

Research report

## Vestibular compensation in the horizontal vestibulo-ocular reflex of the goldfish

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### Abstract

Vestibular compensation is the process whereby vestibular system function is restored following unilateral removal of the vestibular receptors (hemilabyrinthectomy). Vestibular compensation was studied in the horizontal vestibulo-ocular reflex (VOR) of the goldfish. Spontaneous VOR (spontaneous nystagmus) was not observed in the goldfish following recovery from the surgery for hemilabyrinthectomy (a period of about 30 min). However, hemilabyrinthectomy resulted in an acute decrease in the gain of the horizontal VOR to approx. 50% of normal, and an increase in phase lead for mid-range frequencies (0.05 to 0.5 Hz). After 1 week of compensation, VOR gain had increased toward normal, and phase lead had returned to normal levels for mid-range frequencies, but increased above normal at low frequencies. After 1 month of compensation, horizontal VOR gain had recovered its normal value for head rotational velocity up to 60 deg/s, but it appeared to saturate for higher head velocity, and phase lead had decreased to normal, and even slightly below normal, at low frequencies. The results suggest that the goldfish is capable of almost completely recovering both the gain and phase of the horizontal VOR following 1 month of compensation for hemilabyrinthectomy. The extent of compensation in the horizontal VOR of the goldfish is greater than that which has been reported for mammals.

**Keywords:** Hemilabyrinthectomy; Vestibular compensation; Vestibulo-ocular reflex; Goldfish; Histological verification; Magnetic search-coil technique; Frequency response analysis

### 1. Introduction

Vestibular compensation is 1 of the most well-studied paradigms in motor learning [13,24,26]. It is the plastic process whereby the functioning of the vestibular system is brought back toward normal following the removal of one member of the pair of vestibular receptors. This paper reports on the nature and extent of vestibular compensation in the horizontal vestibulo-ocular reflex (VOR) of the goldfish.

The vestibular receptors in most vertebrates comprise the three semicircular canals (lateral, anterior, and posterior), which transduce rotational acceleration, and the two otoliths (utricle and saccule), which transduce linear acceleration [29]. There is one complete set of receptors

on either side of the head. The vestibular receptors are known anatomically as the labyrinth, and their surgical removal from one side is called hemilabyrinthectomy. The vestibular receptors provide the input to reflexes that stabilize posture and gaze, and hemilabyrinthectomy produces severe deficits in the performance of those reflexes [13]. With time, the deficits can be compensated to varying degrees.

The function of the VOR is to stabilize the retinal image during head rotations by producing counterbalancing eye rotations [30]. Compensation for hemilabyrinthectomy has been studied in the VOR, for rotations in the horizontal plane, in mammals which include the guinea-pig [28], rabbit [5], cat [17], monkey [12,31] and human [16]. Hemilabyrinthectomy unbalances the vestibular input to the VOR, and this causes the VOR to produce eye rotations in the absence of head rotations.

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These spontaneous VOR eye rotations are nystagmic, with slow- and fast-phases directed towards and away from the side of the lesion, respectively. Spontaneous VOR (spontaneous nystagmus) is eliminated by the compensatory process in a matter of days in mammals.

The dynamics of the VOR primarily reflect those of the semicircular canals that provide its input (the lateral canals drive the horizontal VOR). As such, the frequency response dynamics of the VOR can be described approx. as those of a high-pass filter [30]. At any frequency ( $\omega$  in rad/s or  $2\pi f$  with  $f$  in Hz), a sinusoidal head rotational velocity of the form  $A \sin(\omega t + \phi)$ , in which  $A$  is the rotational velocity peak amplitude and  $\phi$  is the phase, will produce a VOR slow-phase eye velocity response that will also be roughly sinusoidal. For analysis purposes, either the head or eye velocity sinusoid is inverted to remove the factor of  $-1$  between them, and the VOR amplitude ratio or gain (peak eye velocity/peak head velocity), and phase difference (eye velocity phase – head velocity phase) are calculated. As for a high-pass filter, VOR gain increases and phase lead decreases as frequency increases.

Hemilabyrinthectomy removes one half of the input to the VOR and accordingly reduces VOR gain. It also causes phase lead to increase, presumably by disrupting central VOR neuronal circuitry. In mammals, the compensatory process is able to partially restore VOR gain, but it is unable to reduce phase lead from its increased level acutely post-lesion [5,12,16,17,28,31].

By altering the normal relationship between head rotation and rotation of the visual surround, plastic changes in VOR function were brought about in goldfish that were larger than those that had been reported in any other species [22,25]. It is therefore of interest to see whether the goldfish VOR can also compensate for hemilabyrinthectomy to a greater extent than has been observed in mammalian species. Although compensation for postural deficits following hemilabyrinthectomy have been studied in the goldfish [9,10,18,19], compensation in the VOR has not yet been studied in this species. The purpose of this study was to describe the nature and extent of compensation in the horizontal VOR of the goldfish.

## 2. Materials and methods

### 2.1. Animals

Four comet goldfish (*Carassius auratus*), 15 to 18 cm in length, were purchased locally and kept in large (40 gallon) aquaria. The aquarium room was illuminated with fluorescent lights on a 12-h/12-h day/night cycle and maintained at 21°C. The horizontal VOR was tested

in fish that were intact, and at various times following surgical removal of the vestibular receptors from the left side (left hemilabyrinthectomy).

### 2.2. Surgical procedures

Prior to surgery, each goldfish was immersed for 20 min to 30 min in fish-tank water with 80 mg/l dissolved tricaine methanesulfonate anesthetic (MS-222, Sigma). It was then wrapped in wet gauze and secured in a small restraining tank using contoured body supports. Its mouth was opened over a plastic tube and secured to it with a loop of string. Anesthesia solution was pumped through the tube over the fish's gills (200 ml/m).

The surgical procedure was a modification of the partial hemilabyrinthectomy procedure in goldfish described by Ott and Platt [18]. The overlying skin was scraped away and a drill was used to make an opening in the skull caudal to the eye and just dorsal to the opercular bone articulation. This exposed the vestibular labyrinth. The utricular sac and the three attached semicircular canal ampullae were removed using forceps. The saccule was not accessible using this approach and was not removed. Fluid was cleared from the cavity using gentle suction, and a warm solution of sterile, high gel-strength agar (2% in fish Ringer's) was deposited therein with a Pasteur pipette and allowed to set. The fish was revived in fresh water for about 30 min prior to acute stage experimentation.

The horizontal VOR was tested in each fish pre-op (normal) and immediately (acute), 1 week, and 1 month post-op. Normal, 1 week, and 1 month fish were taken from the aquarium and secured in the restraining tank using body supports and mouth tube as described above. This procedure completely immobilized the fish's body and rigidly coupled its head to the restraining tank. A small coil of Teflon-insulated copper wire (20 turns, 5.3 mm external coil diameter) was attached to the left eye. The eye coil was concentric with the pupil and was attached to the conjunctiva at the perimeter of the iris using ophthalmic suture (6-0 monofilament). The fish eye is relatively insensitive and coil attachment did not require the use of either local or general anesthetic.

### 2.3. Stimulation

The fish and restraining tank were placed within an experimental apparatus that was mounted on board a servo-controlled rotating table (40 ft/lb, Trio-Tech International). The apparatus was equipped with a shock-mounted pump and reservoir of fresh fish-tank water with which the fish was artificially respired for the approx. 2-h duration of each experiment. Magnetic field generating coils and an eye coil preamplifier were also mounted on the apparatus. The rotating table and

experimental apparatus were located inside a Faraday cage. To exclude visual input during experiments the room lights were extinguished, and both the experimental apparatus and the Faraday cage were covered with black plastic shrouds.

Sinusoidal rotational stimuli were delivered at nine different frequencies (0.01, 0.03, 0.05, 0.1, 0.3, 0.5 and 1.0 Hz) at an amplitude setting of 60 deg/s peak rotational velocity. Stimuli at 0.05 and 0.5 Hz were also delivered at amplitude settings of 30 and 90 deg/s peak rotational velocity. Servo reference signals were provided by a high precision, analog function generator (Hewlett-Packard 3326A). The rotator tachometer signal was calibrated during constant velocity rotations by measuring the time intervals between synch pulses that occurred once per revolution.

The dynamics of the entire rotator/apparatus assembly was evaluated by comparing the tachometer signal with the reference signal to the rotator servo. This evaluation revealed a resonance in rotator/apparatus dynamics, which accounted for the fact that rotator velocity was a few deg/s higher at 0.5 and 1.0 Hz than at the lower frequencies, given the same amplitude setting on the function generator for the servo reference signal. The dynamics of the experimental apparatus during rotation were evaluated by mounting a rotational velocity sensor (Watson Industries) on the restraining tank and delivering sinusoidal rotations. The rotational velocity sensor signal was undistorted, and the gain and phase of this signal relative to the rotator tachometer signal were flat, over the stimulus frequency and amplitude ranges employed. This indicated that the apparatus did not contribute its own dynamics over the stimulus range, and that the tachometer signal provided an accurate measure of the rotational velocity of the restraining tank, and so also of the head of the goldfish that was rigidly coupled to it.

#### 2.4. Data acquisition

The eye coil signal was preamplified on board the rotator and transmitted over the rotator slip-rings to a demodulator [23]. Demodulator offset was adjusted to give 0V when the eye position was midway between the maximum and minimum eye positions recorded during several minutes of spontaneous eye movement. This insured that the demodulator worked in its linear range, but did not necessarily provide an accurate measure of the fish's resting eye position. Accurately measuring the angle of resting eye position was not necessary for this study in which the focus was on the relationship between eye and head velocity. Demodulator sensitivity was adjusted to give deflections of approx.  $\pm 1$  V. Demodulator offset and sensitivity settings were locked after adjustment. After each experiment, the eye coil was

detached from the fish's eye, repositioned in the magnetic field at the same place, and calibrated with a protractor.

Goldfish eye position was recorded with the rotator at zero velocity and during the sinusoidal oscillations described above. The eye position signal from the demodulator and the rotator tachometer signal were anti-alias filtered at 25, 150 or 500 Hz, and digitized at 50, 300 or 1000 Hz, for stimuli from 0.01 to 0.1 Hz, 0.3 and 0.5 Hz or 1.0 Hz, respectively. Digitized data were stored on hard disk and tape for later analysis.

#### 2.5. Data analysis

Computerized data analysis routines were developed in the MATLAB programming environment (The MathWorks). The eye position data were digitally differentiated using a modification of the approach described by Bahill and associates [6]. A five-point central difference estimator was applied to the eye position data, and the resulting eye velocity data were then low-pass filtered (single-pole, non-causal) at half the anti-aliasing filter frequency of the sampled eye position data (see above), or at 80 Hz, whichever was smaller. The entire procedure introduced minimal phase distortion and gain attenuation over the ranges of stimulus and sampling frequency employed.

The fast-phases were then edited out of the eye velocity data using an automatic procedure following threshold setting by the user. A nonlinear least-squares routine was used to fit sine waves to the remaining slow-phase eye velocity data and to the rotator tachometer signals. At low frequencies, a relatively large number of fast-phases occurred at the peaks of the eye velocity response. Removing them left fewer points at the peaks than mid-level, and this could cause the fitting procedure to underestimate response amplitude. A graphical interface allowed the user to adjust the amplitude fit manually. The amount of amplitude adjustment never exceeded 10%. Phase measurements were not affected by sparseness of data at the peaks, and were made accurately by the fitting procedure without the need for manual adjustment. The amplitude ratio (gain) and phase difference of the eye velocity response relative to the tachometer signal (head velocity) were then computed from the fitted sine wave parameters. Eye velocity amplitude, gain, and phase, at each stimulus frequency and amplitude, were averaged over all fish tested at each stage of compensation. Mean values are reported along with the standard error of the mean (SEM). Straight lines were fit to the eye velocity or phase versus head velocity data (intensity functions) using linear regression. A nonlinear least-squares technique was used to fit transfer functions to gain and phase data as a function of frequency (frequency responses).

## 2.6. Histology

Lesions were evaluated histologically. Following the last experiment, each fish was perfused transcardially with fish Ringer's followed by formalin. The head was removed and decalcified in a solution of 1.35 M HCl and 0.003 M EDTA (Calex, Fisher) for 4 days. It was then imbedded in egg yolk and frozen sections were cut in a cryostat at 35  $\mu\text{m}$  and mounted directly onto subbed slides. The slides were stained with hematoxylin and counter stained with eosin Y.

## 3. Results

### 3.1. Partial left hemilabyrinthectomy

The results reported are based on measurements taken, in darkness, of the horizontal VOR of four comet goldfish that were intact, and acutely, 1 week, and 1 month after each had undergone surgical removal of the utricle and the three semicircular canal ampullae from the left side (partial left hemilabyrinthectomy). The surgical procedure, which had been adapted from one

described previously [18], spared the saccule. A coronal section through the decalcified skull of a goldfish that had been hemilabyrinthectomized according to this procedure is shown in Fig. 1. On the right side, the utricle (UT) and the ampulla of the lateral canal (LA) can be seen adjacent to the brainstem (BS) and optic tectum (OT). The labyrinth was removed from the left.

Acute VOR experiments began as soon after the surgery as a fish had revived from anesthesia, so that a period of 2 to 3 h had elapsed post-hemilabyrinthectomy before any fish had been returned to the aquarium. The effects of the lesion on posture and swimming at this stage were similar to, but not as severe as, those that have been described immediately post-hemilabyrinthectomy in goldfish [9,18]. Deficits included bending of the body, roll tilt, and spiral swimming toward the lesion (left) side. All of these deficits were compensated with time.

### 3.2. Spontaneous eye movements

Spontaneous horizontal eye movements in darkness were recorded from the left eye in normal fish at again at each stage of compensation. Raw spontaneous eye



Fig. 1. Coronal section through the skull of a goldfish that had undergone a surgical procedure to remove the vestibular receptors (the utricle and three semicircular canal ampullae) from the left side (see Materials and methods). The utricle and the ampulla of the lateral canal can be seen on the right but not on the left. UT, utricle; LA, lateral canal ampulla; CB, cerebellum; OT, optic tectum; BS, brain stem.

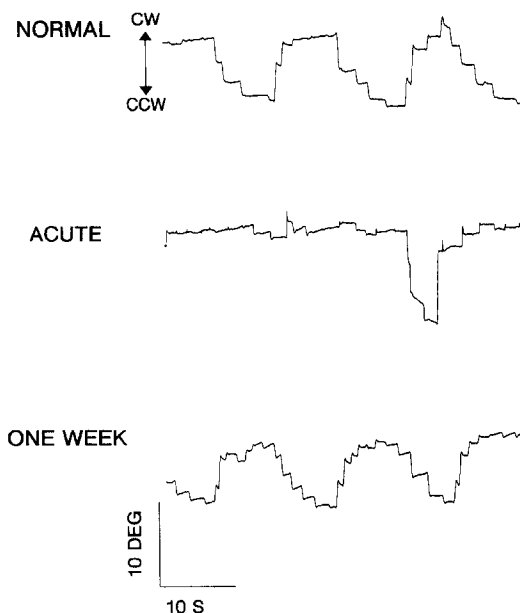


Fig. 2. Spontaneous eye movements, in darkness and in the absence of head rotation, from the same goldfish under normal conditions, and acutely and 1 week following left-side vestibular receptor removal (left hemilabyrinthectomy). All recordings were taken from the left eye, and upward deflections represent clockwise (nasal or leftward) eye rotations. This raw eye position data was low-pass filtered (double-pole, non-causal, 2.5 Hz cut-off) for the purposes of illustration only. The normal searching pattern is disrupted by hemilabyrinthectomy, and although the acute goldfish can make counterclockwise movements with its left eye, this eye is deviated in the clockwise direction. The normal searching pattern was restored after 1 week of compensation. There is a surprising lack of spontaneous VOR in the acute stage.

movement data for one of the fish is shown in Fig. 2. For each trace, upward deflections correspond to clockwise (nasally or leftwardly directed) rotations of the left eye in the horizontal plane. The normal trace shows a quasi-periodic pattern in which it appears that the eyes are scanning the environment in discrete steps. A similar pattern has been reported previously in goldfish for spontaneous horizontal eye movements [11]. The normal scanning pattern is disrupted by hemilabyrinthectomy, and although the acute goldfish can make counterclockwise rotations with its left eye, this eye is deviated in the clockwise direction. The normal scanning pattern was restored after 1 week of compensation.

Eye velocity during fixations in normal goldfish is not zero. Ocular drift during fixation, which has been described previously [11,22], was often nasally directed (clockwise in the left eye) but could also be temporally directed in normal goldfish. Acutely, left hemilabyrinthectomy should have produced intense spontaneous VOR (spontaneous nystagmus) with slow-phases directed toward the side of the lesion (counterclockwise in the left eye). Surprisingly, there was a complete lack of spontaneous VOR in the acute stage of the goldfish. Mean eye velocity during fixation (between saccades) for all fish was within  $\pm 1$  deg/s of zero, regardless of

whether the fish were normal, acute, 1 week, or 1 month post-hemilabyrinthectomy. The possibility that the compensatory process in goldfish could eliminate spontaneous VOR within the time period given for recovery from surgery (about 30 min) is currently being investigated.

### 3.3. Responses to rotation

Horizontal VOR eye movements were evoked by rotating the goldfish sinusoidally in the horizontal plane in darkness. VOR eye movements had a nystagmic pattern, in which the slow-phase eye rotations that opposed the rotation of the head were interrupted by fast-phases that reset the position of the eye in the orbit. The response to a 0.5 Hz rotation at a setting of 60 deg/s is shown in Fig. 3. Upward deflections correspond to counterclockwise head velocities and clockwise eye velocities. The amplitude of the horizontal VOR response decreases acutely and largely recovers after 1 week of compensation. Response amplitude recovers even further after 1 month (not shown).

### 3.4. Intensity functions

The amplitude of the goldfish horizontal VOR eye velocity response, and the phase of this response relative to head velocity, were measured for rotations at 0.05 and 0.5 Hz with peak amplitudes set at 30, 60 and 90 deg/s. This intensity function data was collected in goldfish under normal conditions and acutely, 1 week, and 1 month following left hemilabyrinthectomy.

Peak eye velocity as a function of head velocity is plotted in Fig. 4. Linear regression lines were fit to the data at either frequency (0.05 Hz, dashed lines; 0.5 Hz, solid lines). The slopes of these lines provide estimates of VOR gain (amplitude ratio) at their respective frequencies, and are listed in Table 1. At all experimental stages, gain was lower at 0.05 than at 0.5 Hz, as expected for the VOR high-pass filter. At both frequencies, gain decreased acutely and then increased after 1 week and 1 month of compensation. Intercepts were near zero at the acute stage (0.53 and 0.10 deg/s at 0.05 and 0.5 Hz, respectively), indicating a lack of spontaneous VOR at this stage. The mean intercept, averaged for the eye velocity versus head velocity regression lines at both frequencies for fish at all experimental stages, was also essentially zero ( $-0.61 \pm 0.72$ ).

The relationship between eye velocity and head velocity was linear for data from fish at all stages, with coefficient of correlation ( $r$ ) values ranging from 0.9473 to 0.9986. Although the greatest amount of gain recovery had occurred by 1 month, the responses at this stage departed the most from linearity, and appeared to have saturated at the higher velocity. However, gain had fully

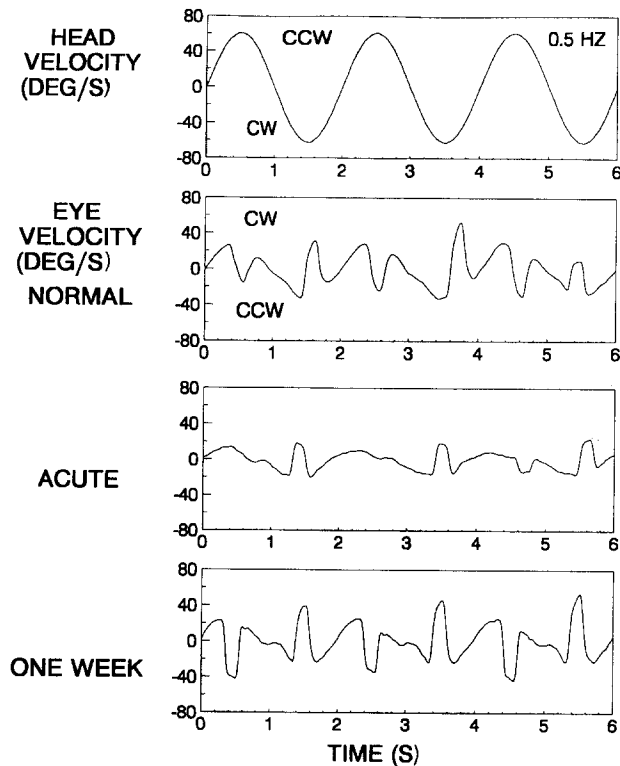


Fig. 3. Horizontal VOR eye movements in the dark, from the same goldfish as in Fig. 2, under normal conditions, and acutely and 1 week following left-side vestibular receptor removal. The sinusoidal stimulus was delivered at 0.5 Hz with a setting of 60 deg/s; positive deflections represent counterclockwise head rotational velocity. Eye rotational velocity was computed from left eye position data (see Materials and methods); positive deflections represent clockwise eye rotational velocity. The normal horizontal VOR response shows the typical alternation of fast- and slow-phases. The amplitude of the response decreases acutely but largely recovers by 1 week.

compensated for the lower and middle velocities. For head rotations at 0.05 and 0.5 Hz and a peak velocity setting of 60 deg/s, mean peak eye velocities in normal fish were  $20.52 \pm 5.63$  and  $37.96 \pm 5.12$  deg/s, respectively. For comparison, the corresponding mean peak eye velocities after 1 month of compensation were  $22.39 \pm 5.91$  and  $39.23 \pm 1.53$  deg/s. These results suggest that the hemilabyrinthectomized goldfish is capable of fully compensating horizontal VOR gain for head rotational velocity up to 60 deg/s, but for higher head velocity, unilateral loss of the vestibular receptors causes some response saturation.

Goldfish horizontal VOR phase lead as a function of peak head velocity is plotted in Fig. 5. Linear regression lines were fit to the data at either frequency (0.05 Hz, dashed lines; 0.5 Hz, solid lines), and the slopes of these lines are listed in Table 1. Phase lead at low frequencies was highly variable in goldfish. Nevertheless, clear trends emerged from the data. For goldfish at the normal, 1 week, and 1 month stages, phase lead is lower at 0.5 Hz than at 0.05 Hz, as expected for the VOR high-pass filter. Also at these stages, phase lead was unrelated to

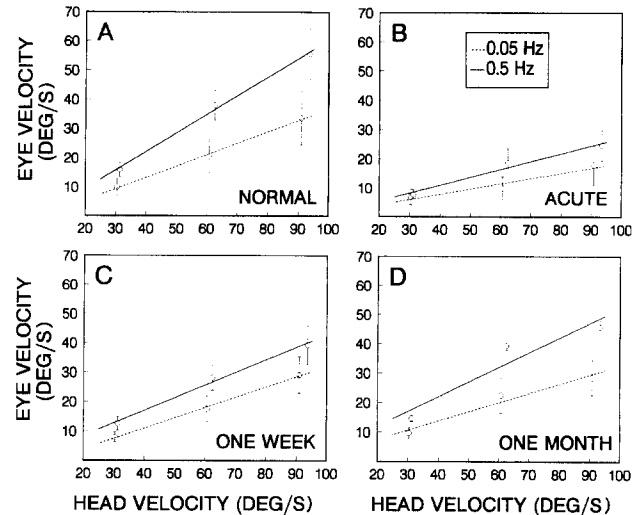


Fig. 4. Mean horizontal VOR eye velocity as a function of head velocity under normal conditions (A) and acutely (B), 1 week (C), and 1 month (D) following left hemilabyrinthectomy. Sinusoidal rotations were delivered at 0.05 or 0.5 Hz with peak rotational velocity settings of 30, 60 and 90 deg/s. Mean data are shown as open circles, and error bars represent the standard error of the mean, in this and subsequent figures. The data were fit with linear regression lines (0.05 Hz, dashed lines; 0.5 Hz, solid lines). The slopes of these lines, which are listed in Table 1, provide an estimate of gain at each frequency and stage of compensation. Gain at 0.05 Hz was lower than that at 0.5 Hz at all stages of compensation. Gain decreased from normal acutely, and gradually recovered with compensation.

Table 1

Slopes of the regression lines fit to the intensity function data for amplitude or phase of the goldfish horizontal vestibulo-ocular reflex at various stages of compensation

	Amplitude		Phase	
	0.05 Hz	0.5 Hz	0.05 Hz	0.5 Hz
Normal	0.39	0.64	-0.07	0.00
Acute	0.18	0.27	0.09	-0.09
One week	0.35	0.43	-0.14	0.02
One month	0.31	0.50	-0.12	0.01

head velocity peak amplitude at 0.5 Hz ( $r$  values ranged from 0.0427 to 0.1965), but tended to decrease as head velocity increased at 0.05 Hz ( $r$  values ranged from -0.4828 to -0.9990). A similar decrease in low frequency VOR phase lead with an increase in head velocity up to 120 deg/s has been reported in rabbits [7], squirrel monkeys [20], and pigeons [4]. These trends are disrupted at the acute stage, where phase lead has increased for both frequencies. Phase lead decreased to normal levels by 1 week and 1 month.

The intensity function data are summarized in Fig. 6, where the linear regression lines that were fit to the data relating horizontal VOR eye velocity or phase and head velocity are re-plotted. Gain, which can be estimated from the slopes of the lines relating eye velocity and head velocity at either frequency (0.05 Hz, Fig. 6A;

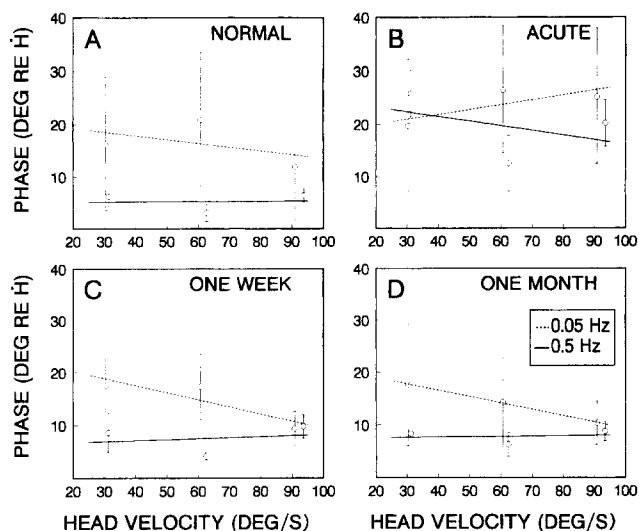


Fig. 5. Mean horizontal VOR phase lead relative to head velocity ( $\dot{H}$ ) under normal conditions (A) and acutely (B), 1 week (C), and 1 month (D) following left hemilabyrinthectomy. Sinusoidal rotations were delivered at 0.05 or 0.5 Hz with peak rotational velocity settings of 30, 60 and 90 deg/s. The data were fit with linear regression lines (0.05 Hz, dashed lines; 0.5 Hz, solid lines), and the slopes are listed in Table 1. Under normal conditions and after 1 week and 1 month of compensation, phase lead at 0.5 Hz was independent of head velocity, and was lower than phase lead at 0.05 Hz, which tended to decrease as head velocity increased. These relationships were disrupted at the acute stage, where phase lead increased at both frequencies.

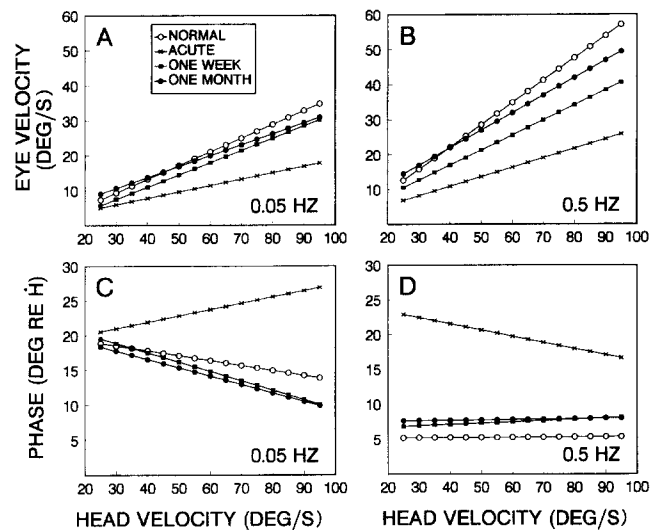


Fig. 6. Comparisons of horizontal VOR eye velocity as a function of head velocity and phase lead relative to head velocity ( $\dot{H}$ ) at various stages of compensation. Linear regression lines are reproduced from Fig. 4 and Fig. 5 for normal conditions ( $\circ$ ) and acutely ( $\times$ ), 1 week ( $\blacksquare$ ), and 1 month ( $\bullet$ ) following left hemilabyrinthectomy. (A and B) The slopes of the lines relating eye and head velocity provide an estimate of gain at each frequency (0.05 Hz, A; 0.5 Hz, B). Gain decreased from normal acutely and then partially recovered after 1 week. After one month, eye velocity matched normal for responses to head velocities near 30 and 60 deg/s, but not 90 deg/s. C and D: Phase lead increased from normal acutely and then decreased again to the compensated state after 1 week.

0.5 Hz, Fig. 6B), decreased from normal (open circles) in the acute stage (crosses), and gradually recovered after 1 week (filled squares) and 1 month (filled circles). At 1 month, eye velocity matched normal up to about 60 deg/s peak head velocity, but diverged at higher velocity (see above). Phase lead at either frequency (0.05 Hz, Fig. 6C; 0.5 Hz, Fig. 6D), increased from normal (open circles) in the acute stage (crosses), and recovered after 1 week (filled squares) and 1 month (filled circles). Phase lead in the compensated state was slightly higher than normal at 0.5 Hz, and slightly lower than normal at 0.05 Hz.

### 3.5. Frequency responses

The phase and gain of the goldfish horizontal VOR eye velocity response was measured for rotations at 0.01, 0.03, 0.05, 0.1, 0.3, 0.5 and 1.0 Hz, with peak amplitude set at 60 deg/s. This frequency response data was collected in goldfish under normal conditions and acutely, 1 week, and 1 month following left hemilabyrinthectomy. Phase and gain data were averaged at each compensatory stage and are plotted in Fig. 7 (phase) and Fig. 8 (gain, transformed by base 10 logarithm). Frequency response data were fit with a transfer function of the following form:

$$\frac{\dot{E}(s)}{\dot{H}(s)} = G \left[ \frac{s\tau}{(s\tau + 1)} \right]^k$$

in which  $\dot{E}$  and  $\dot{H}$  represent eye and head rotational velocity, respectively,  $G$  and  $\tau$  are gain and time con-

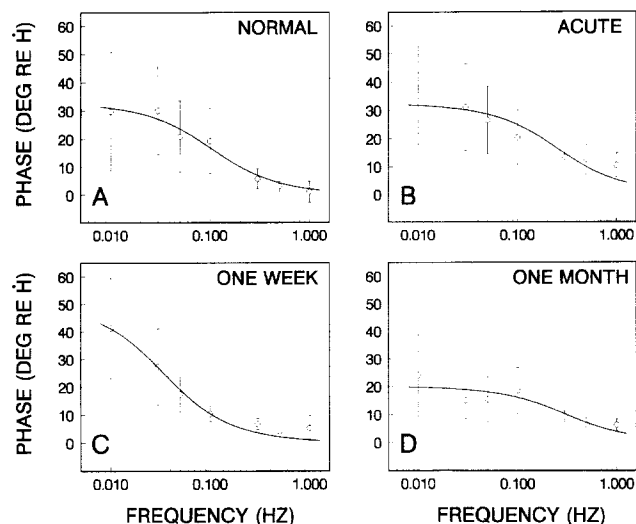


Fig. 7. Mean horizontal VOR phase lead relative to head velocity ( $\dot{H}$ ) as a function of frequency under normal conditions (A) and acutely (B), 1 week (C), and 1 month (D) following left hemilabyrinthectomy. Sinusoidal rotations were delivered at 0.01, 0.03, 0.05, 0.1, 0.3, 0.5 and 1.0 Hz, with peak rotational velocity set at 60 deg/s. A transfer function was fit to the data as described in the text (solid lines), and the best fit parameters are listed in Table 2.

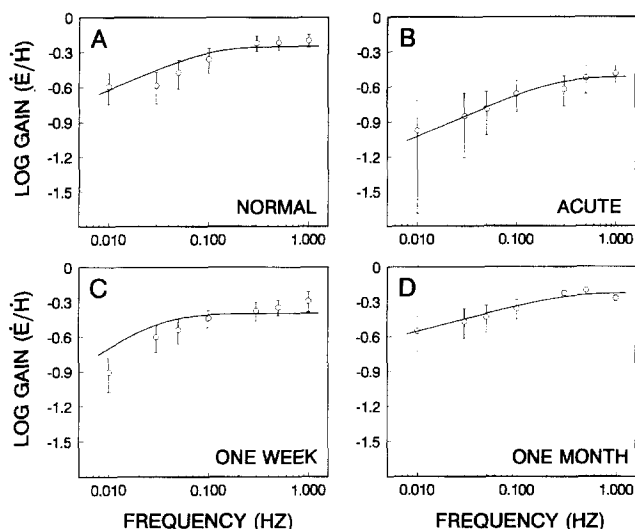


Fig. 8. Mean horizontal VOR gain (eye velocity/head velocity,  $\dot{E}/\dot{H}$ ) as a function of frequency under normal conditions (A) and acutely (B), 1 week (C), and 1 month (D) following left hemilabyrinthectomy. Sinusoidal rotations were delivered at 0.01, 0.03, 0.05, 0.1, 0.3, 0.5 and 1.0 Hz, with peak rotational velocity set at 60 deg/s. A transfer function was fit to the data as described in the text (solid lines), and the best fit parameters are listed in Table 2. Gain values have been log (base ten) transformed.

stants, respectively,  $k$  is an exponent ( $0 \leq k \leq 1$ ), and  $s$  is complex frequency  $j\omega$ , where  $j$  is  $\sqrt{-1}$  and  $\omega$  is frequency in rad/s. This transfer function is based on a high-pass filter term  $[s\tau/(s\tau + 1)]$ . The gain of such a high-pass filter will increase with frequency and then level off at a value of one (log gain zero), and phase lead will drop from 90 to zero deg, and these transitions will both occur at a break frequency that is a function of the time constant ( $f_b = 1/2\pi\tau$  in Hz).

Although phase for the goldfish horizontal VOR clearly drops as frequency increases (Fig. 7), it drops from a level value that is substantially lower than 90 deg. The  $k$  exponent was added to the transfer function to account for the low frequency phase asymptote being less than 90 deg (see Discussion). Similarly, the gain for the goldfish horizontal VOR (Fig. 8) levels off at a value less than 1 (log gain less than zero). The gain constant  $G$  was added to the transfer function to account for the high frequency gain asymptote being less than 1 (log gain less than zero).

The phase characteristic of this transfer function was fit first (Fig. 7) to determine the values of  $\tau$  and  $k$ . Then the  $\tau$  and  $k$  values were frozen, and the gain characteristic was fit (Fig. 8) to determine the value of  $G$ . This was done for averaged frequency response data in goldfish under normal conditions (A) and acutely (B), 1 week (C), and 1 month (D) following left hemilabyrinthectomy. The best fit curves are shown as solid lines in Fig. 7 and Fig. 8, and the best fit parameters are listed in Table 2. Although the frequency response data are plotted as log gain and phase in deg, as per convention,

Table 2

Parameters for the transfer functions fit to the frequency response data of the goldfish horizontal vestibulo-ocular reflex at various stages of compensation

	$G$	$\tau$	$k$
Normal	0.57	1.52	0.37
Acute	0.31	0.61	0.36
One week	0.40	4.72	0.56
One month	0.59	0.52	0.22

$G$ , gain constant;  $\tau$ , time constant;  $k$ , fractional exponent.

they were fit in the more natural forms of untransformed gain and phase in radians. In these forms, the mean squared errors for the fits ranged from 0.0003 to 0.0044 for gain and from 0.0012 to 0.0032 for phase. The best fit curves appear to capture the dynamics of the goldfish horizontal VOR at each stage. The largest discrepancy between the fit and the data occurs for the gain at 1 week of compensation (Fig. 8C). It appears that gain at this stage falls off more rapidly at low frequencies than would be expected from the phase characteristic.

The frequency response data are summarized in Fig. 9, where the best fit curves from Fig. 7 and Fig. 8 are re-plotted. The frequency response results are consistent with the intensity function results at 0.05 and 0.5 Hz (Fig. 4, Fig. 5 and Fig. 6). For gain (Fig. 9A), an acute

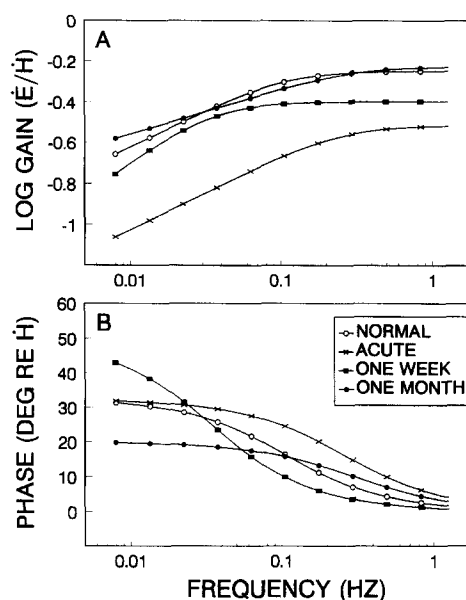


Fig. 9. Comparisons of horizontal VOR log gain and phase as a function of frequency at various stages of compensation. Best-fit transfer function curves are reproduced from Fig. 7 and Fig. 8 for normal conditions ( $\circ$ ) and acutely ( $\times$ ), 1 week ( $\blacksquare$ ), and 1 month ( $\bullet$ ) following left hemilabyrinthectomy. (A) Gain decreases from normal acutely and gradually recovers after 1 week and 1 month of compensation. (B) Phase lead increases from normal acutely, primarily at mid-range frequencies. After 1 week of compensation, phase lead has recovered mid-range, but has increased at low frequencies. Finally, after 1 month of compensation, phase lead decreased to, and even below, normal levels.

decrease (crosses) from normal (open circles) was followed by a partial recovery at 1 week (filled squares) and a full recovery at 1 month (filled circles). The intensity series data showed that eye velocity at 1 month matched normal up to 60 deg/s head velocity, and this is born out by the frequency response results that were collected at a setting of 60 deg/s. For phase (Fig. 9B), at mid-range frequencies including 0.05 and 0.5 Hz, an acute increase in lead from normal was followed by a recovery after 1 week and 1 month of compensation. Changes in phase lead at the low frequencies (0.01 and 0.03 Hz) were surprising. Phase lead changed little from normal acutely and then actually increased after 1 week of compensation. Finally, after 1 month of compensation, phase lead decreased to, and even below, normal levels.

## 4. Discussion

### 4.1. Extent of compensation

Taken as a whole, the data presented above suggest that the goldfish is able to almost completely restore its horizontal VOR following 1 month of compensation for hemilabyrinthectomy. Gain decreased acutely to about 50% of normal, had increased substantially by 1 week, and by 1 month, it had regained its normal level for head rotational velocity up to 60 deg/s. However, VOR gain had not come back to normal for a head rotational velocity of 90 deg/s. Instead it appeared that hemilabyrinthectomy had caused the VOR to saturate at this higher head velocity.

Phase lead at mid-range frequencies (0.05 to 0.5 Hz) increased acutely from normal. After 1 week of compensation, phase lead was brought back to normal mid-range, but phase lead at low frequencies actually increased above normal. Increased low frequency phase lead at 1 week was accompanied by substantially increased gain mid-range, but more rapid gain attenuation at low frequencies. In the monkey, the phase lead and gain attenuation of the horizontal VOR at low frequencies were observed to increase acutely following unilateral plugging of the lateral canal, and to further increase after 1 week and 1 month of compensation [21]. Increases in gain attenuation and phase lead at low frequencies constitute a low frequency deterioration in VOR performance, but this would serve to enhance the rejection by VOR of any low frequency disturbances that might result from unilateral vestibular receptor lesions [21].

The high phase lead at low frequencies that was observed in the goldfish VOR after 1 week was not only reduced to normal, but was actually lower than normal after 1 month of compensation. Gain at 1 month had increased to normal at all frequencies (up to 60 deg/s peak head rotational velocity). A similar decrease in

VOR phase lead with an increase in gain was observed in goldfish that were trained to increase their VOR gain by alteration of the normal relationship between head rotation and rotation of the visual surround [25].

The data presented above suggest that the goldfish is able to recover the normal gain and phase of the horizontal VOR following 1 month of compensation for hemilabyrinthectomy, except at high head velocity where some gain saturation occurs. Following hemilabyrinthectomy in mammals, however, gain is restored to sub-normal levels only, while acute increases in phase lead are not reduced at all [5,12,16,17,28,31]. It therefore appears that the extent of VOR compensation in goldfish exceeds that which has been observed in mammals.

### 4.2. Theoretical considerations

These results bear upon models of the VOR that attempt to provide a framework for understanding vestibular compensation. The VOR is mediated by neurons in the vestibular nuclei (VN), that receive and process inputs from the semicircular canal afferents, and then send them on to the eye muscle motoneurons as VOR commands [30]. Neurophysiological experiments have shown that, under normal conditions, both the VN neurons and the VOR have a higher gain and a lower phase lead at low frequencies than do the canal afferents [8]. It has been suggested that this low frequency enhancement of the VOR is due to temporal integration of the canal signal, which may be brought about by positive feedback loops at the VN level [1,8,15]. The observed low frequency deterioration of the VOR may be due to disruption of these VN feedback loops as a result of hemilabyrinthectomy.

Recently, vestibular compensation has been simulated as learning in a neural network model of the horizontal VOR that incorporated feedback between model VN neurons [2]. Simulated hemilabyrinthectomy resulted in spontaneous VOR, and reduced both gain and temporal integration. The model reproduced the time course of actual compensation, in which spontaneous VOR is eliminated first, and is followed by a gradual increase in VOR gain. In the initial stages of simulated compensation, temporal integration by the feedback loops remained low. This improved compensation, because integration of the imbalance caused by simulated hemilabyrinthectomy would only exacerbate spontaneous VOR and frustrate compensatory adjustments in balance and gain. The network model is also able to recover integration, but only slowly, requiring almost ten times as much learning as for recovery of gain. It appears that compensation in mammals stops at the point where gain is recovered. However, the goldfish is able to restore VOR integration, as in the model, suggesting that its compensatory process is capable of re-establishing the brainstem integration mechanism, perhaps by making

plastic adjustments in synapses at the level of the VN [2,14].

A fractional-order high-pass filter ( $G[\sigma\tau/(\sigma\tau + 1)]^k$ ) was used to model the horizontal VOR in the goldfish. A fractional-order high-pass filter has a low frequency asymptote that can be less than 90 deg ( $k \times 90$  deg where  $0 \leq k \leq 1$ ). This operator captured the relatively low phase lead at low frequencies of the goldfish horizontal VOR. Similar low frequency behavior has been reported in previous studies of the goldfish horizontal VOR [22,25].

A fractional-order high-pass filter can be modeled as a large number of integer-order high-pass filters operating in parallel, where each has its own distinct time constant and is weighted according to the value of its time constant raised to the power  $(-1-k)$  [3,27]. In the range of the high-pass filter break frequencies ( $f_b = 1/2\pi\tau$  in Hz), phase lead for the ensemble will be constant at  $k \times 90$  deg and log gain will increase with log frequency with a slope of  $k$ . For frequencies higher than the highest break frequency in the ensemble, gain will level off at  $G$  and phase will fall to zero. It is possible that the goldfish VOR is mediated by parallel pathways that could constitute such an ensemble of high-pass filters. Changes in the  $G$ ,  $\tau$  and  $k$  values for the fractional-order high-pass filter transfer functions fit to the VOR at different stages of compensation could be modeled as different weightings of the individual integer-order high-pass filters that make up the ensemble.

#### Note added in proof

Subsequent studies revealed that spontaneous VOR (spontaneous nystagmus) can be observed in goldfish immediately following unilateral vestibular nerve section. This spontaneous VOR is eliminated by the compensatory process in 15 min or less. An article describing and modeling this phenomenon has been published (Ratnam, R. and Anastasio, T.J., Evidence for a cooperative learning mechanism in the vestibulo-ocular reflex, *NeuroReport*, 6 (1995) 2129–2133).

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