

DURING movement, the vestibulo-ocular reflex (VOR) normally maintains retinal image stability by making slow-phase eye rotations that counterbalance head rotations. These eye rotations are nystagmic when the on-going slow-phases are interrupted by fast-phase eye rotations that reset eye position. Periodic alternating nystagmus (PAN) is an eye-movement disorder characterized by uncontrollable nystagmus that alternates direction roughly sinusoidally (at about 0.005 Hz). PAN has been observed only in humans with cerebellar disorders and in monkeys with lesions to the cerebellar nodulus and uvula. We show experimentally in intact goldfish that prolonged rotation in darkness for 1 h at specific frequencies (0.05–0.1 Hz) induces PAN, upon which the normal VOR response is superimposed. We show computationally that rotation-induced PAN may result from decreased cerebellar inhibition of VOR brain stem neural pathways.

Key words: Cerebellum; Goldfish; Habituation; Limit-cycle model; PAN; Periodic alternating nystagmus; VOR

Induction of periodic alternating nystagmus in intact goldfish by sinusoidal rotation

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Introduction

Nystagmus is a pattern of eye movement in which on-going, slow-phase eye rotations are interrupted by fast-phase eye rotations that reset eye position. Slow-phase eye rotations can be driven by various oculomotor sub-systems including the vestibulo-ocular reflex (VOR). The VOR maintains retinal image stability by making slow-phase eye rotations that counterbalance head rotations, making it possible to move and see at the same time.¹ Periodic alternating nystagmus (PAN) is a congenital or acquired eye movement disorder characterized by uncontrollable nystagmus that alternates direction roughly sinusoidally with a period of 200–400 s.^{2–4} Various studies have sought to provide a neurophysiological explanation for this disorder. Furman and colleagues⁴ have determined that PAN in humans is caused by lesions of the cerebellar nodulus and uvula (NU). Researchers have observed PAN (with a period of up to 600 s) in monkeys with NU lesions.^{5,6} In monkeys and cats^{5,7–10} NU lesions also prevent habituation which, in intact animals, is a decrease in the amplitude of the low-frequency VOR response brought about by prolonged low-frequency rotational stimulation.

We have shown previously that the VOR also habituates to prolonged low-frequency (0.01 Hz) rotational stimulation in intact goldfish.¹¹ Here we show that prolonged rotation at higher frequencies (0.05–0.1 Hz) induces PAN. This is the first report

of induction of PAN by rotation in an intact animal. Our data can be simulated using a modified version of the limit-cycle model of PAN,^{3,12} in which habituation or PAN result respectively from rotation-induced increases or decreases in NU inhibition of VOR brain stem neural pathways.

Materials and Methods

PAN was studied in 30 intact, experimentally naive comet goldfish (*Carassius auratus*), 10–15 cm in length. Each goldfish was restrained horizontally underwater in a cylindrical tank with its head at the center. Eye movements were measured using the magnetic search coil technique^{13,14} (for technical details see Ref 11.)

The tank was mounted on a horizontal rotating platform. The vertical axis of rotation passed through the center of the tank. Twenty-seven goldfish were rotated continuously for 1 h in darkness at single frequencies between 0.03 Hz and 1.0 Hz. Three additional goldfish were rotated for 1 h at 0.05 Hz with a visible, stationary surround. All stimuli had peak rotational velocities of 60 deg/s. Eye position and rotator (i.e. head) velocity signals were digitized for analysis.

Eye position data were digitally differentiated to compute eye velocity. Fast-phases were removed. The amplitudes of slow-phase eye velocity and head velocity were estimated by fitting least-square sinusoids to the data. To minimize error associated with

a slowly changing baseline due to PAN when it was present, only data segments of ≤ 100 s were fit. VOR gain was calculated as eye velocity amplitude/head velocity amplitude. The gain reduction ratio was calculated by dividing initial gain by the gain after 1 h of rotation.

To quantify PAN, the peak slow-phase eye velocities in both the nasal and temporal directions were measured for each cycle of rotation. Least squares sinusoids were fitted separately to the nasal and temporal sides of the envelope. PAN was considered present if the amplitude of the nasal or temporal envelope sinusoid exceeded a criterion of 10 deg/s, peak to peak. In addition, eye velocity was examined following termination of rotation to determine whether PAN persisted. Data were analyzed and simulated using MATLAB and SIMULINK (The Mathworks, Inc.).

Results

Prolonged rotation of goldfish in darkness at mid-frequencies (0.05–0.1 Hz) could produce a lower-frequency oscillation in slow-phase eye velocity that was superimposed on the normal VOR response (Fig. 1). The resulting periodic alternating nystagmus (PAN) did not occur if a stationary surround was visible. When PAN occurred, it was roughly sinusoidal and varied in period, amplitude, and onset-time, independent of the frequency of rotation.

The period of the PAN oscillations was long relative to those of the imposed rotations, and varied between 2 and 20 min, both between goldfish and during prolonged rotation for each goldfish.

The largest observed PAN amplitude was about 100 deg/s, peak to peak. Typical amplitudes ranged between 30 and 50 deg/s. When PAN occurred, its onset occurred between 0 and 50 min.

PAN persisted until the end of rotation in 10 goldfish, in eight of which PAN continued even after rotation had been terminated (Fig. 2). The peak slow-phase eye velocity of post-rotatory PAN typically reached 25 deg/s. Post-rotatory PAN oscillations could be detected for 100 to > 1000 s, encompassing from 1 to 2.5 cycles. If there were multiple cycles, subsequent cycles usually had shorter periods and reduced amplitudes. Post-rotatory PAN did not occur in any goldfish that did not produce PAN, or in which PAN did not persist until the end of rotation. In these cases, slow-phase VOR eye-velocity decayed linearly to baseline in at most 20 s following termination of rotation.

PAN was most likely to occur during prolonged rotations at frequencies between 0.05 and 0.1 Hz, and progressively less likely as frequency increased and decreased outside this range (Fig. 3). In a previous study¹¹ rotation at 0.01 Hz did not produce PAN, but VOR gain decreased 22 times as a result of habituation. PAN occurred in two of three goldfish tested at 0.03 Hz. In one of these, PAN occurred only while VOR gain was still high, and disappeared as the VOR habituated. VOR gain decreased 2.8 times at 0.03 Hz. Rotations for 1 h at mid frequencies (0.05–0.1 Hz) produced multiple cycles of PAN in 11 of 12 goldfish. VOR gain for these frequencies decreased between 1.3 and 1.8 times. Only one cycle of PAN was observed in each of the two goldfish that showed PAN during prolonged rotation at 0.17 Hz, and VOR gain at this frequency decreased

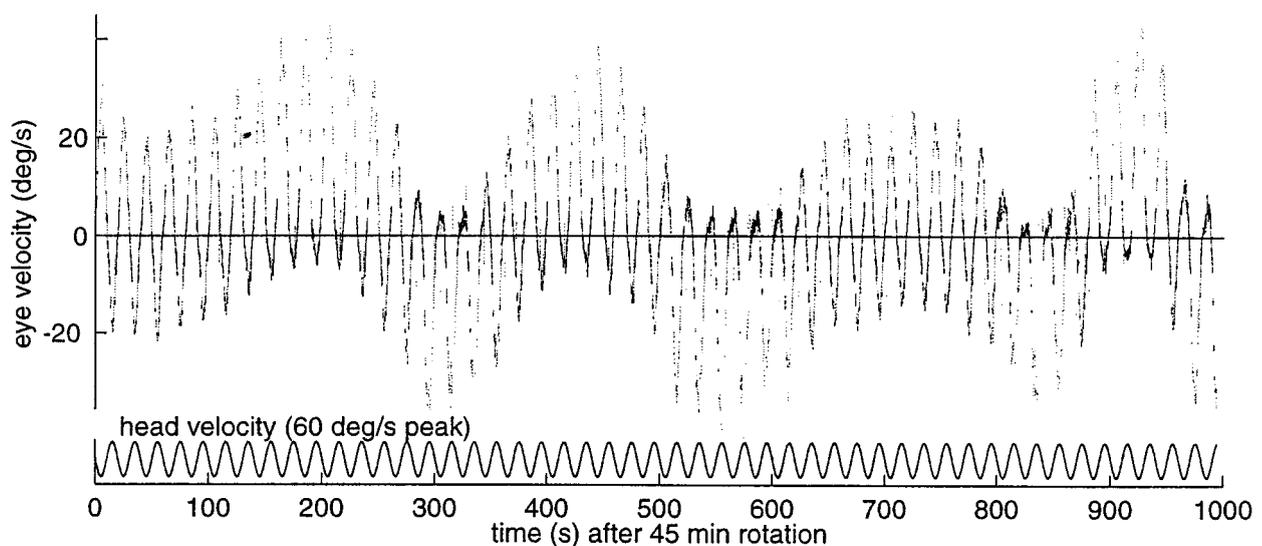


FIG. 1. PAN superimposed on the VOR response after 45 min of continuous rotation at 0.05 Hz. The period and amplitude of PAN varies during the record. Upper trace, slow-phase eye velocity (fast phases removed); lower trace, head velocity (not to scale).

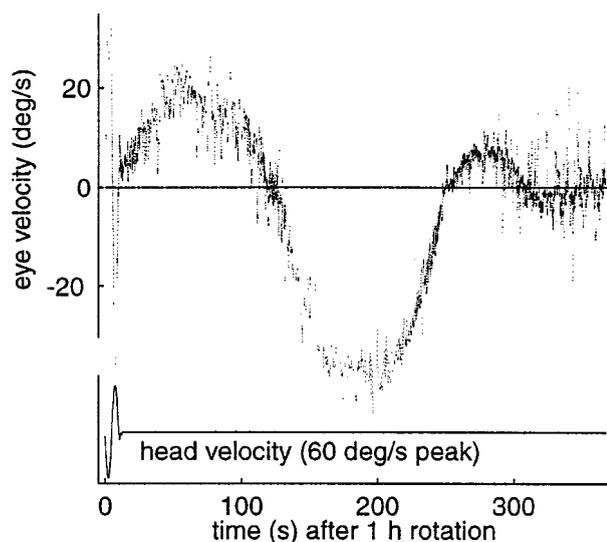


FIG. 2. Persistence of PAN following termination of rotation. The final cycle of 0.1 Hz rotation is shown (lower trace). The first post-rotatory cycle of PAN has a longer period and greater amplitude than the second cycle (upper trace). There is a sharp inflection at the beginning of the second cycle (~260 s).

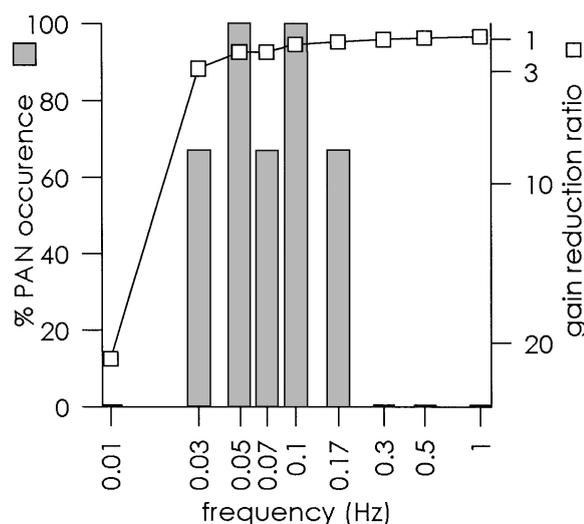


FIG. 3. Comparison of occurrence of PAN and amount of habituation as a function of prolonged rotation frequency. The percentage observed PAN (bars) is the number of goldfish that produced PAN exceeding criterion amplitude (see Materials and Methods) expressed as a percentage of the number of goldfish rotated for 1 h at each frequency. Habituation is expressed as the median gain reduction ratio (boxes) after 1 h of rotation at each frequency. Number of goldfish tested was 14 at 0.01 Hz,¹¹ six at 0.05 Hz and three at each of the other frequencies.

1.1 times. Rotations at higher frequencies (≥ 0.3 Hz) produced neither PAN nor a decrease in gain. Figure 3 shows that PAN is most likely to occur during prolonged rotations at frequencies between those that strongly and those that weakly habituate the VOR.

Human PAN has been simulated using a limit-cycle model by Leigh *et al.*³ This model included a saturation-limited velocity-storage loop and a central adaptation loop, which provided positive and negative feedback, respectively. This second-order system produced sustained, amplitude-limited oscillations, resembling PAN, after the system was made unstable by increasing the gain of the velocity-storage loop. We modified this model to simulate rotation-inducible PAN by adding a variable gain (g_a) to the central adaptation loop (Fig. 4). We further modified the model by adding a threshold non-linearity to the velocity-storage loop, as suggested by Baloh and colleagues.¹⁵ If the non-linearities (i.e. threshold and saturation) are ignored, linear analysis shows that the model will be unstable when $[(1 - g_s)/\tau_s + g_a/\tau_a]$ is negative, and will oscillate with a period of $[2\pi\sqrt{(\tau_s\tau_a/g_a)}]$. The parameters of the linear elements in the model took the following values: $\tau_s = 3$ s, $g_s = 1.05$, and $\tau_a = 30$ s. Initially g_a was set at 1 and the model was stable. The model became unstable and exhibited PAN-like oscillations when g_a was decreased. The unstable oscillations of the linearized system would grow exponentially in amplitude and would persist following termination of rotational stimulation. In the non-linear system, the saturation (± 1.25) limits the amplitude of the unstable oscillations, and the threshold (± 0.0225) causes the oscillations to decay after the stimulus terminates.

The model was used to simulate VOR responses to prolonged rotation at 0.01 Hz, 0.05 Hz and 0.3 Hz (Fig. 5). The goldfish VOR habituates at 0.01 Hz.¹¹ This was simulated by increasing g_a , which reduced the gain of the system at low frequency. Increasing g_a made the model even more stable than it was initially, so PAN-like oscillations could not occur. To simulate PAN at 0.05 Hz, g_a was decreased. This made the model unstable and PAN-like oscillations developed with a time course similar to that observed. PAN persisted for ~2 cycles after the rotational stimulus was terminated. Decay in post-rotational PAN was due to the threshold, which caused a sharp inflection between half-cycles which is also apparent in the data (compare Figs 2 and 5B). To simulate a stationary VOR response at 0.3 Hz, g_a was not modified from its initial value of 1. Because the model is stable with g_a set at 1, PAN-like oscillations did not occur (not shown).

Discussion

The function of velocity-storage is to enhance the low-frequency response of the VOR. Robinson^{16,17} modeled velocity-storage as a positive-feedback loop. This first-order model contained no central adaptation loop, and would become unstable if the

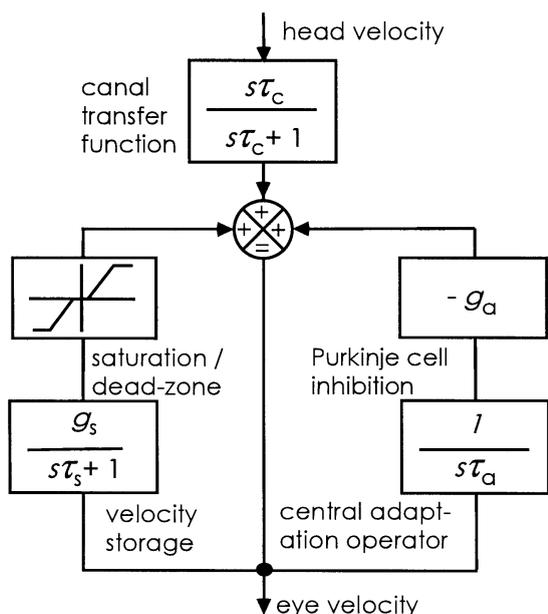


FIG. 4. Model used to simulate PAN. Input to and output from the model represent head and slow-phase eye velocity, respectively. The time constants of the canal ($s\tau_c/(s\tau_c + 1)$) and velocity-storage ($g_s/(s\tau_s + 1)$) elements were set to the value of the canal time constant as determined experimentally in goldfish²⁵ ($\tau_c = \tau_s = 3$ s). The time constant of the central adaptation element ($1/s\tau_a$) was 10 times longer ($\tau_a = 30$ s). The Laplace variable (s) is complex frequency ($s = j\omega$ where j is $\sqrt{-1}$ and ω is frequency in rad/s). The gain of the velocity-storage loop (g_s) is 1.05 while that of the central adaptation loop (g_a) was varied. The central adaptation loop represents in part a negative feedback loop onto vestibular nucleus neurons through inhibitory Purkinje cells of the cerebellar nodulus and uvula. The static non-linearity in the velocity storage loop consists of a threshold (± 0.0225) and a saturation (± 1.25).

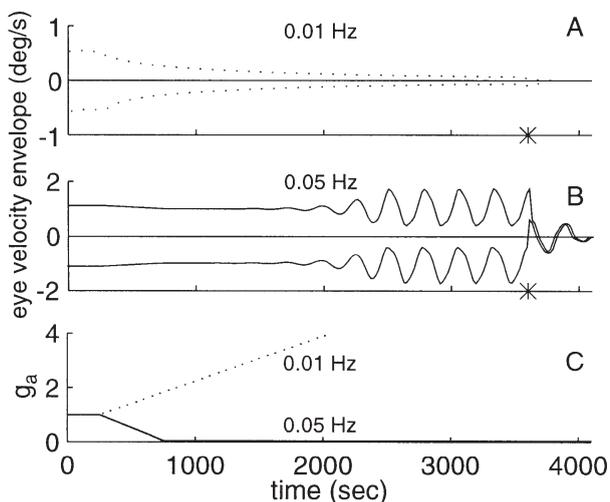


FIG. 5. Model response showing slow phase eye velocity (A, B) and gain of the central adaptation loop (C). The plots in (A and B) show the slow-phase eye velocity envelope (see Materials and Methods). The response to 0.01 Hz rotation shows a gradual decrease in the amplitude of the VOR response (A) as g_a is continually increased (C). Rotation at 0.05 Hz caused development of amplitude-limited oscillations in eye velocity (B) after g_a was decreased to 0.05 (C). (Decreasing g_a to zero would produce an unstable but non-oscillatory first-order system.) The oscillation decayed after the rotation was terminated (* in A, B). Note sharp inflection between half-cycles, as in the data (see Fig. 2).

gain of the positive-feedback loop was too high. In the model of PAN provided by Leigh *et al.*,³ the velocity-storage loop intentionally was made unstable and, with the addition of a saturation element and the central adaptation loop, this second-order model produced amplitude-limited PAN-like oscillations. They suggested that the velocity-storage loop is stable under normal circumstances, and its instability under abnormal circumstances results in PAN. In contrast, we suggest that the velocity-storage loop is unstable under normal circumstances, but this instability is kept in check by the central adaptation loop. With the initial parameter set, our second-order model is stable, and produces low-frequency gain enhancement through a resonance at its low natural frequency. PAN-like oscillations occur in the model when inhibition due to central adaptation is reduced and the model becomes unstable.

The main assumption of our model is that the NU inhibits brain stem VOR pathways, and that habituation and PAN result from increases and decreases in this inhibition, respectively. This assumption is supported by the fact that inhibitory Purkinje cells project from the NU to the vestibular nuclei in many species.¹⁸⁻²² In addition, NU lesions prevent habituation or cause dishabituation,^{5,7-10} while stimulation of the nodulus causes a suppression of the VOR.²³ NU lesions can produce PAN which develops only in darkness,^{5,6} and with less delay if the animal had just been exposed to rotational stimulation.⁵ These results indicate that PAN in nodulo-uvulectomized animals can be suppressed or enhanced by visual or rotational input, respectively, and are consistent with our finding that PAN in intact animals can be induced by rotational stimulation at specific frequencies in darkness.

The central adaptation loop in our model in part represents the NU. The model simulates our results on rotation-induced habituation and PAN with increases and decreases in central adaptation, which correspond to increases and decreases in NU Purkinje cell activation, respectively. The main weakness of the model is its inability to explain our finding that, in most cases, partial habituation and PAN occur simultaneously. This could happen in the real VOR if some of the parallel pathways are inhibited while others are released from inhibition. This in turn could be due to the lateral inhibition among Purkinje cells.²⁴ Increases in activation of some Purkinje cells, leading to habituation of some VOR pathways, would result in the lateral-inhibitory suppression of neighboring Purkinje cells, leading to PAN in other, parallel pathways. The result for the overall VOR would be PAN superimposed on a habituating VOR. This hypothesis will be evaluated in future simulation studies using distributed neural network models.

Conclusion

We show for the first time that PAN, superimposed upon the normal VOR response, can be induced in intact animals by prolonged rotation in darkness at specific frequencies. Our results are consistent with a model in which an unstable, excitatory velocity-storage loop is stabilized by an inhibitory, central adaptation loop, and their interaction produces a low-frequency resonance that enhances the low-frequency response of the VOR. Prolonged rotation at specific frequencies could cause central adaptation to decrease, which produces an unstable, low-frequency oscillation resembling PAN. The central adaptation loop represents in part the cerebellar nodulus and uvula, the removal of which are known to produce PAN.

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