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ASYMMETRIES IN OCULAR AND CERVICAL RESPONSES TO SAGITTAL VESTIBULAR STIMULATIONS

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INTRODUCTION

In cats the eye responses to vertical vestibular stimulations show asymmetries (2, 5, 12, 13) when evoked under particular conditions. The peak velocity and the duration of the upward-directed compensatory slow phases (CSPs) of the vertical vestibulo-ocular reflex (VVOR) exceed those of the downward-directed CSPs when the animals are rotated about the interaural axis in a side-down position (VVOR 90°). In contrast, the CSPs show vertical symmetry when the animal is sagittally oscillated in the upright position (VVOR 0°). We have demonstrated that the lack of dynamic otolithic modulation by gravity in the side-down position is the primary cause of the VVOR 90° asymmetries and that the upward preponderance is characteristic of the pure vertical ampullo-ocular reflex (8, 9).

This preponderance does not seem to be necessary for eye stability, since eye responses are not opposed by gravity. In fact the otolithic modulation abolishes the vertical asymmetry. Therefore it remains to be explained why this upward preponderance exists in the vertical vestibular circuitry. It is possible that these canal-induced asymmetries are, in some way, related to the vestibulo-collic reflex (VCR). Much of the pathway leading from the vestibular system to the oculomotor nuclei is also used to carry vestibular impulses to the motoneurons of the neck (6). While gravity does not offer any significant opposition to ocular movements, it does affect movements of the head. Because of the opposition by gravity to upward-directed head movements, the neck muscle reflex responses have to be more dynamic than those required during downward-directed movements of the head. Because of the common pathway, this preponderance may be reflected in both VCR and VOR.

If this was the case, we should expect to find canal-induced ocular asymmetries when either a laterally or frontally-eyed animal is pitched in the sagittal plane, whereas no disparity in the eye response should be seen during roll stimulation. Such a finding would allow us to relate the presence of vertical upward preponderance to the plane in which gravity presents unidirectional opposition to any moving load. Secondly, we should expect to observe asymmetries in the EMG activation patterns of the extensor and flexor neck muscles during head pitch (sagittal VCR), while no differences in the agonistic and antagonist muscle patterns should be seen during head roll (frontal VCR). Furthermore the sagittal asymmetries should not be suppressed by the otolithic coactivation. Evidence of an upward preponderance in the vertical VCR responses would help to explain the intrinsic asymmetry of the vertical vestibular circuitry.

METHODS

Preliminary surgical procedures. — Experiments were performed on 6 cats and 6 rabbits. The animals were anesthetized with ketamine hydrocloride (Ketalar, Parke-Davis, 25 mg/Kg) and diazepam (Valium, Roche, 2 mg/Kg). The head was held in a stereotaxic apparatus so that the horizontal semicircular canals laid in the horizontal plane (23° nose-down and 12° nose-down with respect to the stereotaxic 0 for cats and rabbits, respectively) and two head-restraining screws were secured with dental cement to smaller screws fixed to the skull. The animals were allowed to recover for at least 3 days before the eye movements were recorded.

Electromyographic recordings were performed in decerebrate cats after the eye movement recording sessions had been completed. Under halothane-nitrous oxide anesthesia the trachea was cannulated and craniotomy was performed. Cerebral tissue was aspirated to decerebrate the animals by a transection in a coronal plane about 5 mm anterior to the superior colliculi. Animals were allowed to recover for at least 1 hour before the electromyographic recording.

Experimental apparatus and stimulations. — The animals were firmly secured inside a cradle placed on a servo-controlled triaxial turn-table (3M 3000 Mangoni, Italy). The head was secured by means of the head-restraining screws to a stereotaxic frame. Vestibular stimulation was delivered by sinusoidally rotating the animals in their sagittal plane (pitch) and frontal plane (roll). Cats and rabbits were also pitched 90° side-down and rolled 90° nose-up. In these positions, modulation of the otolithic input by the gravity vector is eliminated and the response can thus be attributed to stimulation of vertical semicircular canals alone (4).

Eye and table position recording. — Eye position was monitored by an infrared light projection technique (4). The eye was anesthetized (Novesina 0.4%, Sandoz) and a small suction cup bearing a light emitting diode (LED) was attached to the cornea. The LED projected a narrow beam of infrared light onto a photosensitive X-Y position detector (SC-50, United Detector Technology) 5 mm from the tip of the LED.

Ocular torsion recordings in rabbits were obtained by simultaneous use of two photosensors (10). One of them was placed in the sagittal plane and the other in the frontal plane. A second LED, placed perpendicular to the axis of the suction cup, projected the light beam onto the surface of the frontal photosensor. The ocular displacement measured by the frontal photosensor resulted from the combined torsional and vertical eye movements. To obtain torsional recording alone the vertical reading from the sagittal detector was subtracted from the combined response reading. The eye movements transducers were calibrated moving the LED on a model of the animal eye through known horizontal, vertical and torsional angular displacements. They were found to be linear within 5% for eye displacements of ± 15°. Table position was measured by a servo-potentiometer.

Horizontal and vertical components of the eye position and table position signals were displayed on a digital oscilloscope (VKS 22-16, Vuko, Germany) and stored by a magnetic tape recorder (Store 4DS, Racal, UK). The eye position signals were analogically differentiated to obtain the eye velocity signals.

Electromyographic recording. — After cutting the skin, neck muscles were exposed. The edges of incision were anesthetized by Lydocaine (Xylocaina, Byk Gulden) to prevent pain from the skin. Pairs of stainless steel wires insulated except at tip were inserted in several positions in Complexus muscle (extensor and torsional muscle) and in Longus capitis (flexor muscle). Mineral oil at body temperature was employed to prevent cooling and evaporation. The electrode position was verified at the end of experiment. Multiunitary activities were recorded from a neck muscle during each experimental session (8-10 recordings).

The electromyographic activity was differentially amplified and filtered (bandpass filter of 10-3000 Hz). Then electromyographic recording and table position were stored by a magnetic tape recorder (Store 4DS, Racal, UK).

Data analysis. — Signals were digitized off-line using an integrated hardware and software package (ISC-16-E, Computerscope EGA-A System, Electronic Inc., USA) mounted on an AT compatible personal computer (Vectra ES-12, Hewlett Packard, USA).

The gain of the ocular responses was evaluated comparing the compensatory slow phase peak velocity with the table peak velocity. The phase (re 180° + table position) was measured at each half cycle of rotation. Gains and phases were compared by using Student's test.

Asymmetries were evaluated comparing the eye peak velocities during the movements in upward and downward directions (cat) or extorsional and intorsional ones (rabbit).

Electromyographic signals were digitized with a sample period of 640 μsec. High-amplitude artifactual responses were rejected. The digitized data were rectified and 6 to 20 cycles were averaged. Then 43 or 60 points/cycle histogram were computed and a least square procedure was used in order to obtain the first and the second harmonic plus a DC term indicating the average level of electromyographic activity. EMG response timing was determined with respect to table position. Harmonic distortion was calculated as the ratio of second to first harmonic.

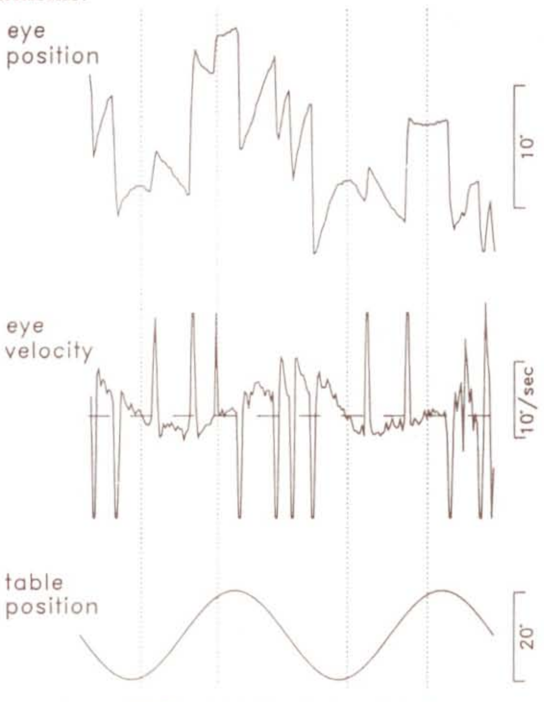


Fig. 1 - *Compensatory slow phases (CSPs) and anticompensatory fast phases (AFPs) in VVOR 90°.*

Upper trace: eye position signal in VVOR 90° at 0.1 Hz, 20° (vertical bar: 10°). Upward deflections represent upward eye movements. Middle trace: eye velocity signal (vertical bar: 10 deg/sec). Horizontal line represents O velocity and vertical lines are traced in correspondence of the 0 velocity signals. Note the different durations of the upward and downward half-period and the relative differences of the slow and fast phases.

RESULTS

1. *The VOR in frontal and sagittal planes.*

Because of the differences in eye implantation, rotation about the interaural axis (pitch) evokes vertical compensatory eye responses in the cats (VVOR) and torsional responses in rabbits (TVOR), while rotation about the longitudinal axis (roll) evokes VVOR in rabbits and TVOR in cats. In the upright position all ocular responses to pitch and roll were nearly symmetric throughout the range of tested frequencies in both rabbits and cats. The asymmetry index, calculated as the ratio of upward peak velocity to downward peak velocity, was, in fact, close to 1.

In contrast, when the animals were pitched in 90° side-down position, the ocular responses of both cats and rabbits showed clear asymmetries. Analysis of eye position in cats shows greater upward directed compensatory slow phases (CSP) than downwards ones (Fig. 1).

Analysis of the eye velocity in VVOR of the cats (Fig. 2) shows that the peak velocities of the upward directed compensatory slow phases were higher and more

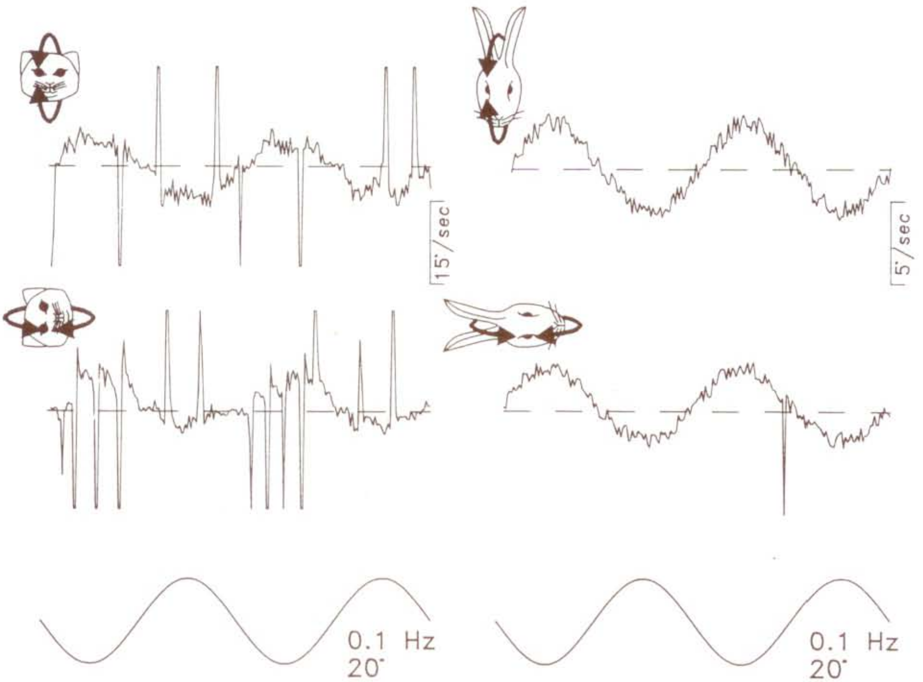


Fig. 2 – *Asymmetry of the compensatory slow phase in the VVORs.*

Left side: eye velocity tracings of VVOR (up) and VVOR 90° (middle) evoked in cats with sinusoidal stimulation at 20°, 0.1 Hz. Upward deflections represent upward eye movements: Down trace: table velocity. The horizontal lines represent 0 velocity. Vertical calibration: 5°/sec. Right side: eye velocity tracings of torsional vestibulo-ocular reflex (TVOR) (up) and TVOR 90° (middle) evoked in rabbits with sinusoidal stimulation at 0.1 Hz, 20°. Upward deflections represent extorsional eye movements. Down trace: table velocity. Vertical calibration 5°/sec.

delayed than those of the downward CSPs (Fig. 2). The asymmetry index was significantly greater than 1 within the 0.05-0.2 Hz frequency range with the highest value at 0.1 Hz (asymmetry ratio >2) (t-test at 0.1 Hz.: $p<0.001$) (Fig. 3). Furthermore in cats the upward-directed slow compensatory responses were more frequently interrupted by AFPs than were the downward ones (Figs. 1 and 2).

In rabbits the TVOR in the side-down position was also asymmetric with a preponderance of extorsional responses over intorsional ones (Fig. 2). However the asymmetry index was lower than that observed in the cats, but still significant (t-test at 0.1 Hz: $p<0.005$) (Fig. 3). AFPs were not observed during upright position, but they did appear during side-down position. Though small and rare, they were consistently more frequent during the extorsional slow phases (Fig. 2).

In contrast to pitch stimulation, roll stimulations with the animal's longitudinal axis perpendicular to the earth (nose-up position), evoked symmetric responses in both cats and rabbits. The asymmetry index was, in fact, close to 1.

2. *The VCR in frontal and sagittal planes.*

EMG of the extensor and torsional muscle (Complexus muscle). — Multiunitary electromyographic responses of the complexus muscle to pitch and roll stimulations ($\pm 15°$, 0.2 HZ) were recorded (8-10 recordings for each muscle). Since this muscle contributes almost equally to both reflexes (3), roll and pitch VCRs can be compared. In cats during sinusoidal pitch stimulation, most of the EMG patterns

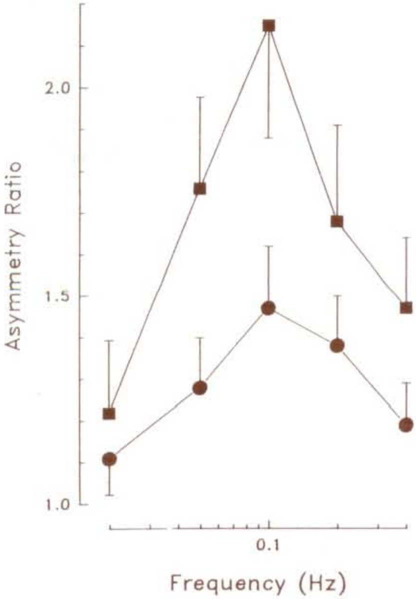


Fig. 3 – *Effects of different stimulation frequencies (0.02-0.4 Hz) on upward downward peak velocity ratios in VVOR 90° in cats (squares) and TVOR 90° in rabbits (circles).*

Vertical bars: standard deviations.

(75%) were characterized by an asymmetry between the activation and inhibition phases. In fact EMG activity increased steeply during downward pitching and decreased gradually during upward pitching. Peak activation led the stimulus (reposition) by about 45° ± 14° while peak inhibition led by 16° ± 8°. Harmonic analysis of the responses showed a high degree of harmonic distortion, as the second harmonic was 20-50% of the first, and the second harmonic max peak led the first harmonic max peak by 35-55° (Fig. 4). The EMG responses of the complexus muscle to the roll stimulation showed a more symmetric behaviour. In most cases the responses had a sinusoidal shape with harmonic distortion of less than 10%. Furthermore, in those responses with distortion higher than 10% (34% of the cases), the time relationship between the first and second harmonics differed from that observed during pitching, with the max peak of second harmonic leading the max peak of the first by 75°-90° (Fig. 4).

EMG of the neck flexor muscle (Longus capitis). — During pitch stimulation the EMG responses showed a high degree of harmonic distortion (>30%). However, in contrast to extensor muscle behaviour, the inhibitory phase was remarkably steeper than the activation phase. Hence the inihibition peak led the stimulus by 54° ± 15°, while the activation peak led by 19° ± 13°. Harmonic analysis reflected this difference revealing an opposite time relationship between the first and second harmonics compared to the extensor responses (Fig. 5).

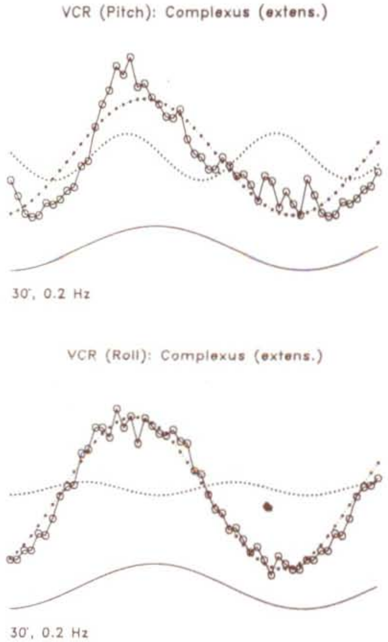
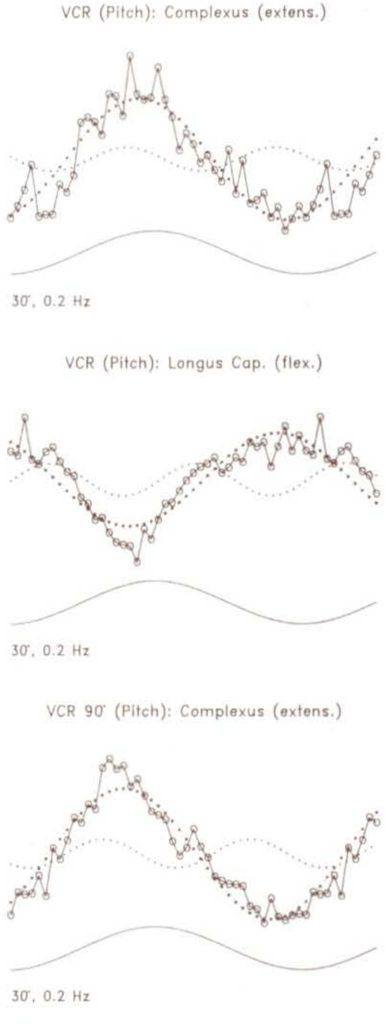


Fig. 4 – *Averaged electromyographic recordings (10 cycles) in Complexus muscle during pitch (upper part) and roll (lower part) vestibular stimulation (±15°, 0.2 Hz).*

The first harmonic (small squares) and second harmonic (dotted curve) are indicated. At the bottom of each panel the table position (continuous curve) is shown.


VCR (Pitch): Complexus (extens.)

VCR (Pitch): Longus Cap. (flex.)

VCR 90° (Pitch): Complexus (extens.)

Fig. 5 - *Effects of different vestibular stimulations on neck muscles.*

Averaged electromyographic responses (10 cycles) of an extensor muscle (Complexus) to pitch stimulation (±15°, 0.2 Hz) in upright position (upper part) and in 90° side-down position (lower part). The middle part of the figure shows EMG response of the flexor muscle (Longus capitis) to pitch stimulation (±15°, 0.2 Hz). In each panel table position (continuous curve) and first (small squares) and second (dotted curve) harmonics are indicated.

The sagittal VCR evoked in side down position. — The sagittal VCR responses were also recorded in animals positioned on their sides so that the vertical semicircular canals were stimulated without coactivating the otolithic receptors. EMG responses (Fig. 5) maintained the same asymmetric behaviour observed in the upright position, i.e., the activation peak was more leading the inhibition peak and the second harmonic was 20-40% of the first one. The timing difference in the VCR responses obtained between VCR 0° and VCR 90° was limited only to the phase of the first harmonic, which was 20° anticipated in side-down position compared to upright position.

Asymmetry in the upward and downward responses of VCR. — To evaluate the timing asymmetry in the VCR, the activation peak phases of the extensor responses and the inhibition peak phases of the flexor responses (extension phase) were compared to the activation peak phases of the flexor responses and the inhibition peak phases of the extensor responses (flexion phase).

At 0.2 Hz of stimulation the EMG extension peak led the stimulus by about 40°-45° while the flexion peak led by 15°-20°. This finding reveals an asymmetry in the VCR: the extensor command is anticipated with respect to the flexor command. At higher and lower frequencies of stimulation the EMG responses were less asymmetric. The asymmetry observed in the EMG activity of the neck muscle during VCR was frequency dependent in a similar way as the VVOR 90° asymmetry was (Fig. 6).

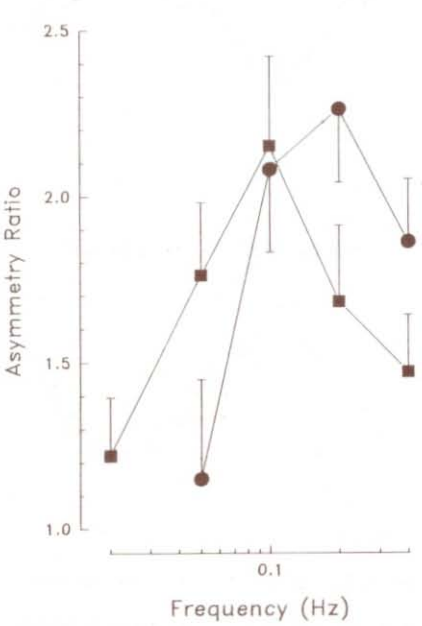


Fig. 6 - *Asymmetry ratio of VVOR and VVCR at different frequencies of stimulation in 90° side-down position.*

Squares: the asymmetry ratio of VVOR (upward eye velocity/downward eye velocity); circles: asymmetry ratio of VVCR (phase of extensor muscle activation peak + phase of flexor muscle inhibition peak/phase of flexor muscle activation peak + phase of extensor muscle inhibition peak). Vertical bars: standard deviations.

DISCUSSION

When cats and rabbits were sinusoidally oscillated in 90° side down position, asymmetrical ocular responses were evoked only in sagittal plane. These asymmetries consisted of a preponderance of upward slow-phases in frontally eyed animals and of extorsional slow-phases in laterally eyed animals. Upward and extorsional slow phases were in fact characterized by higher velocity and longer duration than those of the opposite-directed phases. The eyes were maintained in the central orbital range in spite of these CSP asymmetries by greater and more frequent anticompensatory fast phases. In the upright position no asymmetry was observed during sagittal stimulations in either cats or rabbits. In both directions the gain of the VVOR 0° was almost equal.

It appears that dynamic modulation of the otolithic receptors which occurs in upright position causes a decrease of the upward responses and an increase of the downward ones suppressing vertical and torsional asymmetries. Since gravity does not oppose upward-directed rotatory eye movements, the symmetrizing function of the otolithic reflex seems to be necessary to achieve a good vertical gaze stability. Therefore the canal-induced asymmetries remain an intrinsic feature of vertical reflexes. However, this is present only in the sagittal plane, since it is not present when stimuli are delivered in the frontal plane, regardless of the animal's position or eye implantation type. Thus it would appear that this asymmetry is the result of a specific combination of vertical canal activation. The simultaneous activation of both anterior or the inhibition of both posterior semicircular canals, which occurs during downward head pitching, causes higher gains than those seen by activating posterior canals and inhibiting anterior ones during upward head pitching. Conversely the combined stimulation of the anterior and posterior semicircular canals on one side, caused by roll stimulation, gives rise to symmetric responses. We should attribute the asymmetry of vertical reflexes to the characteristics of spatial orientation of vertical semicircular canals or of the network combining both anterior or posterior canals rather than to their different sensitivity. In fact it has been shown that vertical semicircular canal afferents have almost the same sensitivity to acceleration (1).

Since gravity does not affect the ocular responses, there is no reason for canal-induced asymmetry at level of the eye motility, and in fact, it disappears in upright position in which the otolithic receptors are stimulated. On the contrary we showed that asymmetries were present at level of the VCR in upright as well as in side-down position. The characteristics of neck muscle responses to vestibular stimulation have been extensively studied (3, 6, 7, 11). However, there is no evidence in the literature for an asymmetry in the neck muscles EMG responses to pitch and roll stimulations. In fact, although the harmonic distortion was recognized by other authors they restricted the EMG analysis to the first harmonic (3, 7). In this paper we suggest the role of the second harmonic in determining different time characteristics of the activation and inhibition.

During sagittal stimulation, the lead of the extensor neck muscle activation peak was greater than that of the inhibition peak; this relationship was inverted for the flexor muscles of the neck. In addition, the EMG activity rising was steeper than the decay in the extensor muscles and *vice versa* for the flexor muscles. This was reflected in the results of the harmonic analysis, which showed significant distortion and a characteristic phase combination of the first and second harmonics. The otolithic contribution to the reflex appears to delay the first harmonic phase leaving the response asymmetric. Therefore, the canal induced activation pattern appears to be maintained asymmetrical by the otolithic reflex. Interestingly, in the frontal plane the EMG response was less asymmetric considering the activation and the inhibition peak and fully symmetric considering the two directions of tilt. Therefore, in sagittal plane, the extension command is clearly anticipated to oppose gravity, while the head flexion command which is facilitated by gravity is delayed. This asymmetry would be necessary to oppose the force of gravity, which acts unidirectionally on the head in the sagittal plane, whereas in roll stimulation, in which the gravity acts in both directions, there is no need for a different timing in the two directions of the head response.

SUMMARY

In rabbits and cats vestibulo-ocular reflex (VOR) and vestibulo-collic reflex (VCR) were tested in frontal and sagittal planes. The VOR evoked by pitching the animals in side down position showed asymmetric responses with peak eye velocity higher in upward direction than in downward one. This asymmetry was abolished when pitching the animal in upright position. This indicate that the otolithic coactivation, occurring in upright position, symmetrizes canal-induced vertical responses. Vestibulo-ocular reflex evoked by rolling the animals was also symmetric, no matter the animal position was.

The electromyografic response of neck muscles to sagittal VCR showed an asymmetric behaviour as far as the timing of extensor and flexor activation was concerning. In fact, the increase of extensor muscle activity was steeper than the decrease and the activation peak was more leading than the inhibition peak. The flexor muscles showed an opposite behaviour. This flexion-extension asymmetry was present either with or without otolithic coactivation. On the contrary, the EMG responses to frontal VCR were much more symmetric.

It can be suggested that the upward preponderance is present in the sagittal vestibular circuitry to enhance the upward VCR against a gravity load which opposes head stability during downward head displacements. On the contrary, at level of the eye, in which the gravity does not oppose the responses, the canal-induced vertical asymmetry is cancelled by the otolithic coactivation.

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