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Mechanism of Frequency Selectivity in VOR Motor Learning: Frequency Channel or Waveform Learning?

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Abstract—**The vestibuloocular reflex (VOR), in which cerebellum is intimately involved to produce compensatory eye movements during head motion to stabilize retinal image. The VOR is under adaptive control whose gain (eye velocity/head velocity) can be modified by visual-vestibular mismatch stimuli. It exhibits frequency selectivity in which largest gain change is observed at the training frequency. Currently, we demonstrate in the goldfish horizontal VOR that the gains of VOR at two different frequencies can be modified in the opposite directions simultaneously: high gain at one frequency, low gain at another. We test the mechanism of this frequency selective learning if it is due to frequency channels with modifiable gains or learning of waveform per se.**

I. INTRODUCTION

Due to development, aging, injury, etc., our muscle characteristics are never constant over entire life time. In order to maintain precise motor control, thus the central nervous system must continuously calibrate the motor command to drive the muscles. This distinct function in biological system is called motor learning. The cerebellum is known to play important role in motor control, and intimately be involved in motor learning. The vestibulo-ocular reflex (VOR) is a type of eye movements that stabilizes our vision during head movements, and has been a popular model system to study cerebellar motor learning. The neuronal circuitry subserving the VOR is among the best known in the central nervous system, and is known to be highly preserved from fish to primate. As the VOR has highly linear characteristics producing sinusoidal eye velocity output in response to a sinusoidal head velocity input, its performance is commonly quantified by the simple gain defined as eye velocity / head velocity in the dark, which is usually close to unity. The motor learning of the VOR can be induced by visual-vestibular conflict stimulus which causes an increase or decrease of VOR gain. It has been known that VOR motor learning has frequency selectivity. A largest change in VOR gain is induced at the frequency of visual-vestibular conflict stimulus that induced the learning, thus suggesting existence of the multiple channels each processing a different frequency band [1]. In support, Hirata et al. have demonstrated in monkey vertical VOR that VOR can simultaneously modify its gains at two different frequencies in the opposite directions [2]. Namely, the VOR gain at one frequency can be increased while that at another frequency is decreased. On the other hand, we have shown that goldfish can modify their VOR gains for rightward and leftward head rotation in the opposite directions simultaneously [3]. The same type of asymmetrical learning is possible for upward and downward VOR in monkeys [2]. This type of learning requires nonlinear signal transformation which cannot be explained by models with simple linear gain adjustments. Pharmacological inactivation of cerebellar flocculus precludes VOR motor learning, and abolishes the VOR gain learned relatively in short term (up to 3 hours) [4], [5]. Therefore the nonlinear signal transformations required for the right-left and up-down asymmetrical learning are realized in the cerebellar cortical neuronal circuitry, and the same neural network might be commonly responsible for the frequency selectivity.

Currently, we tested the mechanism of the frequency selectivity in VOR motor learning: if it is due to multiple frequency channels, or it is a learning of waveform.

II. METHODOLOGY

A. Subjects and experimental setup

All experiments were approved by the Chubu University Animal Welfare and Use Committee. Goldfish (*Carassius auratus*, overall length 15-20 cm) were acclimated in the aquaria maintained at 20 °C. During each experiment, goldfish's eye movements were recorded by the scleral search coil technique [6]. Before each experiment, a prefabricated 5 mm dia. eye coil constructed of 100 turns of enamel-insulated copper wire was sutured to the scleral margin of right or left eye under MS-222 anesthesia (Sigma). After this procedure, the goldfish was gently restrained in the cylindrical water tank filled with water at the acclimation temperature. Subsequently, tapered tube delivering aerated water was carefully fitted to the mouth of the fish. The goldfish was acclimatized to the environment in the water tank for 30 minutes prior to any recording procedure. The water tank was placed in the center of the magnetic field generated by two sets of field coils (DNI Inc.) driven by 2 sinusoids with different frequencies for the measurement of horizontal and vertical eye positions. The water tank was attached onto a turn table driven by a servo motor to provide horizontal rotation as vestibular stimulation. An optokinetic visual

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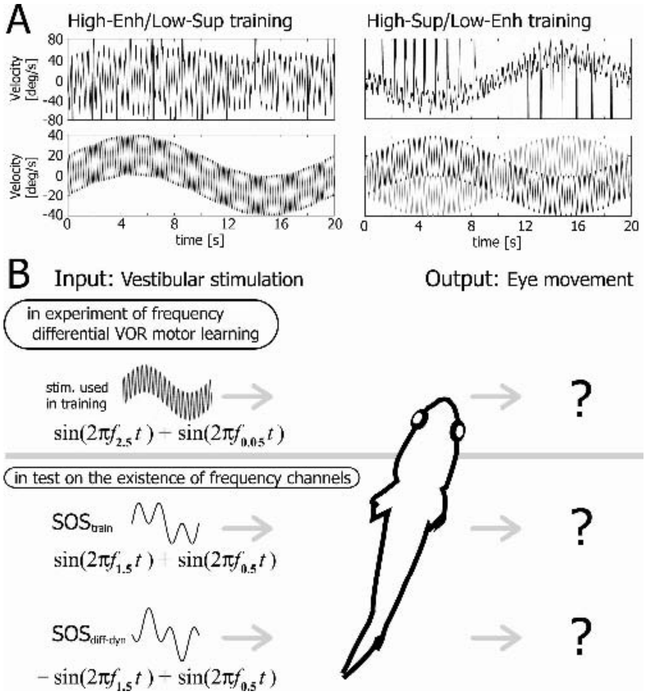

Fig. 1. A: an example of eye movements during frequency differential VOR motor learning by the sum of sines (SOS) stimulus composed of 2.5 and 0.05 Hz sinusoids. Eye velocity (upper column), induced by the combination of vestibular and optokinetic stimulus (black and gray lines in lower column, respectively) is shown. B: Vestibular stimulations employed to evaluate the VOR gains at two frequencies.

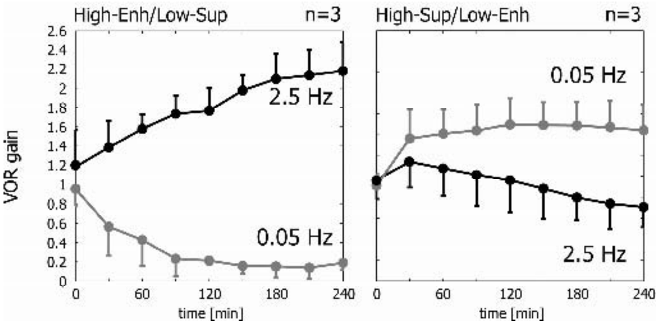

Fig. 2. Time course of the VOR gain trained by frequency differential VOR motor learning. Note that VOR gains at 2.5 and 0.05 Hz changed in the opposite directions simultaneously in both training paradigms. Note also that decrease and increase in VOR gains trained by High-Enh/Low-Sup are greater than those trained by High-Sup/Low-Enh.

stimulus (OKS) was delivered by rotating the servo-controlled planetarium that projects white random dots on the inside wall of the water tank. The rotation axes of the turn table, the center of the water tank and OKS were carefully aligned to the center of the goldfish head at the level of the horizontal semicircular canals. Horizontal and vertical eye positions were calibrated assuming that the gain of the VOR at 0.5 Hz in the light is unity [7]. Vestibular stimulation velocity, OKS velocity, horizontal and vertical eye positions were continuously A/D-converted at a sampling frequency of 1000 Hz each in 16-bits by using a Power 1401 interface (Cambridge Electronic Design) controlled by the Spike2 software to display and storage data.

B. Experimental protocol and training paradigms

After recovery from anesthesia and acclimation to the setup environment, goldfish were exposed in either of the following visual-vestibular interaction stimulation.

In the first experiment, characteristics of low-high frequency differential learning were measured. Vestibular stimulation was delivered as a sum of 2.5 and 0.05 Hz sinusoidal waves with the maximum amplitude of 20 deg/s for each frequency [20 sin(5 π t) + 20 sin(0.1 π t)], whereas OKS was delivered as the combination of the same sinusoids but with the negative sign in one of the 2 components [20 sin(5 π t) – 20 sin(0.1 π t), or – 20 sin(5 π t) + 20 sin(0.1 π t)]. During training by the visual-vestibular mismatch stimulus in which OKS was 20 sin(5 π t) – 20 sin(0.1 π t), the high frequency vestibular component is in phase with the OKS while the low frequency component is out of phase. Thus, to reduce image slip on the retina, the high frequency response of the VOR should be suppressed, whereas the low frequency response should be enhanced. Thus we call this paradigm High-Sup/Low-Enh. In contrast, during training with the OKS – 20 sin(5 π t) + 20 sin(0.1 π t), the high frequency response of the VOR should be enhanced, whereas the low frequency response should be suppressed. Thus it is called High-Enh/Low-Sup. To evaluate the changes in VOR gain during these trainings, the same vestibular stimulus was provided in the dark for 60 seconds every 30 minutes.

In the second experiment, the mechanism of frequency selective VOR motor learning was addressed using low-high frequency differential paradigms. Vestibular stimulation for these learning paradigms was comprised of a sum of 1.5 and 0.5 Hz sinusoids with the maximum amplitude of 20 deg/s for each frequency [20 sin(3 π t) + 20 sin(1 π t)]. In the low-high frequency differential paradigm, OKS with different signs [20 sin(3 π t) – 20 sin(1 π t), or – 20 sin(3 π t) + 20 sin(1 π t)] was combined with the vestibular stimulus. To test if the frequency selective learning is due to multiple frequency channels or waveform adjustments, we employed two types of vestibular stimulations in the dark. One is the same vestibular stimulus used during these learning paradigms (SOS_{train}), and another is composed of the same two sinusoids but the sign of the higher frequency component is flipped [– 20 sin(3 π t) + 20 sin(1 π t)] ($SOS_{diff\text{-}wave}$). These test stimuli were delivered sequentially in the dark for 30 seconds each every 30 minutes. To avoid the possible complexity arising from the decay in VOR gain change [8], [9], $SOS_{diff\text{-}wave}$ was applied first then SOS_{train}.

C. Data analysis

All data analyses were performed on the MATLAB (Mathworks). Horizontal eye velocity traces were calculated from recorded eye position traces by using a low pass differentiation filter. Saccades were then eliminated from the

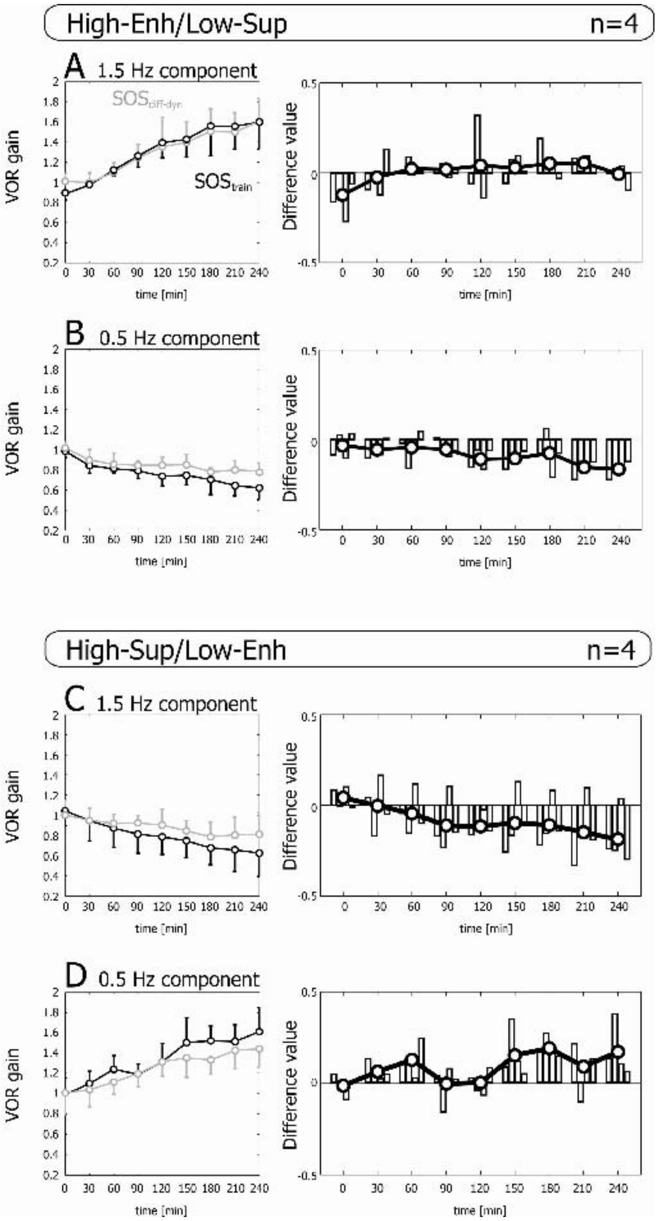

Fig. 3. Left column in A-D: Time course of VOR gain trained by frequency differential VOR motor learning paradigms. VOR gain at 1.5 Hz and 0.5 Hz were evaluated by providing SOS_{train} and $SOS_{diff\text{-}wave}$ (black and gray trace, respectively). Right column in A-D: Differences between VOR gains evaluated by SOS_{train} and $SOS_{diff\text{-}wave}$ for the high and low frequency components calculated by subtracting those evaluated by $SOS_{diff\text{-}wave}$ from those evaluated by SOS_{train}.

eye velocity traces automatically followed by a manual inspection. To estimate VOR gains at two different frequencies, the desaccaded eye velocity traces were reconstructed by a sum of two sinusoids whose frequencies are identical to those contained in the test vestibular stimulus. Amplitudes and phases of these two sinusoids were estimated using the nonlinear least square function in the MATLAB optimization toolbox. VOR gain at low frequency was calculated as the estimated amplitude of eye velocity at low frequency divided by head velocity amplitudes of the corresponding frequency. VOR gain at high frequency was estimated in the same way using the high frequency components.

III. RESULTS

A. *Frequency differential VOR motor learning*

Figure 1A illustrates an example of eye movements during High-Enh/Low-Sup and High-Sup/Low-Enh training. During High-Enh/Low-Sup training, the high frequency component of eye velocity is enhanced while the low frequency component is suppressed. Contrary, during High-Sup/Low-Enh training, the high frequency component is suppressed while the low frequency component is enhanced. These training stimuli induced changes in the high and low frequency response of the VOR differently. Figure 2 illustrates the time course of the VOR gain trained by frequency differential VOR motor learning paradigms. After 4 hours of High-Enh/Low-Sup training, VOR gain at 2.5 Hz increased to 2.18 ± 0.29 (mean ± SD) from 1.18 ± 0.38, while that at 0.05 Hz decreased to 0.18 ± 0.07 from 0.95 ± 0.17. Contrary, after High-Sup/Low-Enh training, VOR gain at 2.5 Hz decreased to 0.70 ± 0.18 from 0.96 ± 0.18 while that at 0.05 Hz increased to 1.44 ± 0.24 from 0.91 ± 0.11. Therefore, as in the vertical VOR in monkey [2], the high and low frequency components of horizontal VOR in goldfish are modifiable in the opposite directions simultaneously. Note that the amount of the gain changes in the high and low frequency component trained by High-Enh/Low-Sup is greater than those trained by High-Sup/Low-Enh.

B. *Test on the existence of frequency channels*

Figure 3 illustrates the time course of the VOR gain trained by High-Enh/Low-Sup (left column in A, B), and High-Sup/Low-Enh paradigms (left column in C, D). In each panel, VOR gains estimated by SOS_{train} (black trace) and $SOS_{diff\text{-}wave}$ (gray trace) were superimposed for a direct comparison. The traces are averages over n goldfish (n=4). Error bars indicate 1 standard deviation. The gains of low (0.5Hz) and high (1.5Hz) frequency components of VOR evaluated by SOS_{train} and $SOS_{diff\text{-}wave}$ both changed toward the trained directions in High-Enh/Low-Sup and High-Sup/Low-Enh paradigms. However the averaged gains evaluated by SOS_{train} indicate always greater changes than those by $SOS_{diff\text{-}wave}$ except for the 1.5 Hz component in High-Enh/Low-Sup training where the difference is close to 0.

The analysis of the difference between gains evaluated by SOS_{train} and $SOS_{diff\text{-}wave}$ for individual samples manifests this phenomenon. Bar graphs in the right column in Figure 3 illustrate differences between VOR gains evaluated by SOS_{train} and $SOS_{diff\text{-}wave}$ in the 4 individual experiments (4 fishes). The values were calculated by subtracting the gain evaluated by $\mathrm{SOS}_{diff\text{-}wave}$ from that by SOS_{train}. Black lines indicate their averages. After 4 hours of training, except for the 1.5 Hz component of High-Enh/Low-Sup paradigm, greater learning level is achieved by the gain evaluation with SOS_{train} in 11 out of 12 cases.

IV. Discussion and Conclusion

It has been demonstrated that frequency differential VOR motor learning can be induced in vertical VOR system in squirrel monkeys. Currently, we extended this to the horizontal VOR in goldfish. We also showed that the amount of the learned VOR gains in High-Sup/Low-Enh paradigm is smaller than that in High-Enh/Low-Sup paradigm at both low and high frequencies. This result suggests the existence of asymmetrical interaction between these two frequencies, namely stronger interaction when the low frequency component is trained toward high gain while the high frequency component is trained toward low gain. Another possibility for this resultant asymmetrical interaction between low and high frequencies is the asymmetrical behavioral performance of goldfish (and monkeys) during the frequency differential VOR learning paradigms. These animals tend to show better performance (less retinal image slip) in High-Enh/Low-Sup paradigm (unpublished observation). Difference in the amount of error signal that drives VOR motor learning may cause the asymmetrical interaction.

Several lines of evidence suggest that the frequency selective VOR motor learning is achieved in cerebellar flocculus [1], [2]. Cerebellar cortical neuronal circuitry constitutes a multi-layered neural network that has a numerous number of granular cells in the hidden layer. Thus the neural network should be able to achieve any waveform transformation [10]. In such a case, the frequency selective VOR learning might not be due to learning in multiple frequency channels, rather it may be due to learning of the waveform that reduces retinal image slip during the training. If there are multiple frequency channels each of which has a gain controller, then the estimated VOR gains after the sum-of-sines training should be identical no matter what type of vestibular stimulus is employed as a test stimulus as long as it contains the same frequency components. We currently showed that greater learning amount is more likely to be found when evaluating with the vestibular stimulus used in the training than that not used. Although the limited number of samples currently we have in hand, this result suggests that the VOR motor learning in cerebellum is not just gain adjustments at multiple frequency channels, but rather it learns to transform the vestibular waveform used in training into a particular waveform that minimizes retinal slip or another error signal driving the VOR motor learning.

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