Topography of saccadic eye movements evoked by microstimulation in rabbit cerebellar vermis

M. Godschalk, J. Van der Burg, B. Van Duin and C. I. De Zeeuw

Department of Anatomy, Faculty of Medicine and Health Sciences,
Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands

1. We investigated saccadic eye movements elicited by microstimulation in the vermis of the rabbit. Scleral search coils were implanted under the conjunctiva of both eyes and a recording chamber was placed over the cerebellar vermis.

2. Conjugate saccadic eye movements were evoked in lobules VI a, b and c and VII of the vermis by currents ranging from 4 to 60 μA. All movements were horizontal with no apparent vertical component.

3. The cortex on both sides of the vermal mid-line could be divided in two zones, dependent on the direction of elicited saccades. In the medial zone saccades were directed ipsilaterally, in the lateral zone contralaterally.

4. We conclude that the topography of saccadic eye movements in the rabbit cerebellar vermis is, unlike in monkey and cat, organized in parasagittal zones.

The cerebellum is organized in sagittal zones of Purkinje cells that are innervated by climbing fibres from a specific olivary subnucleus (Groenewegen, Voogd & Freedman, 1979; Oscarsson, 1979) and that innervate specific parts of the cerebellar and vestibular nuclei (Voogd & Bigaré, 1980). This sagittal organization also holds true for the part of the cerebellum that is involved in compensatory eye movements, i.e. the flocculus and the nodulus (De Zeeuw, Wylie, DiGiorgi & Simpson, 1994). The Purkinje cells in most zones of these regions modulate optimally to optokinetic stimulation about a specific axis in space (De Zeeuw *et al.* 1994). Electrical stimulation in the white matter of each of these zones evokes eye movements about the same axes (Van der Steen, Simpson & Tan, 1991). These axes have a geometry similar to that of the best response axes of the semicircular canals and to the axes about which the three pairs of extraocular muscles rotate the eye (Graf & Simpson, 1981; Simpson, Graf & Leonard, 1981).

The part of the cerebellum that is strongly involved in saccadic eye movements consists of lobules VIc and VII and has been named the oculomotor vermis (Noda & Fujikado, 1987). Electrical stimulation of this area can evoke saccadic eye movements in rabbit (Hoshino, 1921), cat (Cohen, Goto, Shanzer & Weiss, 1965) and monkey (Ron & Robinson, 1973; Fujikado & Noda, 1987; Noda & Fujikado, 1987). The mossy fibres and Purkinje cells in the monkey oculomotor vermis modulate in relation to spontaneous saccades (Llinás & Wolfe, 1977; Kase, Miller & Noda, 1980). Many of these fibres and cells can be direction

selective. However, in contrast to the vestibulocerebellum, it has not been elucidated for the oculomotor vermis whether the Purkinje cells with a specific direction selectivity are located in specific sagittal zones. To find out whether the oculomotor vermis also shows this sagittal organization we mapped the effects of microstimulation in this area of the rabbit. Some of the results have been published in abstract form (Godschalk, Van der Burg & Van Duin, 1992).

METHODS

Subjects

Scleral search coils consisting of five turns of Bioflex wire (type AS632, Cooner Wire Company, Chatsworth, CA, USA) were implanted under the conjunctiva of both eyes according to the method described by Collewijn (1977). Both ends of the wire were tunnelled subcutaneously to the vertex and were soldered to an implant consisting of a connector socket and a head restraint device. Two stainless-steel bone screws used for fixation of the implant were also connected to the socket and

Table 1. Distribution of stimulation sites in the six rabbits

Rabbit no.	454	468	473	503	514	519	Total	Mean threshold (μA)
No. of electrode penetrations	40	22	67	9	36	51	225	
No. of penetrations yielding saccades at $\leqslant 60$ μA	33	11	50	7	25	34	160	
No. of stimulation sites yielding saccades at $\leqslant 60$ μA	41	11	61	7	29	36	185	
No. of stimulation sites yielding saccades at $\leqslant 50$ μA	32	10	47	6	24	32	151	26
Rightward	19	4	24	3	17	17	84	27
Ipsilaterally	17	4	11	2	13	7	54	27
Contralaterally	2	0	13	1	4	10	30	28
Leftward	13	6	23	3	7	15	67	23
Ipsilaterally	12	3	15	3	6	7	46	22
Contralaterlally	1	3	8	0	1	8	21	27
No. of stimulation sites yielding saccades at $\leqslant 10$ μA	8	2	9	0	1	5	25	
Rightward	4	1	2	0	0	4	11	
Leftward	4	1	7	0	1	1	14	

served as indifferent and ground electrodes. Subsequently the implant was fixed to the skull with dental acrylic cement. The occipital bone overlying lobules VI–VIII was removed and a cylindric stainless-steel recording chamber (16 mm in diameter) was implanted on top of the skull.

After surgery and after each session, the cylinder was filled with 0·9% NaCl solution to which a few drops of a 20% chloramphenicol solution was added. No additional antibiotic cover was required. During the first 2 days after surgery, the animals were observed at least 4 times daily. About 2 h after surgery, the animals recovered from anaesthesia and exhibited normal locomotor and grooming behaviour. Therefore, no postoperative analgesia was applied.

Data collection

Up to five electrode penetrations were made during 1–4 h sessions between 2 and 5 times a week. The number of sessions ranged from nine to twenty-six per animal. During the sessions the head of the animal was restrained and oriented in the freeze position (the nasal bone made an angle of 33 deg to the vertical). The animals were observed continuously during sessions and were usually sitting quietly. Whenever the animal made spontaneous body or limb movements for over 1 min, this was taken as an indication of stress and the session was immediately ended.

An Epoxylite-coated tungsten wire electrode was placed in a hydraulic microdrive and lowered through the dura into the brain. The initial impedance of the electrodes was approximately 100 kΩ measured at 1 kHz, but during the penetration the impedance usually dropped to about 30 kΩ. Neuronal activity was recorded with the use of a differential amplifier. At the end of each 100 μm step the amplifier was grounded and the electrode was connected to a stimulating pod. The pod included a biphasic constant-current stimulator (Mitz, Reed & Humphrey, 1984) and a high-compliance stimulus isolation unit. The current was determined by displaying the voltage across an in-line resistor on an oscilloscope. Trains (40–200 ms) of biphasic pulses were delivered at 330 or 600 pulses s^{-1}. Each phase was 0·2 ms in duration (cathodal first) and both phases were symmetrical in amplitude. The current was limited to 60 μA for either phase.

All motor and behavioural effects from stimulation were observed and recorded. In the area explored, most penetrations yielded either eye movements or no movement. In a few penetrations, other movements were elicited in sites below the eye-movement sites. Whenever any non-ocular movement was elicited, stimulation was ended and the electrode withdrawn. Thresholds for all detected eye movements were assessed by a descending series of currents. The threshold was defined as the lowest current by means of which the movement was evoked in at least 50% of the delivered stimuli. The stimulus site in reference to the cerebellar layers was identified by recording Purkinje cell activity as the electrode was advanced. The molecular layer was characterized by a low background activity and by negatively polarized climbing fibre responses firing at relatively low frequencies (0·5–2 Hz); the Purkinje cell layer was characterized by an increase in amplitude of multiple-unit activity; and the granular layer was characterized by positive complex spikes and negative simple spikes.

At the end of the experiments, up to ten lesions (30 μA DC cathodal during 30 s) were made in each animal to demarcate the outlines of the stimulation area.

Data analysis and histology

Both horizontal and vertical eye movements were recorded for both eyes with the use of an electromagnetic eye position measurement system (Skalar Medical, Delft, The Netherlands; for details see Collewijn, 1977). The movements were displayed on-line on a storage oscilloscope and a pen recorder, and stored together with the stimulation parameters on a fourteen-channel instrumentation recorder (Racall Store-14). Subsequently, the data were analysed off-line by means of a data acquisition system (CED 1401+).

Figure 1. Examples of eye movements following microstimulation in one rabbit

Traces represent the horizontal movements of the right eye and are taken from a series of consecutive stimuli of the same site with the same current strength (10 μA; threshold is 8 μA). Traces are plotted in order of initial eye position. Vertical bars represent 10 deg of deviation to the right (R) or left (L). Stimulation train starts at time 0. Note that the amplitude of the elicited eye movements is related to the initial eye position and that the latencies of the eye movements vary considerably. Rabbit no. 473.
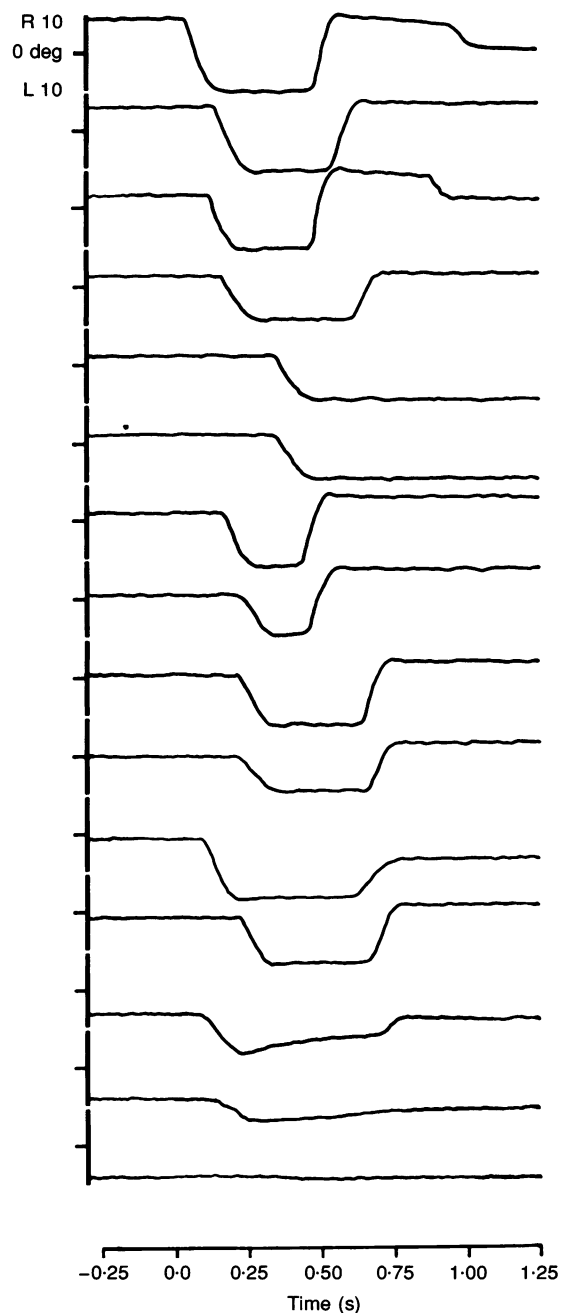
After completion of the recording sessions, the animals were deeply anaesthetized with pentobarbitone and perfused transcardially with a buffered saline solution, followed by a fixative containing paraformaldehyde (2·5%), glutaraldehyde (1·25%) and sucrose (5%) in 0·05 M phosphate buffer. The brain was removed from the skull and the cerebellum and metencephalon were embedded in gelatin. Frozen sections (40 μm) were cut transversally on a microtome and every fourth section was stained with Cresyl Violet. To identify the penetrated lobules a mid-sagittal section was reconstructed from drawings of sections through the cerebellum. In addition, we reconstructed electrode penetration tracks based upon marking lesions and notes taken during the recordings.

RESULTS

Characteristics of eye movements

In the six animals, a total of 225 penetrations were made (Table 1). In 160/225 penetrations, saccadic eye movements could be elicited with currents ≤ 60 μA. These saccades were conjugate and directed in the horizontal plane; no vertical or diagonal eye movements were observed. When the threshold current to elicit saccades dropped and subsequently rose, this stretch of the penetration was called a saccade site. In 25/160 penetrations, two saccade

sites were encountered. In ten of these twenty-five penetrations, the saccades had opposite directions. Thus, with a current $\leqslant 60\,\mu\mathrm{A}$ a total of 185 saccade sites were observed; 151 of these sites had a threshold $\leqslant 50\,\mu\mathrm{A}$, 80 sites $\leqslant 20\,\mu\mathrm{A}$, and 25 sites $\leqslant 10\,\mu\mathrm{A}$ (Table 1). The lowest current that elicited saccades was $4\,\mu\mathrm{A}$. As revealed by the recordings and concomitant analysis of the lesions, the saccade sites with the lowest thresholds were located predominantly in the granular layer and the white matter. The threshold levels were not significantly influenced by the duration of the pulse train, but they appeared to be increased when the alertness of the animal diminished.

The latency of elicited saccades to the start of the stimulation train ranged from 34 to 525 ms (mean $\pm$ s.d., 153·2 $\pm$ 99·6 ms). This large variability existed not only between sites, but also between saccades elicited at the same site with different currents, and even between consecutive saccades elicited at the same site with the same current (Fig. 1). Also, no relation could be observed between the latency and the amplitude of the saccade.

The amplitude of the elicited saccades was generally related to the initial position of the eyes at the start of the stimulus (Fig. 1). When the distance between the initial eye position and the expected final eye position (as observed after previous stimulations at the same site) was small, the amplitude of the elicited saccade was also small or absent. This had the effect, in many cases, that no saccade could be elicited with currents above threshold,
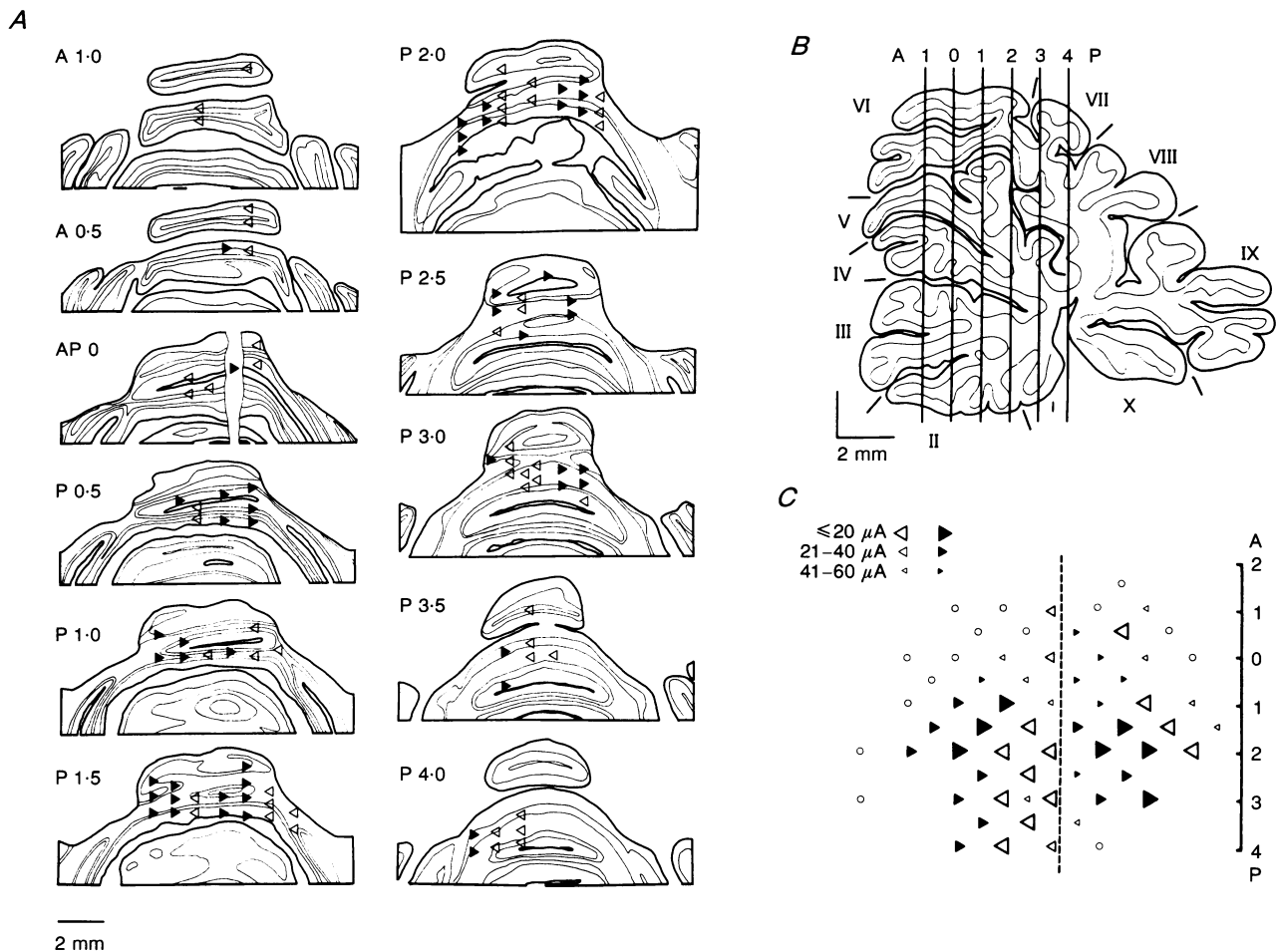

Figure 2. Reconstruction of stimulation sites and penetration tracks of one rabbit

Open triangles represent sites yielding saccades to the left; filled triangles represent saccades to the right. *A*, selected serial sections through the cerebellar vermis showing the approximate sites where saccades were elicited. Saccade sites from deleted sections are plotted on the nearest section shown. *B*, mid-sagittal reconstruction showing the antero-posterior levels of corresponding sections in *A*. *C*, top view of the area explored, showing for each penetration the direction of the elicited saccades and the lowest threshold encountered in that penetration (see upper left corner). Circles represent penetrations in which no eye movements could be elicited with currents $\leqslant 60\,\mu\mathrm{A}$. The dashed line represents the mid-sagittal line. The vertical axis represents the same antero-posterior values as in *A* and *B*. Rabbit no. 473.

when the initial eye position was close to the end-point of a previous saccade (Fig. 1). When the initial eye position was distant from the expected final eye position, the saccade amplitude generally increased with increasing stimulus current. We did not observe a statistically significant increase in amplitude when we increased the pulse frequency from 330 to 600 pulses s^{-1}. In most saccades the amplitude of the eye moving to the temporal direction was larger than that of the other eye (mean difference, 21%; nasal direction taken as 100%). From a selection of 144 stimulations only 16 elicited a higher amplitude in the eye moving to the nasal direction. Consecutive stimulations of a single saccade site could cause opposite effects in this respect.

Topography of saccade sites

Histological analysis of the penetration tracks and the lesions in the brains showed that saccades were elicited in lobules VIa, b and c as well as in lobule VII of the vermis (Fig. 2). No saccades were evoked following stimulation in lobules V or VIII. All saccade sites were within a distance of 3·6 mm from the mid-line. More lateral penetrations (up to 4·4 mm laterally) yielded no saccades.

Of the 151 sites that yielded saccades at a threshold $\leqslant 50 \mu$A, 84 showed conjugate saccades to the right and 67 to the left. Saccades in either direction occurred on both sides of the mid-line. However, the vast majority of the sites yielding ipsilaterally directed saccades (i.e. leftward saccades on the left side of the vermis and vice versa) were closer to the mid-line than contralaterally directed saccades (Figs 2C and 3). The average laterality of ipsilaterally directed saccade sites on the left side was 0·8 mm ($n = 46$) and on the right side 1·0 mm ($n = 54$). For contralaterally directed saccades, these values were 2·0 mm ($n = 30$) on the left side and 2·1 mm ($n = 21$) on the right side. Although some overlap occurred between these groups, an overall pattern emerges of two rostrocaudal

zones of saccade sites on either side of the mid-line: a medial zone with ipsilaterally directed saccades and a lateral zone with contralaterally directed saccades (Fig. 3). We observed no significant differences in latencies, thresholds or amplitudes between the eye movements evoked in the medial and lateral zones.

DISCUSSION

The present study demonstrates (1) that conjugate saccadic eye movements can be elicited by electrical micro-stimulation in lobules VIa, b and c and VII of the vermis of the rabbit, (2) that these saccades occur exclusively in the horizontal plane, and (3) that the saccade sites in the rabbit oculomotor vermis are topographically organized in sagittal zones dependent on the direction of the eye movements.

Hoshino was one of the first to demonstrate horizontal saccadic eye movements evoked by electrical stimulation in the rabbit vermis (Hoshino, 1921); as in the present study, no vertical eye movements were observed. In monkey and cat, electrical microstimulation of lobules VIc and VII in the vermis can also evoke saccadic eye movements (Cohen *et al.* 1965; Ron & Robinson, 1973; McElligott & Keller, 1984; Fujikado & Noda, 1987; Noda & Fujikado, 1987). The major differences with the present study are (1) that they were able to elicit vertically or diagonally directed eye movements, and (2) that they did not observe a lateral zone involved in contralaterally directed saccades. These differences are probably not due to different stimulation parameters. For example, our average threshold current (26 μA for all saccade sites with currents $\leqslant 50 \mu$A) was well within the range of stimulations used by Noda and Fujikado (Fujikado & Noda, 1987; Noda & Fujikado, 1987). Moreover, most of the correlations between different stimulation parameters and different characteristics of the eye responses were very

Figure 3. Composite drawing of the results of microstimulation in five rabbits

From each animal a top view was made of the area explored (as in Fig. 2C), showing for each penetration the direction of the elicited saccades. Open triangles represent sites yielding saccades to the left, and filled triangles represent saccades to the right. Circles represent penetrations in which no eye movements could be elicited with currents $\leqslant 60 \mu$A. When superimposing the individual drawings they were aligned on the mid-sagittal line (represented by the dashed line).
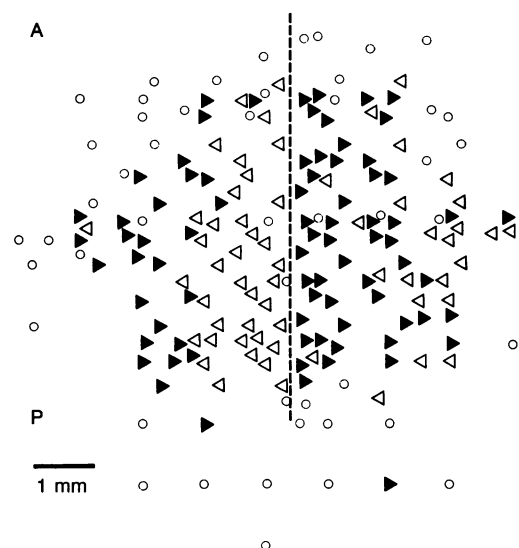
similar to those in the studies in monkey (see also Ron & Robinson, 1973); it was observed that an increasing stimulus current increased the saccade amplitude while the length of the pulse train did not substantially influence the amplitude or the direction and latency of the saccades. On the other hand, it should be noted (1) that the latencies in cat and monkey were generally shorter than in the rabbit and (2) that we, in contrast to the findings by Noda & Fujikado (1987), did not observe a statistically significant change in the saccade amplitude when the pulse frequency changed. This latter correlation may have been blurred by the large variety of saccadic amplitudes in our experiments. Since the amplitude largely depends on the starting point of the saccade, this variety was much larger in our experiments than in the experiments by Noda & Fujikado (1987) in which the monkeys were trained to start each saccade at a fixation point. Nonetheless, these differences cannot explain the major discrepancies between the studies in cat and monkey and the present study in rabbit.

It is unlikely that the presently discovered lateral zone with contralaterally directed saccades is artificial, because the average threshold, latency and amplitude of evoked eye movements in the lateral zone were not significantly different from the ones in the medial zone. In addition, it seems unlikely that the discrepancy is due to the fact that the lateral parts of the vermis in cat and monkey have not been sufficiently explored; most of these studies mention stimulation sites beyond a laterality of 2 mm (Ron & Robinson, 1973; McElligott & Keller, 1984; Fujikado & Noda, 1987; Noda & Fujikado, 1987). Taken together, the species differences elude us. Maybe the discrepancy is related to the different overall organization of the oculomotor system in frontal-eyed vs. lateral-eyed animals. In this respect, it should be noted that the vestibulocerebellum, which is involved in the optokinetic and vestibulo-ocular reflex, is organized very similarly in the rabbit, the cat and the monkey; all three species have separate zones for the horizontal and vertical components of these eye movements (monkey: Balaban, Ito & Watanabe, 1981; cat: Sato, Kanda & Kawasaki, 1988; rabbit: De Zeeuw et al. 1994).

Another possibility is that the oculomotor vermis of the cat and the monkey is in fact more organized in different functional sagittal zones than anticipated. Several findings in the monkey studies (Fujikado & Noda, 1987; Noda & Fujikado, 1987) support this. First, when the stimulation track in the monkey vermis is systematically shifted from the medial to the lateral side, the horizontal component of the saccade increases while the vertical component decreases. Second, the horizontal and vertical components of these saccades may be generated in separate compartments because they show different onset latencies, durations and peak-velocity latencies. Third, the simple spike activity of

direction-selective Purkinje cells modulates in relation to either horizontal or vertical saccades, but rarely to both (Kase et al. 1980).

Future studies, combining different physiological and anatomical techniques, will be required to determine whether the physiologically identified zones in the rabbit correspond to the anatomical zones as defined by Voogd et al. (Groenewegen et al. 1979; Voogd & Bigaré, 1980) and to what extent the oculomotor vermis of the monkey and the cat can also be divided into such sagittal compartments.

REFERENCES

BALABAN, C. D., ITO, M. & WATANABE, E. (1981). Demonstration of zonal projections from the cerebellar flocculus to vestibular nuclei in monkeys (Macaca fuscata). Neuroscience Letters 27, 101–105.

COHEN, B., GOTO, K., SHANZER, S. & WEISS, A. H. (1965). Eye movements induced by electric stimulation of the cerebellum in the alert cat. Experimental Neurology 13, 145–162.

COLLEWIJN, H. (1977). Eye and head movements in freely moving rabbits. Journal of Physiology 266, 471–498.

DE ZEEUW, C. I., WYLIE, D. R., DiGIORGI, P. L. & SIMPSON, J. I. (1994). Projections of individual Purkinje cells of identified zones in the flocculus to the vestibular and cerebellar nuclei in the rabbit. Journal of Comparative Neurology (in the Press).

FUJIKADO, T. & NODA, H. (1987). Saccadic eye movements evoked by microstimulation of lobule VII of the cerebellar vermis of macaque monkeys. Journal of Physiology 394, 573–594.

GODSCHALK, M., VAN DER BURG, J. & VAN DUIN, B. (1992). Topography of saccadic eye movements in the cerebellar vermis of the rabbit evoked by microstimulation. Society of Neuroscience Abstracts 18, 1046.

GRAF, W. & SIMPSON, J. I. (1981). The relations between the semicircular canals, the optic axis, and the extraocular muscles in lateral-eyed and frontal-eyed animals. In Progress in Oculomotor Research, ed. FUCHS, A. & BECKER, W., pp. 411–420. Elsevier, Amsterdam.

GROENEWEGEN, H. J., VOOGD, J. & FREEDMAN, S. L. (1979). The parasagittal zonation within the olivocerebellar projection. II. Climbing fibre distribution in the intermediate and hemispheric parts of cat cerebellum. Journal of Comparative Neurology 183, 551–602.

HOSHINO, T. (1921). Beiträge zur Funktion des Kleinhirnwurmes beim Kaninchen. Acta Oto-Laryngologica, suppl. 2, 1–72.

KASE, M., MILLER, D. C. & NODA, H. (1980). Discharges of Purkinje cells and mossy fibres in the cerebellar vermis of the monkey during saccadic eye movements and fixation. Journal of Physiology 300, 539–555.

LLINÁS, R. & WOLFE, J. W. (1977). Functional linkage between the electrical activity in the vermal cerebellar cortex and saccadic eye movements. Experimental Brain Research 29, 1–14.

McELLIGOTT, J. G. & KELLER, E. L. (1984). Cerebellar vermis involvement in monkey saccadic eye movements: microstimulation. Experimental Neurology 86, 543–558.

MITZ, A. R., REED, D. J. & HUMPHREY, D. R. (1984). A sequential pulse generator for producing true biphasic stimuli. Electroencephalography and Clinical Neurophysiology 57, 587–590.

NODA, H. & FUJIKADO, T. (1987). Topography of the oculomotor area of the cerebellar vermis in macaques as determined by microstimulation. Journal of Neurophysiology 58, 359–378.

OSCARSSON, O. (1979). Functional units of the cerebellum – sagittal zones and microzones. Trends in Neurosciences 2, 143–145.

RON, S. & ROBINSON, D. A. (1973). Eye movements evoked by cerebellar stimulation in the alert monkey. *Journal of Neurophysiology* **36**, 1004–1022.

SATO, Y., KANDA, K. & KAWASAKI, K. (1988). Target neurons of floccular middle zone inhibition in medial vestibular nucleus. *Brain Research* **446**, 225–235.

SIMPSON, J. I., GRAF, W. & LEONARD, C. S. (1981). The coordinate system of visual climbing fibres to the flocculus. In *Progress in Oculomotor Research*, ed. FUCHS, A. & BECKER, W., pp. 475–484. Elsevier, Amsterdam.

VAN DER STEEN, J., SIMPSON, J. I. & TAN, J. (1991). Representation of three-dimensional eye movements in the cerebellar flocculus of the rabbit. In *Oculomotor Control and Cognitive Processes*, ed. SCHMID, R. & ZAMBARBIERI, D., pp. 63–77. Elsevier, Amsterdam.

VOOGD, J. & BIGARÉ, F. (1980). Topographical distribution of olivary and cortico nuclear fibres in the cerebellum: A review. In *The Inferior Olivary Nucleus: Anatomy and Physiology*, ed. COURVILLE, J., DE MONTIGNY, C. & LAMARRE, Y., pp. 207–234. Raven Press, New York.