

**Single Unit and Population Analysis of Saccade-related  
Fastigial Nucleus Activity in the Alert Monkey**

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Fastigial Nucleus Activity in the Alert Monkey**

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# 1. INTRODUCTION

Eye movements of primates can be divided into five types: saccades, smooth pursuit movements, vergence movements, vestibulo-ocular movements and optokinetic movements. This thesis is concerned with the neural control of saccades and in particular with that of horizontal saccades. Saccades are short-lasting, rapid and ballistic eye movements that serve to fix the image of a target on the fovea of the retina. Saccade control is a highly complicated process and involves many centres in various parts of the brain. Horizontal saccades are generated by the so-called saccade generator in the brainstem paramedian pontine reticular formation (PPRF) (Büttner & Büttner-Ennever, 1988; Hepp *et al.*, 1989). The generator's action is based on the interactions between various so-called burst and pause neurones.

The output of the generator activates brainstem motoneurones including the ipsilateral abducens nucleus, which controls the eye muscles, thereby evoking a saccade. Basically, such a saccade would be very inaccurate as to latency, direction, amplitude and duration (as would be clear from judging a number of subsequent saccades to the same target). However, various brain centres outside the PPRF modulate the generator's output in such a way that saccades are highly and consistently accurate. To these centres belong the superior colliculus (SC), which is involved in the regulation of saccade amplitude and direction (Robinson, 1972; Schiller & Stryker, 1972) and, to some extent, in the control of saccade short-latency and accuracy (Wurtz & Goldberg, 1972). The cortical frontal eye fields (FEF) are, in the monkey, also involved in the control of saccade amplitude and frequency, but only in combination with the action of the SC (Schiller & Stryker, 1982). A very important role in controlling saccade properties is exerted by the cerebellum. In the cerebellum, the vermis and especially the lobules VI and VII (the so-called oculomotor vermis; Yamada & Noda, 1987) regulate saccade amplitude, as appears from lesions of the vermis, which lead to step-size error saccade dysmetria (*e.g.* causing a saccade to overshoot its target).

A remarkable role in saccade control is played by the cerebellar fastigial nucleus (FN). The caudal part of the FN lies under the cerebellar vermis, and is also called fastigial oculomotor region, FOR. It is well established that the FOR is crucial for saccade accuracy, its bursting activity being related to essential saccade aspects, viz. direction,

amplitude, latency and duration. However, the precise mechanism by which FOR bursting influences these aspects is under debate. Obviously, the proper functioning of the FOR requires complex inputs from various brain centres, such as the oculomotor vermis, the inferior olive and various brainstem nuclei (for details see 2. BACKGROUND). Whether these inputs include information about the initial eye position at the beginning of a saccade is a matter of controversy, and is one of the main subjects of this thesis research.

Ohtsuka & Noda (1991b) have assumed that the FOR acts without receiving information about the initial eye position. However, because of the great variability in bursting activity within the FOR neurone population, it remains to be seen whether eye position supports saccadic control by at least some FOR neurones, the more so as some authors have reported that a minority of FOR neurones do react to eye position (Fuchs *et al.*, 1993). In order to resolve the above controversy we here report about experiments to definitely determine:

1. whether or not the initial eye position has an effect on the burst for ipsilateral and contralateral saccades
2. if there is a difference between centripetal and centrifugal saccades, and
3. if FOR neurones influence acceleration and deceleration of a saccade, and if so, in which way.

For this purpose, we trained monkeys to perform horizontal saccades to various horizontal and vertical target positions and recorded from saccade-related FOR neurones. We modified and used an algorithm originally described by Hanes *et al.* (1995) and applied by Thier *et al.* (2000) for the oculomotor vermis, to analyse our data obtained from two head-restrained monkeys by single unit recording from individual neurones in the FOR population.

Based on studies on 75 FOR neurones from the two monkeys, our results permit the conclusion that FOR neurones do not receive information about eye position that is relevant to saccade control. We moreover have characterized differences between centripetal and centrifugal saccades, and provide evidence that the FOR may play a clear role in regulating the acceleration and deceleration of a saccade.

## 2. BACKGROUND

In this section the main data from the field of current interest are presented, with particular attention to saccadic eye movements, the anatomy of the FOR, different aspects of the physiology of a saccade, and the central programming of saccadic activity including the (possible) involvement of the FOR.

### A. Eye Movements

As mentioned in the Introduction, there are five types of eye movement: saccades, smooth pursuit movements, vergence movements, vestibulo-ocular movements and optokinetic movements. Below we will concentrate on saccades.

#### A1. What is a Saccade?

Saccades are short, rapid and ballistic eye movements that abruptly shift the point of fixation to keep a moving or moved image focussed on the retina and, in primates, on the fovea. They reveal different amplitudes, ranging from the small movements made while looking at a picture, to the large movements made while gazing around. Saccades are fast eye movements that occur either reflexively, as a result of *e.g.* an optic or acoustic stimulus from the environment (reflexive saccade), or voluntarily, such as during gazing around in a new environment (intentional saccade). In fact, also the rapid eye movements that occur during rapid eye movement (REM) sleep are (reflexive) saccades. During saccades, the eyes move very rapidly and briefly. In primates, saccade velocity can exceed 700 deg/s and a saccade lasts between 15 and 100 ms. Saccade amplitude ranges from 3 arcmin to 90 degrees, but the amplitude of reflexive saccades rarely exceeds 40 degrees. The delay (latency) between the movement of a target and the start of a saccade is generally 200-250 ms but can be as short as 70 ms (Fischer & Boch, 1983). During such a delay, the position of the target with respect to the fovea is computed, *i.e.*, it is calculated how far the eye has to move to fix the target. This difference between the initial and intended eye position is translated into a motor command that activates the extra-ocular muscles to move the eye over the correct distance into the appropriate

direction. The brevity of a saccade maximises the number of targets that can be fixed. Because a saccade lasts only shortly, there is not enough time for visual feed-back to guide the saccade to its target during the movement. Therefore, the brain (and especially the brainstem saccade generator) must specify the command as exactly as possible before the saccade starts. The cerebellum helps to fine-tune this command using external sensory information, and it is essential for making a saccade highly accurate as to direction, amplitude, speed and duration, in a way consistent from moment-to-moment as well as on the long-term. The time-course of a typical saccadic eye movement is shown in Fig. 1.

Because a saccade is ballistic, it will miss the target when the target changes its position during or after the latency period. Consequently, a second saccade has to be made to correct the error. Furthermore, the elasticity of the eye orbit requires force to keep the eye stable in its new position after the saccade. Therefore, at the neuronal level, an additional ‘step’ activation of the agonist muscle is performed to maintain the new eye position.

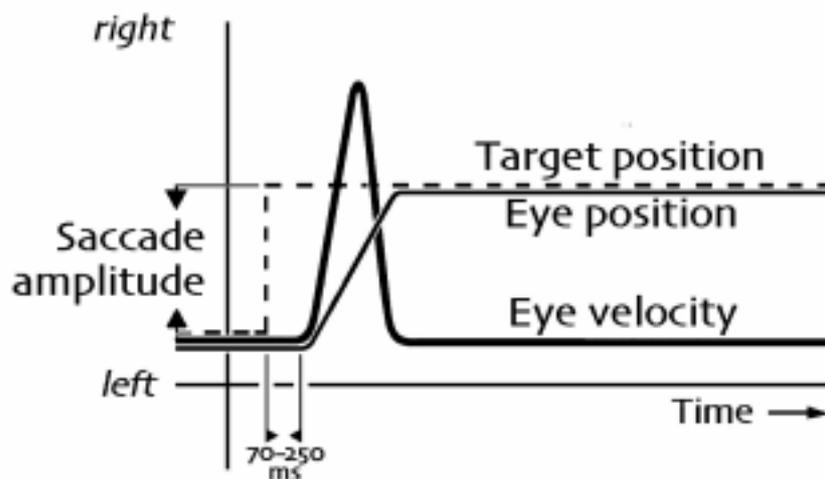


Fig. 1. Time-course of a typical saccadic eye movement. The dotted line indicates the target position, the continuous thin line the eye position and the thick line the eye velocity. When the target moves, there is a latency of 70-250 ms before the saccade starts (modified after Fuchs, 1967).

## **B. Anatomy of the FOR**

### **B1. Structure of the FN**

The FN is the most medial, deep cerebellar nucleus. In monkeys it is located about 2-4 mm lateral to the midline and about 6-9 mm posterior to the interaural line. It extends about 4 mm rostrocaudally, 3 mm mediolaterally and 2.5 mm dorsoventrally (Gardner & Fuchs, 1975).

The FN consists of two parts: a rostral and a caudal, which have different functions. The activity of neurones in the rostral part of the FN is modulated by vestibular stimulation but it does not correlate with eye movements. As a consequence, these rostral FN neurones are named ‘vestibular only’ neurones (Büttner *et al.*, 1991; Siebold *et al.*, 1999). In contrast, neurones in the caudal part of the FN (the FOR) are not influenced by vestibular stimulation but their activity is related to eye movements: saccades, smooth pursuit eye movements (SPEM) and the visual suppression of the vestibulo-ocular reflex (VOR-supp) (Büttner *et al.*, 1991; Ohtsuka & Noda, 1991a).

### **B2. Afferents to the FOR**

Anatomical studies (Noda, 1991) have shown that the FOR receives unilateral projections from the Purkinje cells (P-cells) in the oculomotor vermis and from neurones in the inferior olivary complex (IO), *i.e.*, mainly from the olive (MAO) but in addition from dorsal accessory olive (DAO) neurones. Moreover, a large number of brainstem nuclei innervate the FOR in a bilateral way, such as the pontine nuclei, the nucleus reticularis tegmenti pontis (NRTP), the PPRF, the SC, the vestibular complex (VC), the perihypoglossal nucleus (PHN) and the mesencephalic and medullary reticular formation (Noda, 1991).

### **B3. Efferents of the FOR**

Most efferent fibres of the FOR decussate within the cerebellum, traverse to the contralateral FOR, and proceed via the contralateral uncinate fasciculus to terminate in the brainstem. Their bilateral termination sites include the vestibular nuclei, *viz.* mainly the ventral parts of the lateral vestibular nucleus (LVN) and inferior vestibular nucleus

(IVN), and part of the ventral lateral complex of the thalamus. Furthermore, the FOR projects contralaterally to the NRTP, the pontine nuclei (the dorsomedial pontine nucleus, DMPN, and the dorsolateral pontine nuclei, DLPN), the pontine raphe, the mesodiencephalic junction (rostral interstitial nucleus of the medial longitudinal fasciculus, riMLF, and the medial part of Forel's H Field), the central mesencephalic reticular formation (cMRF), the mediodorsal portion of the medullary reticular formation (DMRF), the periaqueductal gray (PAG), the posterior commissure nucleus, and to the SC (Noda *et al.*, 1990). Ipsilaterally, the FOR projections are limited to a small zone of the reticular formation, namely the rostral end part near the pretectal area and the DMRF (Asanuma & Linden, 1982; Gonzalo-Ruiz & Leichnetz, 1990; Noda *et al.*, 1990). The FOR also projects to the central lateral nucleus of the intralaminar complex. Contralateral fastigial-brainstem fibres mainly arise from the FOR, while the ipsilateral fibres originate mainly from the rostral part of the FOR (Walberg *et al.*, 1962; Matsushita & Iwahori, 1971; Walberg, 1972).

## **C. Physiology of Saccade-related Neurones in the Cerebellum**

In this section attention will be paid to three main types of experimental studies, viz. single unit recordings, lesion studies and electrical stimulation experiments, which have provided information about the physiology of saccade-related neurones in the cerebellum.

### **C1. Single Unit Activity**

#### **C1.1. In the FOR**

There is evidence that FOR neurone burst activity is related to many aspects of saccades, namely saccade direction, amplitude, latency and duration. However, the precise relationships between bursting and these aspects are matter of debate.

The vast majority of FOR neurones produce a burst of action potentials during almost every saccade. However, there is a considerable variability in burst latency, firing frequency and duration, even when saccades have the same direction and amplitude (Ohtsuka & Noda, 1991b; Fuchs *et al.*, 1993). Bursts of FOR neurones start about 8 ms before the onset of a small saccade, irrespective of saccade direction. For contralateral

saccades, burst latency only slightly increases as saccade amplitude increases. For ipsilateral saccades, however, the burst occurs clearly later as saccades are larger, so that for a 20° saccade the burst even begins after saccade onset (though before saccade end). For saccades of about 10°, FOR neurones usually burst earlier for contralateral than for ipsilateral saccades. This pattern of burst timing suggests that the bursts are associated with the beginning of contralateral saccades and with the end of ipsilateral ones (Ohtsuka & Noda, 1990, 1991a,b; Fuchs *et al.*, 1993).

Whereas there is some disagreement as to whether FOR neurones do (Helmchen *et al.*, 1994) or do not (Ohtsuka & Noda, 1992) respond during reflexive saccades in the dark, it is clear that the neurones are active during the fast phases of optokinetic nystagmus (Helmchen *et al.*, 1994) and during spontaneous saccades in the light (Fuchs *et al.*, 1993; Helmchen *et al.*, 1994). Furthermore, there is also no agreement about how strong other aspects of burst activity are related to saccade metrics. One of such aspects is saccade duration. Ohtsuka & Noda (1991b) reported that FOR burst activity and saccade duration correlate very well (correlation coefficient: 0.85–0.97). In contrast, Fuchs *et al.* (1993) concluded that saccade-related bursts of FOR neurones are only weakly correlated to saccade duration (correlation coefficient: < 0.6).

In the FOR population, the tonic level of activity shows no significant correlation with eye position, but in the FOR rostrally to the FOR, there are a few neurones whose activity closely correlates with eye position (Ohtsuka & Noda, 1990).

### **C1.2. In the oculomotor vermis**

Many (71%) oculomotor vermis P-cells reveal saccade-related bursts for either ipsilateral or contralateral saccades or for both (Ohtsuka & Noda, 1995). For ipsilateral saccades, a P-cell burst stops during the second half of a saccade. This absence of inhibitory P-cell activity could cause a depolarisation of cerebellar nuclear neurones (Aizenman & Linden, 1999), which might facilitate the onset of late FOR neurone bursts for controlling ipsilateral saccades. For contralateral saccades, P-cell bursts begin before or early during a saccade, peak near the middle, and continue after saccade end (Ohtsuka & Noda, 1995). This pattern could help produce the so-called post-burst pauses that occur in FOR neurones. About 18% of P-cells pause in case of contralateral saccades. Pause timing is variable from saccade to saccade, with an average mean lead time of 17.5 ms (Ohtsuka & Noda, 1995). The post-burst pauses begin sharply at the same time and could therefore, if

synchronised across many P-cells, trigger the early bursts of FOR neurones controlling contralateral saccades.

A few (11%) P-cells show a burst discharge during contralateral saccades followed by a tonic discharge that is correlated with eye position, with a burst latency relative to saccade onset of  $9.5 \pm 3.9$  ms. The tonic discharge rate of such 'burst tonic cells' is a nonlinear function of the eye position (Ohtsuka & Noda, 1995). Meanwhile, the mechanism by which FOR neurones produce bursts that take place before contralateral saccades, is not clear.

## C2. Lesion Studies

The function of the FOR has been studied in both chemical and surgical lesion experiments. Impairment of the FOR and of the oculomotor vermis produces inaccurate saccades with an abnormally variable amplitude and speed (Robinson *et al.*, 1993). Unilateral inactivation by chemical lesioning of the FOR by injecting the GABA<sub>A</sub> agonist muscimol produces ipsilateral saccades that are too large (gain > 1.2-1.9; normal: 1.0) and contralateral saccades that are too small (gain < 0.6-0.8). Moreover, gains are more variable than normal (*e.g.* standard deviations of saccades to 10° horizontal targets are 1.2-4.8 times larger than normal). After surgical lesioning of the afferents to the oculomotor vermis, both leftward and rightward saccades become hypometric and the postlesion gains are at least twice as variable as normal (Takagi *et al.*, 1998; Barash *et al.*, 1999). Saccades to vertical targets curve strongly, ending 2-9° to the left of their targets. In addition, both ipsilateral and contralateral saccades are slower and much more variable than normal saccades of the same size (Robinson *et al.*, 1993).

Bilateral FOR inactivation by chemical lesioning in monkeys leads to oversized saccades into all directions, with slow and abnormally variable velocity (Robinson *et al.*, 1993), and also impairs the animal's ability to reduce the saccade gain (Robinson & Fuchs, 2001). The dependence of burst timing on saccade direction is fully in line with the results from studies on the effect of lesions on FOR activity. In case of contralateral saccades, a burst occurs early during saccadic eye movement, providing a contralateral drive to accelerate the saccade. Without this drive, the saccade falls short. Later in the saccade, FOR neurones ipsilaterally to the direction of the saccade produce a burst that delivers a drive opposite to the direction of the saccade, to slow down the speed of the saccade. In the absence of this late burst, the saccade does not decelerate properly and

overshoots its target. Somehow, the FOR burst also makes the saccades more consistent and more repeatable.

The dysmetria produced by unilateral FOR inactivation suggests that each saccade lacks a contralateral component and that the net effect of saccade-related FOR activity at one side is to drive the eyes toward the contralateral side.

### C3. Stimulation Studies

Consistent with the above idea that FOR activity drives the eyes to the contralateral side, electrical stimulation of the FOR evokes saccades with large, contralateral components (Noda *et al.*, 1988). Electrical microstimulation inside the oculomotor vermis elicits saccades and also modifies the metrics of visually guided saccades. Contralaterally directed saccades are consistently slowed down in speed and become hypometric, whereas ipsilaterally directed saccades are not affected (Keller *et al.*, 1983).

Microstimulation of the cerebellar vermis evokes saccades whose direction and amplitude are dependent on the position of the eye in its orbit. At a few sites, even the presence or the absence of an evoked saccade depends on the initial eye position. Microstimulation also elicits postsaccadic drifts, whose presence or absence as well as direction are also dependent on the initial eye position (McElligott & Keller, 1984).

In human, transcranial magnetic stimulation (TMS) of the posterior cerebellum produces hypermetric ipsilateral saccades followed by postsaccadic drift, with latencies of 0, 20 and 40 ms. However, for contralateral saccades, only short latency stimuli (near to 0 ms) have an effect, viz. evoking hypometric saccades followed by corrective saccades (Hashimoto & Ohtsuka, 1995).

Microstimulation of the oculomotor vermis and of the ventromedial part of the FOR yields saccades with different horizontal directions, with vermal stimulation leading to ipsilateral saccades and fastigial stimulation eliciting contralateral saccades. Stimulation of the oculomotor vermis inhibits the activity of FOR neurones. Most likely, the cerebellar output signals are projected downstream to saccade-programming circuits where visual information has already been converted into motor command signals (Noda, 1991).

## C4. Conclusion

Without input from the FOR, the saccade machinery produces dysmetric saccades that lack a normal stereotype. The hypometria as revealed by contralateral saccades and the hypermetria of ipsilateral saccades resulting from a unilateral lesion, suggest that FOR activity helps to accelerate contralateral but to decelerate ipsilateral saccades. Obviously, a mechanism outside the medial cerebellum slowly restores mean saccade gain to normal, but no outside mechanism can restore saccade consistency (Barash *et al.*, 1999). The precise relationship between FOR bursting and the control of acceleration/deceleration processes deserves particular attention, as is another aim of this thesis research.

## D. The Central Programming of Saccadic Eye Movements

Three directions can be distinguished in saccadic eye movements: horizontal, vertical and torsional. In this thesis, focus is on horizontal movements. The central programming of horizontal saccadic eye movement involves centres in the brainstem and in the cerebellum (including the FOR). Below we will treat the main models describing the central programming of these movements, with emphasis on the roles of these two essential brain territories.

### D1. Brainstem

Saccadic eye movements are generated by four different types of neurone in different areas of the brainstem, viz. 1. the excitatory immediate premotoneurones (EBNs), 2. the inhibitory medium-lead burst neurones (IBNs), 3. the long-lead burst neurones (LBNs) and 4. the omnipause neurones (OPNs) (Horn *et al.*, 1996). It is generally assumed that the PPRF is essential for the generation of horizontal saccades (Bender & Shanzer, 1964; Cohen *et al.*, 1968; Goebel *et al.*, 1971). In this nucleus EBNs deliver high-frequency bursts of activity to the motoneurones of the extra-ocular eye muscles, via monosynaptic input, 8-15 ms before and also during the saccades, but they are silent during fixation and slow eye movements such as the SPEM and the VOR. The IBNs are situated in the contralateral medullary reticular formation. They project to brainstem motoneurones that control contralateral eye muscles. The LBNs in the brainstem show an irregular, low-

frequent activity ( $\geq 100$  ms) before a saccade-related burst starts. They may stimulate the EBNs and probably do not project directly to motoneurons. The OPNs occur in the nucleus raphe interpositus of the pontine reticular formation. They reveal a reverse firing pattern compared to that of the EBNs, with a high level of tonic activity (more than 100 Hz) being only interrupted for 10-12 ms prior to and during a saccade.

Based on anatomical and physiological studies in monkeys, the following hypothesis is put forward for the roles of these four regulatory components in saccadic eye movement control: during slow eye movements and fixation of the target on the fovea, the OPNs exert a tonic inhibition on both EBNs and IBNs, blocking their firing. During a saccade, the OPNs are inhibited, possibly by polysynaptic inputs from the SC, which might influence the activity of the LBNs. In this way, the inhibition of EBNs and IBNs is deblocked, which allows these neurones to activate the motoneurons of the extra-ocular eye muscles, resulting in a saccade.

Although a theoretical model for SC control of saccades was recently presented by Lefèvre *et al.* (1998), the exact role of the SC in the direct control of the saccade generator in the brainstem is unknown and not the topic of this thesis.

## D2. Cerebellum

Whereas saccades are initiated by the brainstem generator, the cerebellum is crucial for the control (modulation and fine-tuning) of saccadic eye movements. Single-unit and lesion studies have shown that the posterior vermis and the FOR provide a signal to make horizontal saccades fast, accurate and consistent. Moreover, the FOR is also necessary for the recovery of saccadic accuracy after neural or muscular damage that makes horizontal saccades dysmetric.

Below, we will firstly consider the oculomotor vermis into some detail, especially as to the characteristics of the P-cells, and then describe the significance of the oculomotor vermis and the FOR for the determination of eye position. Subsequently, the FOR control of saccades is treated into more detail, with attention to the way FOR neurones influence the saccadic generator and with special emphasis on two possible mechanisms by which they make saccades consistent.

### D2.1. The oculomotor vermis

In the oculomotor vermis, P-cells produce bursts during saccades. From this area saccades can be elicited by electrical stimulation (Noda & Fujikado, 1987). The oculomotor vermis includes the vermal lobule VII and the two most posterior folia of lobule VI. Axons of P-cells in the oculomotor vermis densely terminate in a small, oval region in the ipsilateral FOR and, less densely, in the rostral FOR (Yamada & Noda, 1987).

Thier *et al.* (2000) have gathered evidence that the P-cells act together as one population, which output promotes the determination of saccade duration. Furthermore, changing the duration of the activity of this population output influences saccade amplitude.

### D2.2. Eye position signals in oculomotor vermis and FOR

Cerebellar lesions of the oculomotor vermis (Ritchie, 1976) and the FOR (Vilis & Hore, 1981) cause saccade dysmetria with a size that depends on the starting position of the eye. The abnormal centrifugal saccades are smaller than the centripetal ones, a phenomenon that can also be seen in human patients with infarcts in the posterior vermis (Vahedi *et al.*, 1995). It has been proposed that the posterior medial cerebellum corrects the initial eye position in such a way that centrifugal and centripetal saccades have similar sizes. Fuchs *et al.* (1993) reported that after bilateral inactivation of the FOR, the size of centrifugal saccades to 20° targets is 79% of the size of centripetal saccades. However, others (Ohtsuka *et al.*, 1994) did not find such a difference between the sizes of centrifugal *versus* centripetal saccades. To make this controversy even stronger, some authors conclude that the discharge of FOR neurones has only a weak (Fuchs *et al.*, 1993) or even no relationship at all with the initial eye position (Ohtsuka & Noda, 1991a,b; Ohtsuka *et al.*, 1994).

### D2.3. How does the FOR influence the saccade machinery?

Electrical stimulation of the FOR and the vermal lobules V-VII produces low-threshold, short-latency eye movements. Possibly, the FOR projects contralaterally to EBNs, IBNs and OPNs (Noda *et al.*, 1990; Scudder *et al.*, 2000). IBNs inputs to motoneurones cross, but EBNs projections are ipsilateral. Therefore, FOR activity may accelerate contralateral eye movements via both neurone types. In this case, one would expect that there are late

off-direction bursts produced by IBNs and EBNs. Indeed, most EBNs and some IBNs exhibit a weak, late burst for off-direction saccades (Strassman *et al.*, 1986; Scudder *et al.*, 1988). It appears that FOR neurones receive timing information to produce early bursts for contralateral saccades and late bursts for ipsilateral saccades.

#### **D2.4. How do FOR neurones make saccades more consistent?**

It is clear that FOR neurone bursts make saccades more consistent, although the bursts themselves are very inconsistent as to onset, duration and number of spikes, even in the case of saccades that have very similar characteristics (Fuchs *et al.*, 1993). The saccade-related FOR neurones that project directly to burst neurones in the brainstem premotor saccade-generating network (Scudder *et al.*, 2000) reveal a consistent relationship with the saccade metrics. Two models exist that aim to explain how the variable activity of FOR neurones diminishes the variability in saccade properties. The first model assumes that the saccadic system in the brainstem, including the SC, represents a burst generator which variable output is appropriately corrected by the FOR, resulting in consistent saccades (Robinson, 1995). Possibly, the FOR is able to do so because it receives an efferent feedback about the saccade properties that shapes exactly the FOR output to make all saccades consistently end on target (Lefèvre *et al.*, 1998). The second model implicates that although the output of individual FOR neurones is variable, their combined activity adequately specifies features of each saccade. This would be similar to the way a population of P-cells in the oculomotor vermis accurately specifies the end of a saccade (Thier *et al.*, 2000).

Robinson & Fuchs (2001) schematically presented the central programming of saccadic eye movements, as shown in Fig. 2.

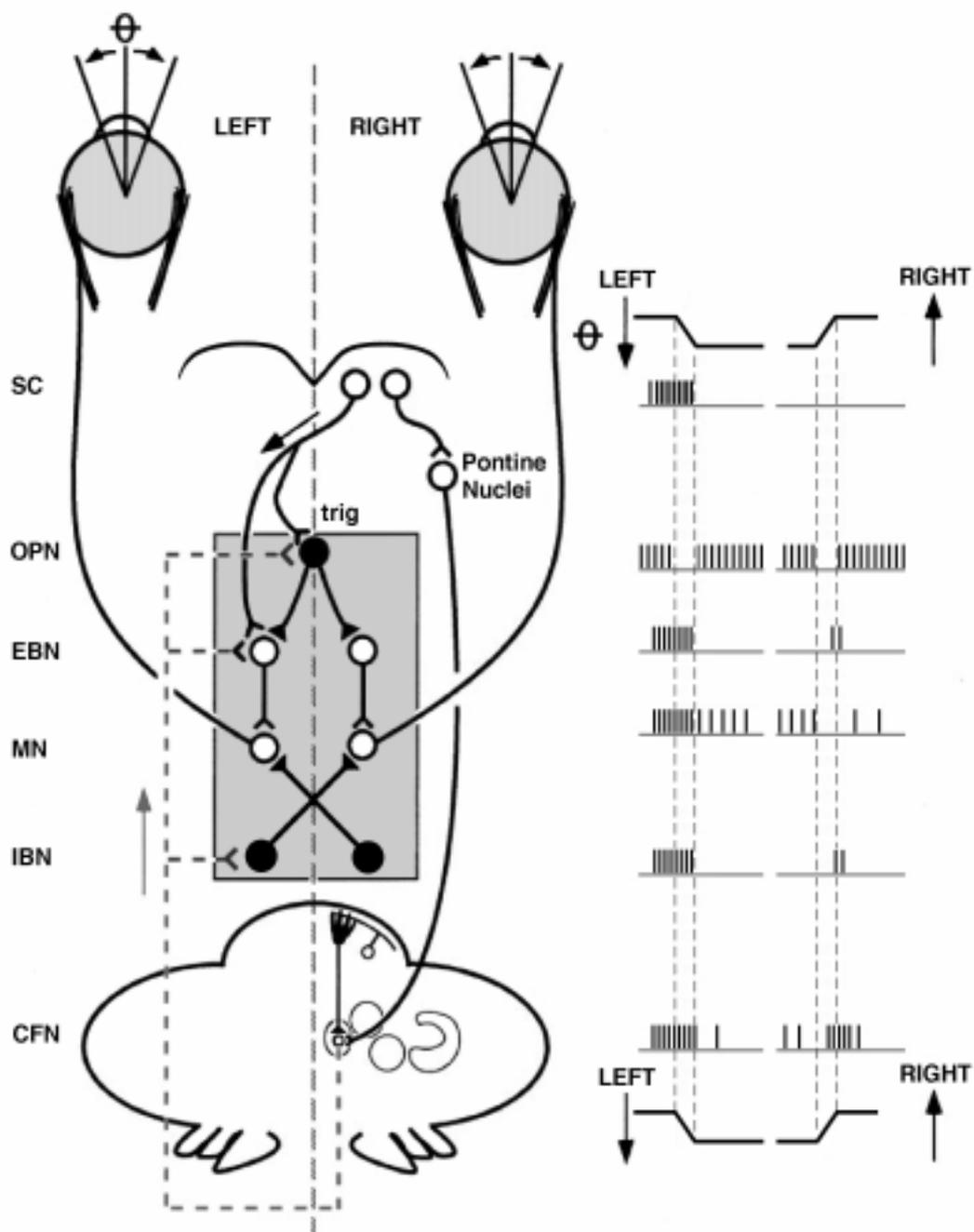


Fig. 2. Schematic representation of the central programming of saccadic eye movements by the brain-stem saccade burst generator (gray box). The generator receives inputs from the superior colliculus (SC) and the cerebellum (dashed lines). SC neurones also control the caudal fastigial nucleus (CFN, also called FOR) via the pontine nuclei. Cerebellar output via the CFN influences three groups of generator neurones, viz. excitatory burst neurones (EBN), inhibitory burst neurones (IBN) and omnipause neurones (OPN). As a result of the interactions between these three groups, motoneurones control left and right eye muscles, leading to saccades. Filled synaptic terminals indicate inhibitory synapses, open terminal symbols mark excitatory synapses (from Robinson & Fuchs, 2001).

### **3. MATERIALS & METHODS**

#### **A. Animals**

Experiments were performed with two conscious, adolescent, male Rhesus monkeys (*Macaca mulatta*), with an age of 4 and 6 years, respectively, each weighing about 4.5 kg. They were fed on dry food and fresh fruits (freely and continuously available unless stated otherwise) and kept in a vivarium at a daily cycle of 16 hrs light / 8 hrs dark, at 20 °C. Before an experiment the monkeys were water-deprived overnight. After the experiment, they were allowed to drink to satiation before going back into their cage.

All experiments were carried out in accordance with the guidelines set by the German national law for animal experimentation, and had been approved by the University committee supervising the handling of experimental animals.

#### **B. Training**

Before surgery, monkeys learned to voluntarily come out of their cages and enter a primate chair. Then they were trained to acclimatise to the experimental environment and sit upright inside a coil frame with the head fixed to the chair by a head holder so as to completely immobilise the head in a painless manner while the body could move freely. The centre of the interpupillary line coincided with the centre of the frame.

Monkeys were trained to follow and fixate on a video screen a small, movable laser light target (measuring 1 degree of visual angle) for 600 ms, which jumped at defined horizontal and vertical positions at unpredictable intervals (1.3 sec to 2.2 sec) over the screen. The distance between the centre of the screen and the eye was 50 cm. The midposition of the eye was determined by repeatedly attracting the monkey's attention to defined fixation points. A correct saccade was recorded when the monkey's eye entered a 3° window area around the target site, and such a successful saccade was contingently rewarded with some fruit-juice.

A 9-point horizontal start position training paradigm, consisting of a 3x3 square grid spaced at 16° intervals, moved the target along the horizontal meridian. In this way,

the monkey could make horizontal saccades ipsilaterally and contralaterally to the recording site, and also centripetal and centrifugal saccades at different vertical starting positions. The monkey's task was to detect a sudden shift in the position of the target, and to make a saccade from the initial spot to the final target within a limited time. Fig. 3 shows the paradigm and the respective eye movements.

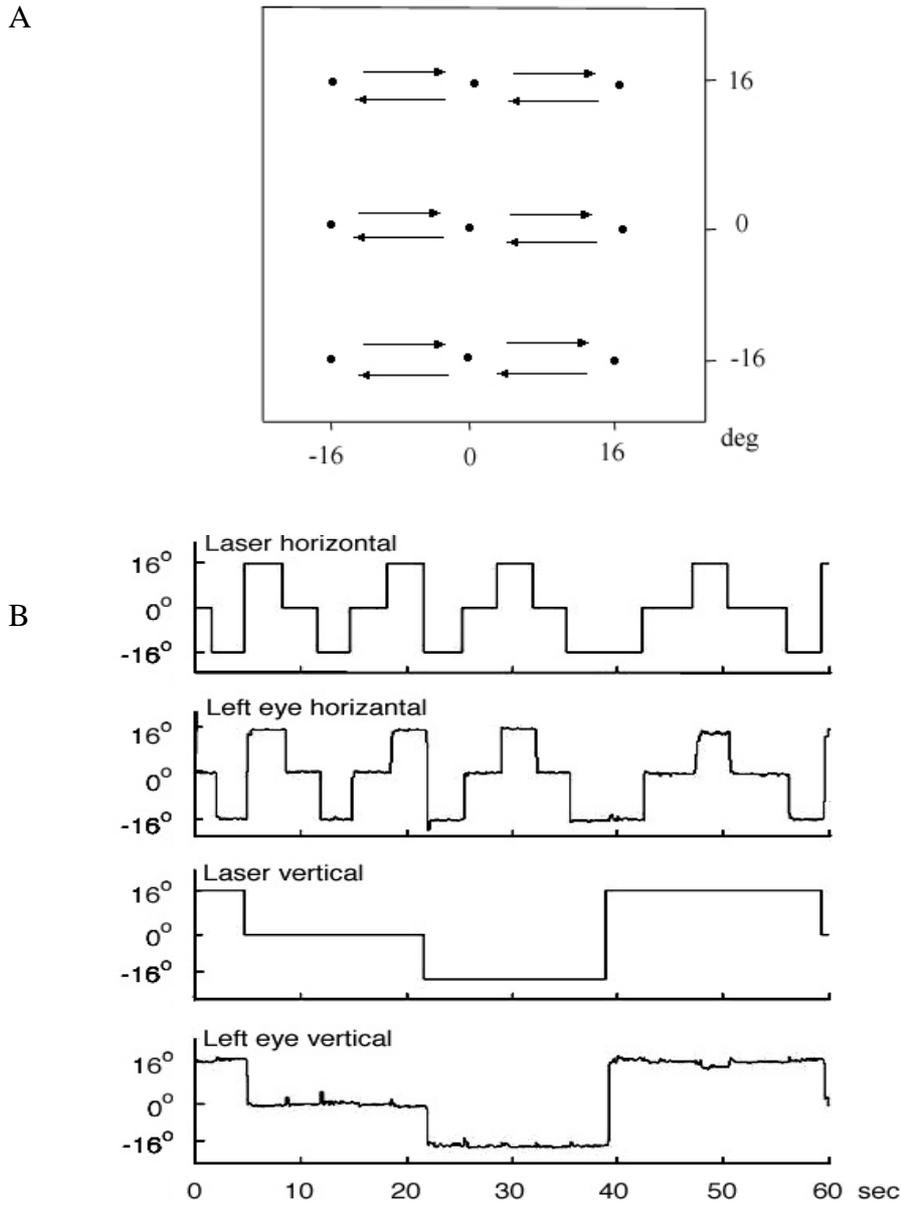


Fig. 3. A. Scheme of the visual guidance paradigm on the video screen. Dots show the different stimulus (laser spots) starting positions, with upper, middle and lower vertical levels at 16 degrees (deg) intervals, and arrows indicate the horizontal directions into which the spots move. B. 2-D eye movements (Left eye horizontal and Left eye vertical) following the onset of target movements (Laser horizontal and Laser vertical), at different vertical levels with 16 degrees intervals, in seconds (sec).

## **C. Surgical Procedures**

Under general pentobarbital sodium anesthesia (intravenous injection) and aseptic conditions, a chamber for single unit activity recording was implanted (co-ordinates: mediolateral: 0 mm, posterior: 7 mm; see primate atlas of Snider & Lee, 1961) through a trephine hole (12 mm diameter) into the skull of the monkey, to allow a vertical approach in the stereotaxic plane to both sides of the FOR. The endocranium was kept intact. Dental cement filled the space between skull and recording chamber, and small bolts were attached to the cranium to stabilise the implant (for details, see Boyle *et al.*, 1985).

To measure eye movements, a self-made dual search-coil was sewed on the sclera of one eye, where the extra-ocular muscles (superior, inferior, medial and lateral rectus) are adhered. The search-coil wires were submerged into the orbit, passed through a hole drilled into the parietal bones, and then showed up at the same side of the operated eye. They were connected to a plug that was attached to the skull with dental cement.

Horizontal and vertical, magnetic, alternating current fields in spatial and phase quadrature were generated around the head, expressing gaze signals along with the target position signal. They were low-pass filtered (50 Hz) and sampled at 1000 Hz, and stored on computer hard disk for off-line analysis. For details on techniques and calibration, see Bartl *et al.* (1996). Spontaneous and visually guided saccades were recorded in darkness.

## **D. Single Unit Activity Recordings**

Single-unit activity was extracellularly recorded with self-made, varnished, tungsten micro-electrodes with an impedance of 2.5-4 M $\Omega$  that was obtained by mechanically opening the isolated tip before recording. The sterilised micro-electrode, which was driven by a hydraulic remote-controlled stepping motor affixed to the top of the recording chamber in a stereotaxic plane, was perpendicularly punctured through the dura and inserted into the FOR by means of a guiding cannula. The position of the electrode with respect to the cerebellar layers was determined on the basis of the characteristic neuronal activity (*e.g.* complex spikes) as the electrode was advanced (for details, see Helmchen, 1995).

The neural signals were amplified and passed through a Schmitt-trigger which generated standard pulses for each discharge, and recorded at a temporal resolution of 20  $\mu$ s. They were observed with an oscilloscope and an acoustic monitor.

## E. Data Collection and Analysis

For off-line quantitative analysis, a computer programme (Wineye; S. Glasauer, LMU, München) automatically identified the onset and the end of the horizontal and vertical components of each saccade, by using an adjustable saccade detection algorithm (speed threshold 50 deg/s, acceleration threshold 100 deg/s<sup>2</sup>). Only horizontal saccades were included that landed 'on target' within a 3° tolerance limit, appearing in a restricted time window after the target jump (80 to 500 ms), and that had a peak speed > 200 deg/s (*cf.* Robinson, 1970).

Subsequently, saccades with similar features (direction, initial eye position) and their related burst activities were quantitatively sorted, using a self-developed (J.F. Kleine, LMU, München) computer-routine programme written in Matlab (Math Software Co., Matwork, USA). The programme plots a raster diagram and a perisaccadic spike density histogram for the sorted saccades, by substituting for each spike a Gaussian function with a width of 5 ms. In each histogram at least 10 saccades were averaged. Histograms were used to relate features of neuronal activity (*i.e.*, burst onset and offset, peak burst activity and latency) to individual saccade parameters. All data were displayed as x-y plots.

The last (first) bin before (after) the time when the neuronal activity exceeded (fell below) the mean + one standard deviation (SD) of the background rate, as determined from the spike histogram in the time interval 500 - 250 ms prior to saccade onset, was designated as burst onset and offset, respectively. The individual saccade-related burst detection data were analysed with another computer routine algorithm (written in Matlab, modified by J.F. Kleine), using Poisson spike train analysis ( $\alpha=5\%$ ). It was originally described by Hanes *et al.* (1995) and recently used by Thier *et al.* (2000) in an oculomotor vermis P-cell discharge analysis.

Statistical significance of differences was assessed with a Mann-Whitney test or t-test for single comparisons, and a Kruskal-Wallis test for multiple comparisons ( $\alpha=5\%$ ).

## 4. RESULTS

### A. Localisation and General Characteristics of Saccade-related FOR Neurones

At the time of the final version of this manuscript, both monkeys were still involved in other experiments. Therefore, a detailed histological reconstruction of the recording sites is not available. However, based on recording patterns in surrounding structures and on the characteristic discharge pattern of the saccade-related neurones it is felt that the neurones recorded were located indeed in the FOR. Rostral to the FOR, in the rostral FN, ‘vestibular only’ neurones (Büttner *et al.*, 1991; Siebold *et al.*, 1999) were recorded. Dorsal to the rostral FN, electrodes clearly passed first through P-cell layers, and then went through several millimeters of white matter where typically no neurones were recorded. Further caudally, the white matter above the FOR became less extensive, and below the FOR P-cells were encountered, in accordance with Büttner *et al.* (1991). FOR saccade-related neurones were clustered in two areas of 2 mm in diameter on the left and right side, separated from each other by 2 – 3 mm across the midline.

FOR neurones were investigated bilaterally, thereby recording simultaneously both neuronal electrical bursting activity and eye movements. In this way, neurones with a discharge activity related to the occurrence of saccades could be readily identified. In total, 75 of such saccade-related FOR neurones were studied, in two monkeys. No preselections were made, so that, regardless of the specific characteristics and the prominence of the saccade-related burst patterns, all units were included that showed a discernible change in their electrical activity during a saccade, provided that they were well isolated and located within the anatomical borders of the FOR.

All FOR neurones were spontaneously active (mean  $\pm$  SD: 45.4  $\pm$  24.2 impulses/s, imp/s), but their discharge activity was far from regular, ranging from 8.4 to 104.3 imp/s, as described previously (Fuchs *et al.*, 1993; Helmchen *et al.*, 1994). Moreover, neurones showed obvious interindividual differences as to their relationship with saccades. Therefore, different classes of FOR neurones were distinguished.

## B. FOR Saccade-related Neurone Classification

Nearly all FOR neurones (N=74/75) showed a burst of electrical activity in relation to a saccade. Of these, 52 neurones exhibited a burst of activity with every saccade occurring, in both ipsilateral and contralateral direction, in a one-to-one fashion. Some neurones (N=21) showed bursts into one direction only (17 neurones bursted only during ipsilateral saccades, 4 neurones bursted only during contralateral saccades) while exhibiting into the opposite direction either a decrease in activity, a pause in activity, or no activity change at all, which is in accordance with previous observations (Ohtsuka & Noda, 1991). The only neurone that did not burst with saccades showed a saccade-related decrease in activity for ipsilateral saccades, but its activity was not modulated during contralateral saccades.

Table 1. Distributions of discharge patterns of ipsilateral and contralateral saccade-related FOR neurones of two monkeys (b, burst neurone; bp, burst-pause neurone; p, pause neurone; pb, pause-burst neurone; pbp, pause-burst-pause neurone).

monkey	ipsilateral						contralateral					
	b	pb	pbp	bp	p	other / no change	b	pb	pbp	bp	p	other / no change
# 60	6	34	-	3	2	-	23	5	4	-	1	1 / 11
# 62	10	15	1	1	3	-	10	2	6	6	-	0 / 6
$\Sigma$	16	49	1	4	5	-	33	7	10	6	1	1 / 17

Quite often (N=50) neuronal bursting was preceded by a pause (pause-burst neurone, PB; Fig. 4A) or by a decrease in activity. Only few neurones (N=5) showed a burst that was followed by a pause (burst-pause neurone, BP; Fig. 4B). A decrease in bursting activity preceding a burst, started a considerable time before the beginning of a saccade (more than 100 ms), with minimal activity occurring shortly before saccade start. One neurone (classified as 'other' in Table 1) was not modulated in its activity during contralateral saccades but showed prominent bursts during ipsilateral saccades. Unlike other cells, it gradually changed its discharge frequency, starting 120 ms before the saccade, having a peak twice the background rate at 40 ms after saccade onset, and then gradually reducing its frequency to the background level within 160 ms.

The respective discharge patterns differed for ipsilateral and contralateral saccades. Contralateral saccades were more often accompanied by a 'pure' burst (N=34) or revealed no activity change (N=17), although complex discharge sequences (PB; BP; or PBP, 4C) were also observed. However, the most frequent combination of discharge patterns was a PB sequence for ipsilateral saccades.

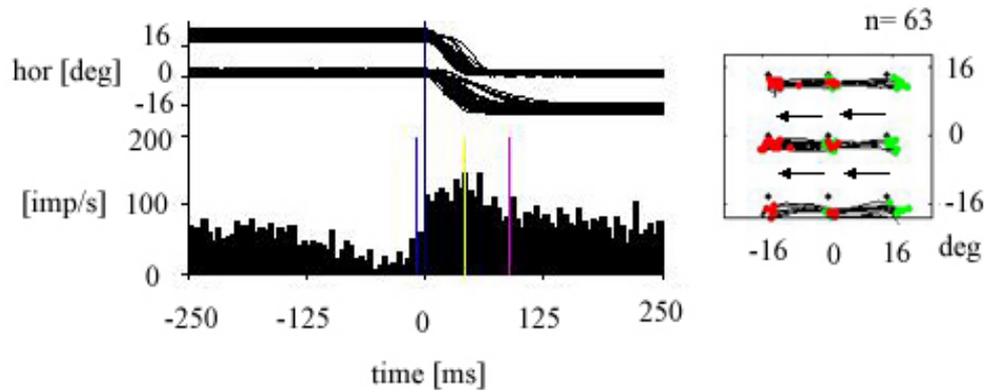
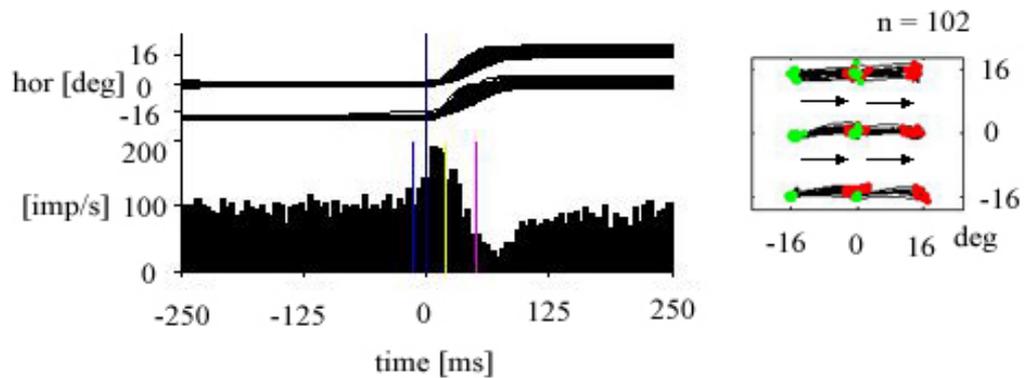
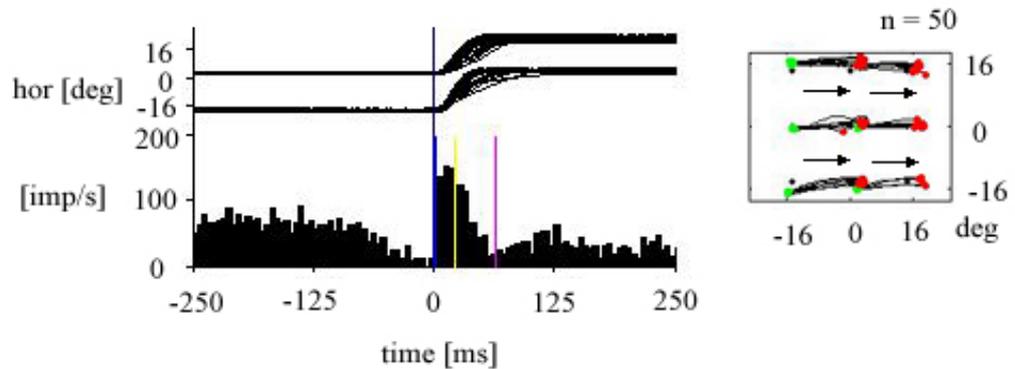
**A****B****C**

Fig. 4. Examples of different combinations of discharge patterns of saccade-related FOR burst neurones. A: pause-burst neurone, ipsilateral saccades; B: burst-pause neurone, contralateral saccades; C: pause-burst-pause neurone, contralateral saccades. Upper panel shows different horizontal (hor) eye positions in degrees (deg). Lower panel presents histogram of spike frequency in impulses/second (imp/s), where 0 milliseconds (ms) at the time axis represents saccade onset. The right panel shows the eye position paradigm, with arrows indicating eye movement direction. n = total number of saccades (above right panel).

### C. Timing of Burst Activity: Ipsilateral *versus* Contralateral Saccades

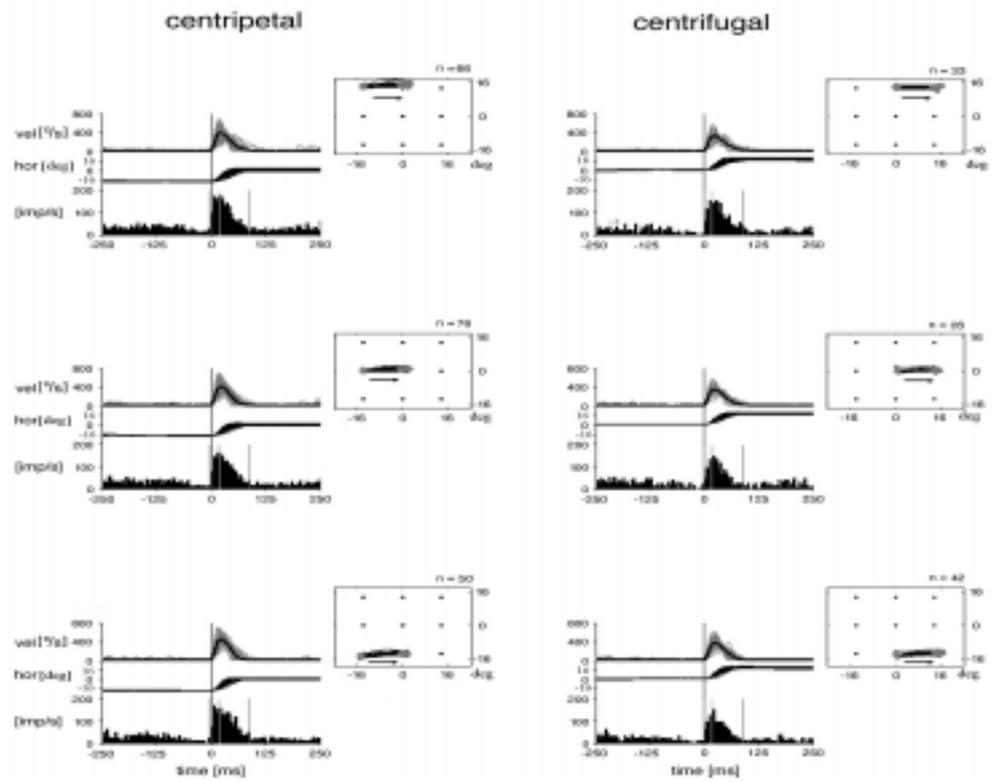
The most conspicuous feature of a FOR neurone is its capacity to change the timing of burst activity, which plays a major role in the determination of saccade direction. This is evident from our spike histogram analyses, as will be shown in the following.

In general, FOR neurones burst earlier to a contralateral saccade than to an ipsilateral saccade. For all FOR neurones, the latency of their bursts (at 16°) is  $11.1 \pm 19.8$  ms before the onset of a contralateral saccade and  $3.0 \pm 18.8$  ms before the start of an ipsilateral saccade, values that differ significantly ( $P < 0.05$ , Wilcoxon). So, bursts generally start before saccades. As a result, the burst offset time (burst end in relation to saccade onset) for contralateral saccades is shorter ( $55.2 \pm 26.7$  ms) than for ipsilateral ones ( $64.1 \pm 25.1$  ms). However, the burst latencies for ipsilateral as well as for contralateral saccades are similar for bilaterally and unilaterally bursting neurones. As to latency, bilaterally bursting neurones start  $9.6 \pm 19.4$  ms (contralateral) and  $3.3 \pm 20.4$  ms (ipsilateral) before saccades, whereas unilaterally bursting neurones have a latency of  $30.0 \pm 16.6$  ms (contralateral) and  $2.0 \pm 13.7$  ms (ipsilateral), values that are not significantly different between the two classes of neurones. Fig. 5 gives a representative example of a FOR neurone, clearly showing the differences between ipsi- and contralateral saccades.

With regard to peak latency, in all bursting neurones examined, peak activity of a burst, *i.e.*, where the firing rate is maximal, is reached earlier after burst onset of contralateral saccades ( $24.4 \pm 22.0$  ms) than of ipsilateral saccades ( $29.2 \pm 19.9$  ms) ( $P < 0.05$ , Wilcoxon). Bilaterally bursting neurones (contralateral:  $25.4 \pm 22.5$  ms; ipsilateral:  $30.2 \pm 20.7$  ms) do not significantly differ from unilaterally bursting ones (contralateral:  $10.5 \pm 5.0$  ms; ipsilateral:  $26.4 \pm 17.6$  ms).

When related to burst onset (and not to saccade onset), peak burst activity and burst duration are similar for saccades in both directions, and also reveal no statistically significant difference between bilaterally and unilaterally bursting neurones. The peak burst amplitudes are  $102.4 \pm 48.6$  imp/s and  $98.2 \pm 42.2$  imp/s for contralateral saccades and ipsilateral ones, respectively. The burst discharge during contralateral saccades lasts  $86.9 \pm 42.5$  ms, which is very similar to that during ipsilateral saccades ( $89.1 \pm 38.6$  ms).

### Contralateral saccades



### Ipsilateral saccades

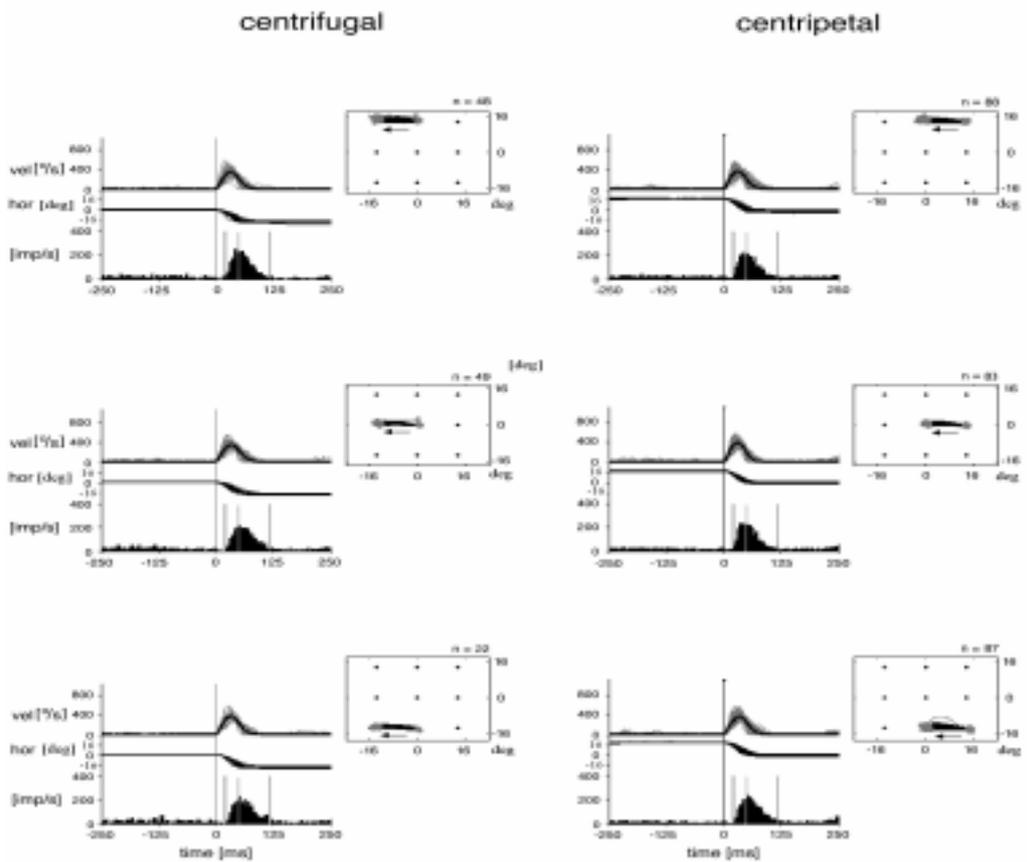


Fig. 5. Example of a typical FOR neurone, clearly showing the differences between contralateral (upper 6 graphs) and ipsilateral (lower 6 graphs) saccades. The neurone starts its bursting activity with the beginning of contralateral saccades. For ipsilateral saccades, the burst starts clearly after the onset of saccades, and the peak activity is later. In each graph, the upper trace shows saccade velocity ( $\text{vel}[^{\circ}/\text{s}]$ ), the dark solid trace presents the average velocity, and the gray traces indicate the velocity of individual trials. The vertical lines within the burst histogram indicate the saccade onset, burst onset, peak of burst and burst offset, respectively. For further explanations, see Fig. 4.

These conclusions are based on spike histogram analyses. Note, however, that the numerical values assigned to peak discharge rates are substantially influenced by the particular method used to quantify them. When calculated according to the criteria applied in individual burst analysis, the obtained peak rates are higher (see below).

#### **D. Spike Histogram *versus* Individual Burst Analysis**

We compared the burst parameters obtained from the perisaccadic spike histograms with the average of the corresponding values obtained from burst analysis of individual trials, in order to validate the results obtained by the computerized burst detection routine. In Fig. 6 the scatter plots are given of the parameters most relevant for the analyses that will be presented below: burst latency, burst-peak latency and burst-peak amplitude. They are derived from the perisaccadic spike histograms and are plotted against the corresponding parameters obtained from the individual burst analyses. It is clear that the two different analytical approaches yield values that are strongly correlated with each other, having highly significant ( $P < 10^{-6}$ ) correlation coefficients:  $r = 0.88$  (burst latency),  $r = 0.86$  (peak latency) and  $r = 0.91$  (peak amplitude). While the peak latency values are lying fairly well around perfect correlation (Fig. 6B), in the three plots marked by the solid line ( $y = x$ ) the two other parameters reveal obvious and systematic differences (Fig. 6A,C): in the spike histogram analysis the onset is assigned to earlier time values and the peak activity is clearly lower than in the individual burst analysis.

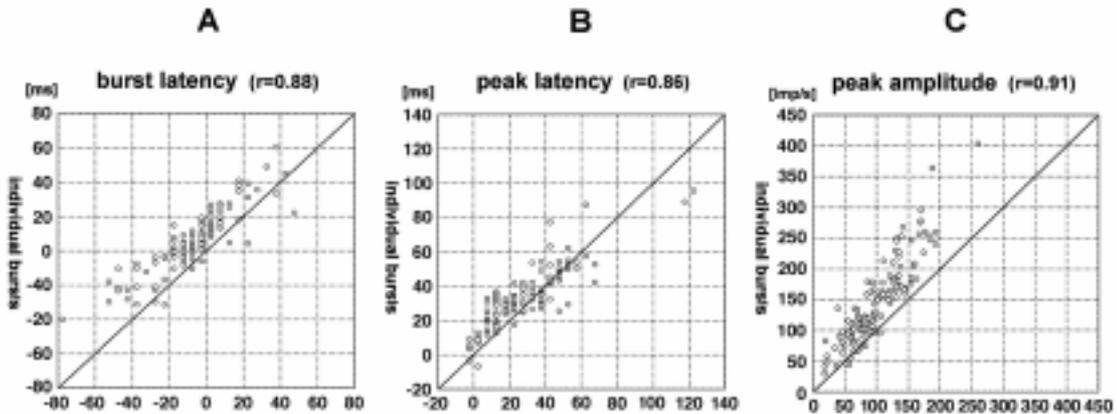


Fig. 6. Significant correlation plots for three burst parameters (A: burst latency; B: burst-peak latency; C: burst-peak amplitude) between data obtained from analyses of individual bursts (average) and corresponding values derived from perisaccadic spike histograms ( $y = x$ , marked by solid line). Each dot represents one FOR burst neurone that is related to ipsilateral or contralateral saccades. A negative value in time axis (A, B) indicates that burst or burst peak starts before the onset of a saccade, whereas a positive value means that these starts are after saccade onset.

## E. Relation Between Burst Parameters and Saccade Kinematics

Although the amplitude of eye movements has been experimentally limited to 16 degrees (target jump amplitude), the velocity profiles of the saccades considerably varied between the two animals examined, their peak velocities extending the permitted range of 200 to 800 deg/s (monkey #60: average  $422.7 \pm 106.2$  deg/s, range 200.6 to 797.3 deg/s;  $N=8170$  saccades; monkey #62: average  $423.3 \pm 117.1$  deg/s, range 200.1 to 797.0 deg/s;  $N=5883$  saccades). This variability, which is possibly mainly due to changes in the alertness level of a monkey during different recordings, enabled a comparison between saccade properties and burst parameters during individual trials.

To carry out such a comparison, we computed correlation coefficients for the relations between saccade peak velocities on the one hand and the various burst parameters derived from the computer-based burst detection algorithm on the other, and tested these coefficients statistically ( $\alpha=5\%$ , Bonferroni-corrected for multiple comparison). This screening procedure provided significant correlations between saccade and burst properties, during ipsilateral bursts for 36/70 units neurones (51%) and during

contralateral bursts for 17/56 units neurones (30%). In Fig. 7 an example of such a correlation is given. It shows burst and peak latency and burst-peak amplitude *versus* saccade peak velocity for the neurone of Fig. 5. This neurone exhibited clear correlations and also illustrates the effects typically observed with increasing saccade speed: the burst amplitude grows, and the burst starts and peaks at shorter latencies (Figs 7, 8). These effects are very similar for ipsilateral and contralateral saccades, although they are more pronounced for the former. Nearly all (34 of the 36 ipsilaterally and 16 of the 17 contralaterally) bursting neurones showed an analogous pattern, with the peak amplitude being positively and/or burst and/or peak latency being negatively correlated with saccade peak velocity. Of these correlations, that of the peak latency was most consistently observed (Fig. 9A).

It should be noted that the above evaluation is based on rigid statistical threshold values applied to data derived from single neurones. This approach is rather conservative and almost certainly leads to an underestimation of the significance of the effects. This is confirmed by a number of observations. In Fig. 9B, for all bursting neurones the correlation coefficients of the relation between peak velocity and the various burst parameters have been plotted *versus* their respective P-values. Clearly, the statistically significant correlation coefficients are all negative for burst and peak latency, and almost all positive for peak amplitude and spikes per burst. However, correlation coefficients that, by themselves, do not reach statistical threshold show the same asymmetrical picture. For ipsilaterally bursting neurones the leftward or rightward shifts of the distributions were, for these four parameters, highly significant ( $P < 0.001$ ), even when the units exhibiting statistically significant correlations were discarded. The same is true for contralateral bursts with respect to burst and peak latency, while the shifts are no longer significant for peak amplitude and spikes/burst after discarding the significant correlation coefficients. The mean correlation coefficients for burst duration are not significantly different from zero, for both ipsilateral and contralateral saccades (Fig. 9B-e)

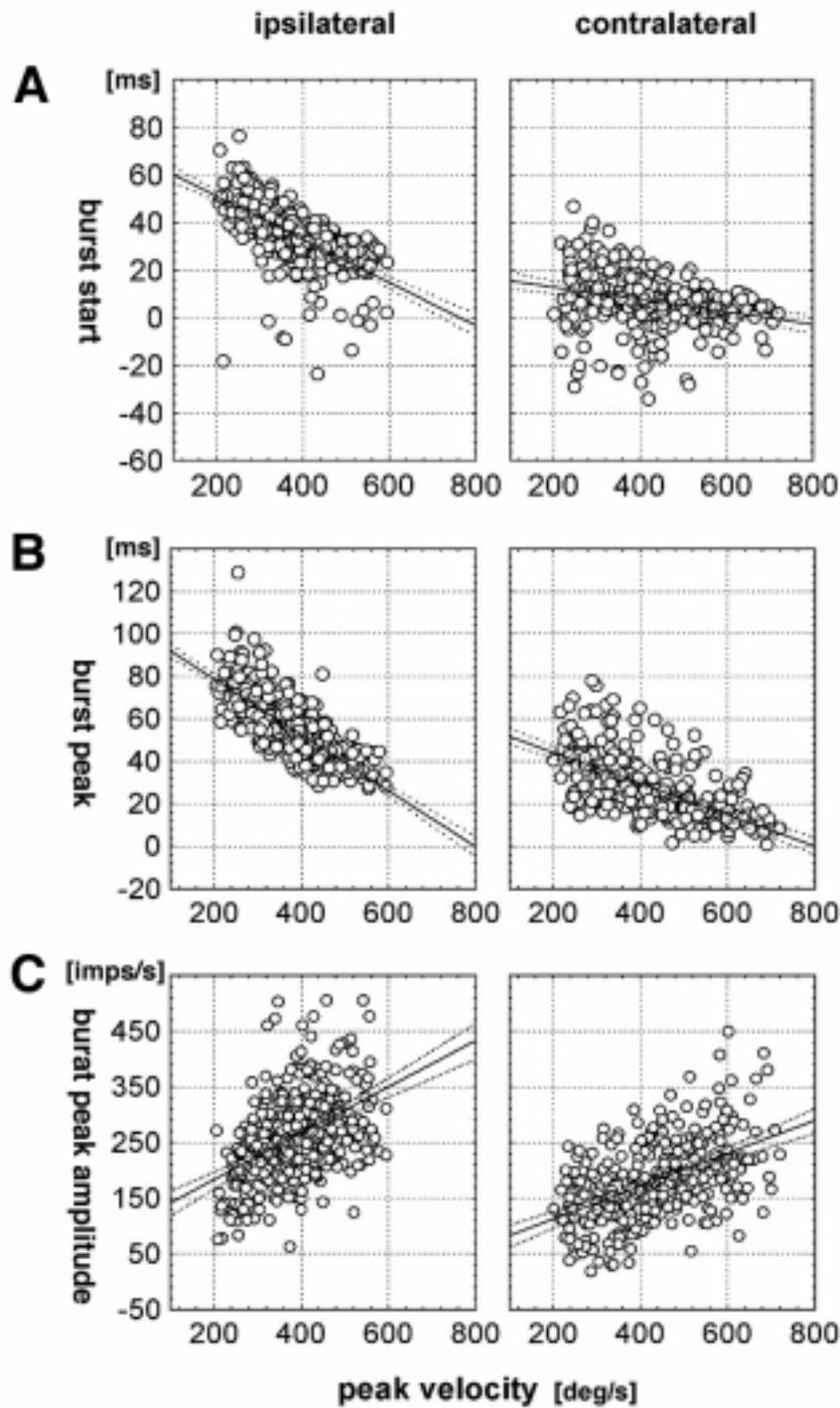
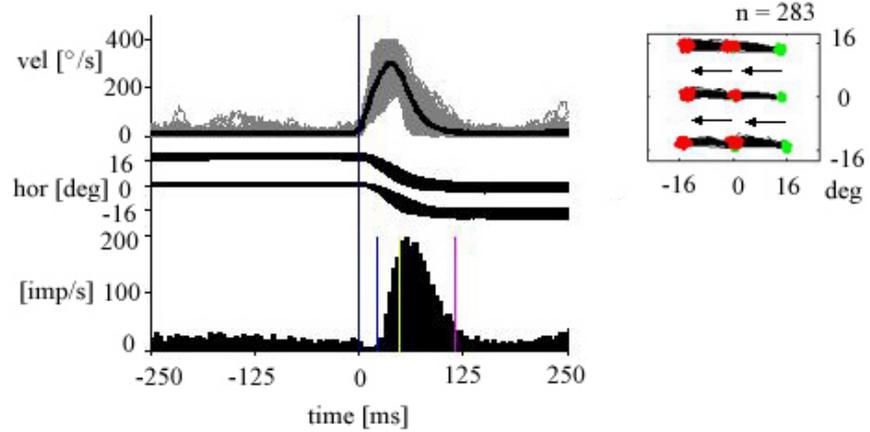


Fig. 7. Scatter plots showing clear and strong correlations between saccade peak velocity (in deg/s) and three burst parameters, for both ipsilateral (left panels) and contralateral (right panels) saccades of the neurone of Fig. 5. This neurone exhibited obvious and systematic differences for these parameters: with increasing saccade speed (in deg/s), bursts start (A, in ms) and peak (B, in ms) with shorter latencies, while burst-peak amplitude grows (C, in imp/s). Open dots indicate saccades. Solid lines are predicted linear regression lines. Dashed lines demonstrate the 95% confidence interval.

**A**

slow (200-400 deg/s)

**B**

fast (500-800 deg/s)

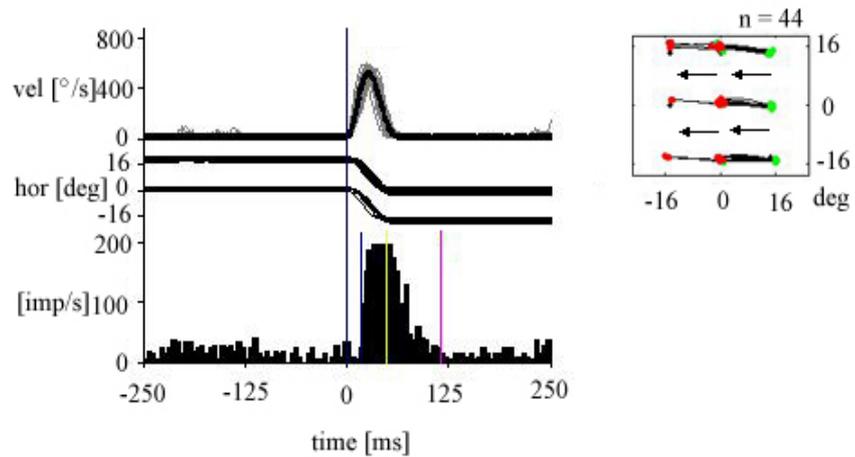


Fig. 8. Same neurone as presented in Fig. 5, illustrating the strong correlation between saccade velocity and the burst parameters during the ipsilateral saccades. A. Bursts with low saccade velocities (200 - 400 deg/s). B. Bursts with high velocities (500 - 800 deg/s). When saccade velocity increases, burst amplitude grows, the burst starts earlier (23.4 ms after the onset of slower saccades, 16.1 ms after the onset of faster saccades) and the burst peak appears with shorter latencies. For explanations of traces and vertical lines, see Figs 4, 5.

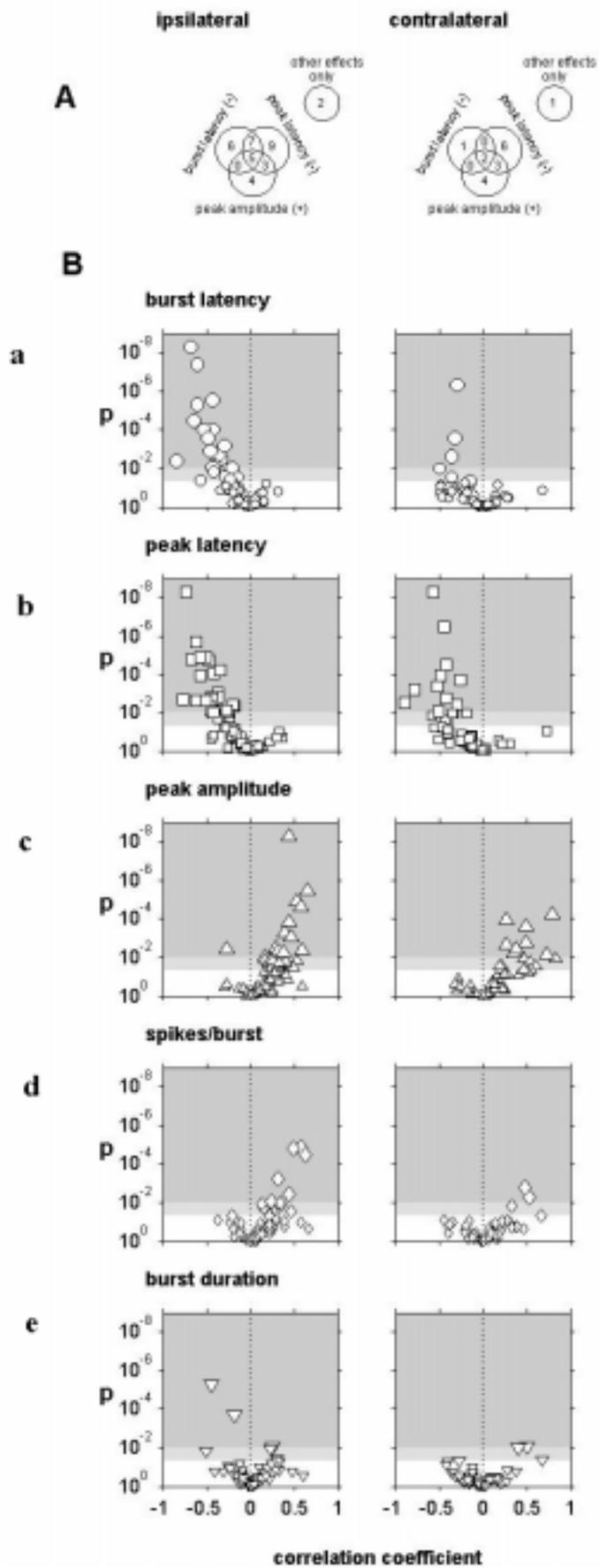


Fig. 9. A. Ipsilateral (34 neurones) and contralateral (16 neurones) saccade-related bursts of FOR neurones, showing analogous patterns of combinations of correlations between saccades, with the peak amplitude being positively and/or burst and/or peak latency being negatively correlated with saccade peak velocity. Numbers in overlapping parts of circles indicate numbers of neurones that reveal significant correlations between the respective parameters. B. Correlation coefficients of the relations between saccade peak velocity and the various burst parameters (a, burst latency; b, peak latency; c, peak amplitude; d, spikes/burst; e, burst duration) plotted *versus* their respective P-values. The statistically significant correlation coefficients are all negative for burst and peak latency, and almost all positive for peak amplitude and spikes/burst, but they are not significantly different from zero for burst duration.

## F. Population Analysis

Above, we showed results based on analyses of single cell discharges. In order to increase our understanding of how the entire FOR neurone population encodes saccade properties, we carried out a population analysis of the same data. The analysis shows that clear differences exist between bursts of ipsilateral and those of contralateral saccades. In monkey #62 (36 neurones) bursts in both directions started before the beginning of saccades (contralateral: -12.5 ms, ipsilateral: -7.5 ms). Subsequently, the burst activity patterns became markedly different. For contralateral saccades, maximal burst activity was observed already within 12.5 ms after saccade onset and then gradually decreased within 25.0 ms to baseline activity. For ipsilateral saccades, however, burst activity gradually increased and reached its maximum value 56.3 ms after saccade onset; baseline activity was reached 75.0 ms after saccade onset. These data clearly reflect the acceleration/deceleration profile of the saccades (Fig. 10); maximal bursting activity for contralateral saccades occurs aligned to the maximal acceleration and occurs for ipsilateral saccades aligned to the maximal deceleration. For monkey #60 (39 neurones) the activity patterns were largely similar.

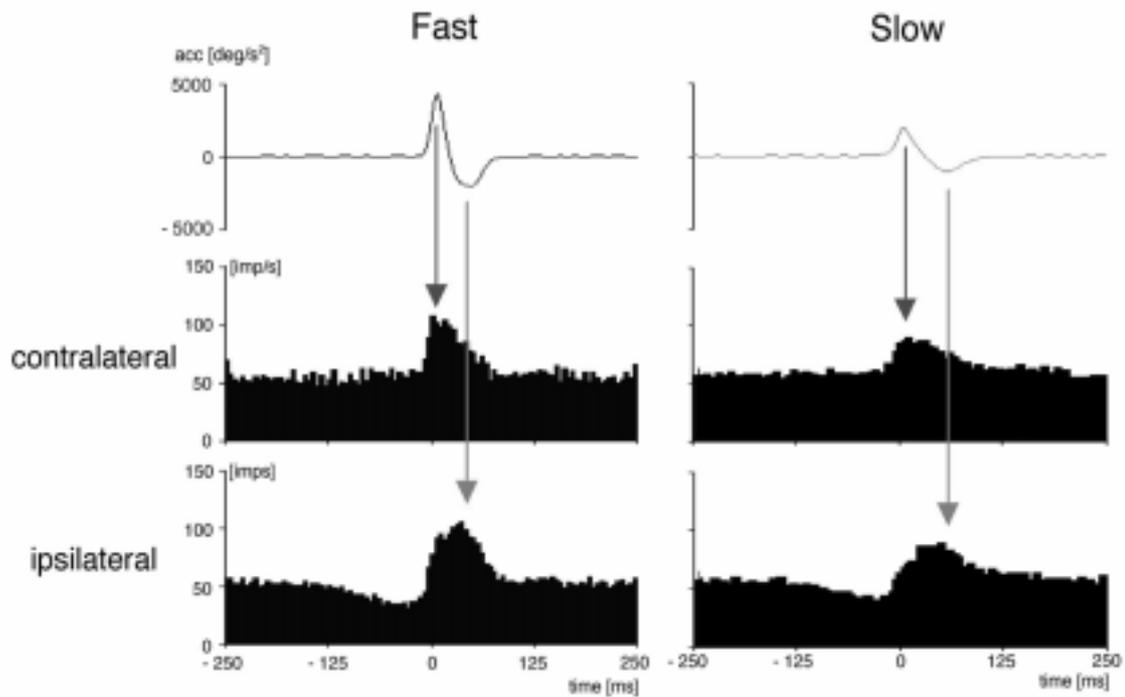


Fig. 10. Peak bursting activities of the FOR neurone population during contralateral and ipsilateral saccades are temporally correlated with the average deceleration and acceleration profile, respectively, of the average saccade (indicated by arrows). Left: average responses to fast saccades with peak velocities from 400-600 deg/s. Right: FOR activity during slow (200-400 deg/s) saccades of the same amplitude. (N=36 units from monkey # 62). For further explanation, see Figs 4, 5.

The above results hold for both slow-speed (200-400 deg/s) and high-speed (400-600 deg/s) saccadic eye movements. The peak of the FOR burst coincides with the end of the ipsilateral saccade deceleration phase, whereas it is linked to the start of the contralateral saccade acceleration phase. However, the comparison of high-speed saccades with slow-speed saccades of the same amplitude, reveals differences in burst profile. Notably, for both ipsilateral and contralateral saccades, during slow saccades peak burst activity is lower and occurs later. This corresponds with the changed acceleration profile: saccades last longer, and acceleration and deceleration values are smaller and occur later.

## **G. Influence of the Initial Eye Position on Saccade-related FOR Bursting**

### **G1. Bursting Activity**

Starting positions of the eye accompanying  $16^\circ$  saccades were presented at three vertical levels, viz. an upper, a middle and a lower one. A possible effect of the initial eye position on FOR bursting activity in relation to saccades was studied on the basis of observing corresponding perisaccadic spike histograms, and by statistical analysis (Kruskal-Wallis; non-parametric analysis of variance) of the burst parameter distributions derived from individual trial analyses at different starting points, sorted according to their horizontal and vertical components.

As judged from both analytical approaches, most neurones (about 80%) do not show any clear correlation between starting position and FOR neurone bursting. This holds for all levels, no matter whether saccades were sorted in terms of horizontal (centrifugal vs centripetal), vertical or both components of the initial eye position. Furthermore, when not single neurones but the entire neuronal population was considered, the spike histograms derived from averaging the responses of all individual neurones were virtually identical for all starting positions (Fig. 11). However, some indications for the existence of such a correlation were seen in the histograms, and this existence was confirmed by the statistical analyses.

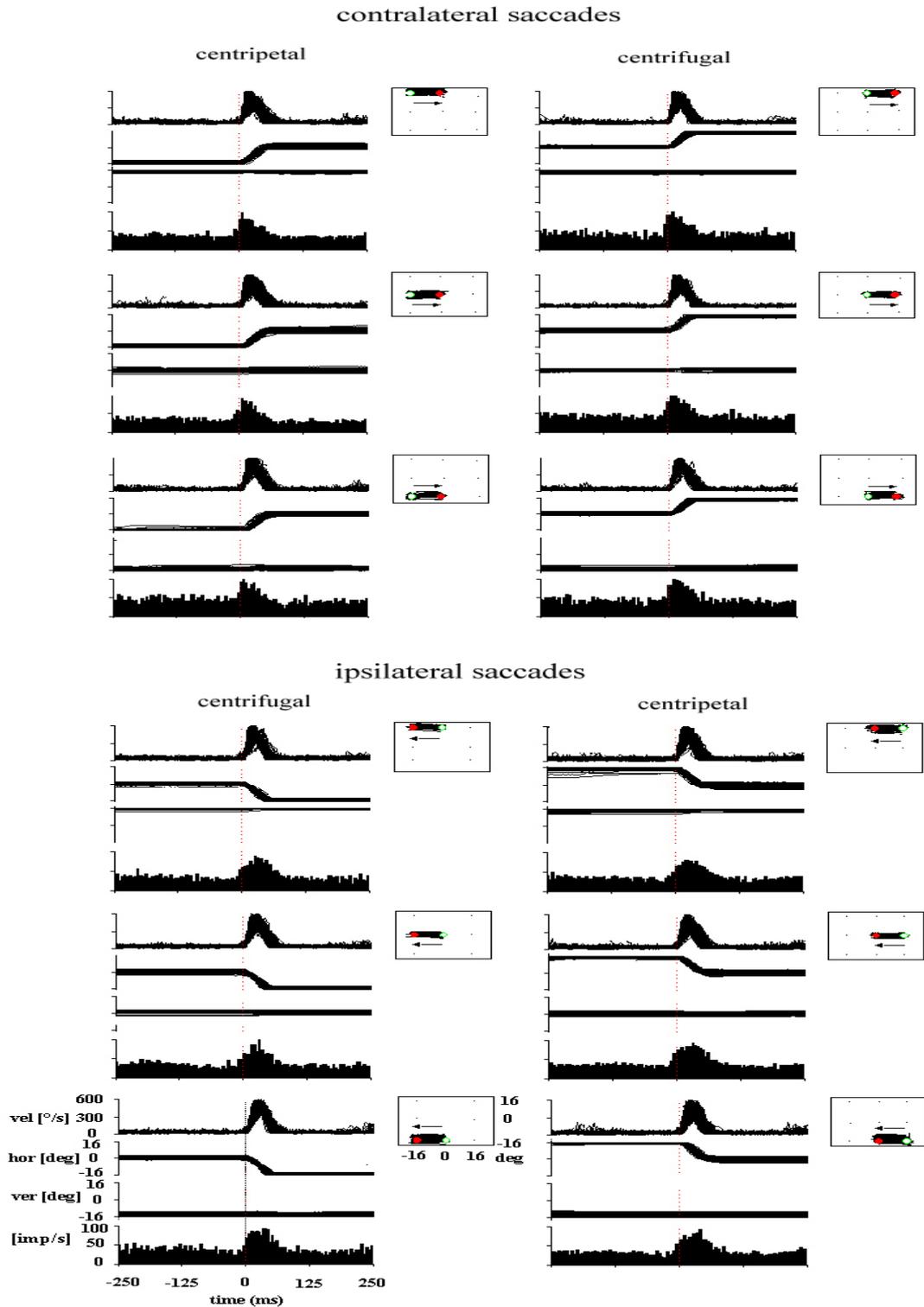


Fig. 11. ‘Population bursts’. Spike histograms derived from averaging the responses of all individual neurones of the FOR neurone population reveal virtually identical patterns for all (horizontal and vertical) starting positions (arrows indicate direction of target movement), so that no evidence appears for the existence of a significant influence of the initial eye position on bursting activity of the FOR population. The vertical dotted line indicates saccade onset. For further explanation, see Figs 4, 5. (N = 36 units from monkey # 62).

### **G1.1. Horizontal Components**

In this section experiments are described to demonstrate a possible causal relationship between eye position and saccade-related FOR bursting activity. Only in a minority of FOR neurones a correlation between eye position and bursting activity appeared, showing differences between centripetal and centrifugal saccades for various burst parameters. However, as will be explained below, these differences might be caused by systematic differences in saccade kinematics, and no evidence for a causal relationship between eye position and FOR bursting could be found.

On the basis of a threshold for statistical significance of  $\alpha=5\%$  (Bonferroni-corrected for multiple comparison), we observed that 10 neurones (8 for ipsilateral, 2 for contralateral saccades) out of 75 neurones revealed differences in burst discharges between centripetal and centrifugal saccades. This is illustrated in Fig. 5: for contralateral saccades, bursts start and peak earlier and show higher peak amplitudes for centripetal than for centrifugal saccades; also for ipsilateral saccades, the bursts start and peak earlier for centripetal saccades, but here the differences are very small and can only be seen when burst onset and burst peak are directly observed using a reference line (see Fig. 5, legend). The example neurone is fairly representative for all neurones observed, as 7/10 neurones (6 ipsilateral, 1 contralateral) show shorter burst or peak latencies or higher peak amplitudes for centripetal saccades than for centrifugal ones. However, it is atypical in that it exhibits these differences more pronounced for contralateral than for ipsilateral saccades.

Thus, in a minority of FOR neurones a correlation exists between eye position and bursting activity. This correlation appears from the fact that most centripetal saccades have shorter burst and peak latencies and higher peak amplitudes than centrifugal saccades. This pattern clearly resembles the asymmetries in the distributions of correlation coefficients of these parameters (Fig. 8B). This similarity is even more obvious in Fig. 12, in which for each ipsilateral and contralateral bursting neurone and for each of the various burst parameters the differences are plotted of the respective parameter means of centripetal and centrifugal saccades *vs* the P-values of the corresponding analysis of variance that has been performed on the parameter distributions.

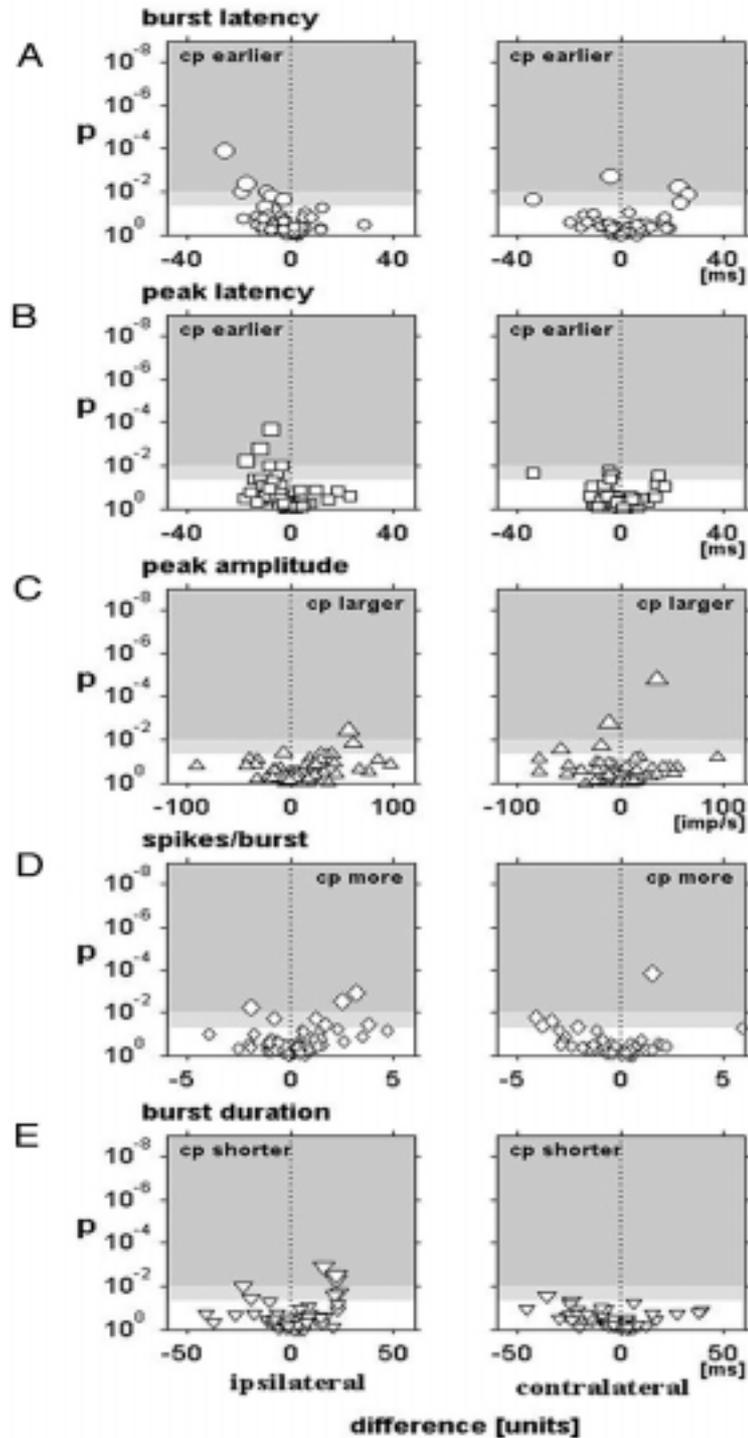


Fig. 12. Asymmetric plots showing the differences between centripetal (cp) and centrifugal (cf) saccades for various burst parameters (means) *versus* the P-values of the corresponding parameters as derived from the analysis of variance. A, burst latency, in milliseconds (ms); B, peak latency, in ms; C, peak amplitude, in impulses/second (imp/s); D, spikes/burst, in number; E, burst duration, in ms. Mainly for ipsilateral saccades, it can be seen that burst (A) and burst peak (B) start earlier for centripetal than centrifugal saccades, and peak amplitude (C) is larger for centripetal saccades, although in general the P-values are low.

In both monkeys, centripetal saccades are clearly faster than centrifugal ones (monkey # 60:  $462.1 \pm 92.4$  deg/s for centripetal,  $430.2 \pm 85.0$  deg/s for centrifugal,  $P < 10^{-5}$  (t-test); monkey # 62:  $463.7 \pm 102.3$  deg/s for centripetal,  $439.1 \pm 95.2$  deg/s for centrifugal,  $P < 10^{-5}$  (t-test; Fig. 13). This indicates that systematic differences in the saccade kinematics are the cause of these differences in burst discharges of centripetal *versus* centrifugal saccades. Such a relationship might mask a causal influence of eye position on FOR bursting activity. To unveil such a possible effect of eye position, we eliminated the influence of saccade speed, in two ways: firstly, we recalculated for all bursting units the Kruskal-Wallis-ANOVA and restricted saccade peak velocities to a mid-range of 400-600 deg/s. Secondly, a multivariate analysis of covariance was carried out on the various burst parameters, for centripetal and centrifugal saccades, in which saccade peak velocity was taken as covariate and compared to the results of the corresponding ANOVA (in which the covariate is not taken into account). With respect to the “typical” eye position effect, both approaches yielded similar results, viz. reducing the number of neurones that revealed a statistically significant difference between centripetal and centrifugal saccades to  $\leq 3$  and increasing the P-value of the respective statistics (“controlled” compared to “uncontrolled” condition) by at least one order of magnitude. Neither approach unveiled any other specific difference occurring with any consistency beyond chance level (5%) between bursts for centripetal and centrifugal saccades.

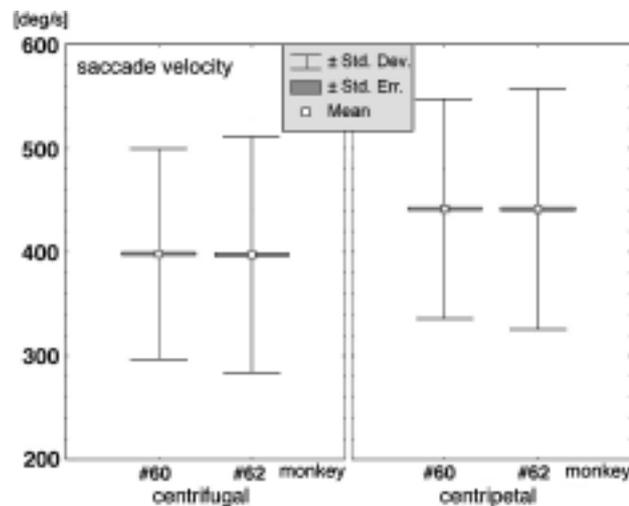


Fig. 13. Plots of centrifugal and centripetal saccade peak velocities (in degrees/sec), with means and standard deviations (Std. Dev.) and standard error of the means (Std. Err.), in monkey #60 (left) and #62 (right), showing that centripetal saccades are consistently faster than centrifugal ones.

In conclusion, the observed differences between centripetal and centrifugal saccades for various burst parameters do not appear to be caused by changes in the initial eye position.

### **G1.2. Vertical Components**

As to a possible influence of the vertical component of the eye position, we applied analogous statistical procedures as for the horizontal component. Given the selected statistical threshold ( $\alpha=5\%$ , Bonferroni-corrected for multiple comparisons), only 7 neurones demonstrated differences in their burst pattern at different vertical positions during saccades. Of these, 2 neurones showed an increased rate of peak activity and more spikes per saccade for lower vertical starting positions, for both contralateral and ipsilateral saccades. For ipsilateral saccades, three neurones exhibited a larger peak amplitude and more spikes/saccade at the middle or the lowest vertical starting position. As to the remaining 2 neurones, only during ipsilateral saccades, one showed a shorter burst latency and the other showed a longer burst duration, both at the upper starting position.

### **G2. Background Discharge**

FOR neurones reveal tonic background activity between saccades. To study a possible influence of eye position, the 9 different starting positions were analysed in a saccade-free time interval, 500 - 250 ms before saccade onset. Analysing the data by means of multiple linear regression, demonstrated statistically significant effects in 31 out of 75 neurones ( $P < 0.05$ ; t-test). The regression coefficients were symmetrically distributed, with a range of  $-0.7$  to  $1.0$  imp/s/deg (horizontal) and a range of  $-0.92$  to  $0.63$  imp/s/deg (vertical). Also, the combined horizontal and vertical regression coefficients were very evenly distributed and did not cluster into any direction, so that these neurones did not appear to form a distinct subgroup. This holds for the entire neuronal sample as well as for the subgroup of neurones that showed a statistically significant individual regression. The net effect of all neurones, calculated by multiple regression across all neurones and corrected for the different numbers of saccades obtained from each cell, was not statistically significant.

## **5. DISCUSSION**

### **A. Saccades in Monkeys**

This thesis research aims to elucidate the neural mechanisms that control saccadic eye movements. Saccades are fast, short-lasting eye movements that enable the animal to focus a moved target on the fovea. In this research we have used the Rhesus monkey, *Macaca mulatta*, as a test animal, because this primate species is relatively easy to handle and to maintain under laboratory conditions, has a strong constitution, and readily learns behavioural tasks. Furthermore, the eye movements of monkeys are very similar to humans (Becker, 1989).

### **B. Analysis Software**

Often, the analysis of individual burst characteristics of FOR neurones is being carried out manually, by the observer (see *e.g.* Ohtsuka *et al.*, 1991a, Fuchs *et al.*, 1993). Obviously, a suitable software programme would improve such analysis, by making it more accurate, more fast and more objective, *i.e.*, independent from the observer's decisions. Here we have applied a software programme that was based on principles described earlier (*e.g.* Poisson spike train analysis; Hanes *et al.*, 1995) and which was recently used by Thier *et al.* (2000), and modified by us (J.F. Kleine) to gain maximum, bias-free information about aspects of neuronal bursting that are of essential interest to this research, *viz.* about the possible relationships between saccade duration, frequency and latency, and saccade onset, offset, amplitude, duration and direction.

## **C. Scientific Questions being Answered**

Neuronal control of saccades is a very complicated process, in which many brain areas are involved, such as the brainstem, where the saccade generator is located, the cortical FEF, the SC, and the cerebellum, which fine-tunes saccade properties like duration and amplitude. Among the various cerebellar areas that are involved in this control, the FOR exerts a particular role as its bursting activity influences many aspects of the saccade, such as direction, amplitude, latency and duration. In this research we have studied these burst parameters, with as the main objective to resolve the mechanism by which FOR burst activity influences saccade properties. Experiments have been performed to answer the following questions:

1. Does the initial eye position have an effect on the burst for ipsilateral and contralateral saccades?
2. What is the difference between centripetal and centrifugal saccades?
3. Do FOR neurones influence acceleration and deceleration of a saccade, and if so, in which way?

## **D. FOR Bursting Differs between Ipsilateral and Contralateral Saccades**

We show that FOR bursts that occur during either ipsilateral or contralateral saccades, clearly differ in many aspects. Although burst latencies for ipsilateral as well as for contralateral saccades are similar for bilaterally and unilaterally bursting neurones, in general FOR neurones burst earlier to a contralateral saccade than to an ipsilateral saccade, and the burst offset time for contralateral saccades is shorter than for ipsilateral ones. As to latency, ipsilateral and contralateral saccades do not differ, but with regard to peak latency, peak activity of a burst is reached earlier after burst onset for contralateral saccades than for ipsilateral saccades.

The question arises why ipsilateral and contralateral saccades are so markedly different in their relationship to FOR neurone bursting. The obvious reason for this difference is explained in the BACKGROUND section. In short, in case of a contralateral saccade, an early FOR burst will accelerate the saccade, without which the saccade would fall short. Later in the saccade, ipsilateral FOR neurones provide an opposite drive that

slows down the saccade. In the absence of this late burst, the saccade would overshoot its target.

## **E. Initial Eye Position Plays no Major Role in the Control of FOR Neurone Activity**

### **E1. Various Brain Centres are Influenced by the Initial Eye Position**

The initial eye position has clear effects on the electrical behaviour of various neuronal centres in the Rhesus monkey. For instance, a majority of the neurones in the lateral intraparietal area (LIP) and in area 7a in the posterior parietal cortex reveal significant influences from the eye position (Andersen, 1990). Furthermore, in 61% of middle temporal area (MT) neurones and in 82% of superior temporal sulcus neurones visual stimulus-induced responses are modulated by the orbital eye position (Bremmer, 1997). For the vast majority of neurones in both the ventral premotor cortex (90%) and the prearcuate cortex (94%), the response to a visual stimulus greatly varies with the gaze angle. These results resemble those found in the posterior parietal cortex, where retinal image location and eye position both affect responsiveness to visual stimuli (Boussaoud, 1993). The responsiveness to visual stimulation of about 50% of the neurones in the prestriate area V3 appears to be influenced by the direction of gaze (Galletti & Battaglini, 1989). Nearly 40% of the neurones in area V6A of the parieto-occipital sulcus are sensitive to eye position (Nakamura, 1999). Effects of different initial eye positions on saccades evoked by electrical stimulation of the SC were investigated in alert monkeys with their head restrained. Following stimulation at 240 out of 367 sites (65%) in the caudal SC, the saccade direction appeared to be influenced by the initial eye position (Azuma, 1996). Groh (1996) recorded from somatosensory neurones in the SC of awake monkeys. The responses of a majority of the cells (25/34; 74%) were significantly affected by eye position.

In the cerebellar vermis, about 20% of the P-cells are sensitive to eye position (Thier *et al.*, 2000). When the initial eye position is changed by experimentally stimulating the FOR just prior to the occurrence of a visually-directed saccade, a monkey

can not compensate anymore for the FOR-stimulated eye movement and the saccade misses its target. This result indicates that FOR output impulses do not act directly on saccade programming circuits where visual information is being converted into motor commands, but project downstream from these circuits, to modulate (fine-tune) these commands (Noda, 1991).

## E2. The FOR

Some years ago, Ohtsuka *et al.* (1991b) proposed that the FOR would function without receiving information about the initial eye position. Others have provided circumstantial evidence for an effect of eye position on saccade activity, as eye position was shown to influence at least a number of FOR neurones (Fuchs *et al.*, 1993). Here, we have studied the possible effects of changing the initial eye position in both horizontal and vertical directions on the main aspects of FOR neurone bursting activity, using our sophisticated analysis software and an extensive behavioural paradigm. Moreover, we have performed population analysis as well as analyses of single neurone bursts, in view of the great variability in bursting activity within the FOR neurone population. As the results show, for most of the neurones studied no statistically significant correlation between the initial eye position and the various aspects of FOR neurone bursting could be detected.

Nevertheless, both the histograms and the statistical analyses revealed that 15-20% of the neurones have a distinct pattern, namely that centripetal saccades mostly have shorter burst and peak latencies and higher peak amplitudes than centrifugal ones. The question arises whether this correlation is due to an effect of eye position on FOR bursting activity or is rather the consequence of the strong correlation between burst properties and saccade properties. The latter possibility is supported by our two approaches in which the factor ‘velocity’ was eliminated. No other specific differences between bursts of centrifugal and centripetal saccades showed up as a result of this elimination, indicating that no effect of eye position, masked by velocity, does exist.

We have analyzed a possible influence of the initial eye position, making both graphical displays and elaborate statistical analyses of FOR bursts. Decreases in saccade-related activity and pauses between saccades have not been subjected to rigorous quantitative evaluations. However, the correlations that appear from the statistical

analyses of burst parameters (which might have been easily overlooked by visual examination of the discharge pattern alone) are also clearly visible in the perisaccadic histograms and raster diagrams, and there is good general agreement between the investigator's impressions derived from the examination of these graphical displays and the results from the objective statistical analyses. Therefore, it is highly unlikely that we have missed additional relevant information that would point to an influence of eye position on saccade-related discharge patterns.

Summarizing, we have found weak but consistent and clearly significant differences in bursts for (ipsilateral) saccades from different horizontal starting positions, resulting in shorter burst and peak latencies, higher burst peak amplitude and larger numbers of spikes/burst for centripetal as compared to centrifugal saccades. However, these differences can not be attributed to an actual influence of eye position *per se*. Rather, they most likely reflect the strong correlation between burst properties and saccade kinematics, caused by the systematic differences in saccade velocities between centripetal and centrifugal saccades. There seems to be no evidence for any other consistent influence of the initial eye position on burst discharges, neither for horizontal nor for vertical position components.

## **F. FOR Bursting does not Differ between Centripetal and Centrifugal Saccades**

Previously, Fuchs *et al.* (1993) reported for the Rhesus monkey that centrifugal and centripetal saccades differ from each other after bilateral inactivation of the FOR, as centrifugal saccades are smaller than centripetal ones. Similar data were reported by Ritchie (1976; cerebellar and oculomotor vermis) and Vilis & Hore (1981). This effect of bilateral FOR inactivation can also be seen in human patients with infarcts in the posterior vermis (Vahedi *et al.*, 1995).

However, the present data, based on an elaborate paradigm involving nine different starting positions at three different vertical levels, show that the starting eye position does not influence bursting in most (96 %) of the FOR neurones, which holds for both centrifugal and centripetal saccades. No differences between the two directions of

eye movement could be found, which is in accordance with results obtained by Ohtsuka *et al.* (1994).

## **G. The relation between FOR bursting and Saccade Properties**

There is no consensus in the literature as to whether and to what degree FOR burst dynamics control saccade characteristics. Ohtsuka & Noda (1991a) supposed that FOR activity especially determines the temporal aspects (start, duration, end) of a saccade, an opinion shared by Thiers *et al.* (2000), but Fuchs *et al.* (1993) concluded that FOR neuronal bursting is only weakly related to saccade metrics. Our data clearly show that activity of FOR neurones correlates with the kinematic properties of saccades, as many neurones reveal statistically significant correlations between bursts and saccade properties and, moreover, the FOR population as a whole shows an obviously consistent pattern of correlations: among saccades that have the same amplitude, the faster ones correlate with saccades that start and peak earlier, have a higher spike frequency and show higher peak burst rates than slower ones. These observations strongly suggest that the FOR neurones accelerate contralateral and decelerate ipsilateral saccades by producing, respectively, early and late bursts (see also Fuchs *et al.*, 1993; Robinson *et al.*, 1993), the more so as they reflect in their shorter burst onsets and peak latencies the faster acceleration-deceleration sequence of fast saccades and in their higher firing frequencies the increased acceleration drive and braking force regulating such saccades.

Therefore, we assume that FOR bursting activity is causally related to saccade velocities, pointing to a role of the FOR not only in the control of temporal but also of kinematic aspects of saccades.

## **H. Acceleration and Deceleration Phenomena Depend on FOR Bursting**

Acceleration and deceleration phenomena are main aspects of a saccade. As outlined in the BACKGROUND, experimentally induced hypometria of ipsilateral and contralateral saccades suggests that the FOR helps to accelerate contralateral and to decelerate

ipsilateral saccades. Our data show for the first time that there is a close relationship between burst peak frequency and acceleration/deceleration phenomena. These data are derived from saccades that have the same amplitude but are grouped on the basis of high-speed and slow-speed saccade samples. More in particular, the data show that for ipsilateral saccades, the peak of the FOR burst coincides with the start of the deceleration phase, whereas for contralateral saccades the FOR burst peak is linked to the start of the acceleration phase. Consequently, we can definitely conclude that acceleration and deceleration phenomena are controlled by FOR neuronal bursting and, more specifically, that they correlate with the peak of the burst discharge, in which acceleration and deceleration are determined by whether the saccade is either contralateral or ipsilateral, respectively.

## **I. Combined Activities of FOR Neurones Adequately Specify Saccade Properties**

As stated in the BACKGROUND, two models theoretically explain how the variable activity of FOR neurones diminishes the variability in saccade properties. The first model implies that the FOR is able to fine-tune the accuracy of the saccadic burst generator in the brainstem because it receives afferent feedback information about the saccade's properties (Robinson, 1995; Lefèvre *et al.*, 1998). The present data, however, favour the second model, which states that in spite of the variable output of individual FOR neurones, their combined activity adequately specifies the properties of a saccade in order to make it accurate. This can be explained as follows. Neurones that reveal a possible influence from the initial eye position do not belong to a homogeneous population, as 14% of them demonstrate clear and varying differences in peak discharge rate during ipsilateral saccades. Nevertheless, when not single neurones but the entire neuronal population is sampled, the resulting average spike pattern is virtually identical for all starting positions, which may well account for an accurate specification of saccade properties. This would mean that the FOR neurones control saccade accuracy in a similar way as the P-cells in the population of the oculomotor vermis, which accurately determine the end of a saccade (see Thier *et al.*, 2000).

## **J. Spike Histogram *versus* Individual Burst Analysis**

We have compared the burst parameters obtained from the perisaccadic spike histograms with the average of the corresponding values derived from the burst detections in individual trials, to validate the results obtained by the computerized burst detection routine. Each of these different analytical approaches yields values that are strongly correlated, but also systematic differences occur: in the individual burst analysis, the burst onset is assigned to later time values and the peak activity is clearly higher than in the spike histograms. The appearance of these systematic deviations may not be surprising, as the definitions and the statistical criteria applied are not identical. In fact, the observed differences can be readily explained from the different characteristics of the methods. Within a noisy signal, as represented by a single spike train, a significant alteration of discharge frequency is more difficult to detect than within a less noisy spike histogram. Using a conservative statistical threshold, a significant change in discharge rate will therefore be detected later in single trials than in a spike histogram analysis, as was observed.

The calculated values for the peak burst rates heavily depend on the amount of smoothing implicit in this particular method, and are largely determined by the bin width of the spike histogram and by the number of consecutive intervals averaged for peak rate calculation in single spike trains. In our case, this latter setting has to compromise between noise reduction and preservation of information, in particular with respect to the temporal location of the burst peak in individual trials. The resultant smoothing effect is therefore lower than in the spike histograms, thus explaining the generally higher numerical values assigned to the peak rate in single trial analysis. Therefore, the observed systematic differences do not disqualify the one or the other analytical approach. The robust correlation between them rather indicates that both methods provide substantial quantitative information about the neuronal discharge properties.

## **K. Perspectives**

Although FOR neurones, as we show, do not receive important information about the initial eye position, they do receive a large amount of neural information from various brain centres (see BACKGROUND). As we have also demonstrated, although the FOR neurone population acts as one unit in saccade control, individual FOR neurones reveal a high variability in their electrical activities. This variability may be due either to differences in their intrinsic properties or to the differential neural inputs they receive. Neuroanatomical and pharmacological studies, especially at the cell physiological, immunocytochemical and ultrastructural level, might help to resolve this issue.

Pharmacology coupled to (electron microscopic) immunocytochemistry may also show whether FOR neurones have all the same or rather different neurotransmitter contents and, hence, act on different targets. The latter issue might be, moreover, studied by neuronal tract tracing, on the basis of the stereotaxic coordinates used in our present study and intracellular (using a multibarrel electrode) dye injection (*e.g.* DiI or horseradish peroxidase) into or nearby the electrically identified neuronal somata.

## **L. Conclusions**

In this thesis research we have identified main properties of a saccade, and have related these properties to FOR neurone bursting. It is shown that ipsilateral and contralateral saccades differ in many respects and that acceleration and deceleration phenomena are controlled by the peak of the FOR neurone burst. On the other hand, we have also demonstrated that the initial eye position does not play a major role in the control of the burst activity of FOR neurones, and centripetal and centrifugal saccades do not differ as to FOR bursting. Modification of the relative contributions of individual neurones to the total population response could provide a mechanism for adaptive control of saccade metrics.

## 6. SUMMARY

A remarkable role in saccade control is played by the cerebellar caudal fastigial nucleus, FN (fastigial oculomotor region, FOR), which lies under the cerebellar vermis. FOR bursting activity is related to many saccade properties, such as direction, amplitude and duration. The precise mechanism by which FOR bursting influences a saccade is under debate. It has been assumed on the basis of microelectrode recordings that saccade-related FOR neurones act without receiving information about the eye position. Similarly, discharge activity of P-cells in the oculomotor vermis does not reveal a prominent eye position-dependency. On the other hand, it is proposed that eye position supports saccadic control via at least some FOR neurones, as some authors found that a minority of FOR neurones do react to a change in horizontal eye position. Likewise, studies on saccade metrics in vermal oculomotor areas indicate that the effects of lesions or of electrical stimulations vary with the starting position of the eye. This suggests that these areas are involved in the neuronal compensation for non-linearities in orbital mechanics. In any case, it is generally agreed that there is a consistent difference in the timing of saccade-related bursts produced by FOR neurones, resulting in shorter latencies for contralateral than for ipsilateral saccades. This pattern of burst timing indicates that the bursts are associated with the beginning of contralateral saccades and with the end of ipsilateral ones.

We have investigated (1) whether or not the initial eye position has an effect on bursts for ipsilateral and contralateral saccades, (2) if there is a difference between centripetal and centrifugal saccades, and (3) if FOR neurones influence acceleration and deceleration of a saccade and, if so, in what way.

For this purpose, a 9-point horizontal starting position training paradigm was applied, using a 3x3 square grid spaced at 16° intervals. Based on observations on 75 saccade-related FOR neurones, our results permit the following conclusions.

(1) Ipsilateral saccades clearly differ from contralateral ones. Individual neurone analysis as well as population burst analysis based on averaging the data across neurones, show that neither the vertical nor the horizontal component of the initial eye position substantially influences saccade-related bursting of the FOR neurone population.

(2) Centripetal and centrifugal saccades do not differ as to FOR bursting.

(3) Although most FOR neurones do not receive information about eye position, they seem to play a crucial role in acceleration and/or deceleration of saccades.

Finally, as the discharge patterns of individual neurones are highly variable, with prominent differences in both latency and amplitude, we propose that modification of the relative contributions of individual FOR neurones to the total FOR neurone population response may provide a mechanism for the adaptive control of saccade metrics in the Rhesus monkey.

## 7. ZUSAMMENFASSUNG

In der Regelung einer Sakkade spielt der Nucleus fastigii (fastigial oculomotor region, FOR), der unter dem okulomotorischen Vermis im Kleinhirn liegt, eine bemerkenswerte Rolle. Das Entladungsmuster der FOR-Neurone beeinflusst mehrere Eigenschaften der Sakkade, wie z. B. Richtung, Amplitude und Dauer. Der präzise Mechanismus des Einflusses des FOR-Burstes auf die Sakkade ist bisher nicht genau bekannt. Basierend auf Mikroelektrodenregistrierungen hat man angenommen, dass sakkadenbezogene FOR-Neurone aktiv sind, ohne über die initiale Augenposition informiert zu sein. Auch geben die Entladungsrate der Purkinje-Zellen im okulomotorischen Vermis keinen Hinweis auf einen deutlichen Einfluss der Augenposition. Andererseits hat man gefunden, dass eine gewisse Anzahl von FOR-Neuronen doch auf eine Änderung der horizontalen Augenposition reagiert. Auch Untersuchungen über die Sakkadenmetrik im okulomotorischen Vermis weisen darauf hin, dass der Einfluss elektrischer Stimulation sich in Abhängigkeit von der initialen Augenposition ändert. Dies könnte darauf hinweisen, dass diese Gebiete eine enge Beziehung zum neuronalen Ausgleich der nonlinearen orbitalen Mechanismen haben. Allgemein akzeptiert ist, dass es einen deutlichen Unterschied des sakkadenbezogenen FOR-Burstes für contralaterale und ipsilaterale Sakkaden gibt. Die Analyse zeigt, dass der Burst mit dem contralateralen Sakkadenbeginn und mit dem ipsilateralen Sakkadenende korreliert.

Es wurde untersucht: (1) ob die Augenposition die neuronale Entladungsrate der FOR-Neurone ipsilaterale und contralaterale Sakkaden beeinflusst, (2) Ob es einen Unterschied des Burstes gibt zwischen zentripetale und zentrifugale Sakkaden, und (3) Wie FOR-Neurone die Akzeleration und Deceleration der Sakkaden beeinflussen.

Zu diesem Zweck wurde trainierten Affen ein Lichtpunkt mit 9 Positionen auf einem 3x3 Gitter mit 16° horizontalen und vertikalen Intervallen dargeboten. Basierend auf der Analyse von 75 Neuronen ergaben sich die folgenden Ergebnisse:

1. Die Bursts für ipsilaterale und contralaterale Sakkaden zeigten klare Unterschiede. Jedoch hatte weder die horizontale noch die vertikale Augenposition einen Einfluss auf die neuronale Entladungsrate der FOR-Neurone.
2. Es fanden sich keine Unterschiede zwischen zentripetalen und zentrifugalen Sakkaden in Bezug auf die FOR-Entladungsraten.

3. Besonders die über mehrere Neurone gemittelten Daten (Populationsanalyse) zeigten einen klaren Bezug der neuronalen Entladungsrate zur Akzeleration und Deceleration der Sakkaden.

Es wurde beobachtet, dass die Burstentladungen einzelner Neurone des FOR eine grosse Variabilität und keine klaren Beziehungen zur Sakkadenstruktur zeigten. Erst die über mehrere Neurone gemittelten Daten (Populationsanalyse) zeigte einen klaren Bezug zwischen Beschleunigung und Verlangsamung der Sakkaden. Dieses Ergebnis ist neu und bestätigt den Bericht anderer Autoren, die früher berichteten, dass im Unterschied zu individuellen Purkinjezellen, die Gesamtpopulation dieser Zellen einen präzisen Zusammenhang von neuronaler Aktivität und dem Beginn und Ende einer Sakkade zeigt (Thier et al., 2000)

## 8. ABBREVIATIONS

BP	burst-pause neurone
cMRF	central mesencephalic reticular formation
DAO	dorsal accessory olive
DLPN	dorsolateral pontine nuclei
DMPN	dorsomedial pontine nucleus
DMRF	medullary reticular formation
EBNs	excitatory immediate premotor neurones
FEF	cortical frontal eye fields
FOR	fastigial oculomotor region
HRP	horseradish peroxidase
IO	inferior olivary complex
IVN	inferior vestibular nucleus
LBNs	long-lead burst neurones
LVN	lateral vestibular nucleus
MAO	medial accessory olive
NRTP	nucleus reticularis tegmenti pontis
NSN	non-typical saccade-related neurone
OPNs	omnipause neurones
PAG	periaquaeductal greygray
PB	pause-burst neurone
PHN	perihypoglossal nucleus
PPRF	brainstem paramedian pontine reticular formation
REM	rapid eye movement
riMLF	rostral interstitial nucleus of the medial longitudinal fasciculus
SC	superior colliculus
SPEM	smooth pursuit eye movements
TMS	transcranial magnetic stimulation
TSN	typical saccade-related neurones
VC	vestibular complex
VOR	vestibulo-ocular reflex

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