# Violation of homogeneity by the vestibulo-ocular reflex of the goldfish

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THE vestibulo-ocular reflex (VOR) allows animals to maintain stable gaze during head rotations by generating compensatory eye rotations. The VOR is typically tested using sinusoidal head rotation, and VOR gain is calculated as the ratio of the amplitude of eye to head rotational velocity. Through habituation, prolonged exposure to lower frequency sinusoidal head rotation in the dark decreases VOR gain. The VOR has been treated and modeled as a linear system. If it is linear, then the VOR must obey the principle of homogeneity: VOR gain at a particular frequency should be the same regardless of head velocity. We examined the habituated goldfish VOR for homogeneity. We found that it violated this basic principle of linear systems and is therefore non-linear. *NeuroReport* 10:3881–3885 © 1999 Lippincott Williams & Wilkins.

**Key words**: Goldfish; Habituation; Homogeneity; Non-linear; Plasticity; Vestibulo-ocular reflex

## Introduction

The vestibulo-ocular reflex (VOR) of vertebrates functions to maintain stable gaze during head movements [1]. To perform this function, the VOR generates eye rotations that counter head rotations. The VOR neural pathway is centered on vestibular nucleus neurons, which receive afferents from the semi-circular canals and in turn send motor control signals to the motoneurons that innervate the eye musculature [1]. Regions of the cerebellum are essential to the performance and plasticity of the VOR [2–6].

The VOR is typically tested using sinusoidal head rotations at various, single frequencies [7–9]. In response, the VOR produces an approximately sinusoidal eye rotational velocity, and can be characterized at any frequency according to its gain (eye velocity amplitude/head velocity amplitude) and phase difference (eye velocity phase–head velocity phase). Through the process of habituation, prolonged exposure to head rotation at a single, lower frequency will decrease VOR gain at that frequency (for review see [10]). It will also cause frequency-specific alterations in VOR gain and phase at other frequencies that have been described using linear system models [11,12]. However, linear system models are inadequate to fully describe the habituated VOR.

Habituation of the goldfish VOR has been shown to exhibit some characteristics that are non-linear. For example, experiments in goldfish have shown that VOR eye rotational velocity during habituation to sinusoidal head rotation is not sinusoidal but is heavily distorted [6]. More importantly, the habituated goldfish VOR violates the superposition principle [5]. The superposition principle states that the response of a linear system to a sinusoidal stimulus at any frequency must be the same whether that stimulus is presented alone or in combination with a stimulus at another frequency [13]. Following habituation of the goldfish VOR at a lower frequency (0.01 Hz), VOR gain at the habituating frequency increased 10-fold when the habituating stimulus was combined with a second stimulus component at a higher frequency (0.3 Hz) [5]. These results demonstrate a severe violation of the superposition principle.

Because the habituated goldfish VOR had already demonstrated a violation of one of the underlying principles of linear systems, we sought to test whether it would also violate the other underlying principle. The principle of homogeneity states that the response of a linear system at any particular frequency should be the same regardless of the amplitude of the stimulus at that frequency [13]. In this study, we find that the habituated goldfish VOR also violates the principle of homogeneity.

## Materials and Methods

Comet goldfish (*Carassius auratus*) were obtained either from hatcheries or local suppliers. Each goldfish was wrapped in moistened gauze and was gently

clamped in an experimental tank by way of contoured body supports. The fish was entirely immersed in water from its home aquarium at 18°C. The head of the fish was centered in the cylindrical experimental tank. The mouth of the fish was opened over a plastic tube and respiration was maintained by pumping water over its gills. A biological digestion filter was installed in series with the return path to the respiration pump to prevent the accumulation of ammonia in the experimental tank. A small (5.3 mm) coil of insulated copper wire (Sokymat, SA, Switzerland) was attached to the sclera of the fish's left eye by means of ophthalmic sutures. All procedures were approved under University of Illinois LACAC protocol number B3R085.

The tank was placed on a servo-controlled, horizontally oriented rotating platform. The center of the tank, and so also the fish's head, were co-linear with the vertical axis of rotation. The tank was centered in an alternating magnetic field (50 kHz) and horizontal eye position was transduced using the magnetic search coil technique [14,15]. Black plastic shrouds were used to cover the chamber and the lights were extinguished so that all experiments were performed in the dark. Eye position and rotator tachometer signals were anti-alias filtered (eight-pole Butterworth, 25 Hz cut-off frequency) and digitized (12 bit A/D, 50 Hz sampling rate). Each coil was calibrated immediately after the experiment at seven angles (± 30 deg.) that covered the range of eye rotational positions (accuracy: 0.1 deg.).

The goal of these experiments was to observe the effect of habituation to head rotation at one frequency and amplitude (peak head velocity) upon the VOR response at the same frequency but at other amplitudes. We wanted to examine the extent to which habituation of VOR gain is amplitude specific. We chose a frequency of head rotation of 0.01 Hz for these experiments because habituation of the goldfish VOR is greatest at and below this frequency [6]. Although 1 h of rotation is required to fully habituate the goldfish VOR at 0.01 Hz, the response can begin to habituate within the first 1 or 2 cycles at this frequency [6]. Thus, we had to habituate goldfish at one amplitude and then briefly retest them at other amplitudes before any amplitude specific habituation at the other amplitudes could take place. The goldfish VOR was habituated with the rotating platform operated sinusoidally at 0.01 Hz with a peak rotational velocity of 30 deg/s. Following 1 h of habituation training at 0.01 Hz and 30 deg/s, the horizontal goldfish VOR was retested for not more than two cycles at 0.01 Hz and each of the following five peak rotational velocities: 7.5, 15,

30, 60 and 90 deg/s. Test amplitudes were randomly presented for each fish.

VOR eye velocity was computed from eye position data by digital differentiation. Normal VOR eye rotations are frequently interrupted by fast eye rotations that serve to reposition the eye in the orbit. These fast-phase eye rotations were automatically eliminated. The resulting VOR eye velocity data were then inspected. Any remaining fast-phase eye rotations were manually removed.

The amount of VOR habituation due to training was measured. Previous research has shown that VOR eye rotational velocity in response to lower frequency sinusoidal stimulation is roughly sinusoidal in experimentally naive and in fully habituated goldfish [5]. Least-squares sinusoids were fit to VOR eye velocity at the beginning and at the end of 1 h of rotation at the habituating frequency and amplitude (0.01 Hz and 30 deg/s). The amplitudes of the least-squares sinusoids were used to compute VOR gain (eye velocity/head velocity). VOR gains at the beginning and at the end of habituation training were compared. To be considered fully habituated, VOR gain had to decrease by 10 times or more. Seventeen of 24 goldfish met this criterion. Only data from these 17 fish were included in the analysis.

Previous research has also shown that the VOR response during habituation to sinusoidal head rotation at a low frequency, and the post-habituation response at nearby frequencies, can be asymmetrical and otherwise heavily distorted [6]. Here we found that the post-habituation VOR response at other amplitudes can also be asymmetrical and heavily distorted (see Results). Because of these distortions, least-squares sinusoids could not be well fitted to post-habituation VOR eye velocity at the other head velocities tested (7.5, 15, 60 and 90 deg/s).

VOR eye velocity data for each of the five test head velocities (including 30 deg/s) from goldfish that met the habituation criterion were passed through a 200 point (equivalent to 4 s at the 50 Hz sampling rate) sliding average filter. VOR response amplitude was determined as the maximum absolute eye velocity returned by the sliding average filter during the first cycle of stimulation. Only the results of the sliding window analyses will be presented.

Despite the smoothing properties of the 200 point (4 s) sliding average filter, the method of choosing the maximal smoothed value as the amplitude of the eye velocity response was still prone to corruption by noise. The predominant source of noise in the VOR response were remnants of fast-phases that remained after editing. The problem of low signal/noise ratio was exacerbated by very low post-habituation VOR response amplitudes at some head

velocities. To aid statistical comparisons and to isolate the noise, eye movements with the head still (head velocity = 0 deg/s), which included slow drifts and fast-phases, were processed as described above and the maximal smoothed value was recorded.

The results to be reported consist of post-habitation VOR response amplitudes at six different head velocities (0, 7.5, 15, 30, 60 and 90 deg/s) and the VOR gains at 30, 60 and 90 deg/s (see Results). These data were not normally distributed. We report the median as the measure of central tendency, and the interquartile intervals provide the associated measures of variability. The various data samples were compared for statistically significant differences using the non-parametric Kruskal–Wallis test for multiple sample groups [16]. When the Kruskal–Wallis multiple samples test indicated statistically significant differences among the samples, the data were further examined with the Kruskal–Wallis selected comparisons test for specific inter-sample differences [16].

## Results

Prolonged rotation of goldfish at 0.01 Hz and 30 deg/s produced a marked decrease in VOR gain. This habituation of the goldfish VOR is similar to that which we have observed previously [5,6,12]. During the first cycles of habituation training, the naive VOR produced eye rotational velocity (Fig. 1A) that nearly compensated for head rotational velocity. Initial VOR gain at 30 deg/s head velocity had a median of 0.571, and the first and third interquartile intervals (IIs) were 0.095 and 0.159, respectively. Goldfish VOR eye velocity was diminished after 1 h of habituation training (Fig. 1B). Median VOR gain at 30 deg/s decreased to 0.080 with IIs of 0.014 and 0.013, respectively.

The post-habituation VOR response was qualitatively much larger at 60 and 90 than at 30 deg/s, but was heavily distorted (Fig. 1C). Asymmetries were observed in the VOR responses at 60 and 90 deg/s in which maximal eye velocity was much higher in one direction than in the other (Fig. 1C). Similar asymmetries have been reported previously during VOR habituation in goldfish, and the directions of asymmetry were found to be independent of stimulus parameters [6]. Here, we observed asymmetries in the post-habituation VOR response to sinusoidal head rotations at amplitudes higher than the habituation amplitude. The post-habituation VOR response at 90 deg/s shown in Fig. 1C exhibited its maximal habituation (minimal eye velocity) during the first stimulus half-cycle and its minimal habitua-
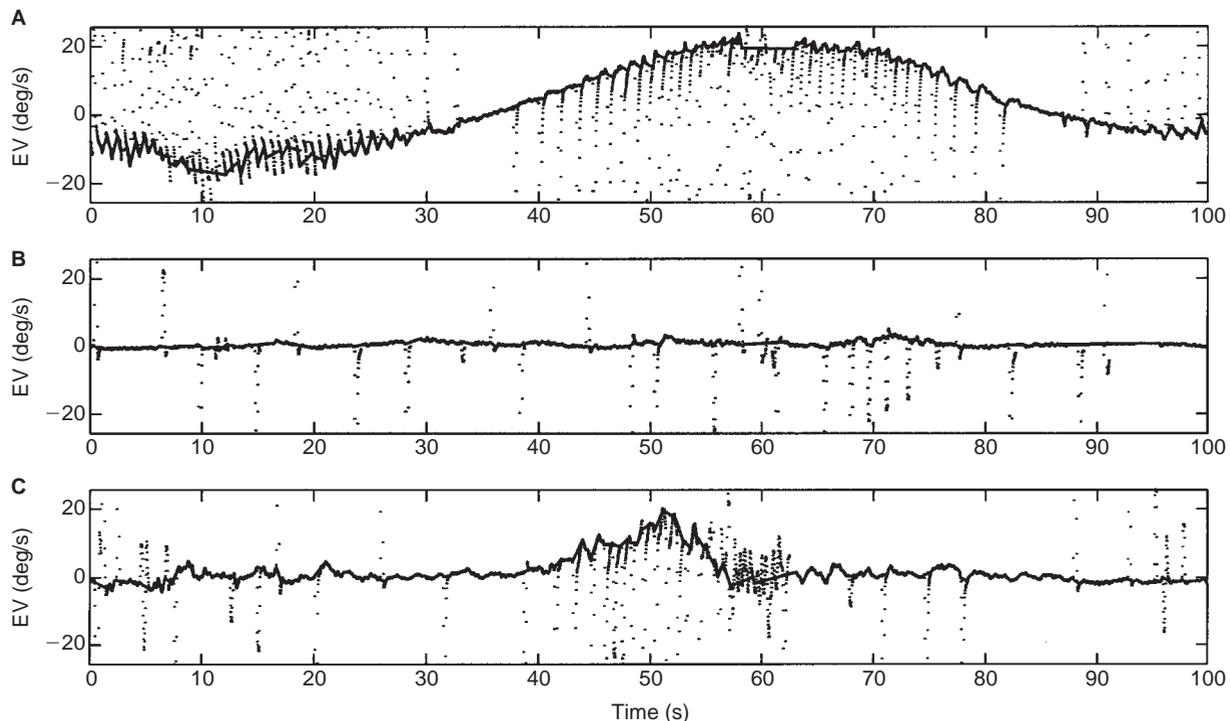


FIG. 1.   Horizontal goldfish VOR before and after habituation and during homogeneity testing. (A) Naive VOR (line) and fast-phase (dots) eye velocities during the first cycle of the habituating stimulus (0.01 Hz and 30 deg/s peak velocity, not shown). (B) Habituated VOR during 0.01 Hz and 30 deg/s stimulation following 1 h of rotation at this frequency and peak velocity. (C) VOR response during 0.01 Hz and 90 deg/s stimulation following habituation at 0.01 Hz and 30 deg/s. Note the higher level of eye velocity between 40 and 60 s. The noise in the habituated VOR eye velocity records (lines in B and C) was reduced by the sliding average filter (output not shown). Positive values represent clock-wise eye rotational velocities. EV = eye rotational velocity (deg/s).

tion (maximal eye velocity) during the second sti-mulus half-cycle. For the entire sample of post-habituation VOR responses at 90 deg/s, maximal eye velocity occurred during the first and second half-cycles on 53% and 47% of trials, respectively. For post-habituation VOR responses at 60 deg/s, max-imal eye velocity occurred during the first and second half-cycles on 41% and 59% of trials, respectively.

We compared the maximal eye velocities at each of the test head velocities (0, 7.5, 15, 30, 60 and 90 deg/s). While there appeared to be little difference between eye velocities at 0, 7.5 and 15 deg/s, there was a steady increase in maximal eye velocities from 30 to 60 to 90 deg/s (Fig. 2). When the maximal eye velocities at 0, 7.5, 15, 30, 60 and 90 deg/s were examined using the Kruskal–Wallis multiple sample group test, we found that there was a statistically significant difference among the samples ($\chi^2 = 76.22$, df = 5, $p < 0.001$). Eye velocities at 0 deg/s were then compared with each of the VOR responses at higher head velocities using the Kruskal–Wallis selected comparisons test. We found no statistically signifi-cant differences between the maximal eye velocities measured at 0 and 7.5 deg/s, or at 0 and 15 deg/s ($p > 0.05$). In contrast, the eye velocities at 30, 60 and 90 deg/s were significantly different from the eye velocities at 0 deg/s ($p < 0.01$). Given that the eye velocities measured at 7.5 and 15 deg/s were not significantly different from that at 0 deg/s, the VOR gains at 7.5 and 15 deg/s were effectively 0.

VOR gains were calculated for the post-habitua-tion responses at 30, 60 and 90 deg/s. At these head velocities there appeared to be an amplitude-depen-dent increase in VOR gain (Fig. 3). There was a statistically significant difference among the VOR gains at these three head velocities ($\chi^2 = 9.69$, df = 2, $p < 0.01$). When the VOR gains at 30 deg/s (median 0.080, IIs = 0.014, 0.013) and 60 deg/s (median 0.098, IIs = 0.039, 0.011) were compared using the Krus-kal–Wallis selected comparisons test, there was no statistically significant difference. However, the VOR gains at 90 deg/s (median 0.136, IIs = 0.022, 0.027) were significantly different from those at 30 deg/s ($p < 0.01$). The VOR gain at 90 deg/s was almost double the gain at 30 deg/s, clearly violating homogeneity.

## Discussion

Following habituation of the goldfish VOR at 0.01 Hz, post-habituation VOR gain was measurably very low at the habituating amplitude of 30 deg/s, but was effectively 0 at lower amplitudes (7.5 and 15 deg/s) and progressively increased at higher am-plitudes (60 and 90 deg/s). The increase in VOR gain at 90 deg/s was highly statistically significant. These results demonstrate that the low-frequency habitu-ated VOR does not show equal gain at all stimulus amplitudes and thus violates the principle of homo-geneity. The nearly two-fold increase in post-habituation gain during the 90 deg/s tests indicates that a fish is able to respond to higher velocity stimuli even after extensive habituation at the same
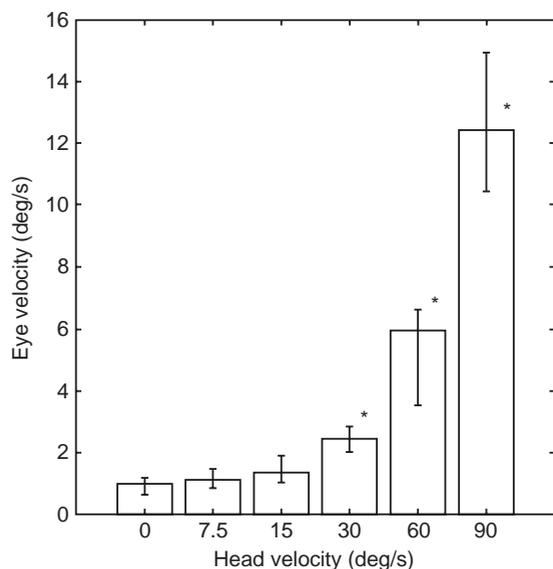


FIG. 2. Comparison of maximal VOR eye velocity at each different head velocity following habituation. Bar height indicates median eye velocity and the error bars indicate the first (bottom) and third (top) interquartile intervals. The *indicates a statistically significant difference in maximal eye velocity at the corresponding head velocity compared to the maximal eye velocity measured at rest (head velocity = 0 deg/s).
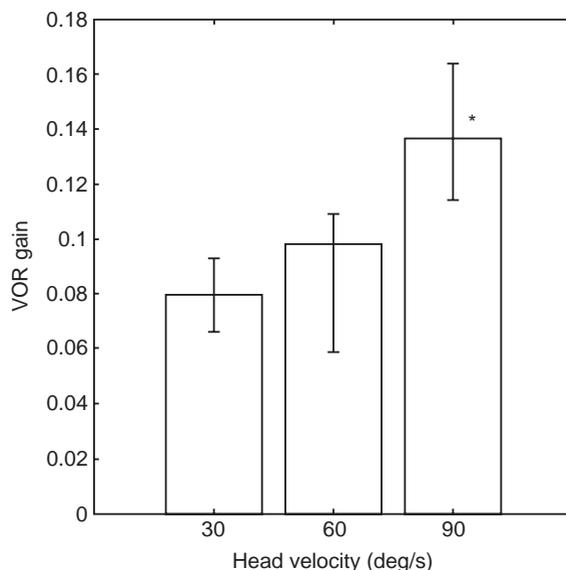
FIG. 3. Comparison of VOR gains at different head velocities following habituation. Bar height indicates median VOR gain and the error bars indicate the first (bottom) and third (top) interquartile intervals. The *indicates a statistically significant difference in VOR gain at 90 deg/s compared with that at 30 deg/s.

low frequency. This work, in which we demonstrate that the VOR violates homogeneity, complements previous work in which we demonstrated that the VOR violates superposition [5]. Failure of the habituated goldfish VOR to obey these two principles of linear systems indicates that it is fundamentally non-linear [13].

It is important to distinguish the violation of homogeneity that we describe here for the habituated goldfish VOR from the saturation non-linearity that has been described for the VOR of naive (normal) animals. VOR eye velocity in normal animals increases linearly with head velocity up to a saturating level of head velocity, above which the VOR plateaus. For example, the normal monkey VOR saturates at 120 deg/s at lower frequencies [7]. The saturation non-linearity decreases VOR gain at higher head velocities, causing a violation of homogeneity. VOR saturation is thought to reflect saturation in the responses of semi-circular canal afferents and vestibular nucleus neurons [7].

The normal goldfish VOR has been shown to be linear out to 90 deg/s at lower and midrange frequencies [9]. The head velocities we employed here in testing homogeneity did not exceed this linear range. Thus, in contrast to the saturation non-linearity, the violation of homogeneity we describe here following habituation is characterized by a VOR gain increase as head velocity increases within the normally linear range of the VOR. Regions of the cerebellum that are known to mediate VOR habituation [3–6] may be responsible for this type of homogeneity violation.

Following 1 h of rotation at 0.01 Hz and 30 deg/s, the post-habituated VOR responses at 60 and 90 deg/s exhibited asymmetries in which eye velocity was greater in one direction than in the other. Similar asymmetries have been observed previously in the VOR response during habituation at 0.01 Hz [6]. The finding that the habituated goldfish VOR violates homogeneity suggests that VOR habituation in goldfish may be amplitude specific. Asymmetries in the post-habituation VOR response at amplitudes higher than the habituating amplitude suggest that a higher head rotational velocity can initiate another amplitude-specific process of habituation, which then asymmetrically reduces VOR gain at the new, higher amplitude.

## Conclusion

The habituated goldfish VOR violated the principle of homogeneity of linear systems by demonstrating a non-uniform VOR gain at stimulus amplitudes different from the habituating amplitude at the same frequency. This violation of homogeneity, combined with the previously demonstrated violation of superposition, indicates that the habituated VOR of goldfish is fundamentally non-linear.

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