

RESEARCH NOTE

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Otolith orientation and downbeat nystagmus in the normal cat

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Abstract Upward drift of the eyes in darkness, influenced by whole body orientation, was studied in 12 cats using electromagnetic search coil and electro-oculographic techniques. Animals were positioned stationary with respect to gravity with 0° tilt (“upright”) or rolled 90° (“on side”), pitched 90° (“on nose” or “on tail”), or inverted 180° (“upside down”). A downbeat quick-phase nystagmus (slow-phase upward in the cat’s orbit) was measured, varying in magnitude with angle of tilt (0.21°/s at 0° tilt; 4.14°/s at 180° tilt). The drift was not present in the light. Upward eye velocities over a range of body orientations in darkness suggest a systematic drive to the eyes which increases with tilt away from upright. The relationship of this behavior to previous models of angular velocity estimation by an otolith-driven central mechanism is discussed.

Key words Vestibulo-ocular reflex · Otolith organs · Gravity · Eye movements · Nystagmus

Introduction

Anecdotal reports of spontaneous upward eye movements have been in the physiologic literature and have been reported in many species for years. Matsuo and Cohen (1984) mentioned in their methods an upward drift in monkeys of around 5°/s, as did Angelaki and Hess (1995). Barmack (1981) inferred a gravity-related upward slow phase in the rabbit, based on vertical vestibulo-ocular reflex (VOR) asymmetry during rotation, and commented on possible saccular roles in such a response. Darlot and Denise (1988) discussed an upward eye movement bias seen in the cat in terms of central mechanisms constructing and storing vertical eye velocity based on canal and otolith signals.

Upward ocular drift has been recognized to the point of selecting animals for study which minimally express this natural eye movement (Matsuo and Cohen 1984; Angelaki and Hess 1995); however, the eye movement itself has not been studied. We have presented preliminary evidence of a consistent upward drift of the eyes (downbeat quick-phase nystagmus) during darkness, which increases in velocity with increases in head tilt from upright (Rude et al. 1991). This drift overwhelmingly favors the same direction in the orbit and ranges from negligible to large velocities, in accordance with the angle of tilt. In this paper we will quantify the upward drift correlated with angle of tilt and measure the reliability of the phenomenon.

Materials and methods

All procedures followed the principles of laboratory animal care set forth by the National Institutes of Health in the *Guide for care and use of laboratory animals* (NIH publication no. 86–23, revised 1985) and were approved by the Institutional Animal Care and Use Committee at Northwestern University. Twelve cats were prepared for chronic electromagnetic search coil recording using sterile surgical procedures under halothane anesthesia (1% halothane and 1:1 N₂O to O₂).

A coil of two to four turns of Teflon-insulated stainless steel wire (50-strand, 0.001 in., Cooner Wire Co., #AS-635) was implanted by the following procedures: The eyelids were retracted using 3–0 silk suture, and a trough was made all around the limbus, 1 mm from the sclerocorneal junction, using a no. 15 blade cutting through the conjunctiva to the level of the sclera. A pocket 3 mm wide and 8 mm deep was blunt dissected temporally into the eye socket to contain a strain-relief “S-curve” of lead wire coming off the coil. The coil and S-curve were inserted in the trough and pocket, respectively, and the free ends of the lead wires were threaded subcutaneously around the side of the head to a connector at the top of the skull, maintaining enough slack to allow untethered movement of the eye. The conjunctiva was then drawn tight over the wounds, partially obscuring the cornea, with 5–0 absorbable purse-string sutures. The conjunctiva retracted to normal positions in 2–3 days postoperatively, allowing the conjunctival flap to heal over the coil in the trough, fixing it firmly to the eyeball. Coil placement was confirmed by post-mortem examination on several cats.

Five of the 12 cats had search coils implanted binocularly to measure convergence. Three of the cats with monocular coils were

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also implanted with electro-oculographic (EOG) electrodes (after Bond and Ho 1970) to determine the suitability of EOG recording for drift measurements. Nylon screw inserts were cemented to the skulls of all cats in the horizontal stereotaxic plane and were used to immobilize the head at an angle flexed 28° nose-down during experimental rotations. Flocillin antibiotic (0.5 ml/kg) and Buprenex analgesic (0.05 mg/kg) were administered prophylactically for 1 day postoperatively under the advice of a veterinarian, and continued if deemed necessary.

Recording was begun as soon as postoperative edema and conjunctival occlusion subsided (usually several days). The cats were placed in a carrier in a normal upright resting position and restrained with a veterinary bag (e.g. J.A. Webster, Inc., model #149002) and foam padding, with attention being paid to the animals' comfort. The head was bolted to a restraining bar which flexed the head at a 28° nose-down pitch from the stereotaxic plane. This places the vertical semicircular canals in a position which minimizes their projection onto the earth-horizontal plane (Ezure and Graf 1984). We call this the upright, 0° tilt position.

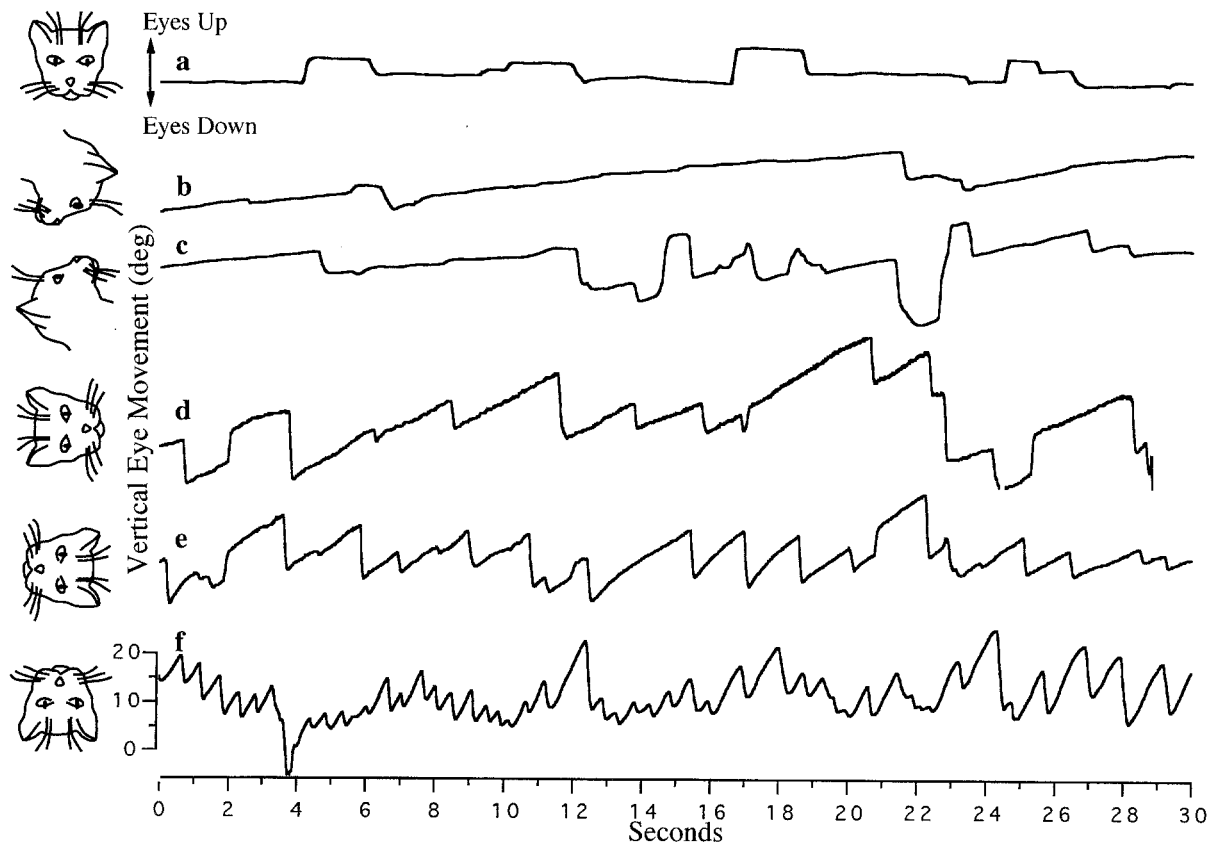
The animal carrier was mounted on a computer-controlled rotator with a servo-driven axis of rotation that passed through the C1–C2 vertebral joint regardless of the body orientation. The static tilts employed were roll or pitch angles from the upright (0°) position to the inverted (180°) position with a minimum of ten replications for each tilt position. Left and right side positions ($\pm 90^\circ$ roll) were interleaved and responses were compared. Before each experiment the cat's VOR was calibrated in the upright position: An interesting fixation target was spotlighted while the cat was oscillated horizontally or vertically for five cycles at 0.1 Hz, with runs at 5°

and 10° amplitude ($\pm \pi^\circ/s$ and $\pm 2\pi^\circ/s$). The averaged ratio of slow-phase eye velocity to head velocity during these trials was called a VOR gain of 1.0 (i.e., eye movement compensating perfectly for head movement), and was used for comparison in the analysis of subsequent trials.

Trials comprised 100-s and 400-s records collected and stored by a Macintosh computer at 1000 Hz, or in early experiments 80 Hz. After each positioning, cats rested 1–5 min before recording began. Saccadic intervals in the eye position record were marked later by visual inspection using a program developed in our department, leaving the slow-phase portion of the data unchanged. Eye velocity was calculated for each continuous eye position record which remained after saccade removal, by a linear regression fit of the eye position. All slow-phase velocities of each cat at each orientation were then averaged, and final averages were calculated across cats. A second method of analysis was performed which gave very similar results, but is not included in this paper: The eye position data from all cats at each orientation were pooled, and the slope of the linear regression fit to each intersaccadic interval was weighted according to the length of the interval [e.g., $(4.0^\circ/s * 1800 \text{ ms})$]. The weighted values at each orientation were summed, then divided by the total time of all slow phases at that orientation to determine drift velocity (e.g., $[(4.0^\circ/s * 1800 \text{ ms}) + (8.0^\circ/s * 200 \text{ ms})] / 2000 \text{ ms} = 4.4^\circ/s$).

In addition to eye movement records in the dark, continuous measurements were taken during light and dark episodes in four cats $\pm 90^\circ$ rolled or 180° inverted. Attempts were made to correlate degree of vergence with eye drift velocity in five cats, although the depth of fixation could not be reliably quantified due to difficulties in training the cats to fixate targets at specific distances.

Fig. 1a–f Six 30-s vertical eye position records (in degrees) from five cats. Upward on the trace indicates upward eye movement in the orbit. Orientations from top to bottom are: **a** upright as defined in methods (0° tilt); **b** facing floor (pitched forward +90°); **c** facing ceiling (pitched backward -90°); **d** lying on right side (rolled +90°); **e** lying on left side (rolled -90°); **f** upside down (rolled 180°). Records are scleral search coil recordings



Results

All cats were able to maintain steady fixation in the light with no visible nystagmus regardless of orientation. In the dark, in all orientations other than upright, there was

Fig. 2 Average across cats of slow-phase eye velocity with standard errors for static orientations of tilt with respect to gravity. Positive eye velocity indicates eye movement upward in the orbit of the cat. *Dotted lines* indicate pitch angles (positive is forward, negative is backward). *Continuous lines* indicate roll angles (positive is rightward, negative is leftward). All 180° angles are identical positions

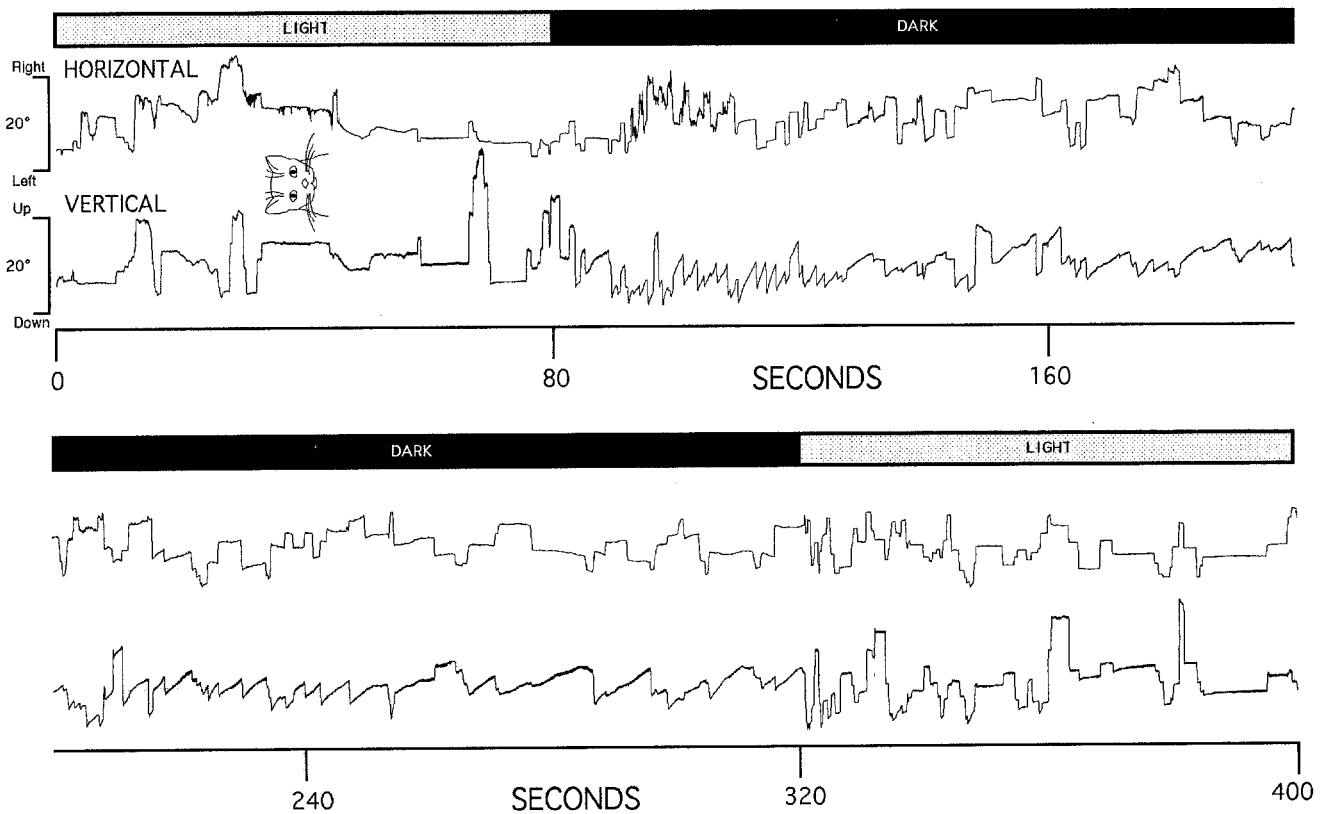
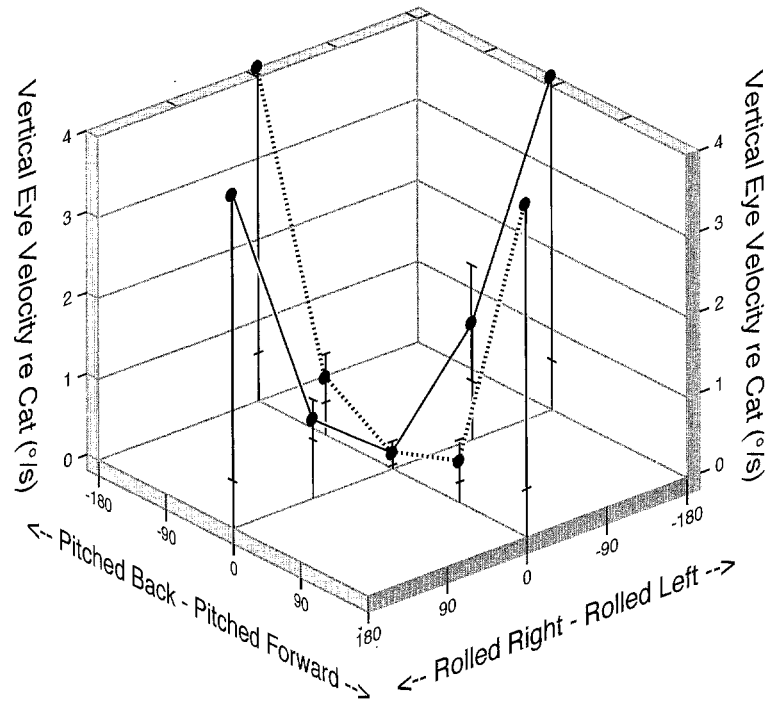


Fig. 3 Continuous 400-s horizontal and vertical eye position record (in degrees) from a cat lying on its right side (rolled +90°). The initial 80 s are in light, followed by 240 s in darkness, and finally 80 s in light again. *Upward* in the vertical trace is upward in the cat's orbit. *Upward* in the horizontal trace is rightward in the cat's orbit. Records are scleral search coil recordings

a persistent slow-phase eye velocity which was upward in the orbit and was found to vary in magnitude according to angle of tilt (Fig. 1). No consistent horizontal drift was seen in any position.

Figure 1 illustrates a 30-s sampling of vertical eye position from several cats showing clear representations of vertical drift in the dark at different whole body orientations. Figure 1a, upright, displays no slow-phase velocity, while orientations pitched nose-down or nose up (Fig. 1b,c), or rolled to the left or right side (Fig. 1d,e), display obvious vertical slow-phase drift upward in the orbit. Figure 1f is a typical record indicating that the vertical drift is largest when the cat is 180° inverted. All records were taken at least 3 min after positioning.

Figure 2 illustrates averaged vertical eye velocities of all cats at each orientation after saccade removal and calculation of drift velocity from regression fits to intersaccadic intervals (as described in Materials and methods). Unweighted averages across cats yielded a mean velocity and standard error of 0.21°/s±0.16 for upright (0° tilt), 1.00°/s±0.21 on right side (rolled 90°), 1.46°/s±0.73 on left side (rolled -90°), 0.55°/s±0.26 on nose (pitched 90°), 0.76°/s±0.28 on tail (pitched -90°), and 4.14°/s±3.50 for the inverted position (180° tilt). Three of the cats in this study that had one scleral search coil also had EOG implants, but it is the coil data which have been used for the averages. However, the drift phenomenon was readily apparent and quantifiable from the EOG records alone, and was easily distinguishable from the DC drift which can accompany EOG recordings.

Four cats were studied during 400-s stationary trials either rolled ±90° or inverted 180° (upside down), comparing eye movements in light and in dark. No vertical drift was seen in an initial 80-s period of normal room illumination. Upward drift became apparent within the first few seconds after visual input was removed, remained present during the 240 s of darkness, and disappeared once more when the lights were turned on for a final 80-s period (Fig. 3). Horizontal slow-phase eye velocity varied nonsystematically around zero.

Although we were unable to train the cats to fixate specific target distances, we were able to distinguish relative changes in binocular vergence angles. No consistent correlation between vergence and drift velocity could be determined.

Discussion

A predominance of upward eye movement in the VOR has been noted for many years. This has been expressed in terms of longer time constants for upward post-rotatory and optokinetic after-nystagmus (Guedry and Benson 1970; Anderson et al. 1979; Darlot et al. 1981; Matsuo and Cohen 1984), better upward versus downward pursuit (Guedry and Benson 1970; Baloh et al. 1983), and a nystagmic beat frequency during upward slow phase VOR which is higher on the side than upright (Benson and Guedry 1971; Barmack 1981; Matsuo and Cohen 1984).

Because the upward eye movements reported here depended on head orientation rather than any visual or dynamic vestibular stimulus, we initially proposed a mechanism by which upward eye velocity is produced in proportion to the reduction of downward gravitational stimulus to the head brought about by increased tilt away from upright (Rude et al. 1994). The model is in keeping with the particular sensitivity of the sacculus to accelerations in the inferior-superior directions (Daunton and Melvill Jones 1973; Fernández and Goldberg 1976). The velocity of upward drift was modelled as

$$V = k * (1 - \cos(\theta))$$

where θ is the angle of the head with respect to the upright position. Thus an upward eye velocity was added linearly to the VOR, specifically a simplified saccular signal [proportional to $-\cos(\theta)$] summed with an offset to produce zero eye velocity when the head was upright. A simple model of this sort can simulate the aforementioned observations of predominance of upward eye movement.

The offset term in the model may reflect an adaptive cancellation of the downward stimulus of gravity (upward linear acceleration) to the sacculus. This cancellation via an offset constant would prevent the reflexive downward movement of the eyes when the animal maintains its normal head posture, while allowing linear VOR responses to changes in vertical acceleration. In addition, an offset which is constant would produce an upward drive to the eyes, dependent on saccular activity, which would be revealed in a cosine-tuned manner as the orientation of the head moves away from upright; since the response of otolith hair cells varies sinusoidally as a function of their orientation with respect to gravity (the cosine rule), the preliminary model of direct saccular control of eye drift predicts a sinusoidal modulation of upward drift velocities across the range of tilts from upright to upside down (0° to 180°). However, the data illustrated in Fig. 2 do not provide strong support for this simple model, in that the upward drift velocities at 90° tilt are too low for the cosine function with offset (1.00°/s and 1.46°/s versus $k=2.07$ °/s predicted from the 180° data).

There is evidence that at least some saccular afferents are not perfectly sinusoidal in their spatial responses (Fernandez and Goldberg 1976; Anderson et al. 1978) and may be distorted 10–30% in their “on” direction. The low observed values of upward drift in the 90° positions could be explained, for example, by a selective contribution from saccular afferents with superiorly directed morphologic polarization vectors and larger excitatory than inhibitory responses. In other words, a more complex scheme, incorporating signals with a greater difference between 90° and 180° responses than between 0° and 90° responses, would be required for the simple model to describe upward ocular drift accurately.

An alternative modelling approach is to pass saccular inputs to a central mechanism controlling eye movements, such as the otolith central velocity estimator, responsible for estimating angular velocity from dynamic

otolith stimulation (Darlot and Denise 1988). This approach predicts that, on the basis of the observed behavior, the saccular signal has a nonlinear tonic effect on this central estimator. Consistent with this idea is the lack of effect of vergence on drift velocity. The linear VOR is strongly dependent on vergence (Schwartz et al. 1989; Paige 1991; Paige and Tomko 1991) while the angular VOR is less so (Viirre et al. 1990). Our analysis of the relative vergence shifts and the associated drifts found no obvious differences between the vergence states. Since the drift velocity does not seem to be strongly dependent on vergence it may be that the vertical drift is not directly related to the linear VOR, but rather is tied more closely to the processing of otolith signals by the central estimator of angular velocity.

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