

Effects of Alertness on Three-Dimensional Eye Movements

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Purpose: To investigate effects of alertness on three-dimensional (3D) eye movements.

Methods: During drowsy and alert periods, 3D eye movements were recorded with dual search coils in three normal rhesus monkeys, and in two of these monkeys after placement of bilateral kainic acid lesions of the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF).

Results: When the three monkeys were in the normal state, the average plane thickness (standard deviation of all rotation vectors from the regressed plane) during drowsy periods (range, 1.2–1.9°) increased significantly compared with the plane thickness during alert periods (range, 0.7–0.9°) in the light ($P < .05$). The plane thickness increased significantly in the monkey with asymmetric bilateral riMLF-lesions ($P < .05$); however, the increase was not significant in the monkey with more symmetric bilateral riMLF-lesions. After the bilateral riMLF-lesions, the plane thickness also increased with drowsiness in both monkeys ($P < .02$), as it had in the normal state.

Conclusion: The implementation of Listing's law is independent of the presence of vertical-torsional burst neurons in the riMLF. The increase in thickness of Listing's plane during drowsy periods is not due to an imprecise signal from saccadic burst neurons to the 3D velocity-to-position integrator. It is possible that this integrator itself controls Listing's law, depending on the state of alertness. **Jpn J Ophthalmol 2000;44:457–462** © 2000 Japanese Ophthalmological Society

Key Words: burst neuron, drowsiness, Listing's law, riMLF, torsion.

Introduction

When the head is erect and not moving, Donders' law states that the amount of ocular torsion during fixation is uniquely defined by the horizontal-vertical gaze direction and, hence, is independent of the path that the eye has traveled to the current position.¹ The exact mathematical relation between ocular torsion and gaze direction is given by Listing's law²: If eye positions are represented by single axis rotations from a reference position, all axes lie in a plane. Rotation vectors³ and

quaternion vectors^{4,5} are oriented parallel to these rotation axes and their length is given by the amount of rotation; therefore three-dimensional (3D) positions of fixation described as rotation or quaternion vectors lie in a plane.

Since in *encéphale isolé* cats, Listing's law is still valid during alert periods but is violated during sleep, Nakayama suggested that Listing's law is not a mechanical property of the eye plant, but implemented by the central nervous system.⁶ A significant decrease in the precision of Listing's law during drowsy periods was also reported in monkeys.⁷ Accordingly, a reduced position sensitivity of burst-tonic units in the periauducens or pericuculomotor area and position-vestibular pause neurons in the medial longitudinal fasciculus was found by extracellular single-neuron recordings in drowsy rhesus monkeys.⁸ Likewise, vertical eye position coding in

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tonic activity in the interstitial nucleus of Cajal (iC) is not preserved during sleep in cats.⁹ These findings also suggest a neural implementation of Listing's law. Since the anatomical geometry of extraocular muscles probably does not change between alert and drowsy periods, the violation of Listing's law during drowsy periods is a consequence of inappropriate supranuclear activity that disturbs the synergistic activity of the six extraocular eye muscles necessary to implement Listing's law. A possible candidate for neural signals that could drive the eye out of Listing's plane during drowsy periods is the 3D saccadic burst generator in the rostral interstitial nucleus of the medial longitudinal fascicle (riMLF); in this region, tonic activity of vertical-torsional short-lead burst neurons could be recorded during drowsy periods in monkeys.⁸

To investigate whether, in fact, the tonic activity of the burst neurons in the riMLF has a role in the generation of torsional eye movements out of Listing's plane during drowsy periods, we have investigated effects of alertness on 3D eye movements before and after bilateral lesions of the riMLF. Parts of the results have been reported in abstract form.¹⁰

Materials and Methods

Preparation

Three rhesus monkeys (TA, CR, AL) were trained to fixate on a light emission diode (LED) according to the paradigm of Wurtz.¹¹ All experimental procedures conformed to the ARVO statement for the Use of Animal in Ophthalmic and Vision Research, and the protocol was approved by the Veterinary Office of the Canton of Zürich. Under aseptic conditions and general anesthesia, a head holder and a stainless cylinder over a trephine hole in the skull were stereotaxically implanted with screws and acrylate cement. In addition, dual magnetic search coils^{12,13} were implanted on the sclera of one eye. Three-dimensional eye positions were measured with an Eye Position Meter 3000 or 3020 (Skalar Instruments, Delft, Netherlands) and calibrated by a procedure described in Hess et al¹⁴.

Experimental Protocol

During experiments, each monkey was seated in a primate chair with the stereotaxic plane tilted 15° nose-down to position the lateral semicircular canals approximately horizontally. Spontaneous eye movements were recorded in 3D (horizontal, vertical, torsional) both in the light and in the dark. Each recorded file consisted of 92 seconds of eye movements.

During each experimental session, we recorded 1–3 files of spontaneous eye movements during alert periods. Thereafter, we turned off the lights and let the monkey fall asleep. Drowsy periods could easily be distinguished from alert periods when we observed eye movement traces on the oscilloscope. Recordings were started when slowly drifting horizontal and vertical eye movements replaced saccades and fixations. Depending on how tired the monkey was, we recorded 1–9 files of eye movements during drowsy periods.

Data Analysis

Coil signals were digitized at 833 Hz, written onto the computer hard disk, and processed off-line. Calibrated 3D eye positions were expressed as eye rotation vectors.³ According to the right-hand rule, the base vectors of the head-fixed coordinate system point forward (x-axis), leftward (y-axis), and upward (z-axis). Positive rotation about the x-axis corresponds to extorsion of the right and intorsion of the left eye; likewise, positive rotation about the y- and z-axes produces downward and leftward movements, respectively.

Eye position data were rotated so that the null vector coincided with primary position as defined by Listing's law. After this rotation, we computed the standard deviation of all rotation vectors from the regressed plane, so-called "plane thickness."

Chemical Lesions in the riMLF

In two monkeys (TA, CR), small lesions were sequentially placed in the riMLF on both sides, with a 1-week interval between the lesions. We used kainic acid (4.8 µg kainic acid for each side; concentrations: 8 or 16 µg/µL), which was injected using microsyringes. Before the micro-injection, the exact localization of the riMLF was determined by single unit recording.¹⁵ After several weeks of data collection, monkeys were deeply anesthetized with pentobarbital sodium (Nembutal®) and perfused transcardially with a phosphate-buffered 4% formaline solution. Histological analysis of frontal frozen sections of the whole brain stem was done with a light microscope.

Results

We fitted first-order linear regressions (planes) to the end points of eye rotation vectors during spontaneous eye movements. The data were recorded before and after bilateral riMLF-lesions were placed in the 3 rhesus monkeys (TA, AL, and CR), during both alert and drowsy periods. Table 1 lists the median and average plane thickness for the files re-

Table 1. Median/Average Thickness of Regressed Plane*

	Before Lesion			After Lesion		
	Light	Dark	Drowsy	Light	Dark	Drowsy
	Mdn/Avg	Mdn/Avg	Mdn/Avg	Mdn/Avg	Mdn/Avg	Mdn/Avg
TA	0.9°/0.9° (3)	0.6°/0.6° (4)	2.0°/2.0° (12)	1.0°/1.1° (9)	0.5°/0.5° (5)	1.3°/1.4° (8)
CR	0.8°/0.8° (7)	0.5°/0.5° (4)	no data	1.0°/1.0° (4)	0.7°/0.7° (3)	1.7°/1.6° (4)
AL	0.9°/0.9° (2)	0.8°/0.8° (1)	1.2°/1.2° (9)			

*Values in parentheses represent the number of analyzed files.

corded under the three conditions (alert in the light, alert in the dark, drowsy in the dark) before and after the lesions.

Three-Dimensional Eye Positions Before riMLF Lesion

When the monkeys were alert, the plane thickness ranged between 0.7–0.9° in the light and 0.5–0.9° in the dark. In all three monkeys, the plane thickness was slightly less in the dark than in the light. During alert periods, spontaneous eye movements consisted of fixations, and horizontal and vertical saccades both in the light (Figures 1A, B, Figure 2A) and in the dark (Figures 1C,D). When the monkeys became drowsy, the characteristics of eye movements changed dramatically. Now, slow drifting eye movements in the

horizontal and vertical directions could be observed (Figures 3A–C). These drifting eye movements are directly related to sleep-specific electroencephalographic (EEG) patterns, as described by Henn et al.⁸ Careful inspection of ocular drift movements during drowsy periods revealed a considerable torsional movement component, corresponding to a violation of Listing’s law (Figures 3B,C). Ocular torsion reached values up to 10° during drowsy periods.

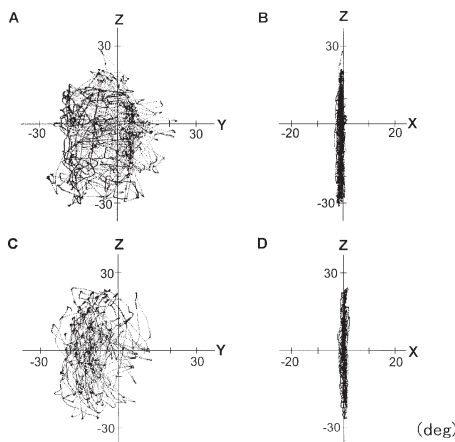


Figure 1. Three-dimensionally recorded 92 seconds of spontaneous eye movements in light (A,B) and in dark (C,D) of monkey CR during awake periods before medial longitudinal fasciculus lesion (A,C) horizontal (Z)–vertical (Y) projection. (B,D) horizontal (Z)–torsional (X) projection. Scales are in degrees. Standard deviation of regressed plane is 0.6° in light and 0.5° in dark. Maximum deviation of torsional components from Listing’s plane is less than 2°.

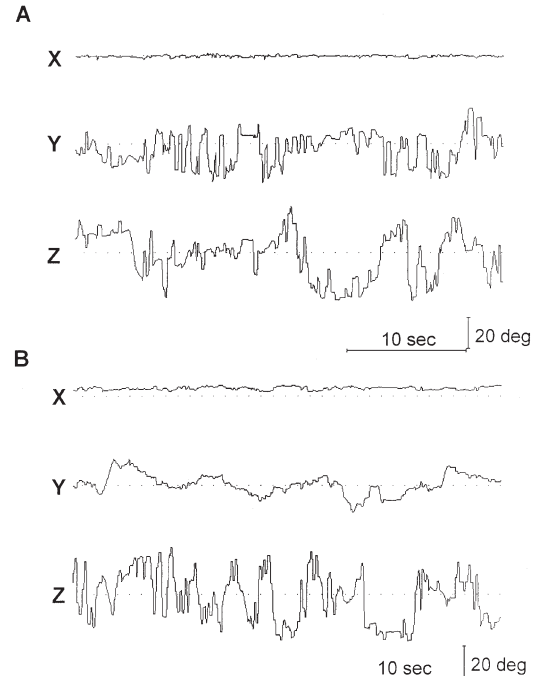


Figure 2. Three components of 36 seconds of spontaneous eye movements of monkey CR during awake periods in light. (A) Before medial longitudinal fasciculus lesion (riMLF) lesion. Torsional components (X) are scattered around zero and vertical (Y) and horizontal (Z) components consist of fixation periods interrupted by saccades. (B) After bilateral riMLF lesion. Lesion was not symmetric and resulted in tonic positive torsional deviation of about 5°.

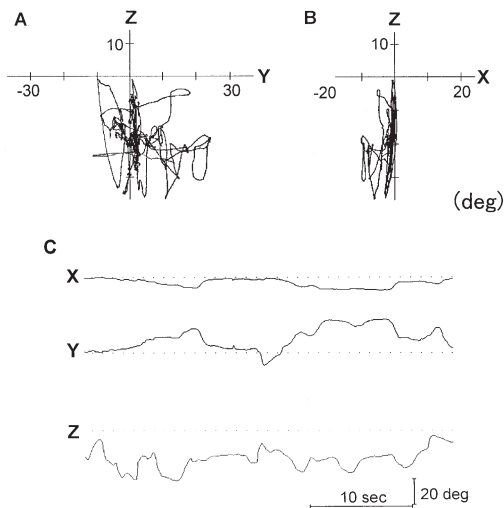


Figure 3. Slow drifting eye movements of monkey AL during drowsy periods in normal condition. (A,B) Three-dimensional distribution of 92 seconds of eye movements are shown with horizontal-vertical (A) and horizontal-torsional (B) projections. Maximum deviation of torsional components is -10° (B). (C) Three components of 36 seconds of eye movements taken from Figures 3A and B are plotted against time.

Both the median and average plane thickness during drowsy periods was 2.0° ($n = 12$) in monkey TA and 1.2° ($n = 9$) in monkey AL. When we assume equal variance, the average plane thickness during drowsy periods was significantly thicker than that during alert periods in the light and in the dark (Student's one-tailed *t*-test; $P < .001$ (light and dark, TA); $P = .031/.018$ (light/dark, AL).

Three-Dimensional Eye Position After Bilateral riMLF-Lesions

Three-dimensional eye movements during alert and drowsy periods after bilateral riMLF-lesions were investigated in two of the monkeys (TA and CR). In monkey TA, we could directly compare eye movement data during drowsy periods before and after the bilateral riMLF-lesions. In monkey CR, only the data after bilateral riMLF-lesions were available, ie, no drowsy data before lesions were taken. The effects of uni- and bilateral riMLF lesions on Listing's plane have been reported elsewhere, including the histological findings of monkeys TA and CR.¹⁵ Unilateral lesion of the riMLF with kainic acid resulted in strong counter-torsional deviation of the eyes and an increased variability of Listing's plane. Bilateral lesions of the riMLF caused a total loss of vertical and torsional rapid eye movements, but the characteristics of Listing's plane were preserved.

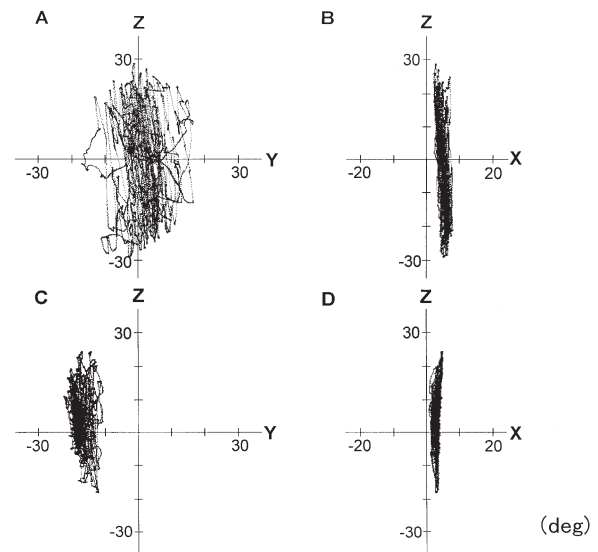


Figure 4. Three-dimensional distribution of 92 seconds of spontaneous eye movements of monkey CR after bilateral medial longitudinal fasciculus lesion. Eye movements in light (A,B) and in dark (C,D) are shown with same configuration as Figure 1. Left and right columns show horizontal (Z)-vertical (Y) projection and horizontal (Z)-torsional (X) projection, respectively. Parts of data in light are also shown in Figure 2B. Tonic positive deviation of torsional components corresponds to positive offset of Listing's plane that is less in dark. Standard deviation of Listing's plane is 1.0° in light and 0.5° in dark.

Monkey TA had symmetrical bilateral riMLF-lesions, and Listing's plane revealed no torsional offset after the lesions (Figure 3 in Suzuki et al¹⁵). Spontaneous eye movements of monkey CR in the light after the bilateral riMLF-lesions are shown in Figures 4A,B and Figure 2B.

The bilateral-riMLF lesions in monkey CR were not symmetrical and resulted in a positive torsional shift of Listing's plane of about 5° in the light. This torsional shift was less in the dark (Figures 4C,D). Rapid eye movements in the vertical and/or torsional direction were severely impaired; however, horizontal rapid eye movements were still preserved (Figure 2B). After bilateral riMLF-lesions, the plane thickness in the light increased to $1.0^\circ/1.1^\circ$ (median/average, $n = 9$) in monkey TA and $1.0^\circ/1.0^\circ$ (median/average, $n = 4$) in monkey CR. The increase in the average plane thickness was significant in monkey CR ($P < .01$) but not significant in monkey TA ($P = .09$).

Figures 5A-C show 3D eye movements during drowsy periods recorded on the same day as the data in Figure 1. Characteristics of eye movements were similar to the eye movements during drowsy periods in the normal monkey (Figure 3). Slow drifting eye

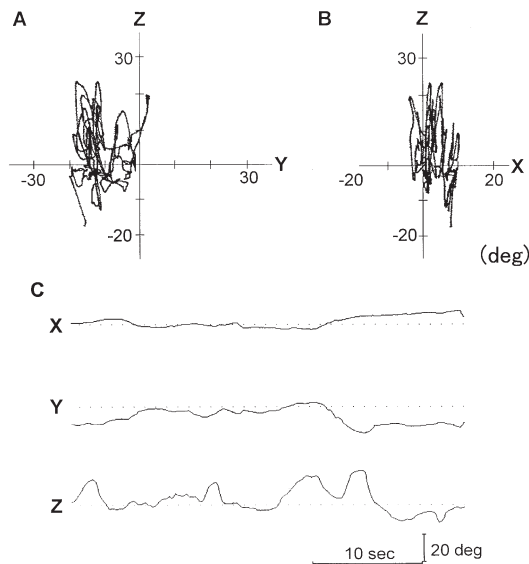


Figure 5. Slow drifting eye movements (92 seconds) during drowsy periods of monkey CR recorded on same day as Figure 4. Configuration of panels is same as in Figure 3. (A) horizontal (Z)–vertical (Y) projection. (B) horizontal (Z)–torsional (X) projection. (C) Three components of 36-second eye movements taken from Figures 5A and B.

movements were observed not only in the vertical and horizontal, but also torsional direction. The plane thickness was 1.8° , ie, nearly double the average plane thickness in the alert condition. The torsional oculomotor range was only 6.0° with alertness; however, it increased up to 15.2° during drowsy periods. The median and average plane thickness during drowsy periods were 1.3° and 1.4° in monkey TA ($n = 8$) and 1.7° and 1.6° ($n = 4$) in monkey CR, respectively. In both monkeys, the average plane thickness during drowsy periods was significantly larger than that during alert periods in the light and in the dark ($P = .014$ and $.007$, light and dark, respectively, in monkey CR; $P = .021$ and $P < .001$, light and dark, respectively, in monkey TA; Student's one-tailed t -test, assuming equal variance).

Discussion

A clear difference in the characteristics of spontaneous horizontal and vertical eye movements between alert and drowsy conditions has been reported in the past. Changes of EEG activity from a high frequency low amplitude pattern to a low frequency high amplitude pattern were accompanied by disruption of fixation and saccade in the rhesus monkey.⁸ The frequency of saccades during spontaneous eye movements was used as an index of alertness in

cats.¹⁶ In this paper, we measured spontaneous eye movements in three dimensions during drowsy and alert periods and observed a clear difference in torsional eye movements similar to that observed in horizontal and vertical eye movements. During drowsy periods, the torsional oculomotor range was still smaller than the horizontal and/or vertical oculomotor range. However, Listing's law was clearly violated. The average plane thickness during drowsy periods was significantly larger than that during the alert period in the light in the monkeys when in the normal condition ($P < .05$). The anatomical arrangement of extraocular muscles and the surrounding soft tissue in the orbit probably restrict the torsional oculomotor range, but cannot reduce rotational degrees of freedom from 3 to 2. Nakayama already pointed out the violation of Listing's law during sleep periods in *encéphale isolé* cats and concluded that the nervous system must provide the one degree of rotational constraint to the eye.⁶ We agree with his arguments. Neural implementation of Listing's law possibly takes place downstream of the deep layer of the superior colliculus.¹⁷ If so, good candidates for this implementation are the 3D burst generator and the 3D velocity-to-position integrator. Of course, the oculomotor system still can modify the orientation of Listing's plane, eg, during convergence,^{18,19} or violate Listing's law, eg, during torsional vestibulo-ocular reflex, with such neural implementation of Listing's law.

Pure burst neurons for torsional and vertical rapid eye movements have been found in the riMLF.^{20–22} We have extensively reported on effects of kainic acid lesion on the riMLF in the alert rhesus monkey and concluded that the burst activity in the riMLF was not necessary to implement Listing's law, because Listing's law retains its validity after the bilateral riMLF-lesions.¹⁵ On the other hand, imprecise fluctuating tonic activity of burst neurons in the riMLF during drowsy periods⁸ could generate slow torsional eye movements drifting out of Listing's plane. To investigate the role of tonic activity of the burst neurons in the riMLF during drowsy periods, we compared the average plane thickness during drowsy periods with that during alert periods in monkeys with the bilateral riMLF-lesions. The violation of Listing's law with slow drifting torsional eye movements was similar to the violation of Listing's law observed in the normal monkeys. The average plane thickness during drowsy periods was significantly larger than that during the alert period in the light ($P < .025$) after the bilateral riMLF-lesions. Hence, fluctuating activity of burst neurons in the

riMLF was not responsible for the torsional and vertical drifting eye movements during drowsy periods. These results agree with our conclusion in the alert monkey that the riMLF is not responsible for the neural implementation of Listing's law.

In contrast to the drowsy period, rapid eye movements can be observed during the REM period of sleep. A very short time constant of vertical velocity-to-position integration suggesting leaky integrator during REM sleep has already been reported in normal cats.⁹ The interstitial nucleus of Cajal (iC) is a part of torsional and vertical velocity-to-position integrator.^{23,24} Fukushima and Fukushima concluded that neurons in the iC were not involved in slow drifting eye movements during sleep periods because no correlation between tonic discharge rates of iC neurons and vertical eye positions has been found.⁹ They also showed preservation of high and regular resting discharge rates of iC neurons during both non-REM and REM sleep in cats. However, the correlation between discharge rates of iC neurons and torsional eye position was not analyzed. Therefore, we conclude that the 3D neural integrator including the iC is a possible candidate for neural implementation of Listing's law for conjugate eye movements.

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