was discontinued, the cat had at least two days of rest before recording continued.

2.6.2 Optically induced motor learning

Once all the baseline data was collected, the cat either wore spectacles or had a canal plug. Cat R wore spectacles when her labyrinths were intact. Cat Z wore spectacles 5 months after her right horizontal canal was plugged. Figure 11 illustrates the spectacles constructed to fit the face of the cat. This ensured the cat could only look through the lenses to see and cause VOR adaptation to occur during head rotations, in the light. Ketamine I.M. sedated the cat, while a mould of the face was made. Dental acrylic was built up along the rim of brass frames to fit the spectacles to the face. Dental acrylic was used to attach a 90° corner post onto the spectacles frame. The corner post was connected to the small head cylinder. The cylinder was then connected to the head holder pedestal. This allowed the cat to wear the
spectacles 24 hours a day. The magnifying and miniaturizing spectacles weighed about 117g and 104g, respectively.

During the spectacle experiments, Cat R wore each magnifying (x2.0) and miniaturizing (x0.25) spectacles three times for 3-day periods. Cat Z wore x0.25 spectacles for one 3-day period. Cat Z did wear x2.0 spectacles, but a surface lesion that appeared on her cornea prevented any motor learning. At least two days passed before another three days of spectacle wearing occurred. However, two days was not long enough for the VOR gain to return to normal and the cat should have been rotated in the light when the spectacles were removed on the third day.

<table>
<thead>
<tr>
<th>Sum-of-sines</th>
<th>Frequency [Hz]</th>
<th>0.25</th>
<th>1.0</th>
<th>2.0</th>
<th>5.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Head Velocity [°/s]</td>
<td>25.0</td>
<td>10.0</td>
<td>10.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Cat R and Cat Z's faces and spectacles were cleaned every day when the spectacles were removed to collect data. Eye movements were collected in the dark while the cats did not wear spectacles.

I measured the eye movements generated in Cat R using velocity pulses and current pulses. From Cat Z, I measured the eye movements generated by velocity pulses, sinusoidal rotations, and current pulses. I recorded from the cats twice a day, before and after one hour of rotations in the apparatus, in the light. The cats wore their spectacles while they were rotated in the light. I rotated the cats using a second type of sum of sine stimulus, in the light. The frequencies and peak table velocity are shown in table 2. Black and white patterns on the walls, audio stimuli, and food were used to maintain the alertness of the cat during these rotations. The rotations in the light decreased the amount of time needed for the VOR gain to reach an asymptote. Previous cats in the same lab (unpublished) that wore telescopic spectacles, but were not rotated in the light with the spectacles on did not reach an asymptote until at least 72 hours passed.

2.7 Controls

2.7.1 Sham surgery

Two control studies were performed on Cat O to separate any potential effects of anesthesia, frequent current stimulation, and drilling and cutting during surgery on the VOR from the effects of the canal plug. I asked the following questions: Did the anesthesia cause the low VOR gain immediately after the canal plug? Did the frequent current stimulation cause the increased VOR gain and/or peak eye velocity evoked by the current pulses, after the canal plug? Did the cutting and vibration due to drilling during the canal plug surgery cause the VOR gain to increase after the canal plug?

The sham surgery followed the same surgical procedure as the canal plug, but after the left incus was exposed, we started suturing the ear closed. Following
the sham surgery, I repeated almost the same experimental protocol as followed after the canal plug surgery, for two weeks. The cat was in the dark during the first 3 hours after the sham surgery, before he was in the light. I recorded 2, 3, 6, 9, 12,.. hours after the sham surgery. Gradually we recorded every 12 hours. I stopped recording from Cat O, two weeks after the sham surgery. I stimulated the cat using velocity pulses, sum-of-sines and current pulses. There were two differences between the sham surgery and the protocol after the canal plug surgery. I omitted the sinusoidal oscillations, and no one was present in the room with Cat O during the current pulse stimulation. Sinusoidal oscillations were eliminated because it took a long time to complete and the sum-of-sines stimulus was quicker to complete and the cat would less likely become sleepy. Second, no one was in the room with Cat O, because he was able to maintain fixation within the ±10° horizontal eye position during the baseline data recording sessions.

2.7.2 Anesthesia control and frequency of stimulation

I subjected Cat O to 2.5 hours of anesthesia. This was the same duration as the sham surgery, but may be too short to compare with the canal plug surgery. I replicated the conditions and frequency of recording following the canal plug for 55 hours, but again eliminated the sinusoidal oscillations. Following the anesthesia, the cat was in the dark until the third hour after the anesthesia was turned off. I recorded 2, 3, 4, 6, 9, 12 and 16 hours after the anesthesia was turned off. Gradually the time between recordings increased to every 6 hours. I stimulated the cat using velocity pulses, sum-of-sines stimulus and current pulses. Someone was always in the experimental room with the cat to make noise.

2.8 Summary of calculations

Figure 12 shows a simplified version of the measurements and calculations needed. Starting from left to right there are two large enclosing rectangles. The front rect-
Figure 12: Flow chart of the data

Starting from left to right, the 1st column shows the stimuli used to activate the VOR pathways. The 2nd column with only one entry, shows the spectral analysis performed by the computer. The 3rd column shows the quantities calculated after each experimental session. This is repeated for many days, when the cat is wearing spectacles or after a canal plug. The 4th column shows the eye velocity traces due to current pulses plotted against the concurrent VOR gains. The 5th column shows a series of time slices of the 3 dimensional plot taken at every 0.1ms. The slope of the linear regression line relating the eye velocity to the gain is found at every 0.1ms. The 6th column shows the plot of the slopes against time. This is the time course of the modification index. In the 7th column the modification index at the time of the peak eye velocity evoked by the current pulse is found. In the last column the correlation coefficient between the peak eye velocity and the VOR gains are calculated.
angle represents the measurements taken during one experimental session when the cat has either been adapting to spectacles or has a canal plug. For example when the cat is wearing spectacles there are two sessions a day, one before and after the one hour of rotations in the light. The rectangle in the back represents the other repetitions for the entire experimental period that lasts for days. Using the same example, Cat R wore spectacles for six 3-day periods. Inside the enclosing rectangle lie the previously mentioned four stimuli: current pulses, velocity pulses, sinusoids and sum-of-sines. Responses to single current pulses and rotations were averaged. Next, calculations were performed on the averaged responses. The peak eye velocity was calculated from the evoked eye response to current pulses. The spectral analysis of the VOR response during the sum-of-sines stimulus was calculated before the VOR gain was calculated. The VOR gain was calculated from the other stimuli. The VOR gain calculated from the sinusoidal and sum-of-sines rotations were similar at the same frequencies. In the case of the velocity pulse and sinusoid stimuli, VOR gains during rightward and leftward head rotations were calculated, and averaged. The VOR gain from the velocity pulse stimulus was calculated as \( \text{Gain}_{vp} = \frac{\text{Average Steady State Eye Velocity}}{\text{Average Steady State Head Velocity}} \) and from the sinusoids as \( \text{Gain}_s = \frac{\text{Peak Eye Velocity}}{\text{Peak Head Velocity}} \) The dynamic index \( \text{DI} = \frac{\text{Peak Eye Velocity}}{\text{Average Steady State Eye Velocity}} \) and phase were calculated from responses to the velocity pulse and sinusoid, respectively. The peak eye velocity, VOR gains and phase will be plotted as functions of time in the results section.

Once all the experiments were completed, all or most of the data represented as thicker arrows were used to do further calculations. To determine if the disynaptic pathway is modifiable during adaptation to spectacles or after a canal plug, the modification index was calculated. The modification index describes the change in eye velocity evoked by current pulses per unit change in VOR gain, \( \text{MI} = \frac{\partial E}{\partial G} \). G stands for VOR gain. The calculation of the modification index required a few
Figure 13: Calculation of the modification index

(A) A 3-dimensional graph of velocity, gain and time is made. All the eye responses evoked from current pulses with their corresponding VOR gains are plotted on the 3-D graph. Two representative eye traces are shown. (B) Cross sections along the time axis are taken every 0.1ms, resulting in 2 dimensional plot of velocity versus gain. Four representative cross sections are shown. A linear regression line was found and the slope is the modification index. MI = dE/dG where G stands for VOR gain and E is eye position. The MI = 0.11, 30.1, 14.5 and 10 °/s for t = 10, 24, 30, 40ms.
The first step requires the plotting of a subset of all the eye velocity traces evoked by the current pulses against the concurrent VOR gains. Recall the VOR gain was calculated from the velocity pulse, sinusoid and sum-of-sines stimuli. Twelve of these 3-dimensional plots will be made using the data collected after the canal was plugged: 1 using the VOR gain calculated from velocity pulses, 7 using the VOR gain calculated from the sinusoids at each frequency (0.5Hz, 1Hz, 2Hz, 5Hz, 6Hz, 7Hz, 8Hz) and 5 using the VOR gain calculated from the sum-of-sines at each frequency (0.5Hz, 1Hz, 2Hz, 4Hz, 8Hz).

The modification index is a function of the parameters of the stimuli. Since one set of data used is from the current pulse, the modification index is always a function of time. In the case when the MI is calculated using the velocity pulse data, the modification index is also a function of the peak head velocity, steady state duration of the pulse. When the modification index is calculated using the sinusoid or sum-of-sines data, the modification index is also a function of frequency and peak head velocity.

Figure 13A shows an example of the two eye velocity traces plotted against the VOR gain. The traces are projected downwards onto the VOR gain and time plane as a visual aid, to show the VOR gains at those times were 0.56 and 0.84. The three axes of the graph are velocity in degree/s, VOR gain which is dimensionless and time in milliseconds. The second step requires taking a series of samples along the time axis from the plot (see figure 13B). This results in two-dimensional plots of eye velocity versus VOR gain. The slope of the regression line relating the momentary eye velocity to the VOR gain is found. The slope of the regression line is the modification index. At each 0.1ms, the modification index was calculated. The units of the MI are (°/s)/dimensionless. Figure 13B shows four examples of the regression line when the time is 10ms, 24ms, 30ms and 40ms. At t=10ms the MI is 0.11°/s, the eye velocity increases by 0.11°/s when the VOR gain increases by one. At t=24ms the MI is 30.1°/s, the eye velocity increases 30.1°/s when the
VOR gain increases by one. At $t=30$ the MI is $14.5^\circ/s$, the eye velocity increases by $14.5^\circ/s$ when the VOR gain increases by one. At $t=40$ the MI is $-10^\circ/s$, the eye velocity decreases by $10^\circ/s$ when the VOR gain increases by one.

The modification index is always a function of time. Recall that the initial onset and peak of the eye movement evoked by the current pulses is probably caused by the activation of the disynaptic VOR pathway. The average time of the peak eye velocity is found. The value of the modification index at the time of the peak eye velocity will be compared to answer the question, whether the disynaptic pathway is modifiable. A significant non-zero value will show the disynaptic pathway is modifiable. The correlation coefficient relating the peak eye velocity to each of the gains was also calculated. The first week of data collected after the canal plug all the data from the spectacle wearing periods were used to calculate the modification index and the correlation coefficient.
3 RESULTS

The modification index quantifies the changes in eye velocity evoked by the activation of the VOR pathways as a function of VOR gain. To calculate the modification index requires several samples of the VOR gain and the concurrent evoked eye movement from current pulses over a period of hours or days. Figure 12 will be shown throughout this section as each step, shown in black is about to be completed. Before I show the results of the spectacle and canal plug experiment two control studies will be shown.

3.1 Controls

3.1.1 Effects of anesthesia and frequency of electrical stimulation of the labyrinth

Immediately after the canal plug surgery, the VOR gain dropped from normal, then quickly increased. To decide if the low VOR gain measured was caused by the anesthesia, and the rapid increase of VOR gain was caused by the frequent electrical stimulation, Cat O was placed on anesthesia for 2.5 hours followed by 2.5 days of intense recordings. After the anesthesia was turned off, he was in the dark during the next 3 hours. It is not known whether Cat O was active in the dark, but he was very active in the light the following 17 hours.

The anesthesia did not have an effect except during the velocity pulse stimulation. The VOR gain during rightward (away from the open-ear sham surgery) but not leftward (towards the open-ear sham surgery) velocity pulse rotations was below normal immediately after the end of the anesthesia (see figure 14A). As previously mentioned, by the time of the control experiment Cat O had compensated for vestibular damage in his right ear. The anesthesia may have caused decompensation, which outlasted the generalized effects on the VOR. In contrast, normal peak eye velocity evoked by the current pulse (figure 14B) and the VOR gain dur-
Figure 14: Anesthesia control
(A) The time course of the VOR gain during velocity pulses. (B) The time course of the peak eye velocity evoked by current pulses. The electrode was located in the right ear. (C) The time course of the VOR gain during sum-of-sines stimulus.
ing sum-of-sines oscillations (figure 14C) was obtained 2 hours after the end of the anesthesia. It is unknown why the VOR gain during leftward velocity pulses and sum-of-sines stimulus between 12-31 hours increased. Frequent electrical stimulation did not increase the VOR gains during horizontal rotations or the peak eye velocity evoked from current pulses. The affects of the anesthesia may only have been seen during velocity pulses, because the VOR response to rightward and leftward head rotations could be separated. Since the VOR response to rightward turns did not recover until the 6th hour after the end of the anesthesia, data collected 12 hours and onwards after the canal plug surgery will be shown.

3.1.2 Sham surgery

In addition to the anesthesia, the drilling and cutting performed during surgery may have affected the VOR. A sham surgery was performed on Cat O. Cat O was in the dark, during the first three hours after the sham surgery. The sham surgery did not show any affect on the VOR during velocity pulses (figure 15A) and sum-of-sines stimulus (figure 15C-F). The VOR gain before and after the sham surgery was the same. It is not known whether the current pulses affected the peak eye velocity evoked by the current pulses, because the stimulating electrode appeared to be changing. Figure 15B shows the time course of the peak eye velocity evoked by 400µA current pulses. The peak eye velocity was plotted showing positive peak eye velocity, instead of the actual negative peak eye velocity. The noisy baseline data seems to indicate the electrode was changing and may have affected the time course of the peak eye velocity after the sham surgery. Damage to the surrounding tissue, change in pH could have caused changes in the electrode.
Figure 15: Sham surgery
The time course of the VOR gain during velocity pulses (A), and peak eye velocity evoked by current pulses (B). The time course of the VOR gain at 0.5Hz, 1.0Hz (C), 2.0Hz (D), 4.0Hz(E) and 8.0Hz (F) during sum-of-sines rotations.
3.2 Eye movements after a canal plug and during optically induced motor learning

Two cats, Cat Z and Cat R were used in this section. Cat Z had a right horizontal canal plug, and did not experience a post-plug nystagmus. She spent the first 3 hours after the surgery in the dark before allowed in the light. During the next 13 hours in the light she was very active and had approximately 1 hour of sleep. Five months after the canal plug surgery, Cat Z wore x0.25 miniaturizing spectacles during one 3-day period. Cat R wore magnifying and miniaturizing spectacles, 3 times each during 3-day periods.

3.2.1 VOR response to velocity pulses

After the canal plug surgery and during the spectacle experiments the VOR response to velocity pulses changed in two ways. The VOR response during the eye velocity during the steady-state portion increased or decreased from normal. This is shown in figure 16. This was measured by calculating the VOR gain. After the canal plug, the VOR gain decreased by 60%, then increased to about 80% of normal in two days (see figure 17A). The VOR gain did not return to preplug values and averaged to 77% of the normal, 30 days after the surgery (see figure 17B). The VOR gain during contralesional rotations was higher than during ipsilesional rotations. The decrease gain between 10-30 days post plug was probably caused by sleepiness. The data collected during this period was not included in the calculations of the modification index or the correlation coefficients later. When the cats wore x2.0 and x0.25 spectacles the VOR gain increased and decreased, respectively. Most of the gain changes occurred during the first day of wearing spectacles. After the 3-day period, the average VOR gain increase and decrease by about 30% and 60% of normal, respectively in Cat R (see figure 17C).
Figure 16: VOR response to velocity pulses
The VOR response to contralesional (A) and ipsilesional (B) rotations before and 12 hours after a canal plug surgery. The VOR response before and 73 hours after wearing x2.0 (C) and x0.25 (D) spectacles is shown. The solid line is the head velocity. The dashed line is the pre-plug/spectacle eye velocity. The dotted line is the post-plug/spectacle eye velocity. Positive y-axis is always considered to be in the rightward direction.
Figure 17: VOR gain during velocity pulses

The time course of the VOR gain during the first 9 days (A) and 110 days (B) after a canal plug surgery. The time course of the VOR gain of Cat R (C) and Cat Z (D) during optically induced motor learning.
Figure 18: Dynamic index

(A) The dynamic index versus VOR gain 30-110 days after a canal plug surgery. The dynamic index versus VOR gain during velocity pulses in Cat R (B) and Cat Z (C). The dynamic index shown is an average of rightward and leftward head turns. The baseline data is shown with 1 standard deviation error bar.

In Cat Z, the VOR gain decreased by about 50% (see figure 17D).

The second way the VOR response changed was the initial overshoot in the eye velocity became exaggerated or diminished. This can be seen in figure 16. During optically induced motor learning to x2.0 spectacles, Cat R’s VOR response to contralesional rotations showed an increased overshoot. Similarly, during ipsilesional rotations or during the adaptive period to x0.25 spectacles of Cat R, the VOR response showed a decrease overshoot. This was measured by calculating the dynamic index and plotting it against the VOR gain. Figure 18A shows the dynamic index was higher during contralesional rotations than during ipsilesional rotations. The asymmetry of the dynamic index did not recover during the 3.5 months of recording. During optically induced motor learning to x2.0 and x0.25 spectacles, the dynamic index increased and decreased, respectively (Student’s t-test, p < 0.001, n=8) in Cat R. In contrast, the dynamic index from Cat Z did not vary from values collected before wearing spectacles (see figure 18C).
3.2.2 VOR response to sinusoids

The frequency response of the VOR was found by oscillating Cat Z using sinusoids (0.5-8.0Hz) with a peak head velocity of 10°/s. The VOR was linear within these parameters [41]. She was oscillated before and after her canal plug surgery, then during her x0.25 spectacle wearing period and the spectacles was temporarily removed during recording. The normal VOR gains during the low frequencies were higher than during the high frequencies (see figure 19A). Figure 19B shows the corresponding phase at the various stimulating frequencies. A small phase lead was present at 0.5Hz. During the other frequencies, a phase lag was present. The phase lag increased as the frequency increased.

The VOR gain increased during the first 6 days after the canal plug surgery (figure 20A and figure 20B). Figure 20C and figure 20D shows the time course of the phase during the first 20 days after the canal plug. Immediately after the canal plug, an increased phase lead occurred at all the frequencies.
Figure 20: The time course of the VOR gain and phase during sinusoidal oscillations after a canal plug surgery. The first 20 days after the canal plug surgery is shown. The VOR gain time course during 0.5Hz (A) and 8.0Hz (B) sinusoidal oscillations. The VOR phase time course during 0.5Hz (C) and 8.0Hz (D). The points left of the dotted vertical line were used to calculate the modification index.
After plugging the canal, the VOR response during sinusoidal rotations between 2.0-8.0Hz became asymmetric. The VOR gain during ipsilesional and contralesional head rotations were no longer the same. Just like the VOR response during velocity pulses, the VOR gain during ipsilateral head rotations was smaller than contralateral head rotations. Figure 20A and figure 20B shows the time course of the VOR gain during sinusoidal rotations at 0.5Hz and 8.0Hz. The VOR gain was asymmetric at 8.0Hz and not at 0.5Hz. The Student's t-test showed during the first 29 days after the canal was plugged, the VOR gain was symmetric during sinusoidal rotations at 0.5Hz and 1.0Hz ($p > 0.1$, $n=22$) and asymmetric during rotations between 2.0-8.0Hz ($p < 1.0 \times 10^{-5}$, $n=22$). The asymmetry was more prominent at high frequencies.

Figure 21 shows the average VOR gain and phase at least 30 days after the canal plug surgery. 30 days was chosen, because the time course of the VOR gain after a canal plug in a previous cat from this lab could be approximated by two exponential functions. The shorter time constant ranged from 28-42 hours and the longer time constant ranged between 20-30 days. Between 30-154 days after the canal was plugged the VOR gain was symmetric during rotations between 0.5-2.0Hz (Student's t-test $p > 0.01$, $n=11$) and asymmetric during rotations between 5.0-8.0Hz (Student's t-test $p < 0.01$, $n=11$). The compensated VOR gain during rotations at 2.0-8.0Hz was not significantly different from the normal VOR gain before the canal was plugged (Student's t-test $p > 0.01$, $n=11$). However the compensated VOR gain during low frequency rotations at 0.5-1.0Hz was significantly lower than the normal VOR gain before the canal was plugged (Student's t-test $p < 0.002$, $n=11$). In the worst case, at 0.5Hz the VOR gain returned to 80% of normal. A decrease phase lag ranging from 5°-15° was present at all the frequencies after the canal was plugged (see figure 21B).
Figure 21: VOR frequency response after a canal plug surgery

The average VOR gain (A) and phase (C) at each frequency during sinusoidal rotations of Cat Z calculated from data collected at least 30 days after the canal plug surgery (n=11).

When Cat Z wore x0.25 spectacles, most of the gain changes occurred during the first hour of wearing spectacles. Figure 22 shows the average VOR response after wearing the spectacles for at least 2 days. After wearing x0.25 spectacles, the VOR gain showed larger changes at the low frequencies than at the high frequencies. The gain decreased to between 56%-18% of baseline gain. The phase lead increased between 3°-24° except at 0.5Hz in which the phase lead decreased by 4° during optically induced motor learning to x0.25 spectacles. The greatest phase lead increase occurred at the high frequencies.

### 3.2.3 VOR response to sum-of-sines

The sum-of-sines stimulus was used in case the cat could not stay awake long enough to be rotated at each of the sinusoidal frequencies, immediately after the canal plug surgery. Sum-of-sines data were not obtained before Cat Z's
Figure 22: The VOR response to sinusoidal rotations after wearing spectacles for two days. Average VOR gain and phase during sinusoidal oscillations after Cat Z wore x0.25 spectacles for at least 2 days (n=3). The VOR gain and phase during Ipsilesional and contrallesional rotations were averaged. Standard deviations are shown.

canal was plugged. Cat Z was able to stay awake immediately after the canal plug surgery. Figure 23A shows the stimulus and VOR response 12 hours and 6 days after the plug. Twelve hours after the canal was plugged, the VOR gains during sum-of-sines rotations ranged from 0.38 at 0.5Hz, to 0.51 at 8.0Hz (figure 23B to figure 23D). The time course of the VOR gain was similar to the sinusoids. For both stimuli, the VOR gain increased and reached a peak 6 days after the plug. The correlation coefficient relating the VOR gain during sinusoids and sum-of-sines stimulation ranged from 0.6-0.8 (n=25). The average VOR gain 30 days after the canal plug surgery (n=5) was the same as the data collected from sinusoidal oscillations (see figure 23E and figure 21A).
Figure 23: The VOR response to sum-of-sines stimulation, after a canal plug surgery.
(A) Eye and head velocity trace at 12 hours and 6 days after the canal plug surgery. The time course of the VOR gain during the first 20 days after the canal plug surgery 0.5Hz (B), 8.0Hz (B), 1.0Hz (C), 2.0Hz (C) and 4.0Hz(D). The average VOR gain taken at least 30 days after the surgery (E). Points left of the dotted vertical line was used to calculate the modification index.
Figure 24: Eye movement evoked by current pulses

(A) Averaged evoked eye velocity responses to current pulses (300µA) before and at least 72 hours after Cat R wore x2.0 and x0.25 spectacles. (B) Averaged evoked eye movement to current pulses (400µA) before and 5 days after the canal plug surgery, in Cat Z is shown. The small vertical line is the time of the current pulse. The entire time course (C) and the first 20 days (D) of the peak eye velocity evoked by current pulses in Cat Z after a canal plug surgery. Points left of the dotted vertical line were used to calculate the modification index.
Table 3: Latency of the evoked eye response after the current pulse

<table>
<thead>
<tr>
<th>Cat</th>
<th>experiment</th>
<th>latency of the onset</th>
<th>latency of the peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>spectacles</td>
<td>7.3ms±0.1SD</td>
<td>14.1ms±1.1SD (n=22)</td>
</tr>
<tr>
<td>Z</td>
<td>spectacles</td>
<td>6ms±1SD</td>
<td>13ms±1SD (n=9)</td>
</tr>
<tr>
<td>Z</td>
<td>canal plug</td>
<td>7.1ms±0.5SD</td>
<td>13.8ms±0.5SD (n=14)</td>
</tr>
</tbody>
</table>

3.2.4 Evoked eye movement caused by current pulses

All the data collected from the rotational stimuli have been shown above. The last set of data needed to calculate the modification index is the eye movements induced by current pulses. The biphasic current pulse was applied at 10ms. The cats were stimulated at twice the threshold current, 300μA for Cat R and 400μA for Cat Z. The peak eye velocity evoked by the current pulse increased or decreased by about 2°/s from normal, during optically induced motor learning (see figure 24A). In contrast, five days after the canal plug, the peak eye velocity increased by about 6 times the normal peak eye velocity (see figure 24B). The normal peak eye velocity was 2.4°/s±1.4SD (n=14). The peak eye velocity did not remain high, but decreased to an average of 9.7°/s±1SD (n=5), about 4 times the normal peak eye velocity between 30-110 days after the canal plug surgery (see figure 24C). The peak eye velocity stimulated by the current pulse between 3-15 days after the canal plug surgery was significantly different (Student’s t-test 400μA, p < 0.02, n=13, see figure 24D).

Table 3 shows the latency of the evoked eye movement and the peak eye velocity after the current pulse. In general, after the onset of the head velocity, 7ms pass before the eye moves, and 14ms pass before the eye velocity peaks. The modification indices at the time of the peak eye velocity will answer the question whether
the disynaptic pathway is modifiable.

3.3 Modification index

To determine if the disynaptic pathway was modifiable during motor learning, a metric called the modification index ($MI$) was calculated.

\[ MI(t) = \frac{d\dot{E}}{dG}(t) \]

where $\dot{E}$ is the eye velocity and $G$ is the VOR gain. To calculate the modification index, either we used all the data while the cat wore spectacles or the first week of data after the canal plug surgery. One week was chosen because the VOR gains from the various stimuli and the peak eye velocity evoked from the current pulses were generally increasing. In the case of the canal plug surgery, the eye velocity traces corresponding to the points left of the dotted vertical line in figure 24D were used to calculate the modification index. The VOR gains used to calculate the modification index were either from the velocity pulses, sinusoids or sum-of-sines rotations. The VOR gain calculated from the sinusoids and the sum-of-sines were used to determine if the evoked eye velocity from the current pulse was correlated with changes of the VOR gain at a particular frequency. The data points left of the vertical dotted line in figure 17A, figure 20A-B, and figure 23B-D were used. The evoked eye velocity from the current pulses was plotted against the concurrent VOR gains.

At every 0.1ms, the slope of the regression line relating eye velocity and gain was found. Figure 25 shows cross sections along the time axis every 5ms, starting 5ms before the current pulse to 35ms after the current pulse when the VOR gain was calculated from velocity pulses. At $t=0$ the current pulse starts. Figure 25A shows the series of cross sections from Cat Z after the canal plug. Figure 25B shows the series of cross sections from Cat R during optically
A. Recovery from a Canal Plug

Figure 25: Calculation of the modification index: Time slices
Cross sections along the time axis and the regression line are shown every 5ms. The current pulse was applied at t=0ms. (A) The regression lines after the canal plug surgery from Cat Z during 400μA. (B) The regression lines during optically induced learning from Cat R during 300μA.

61
Figure 26: The modification index using VOR gain from velocity pulses. The VOR gain was calculated from the velocity pulse stimulus. (A) The modification index of Cat R during optically induced motor learning (x2.0 and x0.25 spectacles) is shown. (B) The modification index of Cat Z during optically induced motor learning (x0.25 spectacles) is shown. (C) The modification index of Cat Z, calculated from data collected up to 1 week after the canal plug surgery. The vertical line at 10ms indicates the current pulse stimulus. An eye velocity trace evoked by the current pulse is also shown.

Induced motor learning. 5ms before, during and after the current pulse the eye velocity remained relatively constant at 0°/s, during the days the VOR gain was recalibrating. Therefore, the slope of the regression line or the modification index is about zero. 10ms after the current pulses and onwards the slope of the regression line was non-zero. The modification index was larger following a canal plug than during the spectacle experiments. The difference is best seen at t=15ms. This was also seen when the modification index was calculated using the VOR gain calculated from sinusoids and sum-of-sines.
The differences between the modification index after a canal plug and during optically induced motor learning is easier in figure 26. Figure 26 shows the time course of the modification. Figure 26A and figure 26B show the modification index for optically induced learning from Cat R and Cat Z. Figure 26C shows the modification index from Cat Z after the canal plug. The current pulse is shown at 10ms. An evoked eye velocity trace from current pulses is also shown. During the first 5ms before and after the current pulse the modification index was zero. The peak modification index after the canal plug is about 5 times greater than during optically induced motor learning. The initial onset and the peak of the eye velocity is thought to be cause by the VOR disynaptic pathway [7]. The modification index at the time of the peak eye velocity is less than 4°/s during optically induced motor learning. In contrast, the modification index is about 30°/s after a canal plug, at the time of the peak eye velocity. When the VOR recovers from a plugged canal the disynaptic pathway increases transmission more than during optically induced motor learning.

To determine if the modifiability of the disynaptic pathway was frequency dependent, I calculated the modification index using the VOR gain calculated from each of the frequencies of the sinusoidal oscillations and from the sum-of-sines oscillations. Figure 27 shows the time traces of the modification index between 0.5-8.0Hz, when the gain was calculated from sinusoidal oscillations of particular frequencies. The modification index at the time of the evoked peak eye velocity was greater after a canal plug than during optically induced motor learning. The modification index at the evoked peak eye velocity ranged from about 14-30°/s between 0.5-8Hz after the canal plug (see figure 27A). All of the modification indices were less than 20°/s except at 5Hz. The modification index during optically induced motor learning was variable across all frequencies (see figure 27B). The
Figure 27: The modification index using VOR gain from sinusoidal rotations. The time course of the modification index calculated when the VOR gain was calculated from sinusoidal oscillations of particular frequencies. (A) The modification index calculated after the canal plug in Cat Z (n=14). (B) The modification index calculated from Cat Z during the spectacle experiments (x0.25)(n=9). An evoked eye movement from the current pulse is shown as a dotted line. The vertical line at 10ms represents the current pulse stimulus.
variability may have been due to the small number of data points (n=9) used to calculate the modification index. At 8.0Hz the modification index at the time of the peak eye velocity was greater than at the rest of the frequencies. At 7Hz the modification index became negative during the activation of the disynaptic pathway. It is unknown why the modification index would become negative at 7Hz and not at the other frequencies. This may be a peculiarity of the cat. When Cat Z wore spectacles some damage to her cornea was present. The damage to her cornea may have contributed to the negative modification index seen at 7Hz. Further studies are needed to verify if this data is unique only to Cat Z.
Figure 28 shows the time course of the modification index, when the VOR gain was calculated from the sum-of-sines stimulus after the canal plug. The modification index at the peak of the evoked eye movement ranged between 10-20°/s. The modification index did not depend on frequency.
Figure 29: The modification index at the peak eye velocity

The modification index at the time of the peak eye velocity evoked by the current pulse, from all the modification indices shown.

The modification index at the time of the peak eye velocity was greater after a canal plug than during optically induced motor learning. The modification index did not show a preference for high or low frequencies after the canal plug surgery.

In addition to calculating the modification index, the correlation coefficient between the peak eye velocity and the VOR gain during velocity pulses, sinusoids and sum-of-sines rotations was calculated (see figure 30). The correlation coefficient was calculated to show whether the changes in the peak eye velocity evoked by the current pulse was related to the concurrent changes in the VOR gain. The correlation coefficient was calculated using the data collected during the first week.
Figure 30: Correlation coefficients

The correlation coefficient relating the peak eye velocity evoked by current pulses and VOR gain during velocity pulses, sinusoids and sum-of-sines after a canal plug and while the cats wore spectacles.

after the canal plug surgery (Cat Z n=13), and all the data during optically induced motor learning (Cat R n=22, Cat Z n=9). This is the same data set used to calculate the modification index. The correlation coefficients after the canal plug were mostly between 2–4 times greater than while Cat Z wore spectacles.
4 DISCUSSION

At the beginning of this study, I asked the question whether the disynaptic pathway was modifiable after a canal plug and transmitted high frequencies. The results showed the disynaptic pathway was more modifiable after a canal plug than during optically induced motor learning. This could be seen in figure 26 and figure 29 by the larger modification index value and in figure 30 by the higher correlation coefficient value. High frequencies were also found to be transmitted along the disynaptic pathway, although the modification of the disynaptic pathway after a canal plug did not show a preference to high or low frequencies. Figure 29 and figure 30 showed the modification index and correlation coefficients were larger after the canal plug than during the spectacle experiments. The study exhibited evidence suggesting the neural mechanisms during optically induced motor and after a canal plug had similarities and differences. In addition, further division of the VOR frequency response during high and low frequency stimuli was prominent. This can be seen in figure 21 and figure 22. Figure 21 shows the recovery of the asymmetry at low frequencies but not at high frequencies and the recovery of the gain at high frequencies but not at low frequencies. Figure 22 also shows the VOR gain changes more at low frequencies than at high frequencies during optically induced motor learning.

The modification index showed the disynaptic pathway was more modifiable after a canal plug than during adaptation to spectacles. The earliest portion of the evoked eye response to the current pulse which includes the onset and initial peak was caused by the activation of the disynaptic pathway [7]. The modification index relates the change in horizontal eye velocity to changes in the VOR gain. During the activation of the disynaptic pathway, the modification index was greater after a canal plug than during optically induced learning. The small changes in the disynaptic pathway during optically induced motor learning were also found by Broussard at al. [7]. In addition, the modification index during optically induced
motor learning was larger after the activation at latencies longer than the disynaptic pathway. This was also seen by Broussard et al. [7].

The large changes in the disynaptic pathway following an injury suggest a different mechanism from that used during optically induced motor learning was employed by the VOR to recover. The independent methods of motor learning are seen by the ability of the VOR to continue learning after recovering from a canal plug. Five months after the canal plug surgery, the VOR had compensated but optically induced motor learning could still occur. This was also seen in other cats in the lab (unpublished). Two previous cats in the lab wore x2.0 and x0.25 spectacles before and after compensating from a unilateral canal plug. The gain changes before and after the canal plug surgery were about the same when the cat wore x2.0 spectacles. The gain change after compensating from the x0.25 spectacles was less than before the plug.

The disynaptic pathway is more modifiable after a plugged canal than during optically induced motor learning. Two disynaptic pathways exist, either including the FTNs or the PVPs as the secondary vestibular neurons. The FTNs have been found to increase or decrease firing activity when the VOR adapts to telescopic lenses and are probably involved during VOR adaptions [38]. The disynaptic pathway containing the PVPs may contain a site of learning after a canal has been plugged.

During optically induced motor learning, the VOR gain during low frequencies changed more than during high frequencies. This was seen in monkeys [36] [56] and in other cats from the same lab (unpublished). Although my cat (Cat Z) was only able to adapt during x0.25 spectacles after the canal plug surgery, other cats in the lab were able to adapt to x2.0 and x0.25 spectacles after their canal plug surgery. Their VOR was also able to adapt more so at the low frequencies than at the high frequencies. In contrast, the VOR gain increased across all frequencies after a canal plug surgery and only recovered at high frequencies. The recovery
mechanisms following a canal plug surgery may encourage changes to occur during high frequency VOR, while concurrently hampering the full recovery of the VOR low frequency response. Frequency-selective channels in the VOR may exist [34] [56].

The increase VOR gain during high frequencies after a canal plug, seen in figure 20 is probably due to a number of causes. Some of the VOR gain occurring at high frequencies is caused by residual functioning of the plugged canal. A VOR gain of 0.2 during 8Hz, but not at 2Hz sinusoidal oscillations was found to occur after a cat had a bilateral horizontal canal plug ([6] in review). The existence of a VOR gain at the high frequency supports one of the predictions made by Rabbit's model of the semicircular canals [55]. If the primary afferents are not damaged during the canal plug surgery, then at high frequencies the flexion, in the walls of the canal allows the vestibular hair cells to react.

Since the horizontal and vertical semicircular canals are not quite orthogonal [3], the vertical canals may contribute to the VOR response during rotations about a vertical axis. However, no contribution from the vertical canals was observed at frequencies between 2-8Hz in one cat after a bilateral canal plug ([6] in review). The VOR gain was found not to depend on the pitch angle (rotation about the interaural axis) either, at 2Hz nor at 8Hz. At frequencies below 1Hz the vertical canals contribute the horizontal VOR [1]. The remaining component of the VOR gain is caused by something else.

Primary vestibular afferents exhibit an enhanced high frequency gain [24]. Neurons in the medial vestibular nucleus have been shown able to transmit high and low frequencies. Each neuron acts as a low pass filter, and the corner frequency is related to the spontaneous firing rate of the neurons. One such experiment was performed in rat brain slices [15].

In addition, the quick VOR gain increase after the canal plug was partly due to the hyperactivity of the cat immediately after the surgery. Exercise decreases
the recovery time of vestibular compensation after a unilateral vestibular lesion [28]. The balance normally seen during locomotion is regained sooner if physical exercise is performed.

Although these experiments were unable to show that the modification of the disynaptic pathway was more modifiable at high frequencies than during low frequencies, they do not rule out that possibility. The VOR gain fully recovered at high frequencies, and partially recovered at low frequencies, after the canal was plugged. This suggests the recovery of the VOR gain during high and low frequency oscillations require at least one element that is not shared during recovery. VOR gain asymmetry was able to recover during low frequency oscillations, but not during high frequency oscillations after a canal plug surgery. This suggests information from both vestibular nuclei is needed to regain VOR symmetry during low frequencies but not at high frequencies.

Information about the contralateral vestibular nuclei can be transmitted over the vestibular commissure. The feedback loops composed of the type I and type II neurons across the vestibular commissure [61], may provide the communication needed to regain VOR symmetry during low frequencies. Immediately following a labyrinthectomy, the vestibular imbalance can be seen by the presence of a spontaneous nystagmus and a postural tilt. The re-establishment of the spontaneous resting rate of type I neurons on both sides of the vestibular commissure, [63] [64] and the sensitivity of the ipsilesional neurons [51] after a labyrinthectomy quickly reduces the amount of static disturbance. The ability of the type I and type II neurons to regain symmetry may aid in the recovery of the VOR during low frequencies. More time may be needed for these neurons to recover and regain their dynamic properties and aid the VOR. Since neurons in the medial vestibular nucleus can transmit high and low frequencies, neurons involved in the commissural pathway may either only transmit low frequencies and/or the commissure contains some other effective low pass filter. Galiana and Outerbridge's (1984) [23] model
included a low pass filter as part of the commissure. The nucleus prepositus hypoglossi has been suggested as being the neural integrator in the horizontal VOR [42]. The nucleus prepositus hypoglossi does have the appropriate connections, receiving information from the contralateral vestibular nerve at disynaptic latencies [2]. In addition, recently, fast mechanical indentations of the cupula have been recorded on the contralateral side as a low frequency signal [13].

The regained symmetry of the VOR during low frequency stimulation may involve the vestibular commissure. The non-recovery of the VOR symmetry during high frequencies suggests communication between the vestibular nuclei does not occur. The neurons in the medial vestibular nucleus are able to transmit high and low frequencies. Signals entering the vestibular commissure are either filtered by a low pass filter or are only low frequency signals. The high frequencies may be transmitted through the disynaptic pathway, bypassing the commissural route.

In 1986, Lisberger and Pavelko [36] found low VOR gains during velocity pulses to be associated with high dynamic indices. We found this was not case in Cat R, but was so in Cat Z. Other data (unpublished) collected from this lab have shown data showing the same trend as Cat R. There does not seem to be a clear relationship associating the VOR gain and the dynamic index. The findings of Lisberger and Pavelko probably represent the trend seen the majority of the time, while results similar to Cat R are seen the minority of the time. This difference may manifest itself as other subtle peculiarities in the data collected.

After Cat Z wore x0.25 spectacles the frequency response of the VOR showed an increase phase lead at most of the frequencies (see figure 22). The phase lead was greatest at the high frequencies. This was also seen by Lisberger et al. (1983) [34] when monkeys wore x0 spectacles. Primary afferents with an average high phase leads [17] may provide the input for the high frequencies.

One problem encountered during the course of the experiment was the sleepiness of the cat. Some of the data showed exhibit variability. This was probably
caused by sleepiness. Most of the variable data after the canal plug surgery oc-
curred after the first week. These data were not included in the data used to calcu-
late the modification index or the correlation coefficients, and would not affect our
results.

In summary, different mechanisms are employed by the VOR during optically
induced motor learning and after a plugged canal. The disynaptic pathway is mod-
ifiable after surgically plugging a canal. The disynaptic pathway was modifiable at
all frequencies. The VOR appears to process low and high frequencies differently.
Glossary

**ampulla**  An enlargement of the semicircular canals that contains the sensory epithelium.

**disynaptic pathway**  Shortest VOR pathway consisting of 3 neurons.

**dynamic index**  Ratio of the peak to the steady state eye velocity during a velocity pulse stimulus.

**EHV**  Eye head velocity neuron in the vestibular medial nucleus, that is thought to contain FTNs.

**FTN**  Flocculus target neuron.

**modification index**  Ratio of the eye velocity evoked by a current pulse per unit gain change.

**nystagmus**  Side to side movement of the eyes consisting of a quick movement to one side followed by a slower movement to the other side.

**phase**  In this study, it is the angle the eye velocity leads the head velocity.

**Purkinje cell**  The sole output of the cerebellum flocculus, that projects to the FTNs.

**PVP**  Position vestibular pause neuron.

**saccade**  An eye movement that quickly brings the target object to the fovea.

**threshold current**  The smallest current applied to the labyrinth to evoke an eye movement.

**type I**  A vestibular neuron that increases activity during ipsilateral rotations, and decreases activity during contralateral rotations. See also type II.
type II A vestibular neuron that decrease activity during contralateral rotations, and increases activity during contralateral rotations. See also type I.

VOR gain Ratio of the eye velocity to the head velocity. During a velocity pulse stimulus, the gain is the ratio of the averaged eye velocity to the averaged head velocity during the steady state portion. During a sinusoid stimulus, the gain is the ratio of the peak eye velocity to the peak head velocity.
References


[8] DM. Broussard, C. DeCharms, and SG. Lisberger. Inputs from the ipsilateral and contralateral vestibular apparatus to behaviourally characterized ab-


