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# Three-dimensional extraocular motoneuron innervation in the rhesus monkey

# I: Muscle rotation axes and on-directions during fixation

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**Abstract** The rotation axis for each of the six extraocular muscles was determined in four eyes from three perfused rhesus monkeys. Measurements of the locations of muscle insertions and origins were made in the stereotaxic reference frame with the x-y plane horizontal and the x-z plane sagittal. The computed rotation axes of the horizontal recti were close to being in the x-z plane at an angle of about 15° to the z axis. The rotation axes of the vertical recti and the obliques were close to being in the x-y plane at an angle of about  $30^{\circ}$  to the y axis. In five alert rhesus monkeys, we simultaneously recorded extraocular motoneuron activity and eye position in three dimensions (3D). The activity of 51 motoneuron axons was obtained from the oculomotor (n=34), trochlear (n=11), and abducens nerve (n=6)during spontaneous eye movements. To extend the torsional range of eye position, the animals were also put in different static roll positions, which induced ocular counterroll without dynamic vestibular stimulation. Periods of 100 ms during fixation or slow eve movements ( $<10^{\circ}/s$ ) were chosen for analysis. For each motoneuron, a multiple linear regression was performed between firing frequency and 3D eye position, expressed as a rotation vector, in both stereotaxic and Listing's reference frame. The direction with the highest correlation coefficient (average  $R=0.94\pm0.07$ SD) was taken as the on-direction. Each unit's activity

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could be unequivocally attributed to one particular muscle. On-directions for each motoneuron were confined to a well-defined cone in 3D. Average on-directions of motoneurons differed significantly from the corresponding anatomically determined muscle rotation axes expressed in the stereotaxic reference frame (range of deviations: 11.9° to 29.0°). This difference was most pronounced for the vertical recti and oblique muscles. The muscle rotation axes of the vertical rectus pair and the oblique muscle pair form an angle of  $58.3^{\circ}$ , whereas the corresponding angle for paired motoneuron on-directions was 105.6°. On-directions of motoneurons were better aligned with the on-directions of semicircular canal afferents (range of deviation: 9.4–18.9°) or with the anatomically determined sensitivity vectors of the semicircular canals (range of deviation: 3.9–15.9°) than with the anatomically determined muscle rotation axes, but significant differences remain to be explained. The on-directions of motoneurons were arranged symmetrically to Listing's plane, in the sense that the torsional components for antagonistically paired muscles were almost equal, but of opposite sign. Thus, the torsional components of motoneuron on-directions cancel when eye movements are confined to Listing's plane. This arrangement simplifies the neuronal transformations for conjugate head-fixed voluntary eye movements, while the approximate alignment with the semicircular canal reference frame is optimal for generating compensatory eye movements.

**Key words** Extraocular muscle · Extraocular motoneuron · Position coding · Three-dimension · On-direction

# Introduction

Six extraocular muscles work in synergy to move the eye with three rotational degrees of freedom. The axis of rotation for a single muscle is the axis about which the eye rotates when only that muscle contracts, and it is defined by the anatomical arrangement of the muscle and the eyeball in the orbit. On the other hand, the tonic on-direction of a single extraocular motoneuron is the direction of the gradient of its innervation, which can be obtained by fitting motoneuron activity during fixation as a function of eye position. However, one does not expect that the axis of rotation for a single muscle coincides with its tonic innervation on-direction, because the six axes of rotation for each extraocular muscle are not strictly orthogonal to each other and mechanical restrictions by tissue compartments in the orbit do not allow the muscles to take the shortest path between their origin and insertion (Simonsz et al. 1985; Miller and Robins 1987; Demer et al. 1995).

Earlier studies of motoneuron on-directions were essentially one-dimensional (1D), seeking the correlation between extraocular motoneuron activity and horizontal or vertical eye movements (Fuchs and Luschei 1970; Robinson 1970; Schiller 1970; Keller and Robinson 1972) or trying to find the direction in which the activity showed the best linear fit with eye position (Henn and Cohen 1973). Subsequently, two-dimensional (2D) isofrequency curves, which showed a complex relation between activity and eye positions, were determined (Hepp and Henn 1985). While it is likely that the positions measured in these studies were in Listing's plane, neither the 1D nor 2D studies determined the orientation of primary position in a stereotaxic reference frame.

An important, but unsolved question concerning the sensory-to-motor transformation from visual and vestibular input to oculomotor output is whether the innervation on-directions of extraocular motoneurons are related more closely to the "sensory" semicircular canal system, the "visuo-motor" intrinsic reference frame of the saccadic system, or the "motor" extraocular muscle reference frame. To answer this question, precise three-dimensional (3D) measurements of eye movements are essential. In addition, the innervation on-direction of extraocular motoneurons in 3D space is also important to interpret innervation on-directions in burst neurons of the saccadic system (e.g., Hepp et al. 1989) or to determine 3D transformations in neuronal populations responsible for the vestibulo-ocular reflex (VOR) (e.g., Ezure and Graf 1984).

When characterizing on-directions in 3D space, reference frames have to be clearly defined (e.g., Soechting and Flanders 1992). Muscle rotation axes have to be expressed in anatomically defined coordinates, e.g., in a stereotaxic reference frame. Another important reference frame is the intrinsic one, defined functionally by Listing's law (Helmholtz 1867). The orientation of primary position in a stereotaxic reference frame can be variable for each subject. However, according to Listing's law, all conjugate head-fixed eye positions expressed in Listing's reference frame can be reached by rotations from primary position about the axes, which lie in a plane that is perpendicular to the gaze direction in primary position. Listing's law has been found to be valid during fixations (Helmholtz 1867; Ferman et al. 1987a), saccades (Ferman et al. 1987b; Tweed and Vilis 1990), and smooth pursuit (Haslwanter et al. 1991; Tweed et al. 1992).

We recorded neural activity from single axons in the 3rd, 4th, and 6th cranial nerves and calculated tonic 3D

innervation on-directions of single extraocular motoneurons both in stereotaxic and Listing's reference frames. We also anatomically determined the axis of rotation for each of the six muscles in a stereotaxic reference frame. Tonic innervation on-directions of extraocular motoneurons were then compared with the axes of rotation of extraocular muscles, the on-direction of semicircular canal afferents (Reisine et al. 1988), and the sensitivity vectors of semicircular canals (Reisine et al. 1988).

Innervation on-directions of extraocular motoneurons were antagonistic for the pairs of horizontal rectus, vertical rectus, and oblique muscles. They were better aligned with on-directions of semicircular canal afferents or with the sensitivity vectors of semicircular canals than with the axis of rotation for single muscles in a stereotaxic reference frame. In Listing's reference frame, the innervation on-directions were arranged symmetrical to the origin of the reference frame. Part of this work has been presented in abstract form and in a conference report (Reisine et al. 1985; Simpson et al. 1986; Suzuki et al. 1992, 1994a; Henn et al. 1994).

# **Materials and methods**

#### Animal preparation

Five rhesus monkeys (*Macaca mulatta*, 3.3–4.2 kg body weight) were first trained in the paradigm of Wurtz (1969) to fixate a light emitting diode. Then, two operations were performed: following anesthesia by pentobarbital sodium (i.p. 30 mg/kg body weight), the monkey was intubated and given a mixture of NO<sub>2</sub>-O<sub>2</sub> in a closed respiratory system. Haloperidol was added as necessary. Rectal temperature and heart rate were continuously monitored. In the first operation, we implanted four stainless steel bolts to fixate the head during the experiments and a stainless cylinder (inner diameter: 19 mm) over a trephine hole in the skull. In the scond operation, a dual magnetic search coil (Robinson 1963; Hess 1990) was implanted. It was fixed on the sclera between the limbus and the rectus muscle insertions with 8–0 Prolene mono-filament sutures and then covered by conjunctiva.

Another three rhesus monkeys (no. 92, 99, and 100) were deeply anesthetized with pentobarbital sodium and perfused transcardially with a phosphate-buffered 4% formalin solution to perform anatomical measurements of extraocular muscle geometry. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the Veterinary Office of the Canton of Zürich.

#### Calibration of 3D eye position

Eye positions were measured with the dual search coil in a twofield magnetic coil frame (Eye Position Meter 3000; Skalar Instruments, Delft, Netherlands). Calibration was performed as described by Hess et al. (1992). In brief, the in-vitro calibration obtained before implantation gave the magnitudes of the two coil-sensitivity vectors and their relative angle. In daily in-vivo calibrations, the monkey had to fixate light spots in different positions. From the voltages of four coil signals measured at these eye positions, we calculated the orientation of both coil vectors and voltage offsets.

#### Experimental protocol

During the in-vivo calibration and single-unit recordings, the monkey sat in a primate chair on a turntable that had three motorized



Fig. 1A-C Example of 3D eye-rotation vectors in Listing's reference frame during periods of fixation (monkey Cr). Eye rotations about the x-, y-, and z-axes are torsional, vertical, and horizontal, respectively. Movement of the upper ocular pole to the right, downward movement and leftward movement are positive, in accordance with right-hand rule. For ease of interpretation, rotation vector components are expressed in degrees. The monkey made spontaneous eye movements in the light, while upright or in static roll 40° right- or left-ear-down positions. A Eye positions projected onto the y-z-plane. The oculomotor range is about  $\pm 30^{\circ}$  in both the horizontal and vertical directions, and its center is near primary position, i.e., the origin of the coordinate system. B Eye positions projected onto the x-z-plane. The three distinct data clouds orthogonal to the x-axis correspond to the three head positions, resulting in different amounts of ocular counterrolling. C Eye positions projected onto the x-y-plane

axes (Acutronic, Jona, Switzerland). The head of the monkey was fixed such that Reid's plane (plane through the middle of the outer acoustic meatus and the lower orbital rim on both sides) was 15° nose-down relative to the horizontal plane of the field coil cube, and the midsagittal plane of the monkeys head coincided with the midsagittal plane of the field coil cube. The center of the interaural line was located at the common intersection of the three rotation axes of the turntable.

Extracellular single-unit activity was recorded with varnished tungsten electrodes (impedance:  $1-2 \text{ M}\Omega$  at 1000 Hz), which were advanced according to stereotaxic coordinates to the 3rd, 4th, and 6th nerve rootlets. To determine motoneuron on-directions in 3D during fixation, it was necessary to obtain eye position data in 3D. Since Listing's law restricts the rotatory degrees of freedom of eye position from three to two, we also recorded motoneuron activity in different static roll positions, which induced ocular counterrolling. With different roll positions of the head, Listing's plane is shifted counter-torsionally to the head (ocular counterroll; Haslwanter et al. 1992; Suzuki et al. 1997). When we encountered fiber activity of a putative motoneuron axon, spontaneous eye movements were recorded with the animal upright and in static roll positions (up to 30 or 40° right- or left-ear down), which increased the torsional range of eye positions up to  $\pm 8^{\circ}$  relative to Listing's plane with the head erect (Fig. 1). Single-unit activity, four channels of coil signals, and the turntable position were digitized at a frequency of  $\bar{8}33$  Hz with 12-bit resolution and stored on a computer harddisk for off-line processing.

#### Motoneuron identification

All motoneuron recordings were made from nerve rootlets, which are thought to contain only axons innervating extraocular muscles. The relative stereotaxic localization of oculomotor structures was consistent among animals. The values of absolute localization sometimes needed correction after certain landmarks were identified. One of the useful landmarks was the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF), which contains short-lead burst neurons with vertical and ipsi-torsional (extorsion of the ipsilateral eye and intorsion of the contralateral eye) rapid eye movement on-directions (Vilis et al. 1989). The bilateral paramedian location of the nucleus also gives a clear indication of the midline. Oculomotor nerve fibers were reliably found 3-4 mm below the riMLF. Over a depth of a few hundred micrometers, fiber activity from all types of motoneurons originating in the oculomotor nuclei was intermingled. Trochlear nerve-fiber activity was recorded 4-5 mm beneath the deep layers of the superior colliculus and 1-3 mm lateral from the midline. Abducens nerve fibers were localized 4-5 mm below the trochlear nerve fibers, anterior and slightly lateral to the paramedian pontine reticular formation.

After completion of the series of single-unit recordings, selected brainstem areas were inactivated with kainic-acid lesions to study oculomotor deficits. The results of these experiments have been reported elsewhere (Suzuki et al. 1995). At the conclusion of all experiments, the animal was deeply anesthetized with pentobarbital sodium and perfused transcardially with a phosphate-buffered 4% formalin solution. Frozen sections of the brainstem were cut in the coronal plane and stained with cresyl violet. Histological analysis was performed with light microscopy to confirm the relative locations of the recording sites in stereotaxic coordinates.

#### Data analysis

Rotation vectors were used to describe eye positions in 3D (Haustein 1989). They were computed as described by Hess et al. (1992) from the voltages induced in the dual search coil. The coordinates for the rotation vectors were first expressed in relation to the field coil cube, with the x-axis pointing straight ahead, forming a right angle with the horizontal axis (y-axis, interaural) and the vertical axis (z-axis), with signs according to the right-hand rule. All data were referred to the right eye. Thus, positive values correspond to movement clockwise (CW), downward, and leftward from the subject's point of view (Tweed and Vilis 1987). Rotation vectors are directed along the axis about which the eye would have to rotate from a defined reference position to the current position; the vector length, *r*, relates to the amount,  $\alpha$ , of rotation, expressed as  $r=\tan(\alpha/2)$ , e.g., r=0.05 corresponds to 5.72°.

Eye-position data obtained during recording of units projecting to the left eye were referred to the right eye by changing the signs for the x- and z-, but not for the y-components of the rotation vectors. Then, the rotation vectors were transformed from the coil reference frame to the stereotaxic reference frame and Listing's reference frame.

#### Listing's reference frame

At the start of each experimental day, we collected spontaneous eye movements over 92 s with the monkey upright and fitted a plane to the data cloud of rotation vectors in the field-coil reference frame. The three parameters of the plane gave us the necessary three parameters for the transformation by which all rotation vectors of this experimental day had to be rotated in order to align primary position with the origin of Listing's reference frame (Tweed and Vilis 1990).

#### Stereotaxic reference frame

Rotation vectors were transformed such that Reid's plane coincided with the x-y-plane of the stereotaxic reference frame. Interactive computer programs identified rapid and slow eye movements using velocity and acceleration criteria. Fixation periods were accepted when their duration was longer than 150 ms. Unit activity, together with eye position, was examined on a display, and periods with a regular firing rate were chosen with a cursor for further analysis. Bins of 100 ms were selected in the middle of these fixation periods to determine firing frequency, with a maximal resolution of 10 Hz. Periods of activity were discarded when the eye moved more than 1° or, because motoneurons display a threshold of about 20 Hz (Hepp and Henn 1985), when fewer than three spikes were encountered in 100 ms. For quantitative analysis, we related the x-, y-, and z- components of the rotation vectors in both the stereotaxic and Listing's reference frame to the tonic unit activity during fixation. Data from different static roll positions were pooled for each unit, and the following calculations were performed:

*Linear regression in 3D.* On-directions were calculated by multiple linear regression of firing frequency, f, as a function of the fixation position r=(x, y, z) in both the stereotaxic and Listing's reference frame:  $f=c_0+c_1x+c_2y+c_3z$ . The direction of  $c=(c_1, c_2, c_3)$  is defined by the unit vector (denoted by "^")  $c^{-}=c/|c|$  and is called the on-direction of the motoneuron. Similar methods have been used to determine the saccadic on-direction of motoneurons in 2D space (Henn and Cohen 1973; Delgado-Garcia et al. 1986). For each of the three components of the on-direction unit vector, confidence intervals were calculated with standard statistical methods.

Iso-frequency surfaces in 3D. Data were sorted in non-overlapping bins of firing frequencies (50 Hz width) and correlated with eye positions in 3D space. Data points clustered in layered clouds that could be approximated by second-order surfaces in the 3D space of rotation vectors. Within each bin of firing rate, least square fits were calculated using the following equation for a second order surface:

#### $x = a_0 + a_1 y + a_2 z + a_3 y^2 + a_4 y z + a_5 z^2$

Since on-directions of horizontal motoneurons have relatively small torsional and vertical components, a better fit was found using the following equation:

#### $z = b_0 + b_1 x + b_2 y + b_3 x^2 + b_4 x y + b_5 y^2$

The direction perpendicular to the surfaces in the center of the oculomotor range above threshold can be taken as another measure of motoneuron on-directions.

**Table 1** Insertions of extraocular eye muscles of four eyes (*i92*, *i99r*, *i99l*, *i100*) and on the idealized rhesus eye (*iid*). *MR* Medial rectus muscle, *LR* lateral rectus muscle, *SR* superior rectus muscle,

Anatomical measurements of the eye plant

The eye rotation axis for each of the six extraocular muscles [lateral rectus (LR), medial rectus (MR), superior rectus (SR), inferior rectus (IR), superior oblique (SO), and inferior oblique (IO)] was determined from anatomical measurements made on four eyes from three perfused rhesus monkeys (nos. 92, 99, and 100). The measurements were made with the eye in the anatomical position of rest; the eye was kept inflated by injection of saline into the vitreous cavity. The rationale for calculating the muscle rotation axes was that used since the time of Volkmann (1869; see, e.g., Robinson 1975; Miller and Robins 1987) and assumes that the eye rotates about the center of the globe and that the path of each muscle from its insertion to its point of tangency follows a great circle when the eye is in the anatomical position of rest. All measurements were referenced to a marker fixed in the skull between the eves. The muscle insertion was taken to be the midpoint along the line of insertion. To determine the location of the center of the globe, a sphere was fitted to nine points on the globe - the seven insertion points (including the accessory abducens muscle, whose coordinates we also measured, but which are not used in this paper; see Simpson et al. 1986) plus two additional points chosen at random on the more posterior surface of the globe. After measurements of the insertions, the eye was removed and the origins of the detached muscles (the trochlea for the SO) were measured. From these measurements, two vectors were determined for each muscle, one from the center of the globe to the insertion (i) and one from the center of the globe to the origin (o). The normalized crossproduct  $m=(i \times o) \land$  of these two vectors provides the rotation axis for each muscle when the eye is in the anatomical resting position.

From the data of the four eyes (k=no. 92, 99 l, 99r, and100), we computed for each muscle ( $\mu$ =MR, LR, SR, IR, IO, SO) the average rotation axis,  $m_{\mu\alpha\nu}$ =(mean( $m_{\mu k}$ )) $\wedge$  (i.e., averaged over all monkeys), and the geometry of an idealized rhesus eye. The *idealized radius* was taken to be the average length of all insertion vectors,  $r_{id}$ =mean( $|i_{\mu k}|$ ), the *idealized insertion* and *origin* of muscle  $\mu$ were defined as  $i_{\mu id}$ =(mean( $i_{\mu k} \wedge$ )) $\wedge$ \* $r_{id}$  and  $o_{uid}$ =mean( $o_{uk}$ ).

# Results

Axes of rotation of extraocular muscles

Tables 1 and 2 show the results of the anatomical measurements made on one eye in monkeys 92 and 100 and on both eyes in monkey 99, all referred to the right side. From these values of the origins and insertions, the average axes of rotation  $m_{\mu\alpha\nu}$  were computed. The x, y, and z components of  $m_{\mu\alpha\nu}$  are represented in column EOM in Table 3, together with the average on-directions of the semicircular canal afferents (CA) and semicircular canal sensitivity vectors (CS) of monkeys 99 and 100 from Reisine et al. (1988).

*IR* inferior rectus muscle, *IO* inferior oblique muscle, *SO* superior oblique muscle

	i92		i991			i99r			i100			iid			
	X	Y	Ζ	X	Y	Z	X	Y	Z	X	Y	Ζ	X	Y	Z
MR	4.2	8.0	-0.5	5.6	8.1	0.5	5.4	8.3	0.0	4.6	9.2	0.9	4.86	8.25	0.22
LR	1.6	-8.9	0.0	3.9	-9.0	-0.5	3.6	-9.0	-0.7	2.7	-10.0	-0.6	2.91	-9.11	-0.44
SR	2.6	-1.2	8.3	4.2	-2.3	8.1	3.8	-2.3	8.2	4.0	-2.6	9.1	3.71	-2.14	8.57
IR	3.7	-0.7	-8.1	4.5	-0.7	-8.3	4.8	-0.5	-8.2	3.8	-1.7	-9.4	4.22	-0.91	-8.55
IO	-8.1	-3.7	-0.7	-7.7	-5.2	-1.1	-7.8	-5.3	-1.3	-9.4	-4.7	-0.7	-8.27	-4.74	-0.95
SO	-6.1	-0.4	6.5	-5.3	-3.1	7.6	-4.9	-2.7	7.7	-5.7	-2.5	8.1	-5.53	-2.19	7.51

Table 2 Origins of extraocular eye muscles of four eyes (*o92*, *o99r*, *o99l*, *o100*) and of the idealized rhesus eye (*oid*). Abbreviations are as in Table 1

	092		o991		o99r		o100			oid					
	X	Y	Ζ	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
MR	-16.2	10.3	-3.9	-18.4	12.3	-6.1	-16.3	10.4	-4.2	-20.3	12.1	-6.3	-17.80	11.28	-5.13
LR	-17.4	6.1	-3.9	-19.4	7.6	-5.1	-18	7.4	-5.3	-21.5	8.5	-6.3	-19.08	7.40	-5.15
SR	-16.7	7.9	-0.7	-19.5	9.3	-2.2	-16.5	8.7	-0.7	-20.5	10.7	-2.8	-18.30	9.15	-1.60
IR	-17	8.6	-5.1	-19.2	9.8	-7.8	-16	8.2	-7.5	-20.4	10.3	-7.9	-18.15	9.23	-7.08
IO	5.0	1.1	-11.2	3.7	2.1	-11.9	5.3	2.9	-12.4	5.2	2.2	-11.7	4.80	2.08	-11.80
SO	7.8	6.1	8.4	6.7	7.0	8.6	6.6	7.3	8.7	7.2	6.4	9.4	7.08	6.70	8.78

**Table 3** Normalized average unit vectors of extraocular muscle rotation axes (EOM), normalized average on-directions of extraocular muscle motoneurons (MN), negative semicircular canal afferent on-directions (CA; Reisine et al. 1988), and negative anatomical sensitivity vectors of semicircular canals (CS; Reisine et al.

1988) in stereotaxic coordinates. Values for paired muscles and paired semicircular canals are also listed. *Rlc* and *llc* Right and left lateral canal, *rac* and *lac* right and left anterior canal, *rpc* and *lpc* right and left posterior canal. Other abbreviations are as in Table 1

	Extraocular muscles (EOM)			Extraoci (MN)	raocular motoneurons Semic N) Semic			cular canal tions (CA	l afferent )	Semicircular canal sensitivity vectors (CS)		
	X	Y	Z	Х	Y	Z	X	Y	Z	Х	Y	Z
MR/rlc	-0.211	0.102	0.972	-0.499	0.026	0.866	-0.196	0.019	0.980	-0.245	-0.016	0.969
LR/llc	0.310	0.148	-0.939	0.463	0.018	-0.886	0.214	-0.074	-0.974	0.252	-0.110	-0.962
SR/rac	-0.444	-0.895	-0.029	-0.805	-0.572	-0.157	-0.577	-0.759	-0.301	-0.694	-0.701	-0.162
IR/lpc	0.414	0.903	0.112	0.668	0.683	0.295	0.586	0.786	0.198	0.665	0.710	0.233
IO/lac	0.493	-0.869	0.048	0.767	-0.595	0.239	0.614	-0.709	0.346	0.656	-0.726	0.206
SO/rpc	-0.555	0.813	-0.178	-0.794	0.559	-0.239	-0.627	0.734	-0.262	-0.640	0.727	-0.248
MR&LR/rlc&llc	-0.263	-0.023	0.964	-0.481	0.004	0.876	-0.205	0.047	0.978	-0.249	0.047	0.967
SR&IR/rac&lpc	-0.430	-0.900	-0.071	-0.741	-0.632	-0.227	-0.582	-0.774	-0.250	-0.680	-0.706	-0.198
IO&SO/lac&rpc	0.526	-0.843	0.113	0.781	-0.577	0.239	0.621	-0.722	0.304	0.648	-0.727	0.227

We assumed that these eye rotation axes represent values for the eye close to the primary position. To check this assumption, we measured in one monkey (Cr) the eye position in the perfused skull with the search coil still in place and compared this value to the primary position obtained during the experiments. The anatomical position of rest after perfusion was close to primary position, as determined by Listing's plane. After perfusion, the x-, y-, and z-rotation vector components of the anatomical resting position in Listing's reference frame were 0.7, 3.7, and  $1.8^{\circ}$ , respectively. In the stereotaxic reference frame, the rotation vector after perfusion was 1.0, 19.1, and  $2.6^{\circ}$ , i.e., rotated upward by about  $20^{\circ}$ .

For the idealized rhesus eye, we obtained  $r_{id}$ =9.58 mm and values of  $i_{\mu id}$  and  $o_{\mu id}$ , as shown in Tables 1 and 2. The maximal angle between  $m_{\mu av}$  and  $m_{\mu id}$ = $(i_{\mu id}*o_{\mu id})$ was 0.3°.

Qualitative characteristics of 3D motoneuron firing patterns and data selection

Every motoneuron axon in our database could be associated with a particular extraocular muscle, knowing the recording site and the qualitative relation between the neural activity increase and the direction of eye rotation. Even during the experiment, one could classify most of the motoneurons by the recording site and by observing the change of neuronal activity in relation to the direction of eye rotation. Referring to the right side, medial rectus neurons had their main directional component in the positive z-direction with a small negative x-component [counter-clockwise (CCW)] component, while abducens neurons displayed a large negative z-component with a small positive x-component [clockwise (CW)]. In the oculomotor nerve, the only fiber type with a positive y-component clearly belonged to inferior rectus motoneurons; the torsional component was always positive (CW). Neurons with negative y components could be classified by the different tonic activity encountered with ocular counterrolling in the different static roll positions. Superior rectus and inferior oblique neurons showed an increase in tonic activity with intorsional (CCW) and extorsional positions (CW), respectively. Trochlear units all displayed a positive y-component and a negative xcomponent.

Units were selected for quantitative analysis when activity in at least two different static roll positions could be recorded and data could be obtained for at least three different bins of firing frequency (50 Hz wide), each with 10 or more fixation periods. Recordings from 51 axons were quantitatively analyzed (Table 4): 34 from the oculomotor nerve (five monkeys), 11 from the trochlear nerve (three monkeys), and six from the abducens nerve (four monkeys).

**Table 4** List of motoneurons upon which the analysis is based. Parameters given are: (1) number of fixation periods, (2) correlation coefficients of the multiple linear regressions determining motoneuron ondirections, and (3) average on-directions in Listing's coordinates and stereotaxic coordinates. Abbreviations are as in Table 1

Туре	Side	Unit	Sample	Correlation	On-direction (X, Y, Z)			
			size	coefficient	Stereotaxic frame	Listing's frame		
MR	R	So61-2 So62-2* So62-3 Ca129-2 Ca129-5* Ta21-2 Ta33-4 Cr03-2	504 271 98 121 344 268 373 552	$\begin{array}{c} 0.993 \\ 0.969 \\ 0.964 \\ 0.963 \\ 0.964 \\ 0.954 \\ 0.954 \\ 0.985 \\ 0.986 \end{array}$	$\begin{array}{c} (-0.26, \ 0.01, \ 0.97) \\ (-0.53, \ 0.00, \ 0.85) \\ (-0.50, \ 0.02, \ 0.87) \\ (-0.57, \ 0.09, \ 0.81) \\ (-0.47, \ 0.16, \ 0.87) \\ (-0.64, \ 0.00, \ 0.76) \\ (-0.61, \ -0.03, \ 0.79) \\ (-0.32, \ 0.06, \ 0.95) \end{array}$	$\begin{array}{c} (-0.12, 0.01, 0.99) \\ (-0.39, -0.04, 0.92) \\ (-0.36, 0.00, 0.93) \\ (-0.36, 0.05, 0.93) \\ (-0.25, 0.11, 0.96) \\ (-0.42, -0.05, 0.91) \\ (-0.39, -0.06, 0.92) \\ (-0.18, 0.05, 0.98) \end{array}$		
	L	So81-3 So81-5 Al22-4 Ta25-5 Ta25-8 Ta28-2 Ta56-5 Cr12-3*	276 244 307 319 490 466 153 111	0.99 0.989 0.934 0.552 0.981 0.95 0.968 0.913	$\begin{array}{c} (0.18,-0.01,-0.98)\\ (0.25,-0.02,-0.97)\\ (0.80,0.09,-0.60)\\ (0.70,0.03,-0.71)\\ (0.46,0.03,-0.89)\\ (0.56,0.06,-0.83)\\ (0.57,-0.04,-0.82)\\ (0.42,-0.06,-0.91) \end{array}$	$\begin{array}{c} (-0.03, -0.02, -1.00) \\ (0.03, -0.03, -1.00) \\ (0.75, 0.02, -0.66) \\ (0.51, -0.01, -0.86) \\ (0.22, 0.03, -0.98) \\ (0.34, 0.04, -0.94) \\ (0.34, -0.06, -0.94) \\ (0.28, -0.09, -0.96) \end{array}$		
LR	R	Cr53–10 Cr60–3	459 456	0.976 0.986	(0.38, 0.01, -0.92) (0.48, 0.10, -0.87)	(0.19, -0.01, -0.98) (0.19, 0.08, -0.98)		
	L	Ta42–5* Ta44–4 Cr43–3 Cr43–4	101 205 131 72	0.903 0.952 0.969 0.97	(-0.57, -0.01, 0.82) (-0.63, -0.09, 0.78) (-0.12, 0.02, 0.99) (-0.55, 0.07, 0.83)	(-0.32, -0.08, 0.94) (-0.35, -0.08, 0.93) (0.10, 0.01, 1.00) (-0.36, 0.05, 0.93)		
SR	R	Ca129–3 So90–5 Al45–2	1742 131 106	0.962 0.919 0.972	$\begin{array}{l}(-0.88,-0.45,-0.18)\\(-0.61,-0.78,-0.15)\\(-0.77,-0.60,-0.19)\end{array}$	(-0.85, -0.52, 0.05) (-0.62, -0.79, -0.06) (-0.78, -0.63, -0.03)		
	L	So81–4 Al22–3 Cr09–1	180 122 202	0.953 0.944 0.94	$\begin{array}{l}(0.80, -0.55, 0.23)\\(0.88, -0.47, 0.03)\\(0.83, -0.54, 0.16)\end{array}$	$\begin{array}{c} (0.84, -0.54,  0.08) \\ (0.83, -0.55,  0.05) \\ (0.83, -0.56,  0.02) \end{array}$		
IR	R	So62–1 Al20–1 Ta23–8 Ta24–6	423 175 200 1375	0.971 0.946 0.939 0.944	$\begin{array}{c} (0.82,0.56,0.13)\\ (0.57,0.75,0.34)\\ (0.69,0.66,0.30)\\ (0.74,0.61,0.27) \end{array}$	$\begin{array}{c} (0.80, 0.61, 0.01) \\ (0.62, 0.74, 0.25) \\ (0.76, 0.63, 0.12) \\ (0.81, 0.59, 0.06) \end{array}$		
	L	Ca135–1	425	0.842	(-0.47, 0.79, -0.40)	(-0.58, 0.76, -0.30)		
ΙΟ	R	So46–1 Al37–6	492 445	0.942 0.96	(0.81, -0.53, 0.27) (0.84, -0.49, 0.23)	(0.85, -0.51, 0.13) (0.85, -0.52, 0.05)		
	L	Ta25–6 Ta25–7 Ta28–3 Ta56–2 Ta56–3	428 548 459 121 346	0.986 0.938 0.982 0.951 0.972	$\begin{array}{c} (-0.73, -0.64, -0.26) \\ (-0.70, -0.68, -0.23) \\ (-0.78, -0.59, -0.23) \\ (-0.76, -0.61, -0.22) \\ (-0.75, -0.62, -0.23) \end{array}$	$\begin{array}{c} (-0.79, -0.61, -0.08) \\ (-0.75, -0.66, -0.07) \\ (-0.81, -0.59, -0.05) \\ (-0.78, -0.63, -0.01) \\ (-0.77, -0.64, -0.03) \end{array}$		
SO	R	Al28–1 Ta40–2 Cr44–7 Cr45–6 Cr45–9 Cr45–10	691 222 199 331 301 265	0.829 0.913 0.848 0.955 0.927 0.939	$\begin{array}{l} (-0.54, 0.81, -0.23) \\ (-0.78, 0.54, -0.31) \\ (-0.87, 0.44, -0.24) \\ (-0.87, 0.41, -0.26) \\ (-0.84, 0.47, -0.26) \\ (-0.86, 0.44, -0.24) \end{array}$	$\begin{array}{c} (-0.56,0.81,-0.15)\\ (-0.83,0.55,-0.05)\\ (-0.90,0.44,-0.08)\\ (-0.90,0.42,-0.08)\\ (-0.87,0.49,-0.09)\\ (-0.89,0.45,-0.06)\end{array}$		
	L	Al32–2 Al32–5 Ta39–6 Cr48–3 Cr59–1	222 459 237 374 902	0.949 0.886 0.983 0.958 0.949	$\begin{array}{c} (0.80, 0.57, 0.19) \\ (0.88, 0.46, 0.13) \\ (0.68, 0.69, 0.25) \\ (0.74, 0.64, 0.22) \\ (0.76, 0.60, 0.25) \end{array}$	$\begin{array}{c} (0.83, 0.55, 0.07) \\ (0.89, 0.45, 0.00) \\ (0.72, 0.69, 0.05) \\ (0.76, 0.64, 0.06) \\ (0.79, 0.61, 0.05) \end{array}$		

# Stability of Listing's plane

thickness of Listing's plane remained essentially unchanged.

In all five animals, the primary position and the shape and orientation of Listing's plane remained stable from day to day, as long as measurements were made with the same coil. In one animal in which the coil had to be replaced twice, primary position shifted, but the shape and As a daily routine, eye-position data were taken with the animal in an upright position. Listing's plane had an extension in the torsional direction ("thickness of Listing's plane"), which we quantified by the standard deviation (SD) of the distance between all data points and the **Fig. 2A–E** Example of a typical superior-oblique-muscle unit (Cr45–10). **A–D** Motoneuron firing frequency as a function of the three-dimensional eye position in the direction  $[-\sin(\alpha), \cos(\alpha), 0]$  for  $\alpha=0^{\circ}$ ,  $30^{\circ}, 60^{\circ}, 90^{\circ}$ , respectively. **E** Motoneuron firing frequency as a function of the three-dimensional eye position in the on-direction [-0.899, 0.453, -0.064]. Eye position is shown in half-radians



**Table 5** Normalized 3D average on-directions in Listing's reference frame of six extraocular motoneuron pools and their average 95% confidence intervals. *n* Number of motoneurons. Other abbreviations are as in Table 1

	n	Normaliz	ed average o	n-direction	Average 95% confidence interval [°]				
		X	Y	Z	X	Y	Z		
MR	16	-0.314	-0.003	0.949	9.5	2.1	2.3		
LR	6	0.223	-0.005	-0.975	13.8	3.1	2.9		
SR	6	-0.798	-0.602	-0.034	12.1	2.9	3.6		
IR	5	0.723	0.675	0.150	11.1	2.6	4.0		
IO	7	0.801	-0.595	0.061	5.2	1.7	2.3		
SO	11	-0.824	0.563	-0.070	5.6	2.4	2.4		

best-fit plane. Typically, the SD was less than 1°. In individual animals, the location of primary position showed variations in day-to-day measurements with a SD of  $1.9^{\circ}$  for the horizontal component (range:  $0.9-2.3^{\circ}$ ) and  $2.2^{\circ}$  for the vertical component (range:  $1.5-3.3^{\circ}$ ) over an observation period of 6-16 weeks (average: 11 weeks). The exact locations of the primary position, the center of the oculomotor range, and the eye position during gaze straight ahead were similar among the different monkeys (Suzuki et al. 1994b).

On-directions determined by multiple linear regression analysis

Figure 2 shows the data of a typical SO unit (Cr45-10). In a one-dimensional linear regression analysis, the correlation coefficients (*R*) between firing frequency and the component of eye position, *r*, in Listing's reference frame in the direction *e*, i.e., the dot product *r*·*e*, were R=0.717 (n=265) for e=[0, 1, 0] (downward direction) in A, R=0.799 for  $e=[-\sin(\pi/6), \cos(\pi/6), 0]$  (CCW torsion and downward) in B, R=0.897 for  $e=[-\sin(\pi/3), \cos(\pi/3), \cos(\pi/3)$ 

0] (CCW torsion and downward) in C, and R=0.169 for e=[-1, 0, 0] (CCW torsion) in D. However, multiple linear regression gave a much higher correlation coefficient R=0.939 for the on-direction  $e_{\rm on}=(-0.899, 0.453, -0.064)$ , which is about as CCW torsional as in Fig. 2C and, in addition, has a small abducting horizontal component. Firing frequency plotted against  $r \cdot e_{\rm on}$  is shown in Fig. 2E.

For each of the 51 units, the direction with the maximum correlation coefficient was determined. The average was  $R=0.94\pm0.07$  SD (Table 4). Figure 3 shows the on-directions and confidence intervals for all units in Listing coordinates. Forty-six units (90.1%) had a correlation coefficient R>0.90. The average confidence intervals at P<0.05 for the torsional, vertical, and horizontal components were 9.00, 2.40, and 2.70, respectively (Table 5). Each neuron could unequivocally be assigned to one motoneuron pool, and the on-directions for the different pools of motoneurons did not overlap.

Average on-directions of motoneurons differed significantly from corresponding anatomically determined muscle rotation axes expressed in the stereotaxic reference frame (range of deviations: 11.9–29.0°). This difference



**Fig. 3** On-directions in Listing's reference frame of all 51 motoneurons projected onto the x-y- (**A**), x-z- (**B**), and y-z-planes (**C**). For each neuron, the normalized on-direction vector, together with its confidence interval at P < 0.05, is given. Non-overlapping of motoneuron groups is evident. Abbreviations are given in Table 1

was most pronounced for the vertical recti and oblique muscles. The muscle rotation axes of the vertical rectus pair and the oblique muscle pair form an angle of 58.3°, whereas the corresponding angle for paired motoneuron on-directions was 105.6°. On-directions of motoneurons



**Fig. 4A–C** Iso-frequency surfaces of a medial rectus motoneuron (Cr03–2). The bins relate to the center value of frequency (i.e., the 50-Hz bin includes the range 25–75 Hz, the 100-Hz bin includes the range 75–125 Hz, etc.). The *arrowhead line* indicates the on-direction of the unit as calculated by multiple linear regression. **A, B** y-za and x-z planes, receptively. Rotation-vector components are given in degrees. The surfaces show little curvature, and – to a first approximation – are shifted in parallel with higher frequencies in the direction of the z-axis, with a negative x-component and a small, positive y-component. C Firing frequency versus eye position along the direction. Eye position is shown in degrees

were better aligned with the on-directions of semicircular canal afferents (range of deviation:  $9.4-18.9^{\circ}$ ) or with the anatomically determined sensitivity vectors of the semicircular canals (range of deviation:  $3.9-15.9^{\circ}$ ).

Motoneurons showed differences in threshold and average firing frequency, and the animals had different oculomotor ranges. The slope k (=firing frequency/ length of rotation vector) in the on-direction was significantly correlated with the threshold (*thr* in rad/2): k=1116+2149\*thr, R=0.770 (n=51). Four units, marked with an asterisk in Table 1, had a high threshold (>100) for firing activity during fixation and could therefore only be tested over a small range of eye positions. These units, however, were not omitted from the calculations of the average on-directions of motoneuron pools (see below).

The on-directions of the horizontal recti motoneurons (MR, LR), the vertical recti motoneurons (SR, IR), and the oblique motoneurons (SO, IO) were approximately antagonistic to one another and paired on-directions were roughly orthogonal to each other. This can be seen

Fig. 5A, B Comparison of motoneuron on-directions (MN) in the stereotaxic reference frame with the anatomically measured geometry of the extraocular muscles (EOM) and the semicircular canals (CS). Normalized vectors of MN (lines with unmarked ends), EOM (lines with open circles), and CS (lines with asterisks) are shown. Values of EOM and CS are from Table 3. For values and abbreviations, see Table 3. A x-y-proiection as seen from above. **B** x-z-projection as seen from the right side



**Table 6** Differences between average on-directions of extraocular motoneurons (*MN*), anatomical rotation axes of extraocular muscles (*EOM*), negative semicircular canal afferent on-directions (*CA*), and negative sensitivity vectors of semicircular canals (*CS*). In *columns 2* and *3*, the following correspondences between muscles and semicircular canals are given: MR-rlc, LR-llc, SR-rac, IR-lpc, IO-lac, SO-rpc. Abbreviations as in Tables 1 and 3

	Differences of means							
	MN-EOM [°]	MN–CA [°]	MN–CS [°]					
MR	18.2	18.6	15.9					
LR	11.9	16.1	14.8					
SR	29.0	18.9	9.8					
IR	22.1	9.4	3.9					
IO	24.9	12.6	10.0					
SO	20.4	14.0	13.1					
MR&LR	13.6	17.1	14.5					
SR&IR	25.4	12.3	5.7					
IO&SO	22.5	12.9	11.5					

in the projections on the x-y- (Fig. 3A), x-z- (Fig. 3B), and y-z-planes (Fig. 3C) in Listing's reference frame. The z-components of the on-directions of the vertical recti motoneurons and the y-components of the on-directions of the horizontal recti motoneurons were very small (Fig. 3C). The horizontal recti motoneurons had small, but significant torsional components (extorsional for LR and intorsional for MR), which were roughly opposite in the x-z-plane (Fig. 3B). The numerical values of the average on-directions and 95% confidence intervals are given in Table 5.

#### Iso-frequency surfaces

As a qualitative measure of on-directions, iso-frequency surfaces were determined for all 51 units. Figure 4A and B shows the projection of the iso-frequency surfaces of a medial rectus motoneuron (Cr03-2) onto the x-, z-, and y-z-plane. Iso-frequency surfaces were approximately parallel.

The average direction perpendicular to the surface could be taken as a measure of the on-direction. These on-directions gave values that were similar to those resulting from the multiple regression analysis (Fig. 5C) of motoneurons with low thresholds, in which data points were homogeneously distributed. For the statistics, we did not use the orientations of iso-frequency surfaces.

On-directions of motoneurons compared with muscle rotation axes

The on-directions of motoneurons determined by multiple linear regression analysis in stereotaxic coordinates were compared to the orientation of muscle rotation axes (Table 6, Fig. 5). Thin long lines with open circles (°) and asterisks (\*) in Fig. 5 show the muscle rotation axes of the right eye and the sensitivity vectors of the semicircular canals (Reisine et al. 1988) in the stereotaxic reference frame as seen from above (Fig. 5A) or from the right side (Fig. 5B), respectively. The rotation axes of the vertical recti and oblique muscles deviated considerably from orthogonality. However, they were about approximately perpendicular to the rotation axes of the horizontal muscles.

In each motoneuron group, individual motoneuron ondirections scattered in both projections. The on-directions of the vertical recti and oblique motoneurons clearly have more torsion than do the corresponding muscle rotation axes, and, for IR and SO, they are even more torsional than the corresponding semicircular canal sensitivity vectors. The on-directions of the horizontal motoneurons are more torsional than both the corresponding muscle rotation axes and the semicircular canal sensitivity vectors. Numerical values are given in Tables 3 and 6.

# Discussion

#### Muscle rotation axes

Our measurements of the muscle insertions and origins in four eyes were rather consistent, as can be seen from the small standard deviations of the muscle rotation axes for the four eyes. From the data listed in Tables 1 and 2, one can compute the average standard deviations ( $\sigma$ ) of the x-, y-, and z-components of the muscle rotation axes of the six muscles and find that  $\sigma_x=0.060\pm0.039$ ,  $\sigma_{v} = \sigma_{z} = 0.039 \pm 0.027$ . There are differences between our muscle rotation axes and those of Miller and Robins (1987). After re-normalizing the insertions published by Miller and Robins to make their length equal to their mean radius of the eye of 9.3 mm, we compared the rotation axes of antagonistic muscle pairs (values of Miller and Robins in square brackets):  $\angle (m_{\rm MR}, m_{\rm LR}) = 164.4^{\circ}$  $[170.2^{\circ}], \ \angle(m_{\rm SR}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm SO}, m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ \text{and} \ \angle(m_{\rm IR}) = 175.0^{\circ} \ [175.4^{\circ}], \ (m_{\rm IR$  $m_{\rm IO}$ )=171.1° [145.9°]. We were unable to resolve the discrepancy between our and their value for  $m_{SO}$ . However, our paired muscle rotation axes  $[m_{MLR}=(m_{MR}-m_{LR})\wedge;$  $m_{\rm SIR} = (m_{\rm IR} - m_{\rm SR}) \land; m_{\rm ISO} = (m_{\rm IO} - m_{\rm SO}) \land]$  are in good agreement with those of Miller and Robins.  $m_{MLR}$  has practically no vertical component, and  $m_{\rm SIR}$  and  $m_{\rm ISO}$  almost no horizontal component:  $\angle(m_{\text{MLR}}, \text{ y-axis})=91.3^{\circ}$  [90.8°],  $\angle(m_{\text{SIR}}, \text{ z-axis})=94.0^{\circ} [94.4^{\circ}], \text{ and } \angle(m_{\text{ISO}}, \text{ z-axis})=83.5^{\circ}$  $[90.9^{\circ}]$ .  $m_{\rm MLR}$  has a large z- and a significant x-component:  $\angle (m_{\text{MLR}}, \text{ z-axis}) = 15.3^{\circ} [16.8^{\circ}]$ . Both  $m_{\text{SIR}}$  and  $m_{\text{ISO}}$ have a larger y- than x-component:  $\angle (m_{\text{SIR}}, \text{ y-axis}) = 154.2^{\circ}$ [150.6°] and  $\angle(m_{\rm ISO}, \text{ y-axis})=147.5^{\circ}$  [145.0°]. Clearly,  $m_{\rm SIR}$  and  $m_{\rm ISO}$  are not orthogonal:  $\angle(m_{\rm SIR}, m_{\rm ISO})=58.3^{\circ}$ [64.0°]. However,  $m_{\rm MLR}$  is practically orthogonal to the plane spanned by  $m_{\text{SIR}}$  and  $m_{\text{ISO}}$ :  $\angle (m_{\text{MLR}}, m_{\text{SIR}} \times m_{\text{ISO}}) = 4.8^{\circ}$ [13.8°].  $m_{\text{MLR}}$  is orthogonal to  $m_{\text{SIR}}$ : 86.2° [85.5°] and to  $m_{\rm ISO}$ : 90.5° [99.7°]. We conclude from our data and those of Miller and Robins that the rotation axes  $m_{SIR}$  and  $m_{ISO}$ are roughly orthogonal to  $m_{\rm MLR}$  and are about 30° torsional relative to the stereotaxic y-axis.

To compare muscle rotation axes in the perfused eye with neural firing patterns in the alert monkey, one should know how the anatomical position of rest relates to the primary position of Listing's reference frame. After perfusion of monkey Cr, we measured the position of the eye using the pre-perfusion calibration and primary position. Our findings are consistent with the absence of a strong torsional eye-position component after perfusion. However, the eye was rotated upward by about  $20^{\circ}$ . In the future, anatomical measurements and physiological recordings should be made in the same monkey.

#### Motoneuron recordings

As we had put our animals in different static roll positions, we could sample activity from motoneuron rootlets of extraocular muscles not only over the horizontal and vertical oculomotor range in Listing's plane, but also for different torsional eye positions. In this way, we could determine the on-directions in 3D and compare them to the anatomically defined rotation axes of the eye muscles, the sensitivity planes of the semicircular canals, or to the functionally defined orientation of Listing's plane.

Relation between motoneuron activity and eye position in 3D

After quantitative analysis, unit activity could be unequivocally attributed to one of the motoneuron pools of the six extraocular muscles (Fig. 3). The relative positions of oculomotor landmarks and motoneuron rootlets were verified by histological examination. In an earlier 2D study, a paradigm was chosen in which the animal was trained to pursue a target moving slowly along a path approximately orthogonal to the 2D on-direction of the motoneuron, with all movements confined to Listing's plane (Hepp and Henn 1985). The distribution of data points was more uniform with that paradigm, but it took considerably longer to test one neuron. The novel aspect of the present study is an analysis that takes data points into account which are distributed over much of the 3D oculomotor range.

#### Choice of 3D coordinates

To understand the control of 3D ocular rotations, it is crucial to determine the reference frame that best reflects functional properties. Eye positions were measured in the field-coil reference frame and were then transformed to the stereotaxic reference frame, which differed by the  $15^{\circ}$  head pitch applied to position the animal's head during the experiments. The stereotaxic reference frame is derived from bony anatomical landmarks, which are easy to measure, but lack functional significance. We have converted our data to coordinates in the stereotaxic reference frame to allow comparisons with data from the literature on muscle rotation axes and semicircular canal geometry.

Eye positions were also transformed to Listing coordinates, which are functionally defined for voluntary conjugate eye movements. Listing's plane represents one of the outstanding geometrical constraints on oculomotor behavior. In the tested animals, Listing's plane was found to have a very stable orientation over the experimental period, which lasted up to several months.

# Coding of 3D on-directions

The relation between motoneuron activity and 3D static eye positions was investigated by multiple linear regression analysis and least square fits for quadratic iso-frequency surfaces. In our sample of 51 neurons, we found an average correlation coefficient of  $R=0.94 (\pm 0.07 \text{ SD})$ between firing rate and the rotation vector. Thirty units (59%) had coefficients higher than 0.95 (Table 4). When data for each animal were analyzed separately, the variability of the on-directions was even smaller (data not shown). Figure 3 shows how the on-directions for motoneurons of different extraocular muscles cluster in nonoverlapping distributions. Thus, no ambiguity arose in matching a motoneuron to its respective muscle. Confidence intervals were about two times larger for the torsional than for y- and z-components (Fig. 3 and Table 5). This difference probably is a result of the significantly smaller range of rotation in the torsional component than in the vertical or horizontal components (Fig. 1).

Earlier measurements of iso-frequency curves represented all possible eye positions that the eye can reach during a particular level of activity in a motoneuron (Hepp and Henn 1985). The local on-direction of a motoneuron could then be defined as the innervation gradient, which is orthogonal to the respective iso-frequency curve. In generalizing to 3D space, iso-frequency curves become iso-frequency surfaces (Fig. 4). With increasing firing frequency, a set of approximately parallel surfaces emerges. For lowthreshold units, for which data are available for at least three different roll positions, the direction orthogonal to the surface at the center of the oculomotor range can be taken as the direction of the innervation gradient, i.e., the on-direction of the motoneuron. Mean 3D on-directions derived from iso-frequency surfaces and from calculations of multiple linear regression closely corresponded. However, local on-directions changed with 3D eye position (Fig. 4), as they did in the 2D analysis of iso-frequency curves in Listing's plane (Hepp and Henn 1985).

Our findings showed the importance of 3D investigation of the motoneuron on-direction even for the horizontal rectus muscles, since one of the striking results was the relatively strong torsional components found for medial and lateral rectus motoneurons (Fig. 3B). Such torsional components had already been reported with the use of electromyography in decerebrated cats (Baker et al. 1988; Baker and Peterson 1991), but they had not been determined in alert animals.

# Comparison of motoneuron on-directions in 3D with rotation axes of extraocular muscles

As can be seen from Table 3, the on-directions of MR  $(e_{\rm MR})$  and LR  $(e_{\rm LR})$ , SR  $(e_{\rm SR})$  and IR  $(e_{\rm IR})$ , and IO  $(e_{\rm IO})$  and SO  $(e_{\rm SO})$  are close to antagonistic:  $\angle (e_{\rm MR}, e_{\rm LR})=176.2^{\circ}$ ,  $\angle (e_{\rm SR}, e_{\rm IR})=167.1^{\circ}$ , and  $\angle (e_{\rm SO}, e_{\rm IO})=177.1^{\circ}$ . The motoneuron on-directions of the horizontal recti have only small y-components:  $\angle (e_{\rm MR}, y\text{-axis})=88.5^{\circ}$  and  $\angle (e_{\rm LR}, y\text{-axis})=89.0^{\circ}$ , but the angles with the y-axis are somewhat larger than those of the muscles (the muscle data are in square brackets):  $\angle (e_{\rm MR}, z\text{-axis})=30.0^{\circ}$  [13.6°] and  $\angle (e_{\rm LR}, z\text{-axis})=152.4^{\circ}$  [159.9°]. From Table 6 one finds that  $\angle (e_{\rm MR}, m_{\rm MR})=18.2^{\circ}$  and  $\angle (e_{\rm LR}, m_{\rm LR})=11.9^{\circ}$ .

The deviations between the on-directions of the vertical recti and obliques and the corresponding directions of muscle rotation axes are about twice as large according to Table 3:  $\angle(e_{\rm SR}, y\text{-axis})=124.9^\circ$ ,  $\angle(e_{\rm SR}, m_{\rm SR})=29.0^\circ$ ;  $\angle(e_{\rm IR}, y\text{-axis})=46.9^\circ$ ,  $\angle(e_{\rm IR}, m_{\rm IR})=22.1^\circ$ ;  $\angle(e_{\rm IO}, y\text{-axis})=126.5^\circ$ ,  $\angle(e_{\rm IO}, m_{\rm IO})=24.9^\circ$ ;  $\angle(e_{\rm SO}, y\text{-axis})=56.0^\circ$ ,  $\angle(e_{\rm SO}, m_{\rm SO})=20.4^\circ$ . These discrepancies do not decrease when one compares antagonistically paired motoneuron ondirections with the corresponding muscle pairs, as can be seen in Table 6. Most conspicuous is the comparison between  $\angle(e_{\rm SIR}, e_{\rm ISO})=105.6^\circ$  and  $\angle(m_{\rm SIR}, m_{\rm ISO})=58.3^\circ$ . However,  $e_{\rm MLR}$  is still almost orthogonal to  $e_{\rm SIR} \times e_{\rm ISO}$ :  $\angle(e_{\rm MLR}, e_{\rm SIR} \times e_{\rm ISO})=11.7^\circ$ . The symmetry relative to the origin of the x-, y-, and z-axes is less clear in the stereotaxic reference frame than in the Listing's frame (Fig. 3).

In our subsequent paper (K. Hepp, Y. Suzuki, D. Straumann, V. Henn, in preparation), we shall explain the large differences of the motoneuron on-directions and axes of rotation of the vertical recti and obliques using the fact that these muscles form a non-orthogonal reference frame (Pellionisz and Llinas 1980). In addition, a quantitative analysis of position-dependent variations of on-directions will be given and compared to the predictions from different models of the idealized rhesus eye plant.

# Comparison of 3D motoneuron on-directions with semicircular canal geometry

The vestibular reference frame, defined either by the planes of the semicircular canals or, more physiologically, by the sensitivity directions of the semicircular canal afferents, is, in the middle frequency range, related to head angular velocity and, through the vestibulo-ocular reflex (VOR, where the torsional gain is only about 60% of the horizontal and vertical gains), to eye angular velocity. These coordinates are only indirectly related (via brainstem integrators) to the motoneuron on-directions during fixation. However, as one can see from Tables 3 and 6, the geometric correspondence between the vestibular reference frame and the motoneuron on-directions is much better than between the latter and the muscle rotation axes.

# Semicircular canal afferent activity

Reisine et al. (1988) compared the orientations of canals in the rhesus monkey with the on-directions of afferent fibers in the vestibular nerve. The angle between sensitivity vectors of semicircular canals and the corresponding afferent on-direction ranged from 1.5 to 11.7°. The angle between the motoneuron on-direction and the corresponding semicircular canal afferent on-direction ranged from  $9.4^{\circ}$  to  $18.9^{\circ}$  (Table 6).

#### Semicircular canal sensitivity vectors

Reisine et al. (1988) measured the orientations of the semicircular canals in the rhesus monkey. The three pairs of semicircular canal sensitivity vectors were found to be orthogonal to each other (range:  $88.5-93.2^{\circ}$ ). They also measured a 14° upward inclination of the lateral canals from the stereotaxic horizontal plane, but this finding was based on only two animals. Blanks et al. (1985) measured ten animals (*Macaca mulatta*) and found a surprisingly large variation in orientation, with an average inclination of the lateral canals of  $22^{\circ}\pm 3.5$  SD. In considering whether a relation exists between canal orientations and Listing's plane, one has to keep such variations in mind. Figure 5A and B shows the average sensitivity vectors of the semicircular canals in the two rhesus mon-

keys of Reisine et al. (1988). The signs were reversed to aid comparison of them with the on-directions of motoneurons, because the eye movements induced by the VOR are compensatory to head movements. The roughly orthogonal arrangement of motoneuron on-directions resulted in a closer alignment with the sensitivity vectors of the semicircular canals than with the muscle rotation axes. However, deviations between motoneuron on-directions and canal sensitivity vectors were still prominent and ranged from  $3.9^{\circ}$  to  $15.9^{\circ}$  (Table 6). The on-directions of push-pull motoneuron pairs were compared with similarly paired muscle and semicircular canal geometry (Table 3). A significant reduction of the misalignment between motoneuron on-directions and the semicircular canals was not observed (Table 6).

Listing's reference frame as an intrinsic reference frame

The symmetry of motoneuron on-directions relative to Listing's plane is striking (Fig. 3). The question arises whether some fixed geometrical relation exists between the functionally defined Listing's reference frame and the anatomically defined orientation of the semicircular canals. This problem has been investigated in humans, but, so far, a correlation in individual subjects has not been established (Probst-Müller et al. 1996). As discussed above, there are several experimental uncertainties and a rather large variation of canal and muscle orientations, so that only a statistical relation can be established between Listing's plane and canal orientation.

Directional coding in premotor oculomotor structures is closely related to the orientation of Listing's plane. The riMLF contains burst neurons with vertical and torsional rapid eye-movement on-directions (Vilis et al. 1989; Crawford and Vilis 1992; Hepp et al. 1994), and the vertical-torsional integrator located in the interstitial nucleus of Cajal also shows a symmetrical organization with respect to Listing's plane (Crawford et al. 1991; Crawford and Vilis 1993).

All visual information is topographically mapped in 2D layered structures, starting from the retina, and repeated at different cortical levels as well as in the superior colliculus. In another investigation, we found an essentially 2D representation of eye movement vectors in Listing's plane in the deep layers of the superior colliculus (van Opstal et al. 1991; Hepp et al. 1993), which is an example of where Listing's plane is neuronally coded at a visual-oculomotor interface. In this way, the visual system imposes its 2D organization as an intrinsic reference frame onto the 3D spatial organization of the eye muscles for conjugate eye movements.

The on-directions of extraocular motoneurons are organized in a reference frame that is symmetrical relative to Listing's plane, which simplifies the neural control of voluntary eye movements. On the other hand, motoneuron on-directions are also closely related to the geometry of the vestibular system. This organization makes multidimensional transformation from two sensory inputs to a single motor output more efficient.

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