Exp Brain Res (2003) 149:83–95
DOI 10.1007/s00221-002-1337-9

RESEARCH ARTICLE

D. F. Kutz · P. Fattori · M. Gamberini · R. Breveglieri · C. Galletti

Early- and late-responding cells to saccadic eye movements in the cortical area V6A of macaque monkey

Received: 1 June 2002 / Accepted: 29 October 2002 / Published online: 15 January 2003

Abstract The cortical area V6A, located in the dorsal part of the anterior bank of the parieto-occipital sulcus, contains retino- and craniocentric visual neurones together with neurones sensitive to gaze direction and/or saccadic eye movements, somatosensory stimulation and arm movements. The aim of this work was to study the dynamic characteristics of V6A saccade-related activity. Extracellular recordings were carried out in six macaque monkeys performing a visually guided saccade task with the head restrained. The task was performed in the dark, in both the dark and light, and sometimes in the light only. The discharge of certain neurones during saccades is due to their responsiveness to visual stimuli. We used a statistical method to distinguish responses due to visual stimulation from those responsible for saccadic control. Out of 597 V6A neurones tested, 66 (11%) showed responses correlated with saccades; 26 of 66 responded also to visual stimulation and 31 of 66 did not; the remaining 9 were not visually tested. We calculated the response latency to saccade onset and its inter-trial variance in 24 of 66 neurones. Saccade neurones could respond before, during or after the saccade. Neurones responding before saccade-onset or during saccades had much higher latency variance than neurones responding after saccades. The early-responding cells had a mean latency (±SD) of –64±62 ms, while the late-responding cells a mean latency of +89±20 ms. The responses to saccadic eye movements were directionally sensitive and varied with the amplitude of the saccade. Responses of late-responding cells disappeared in complete darkness. We suggest that the activity of early-responding cells represents the intended saccadic eye movement or the shift of attention towards another part of the visual space, whereas that of late-responding cells is a visual response due to retinal stimulation during saccades.

Keywords Superior parietal lobule · Parieto-occipital cortex · Gaze-shift · Response latency · Visual response

D. F. Kutz (✉) · P. Fattori · M. Gamberini · R. Breveglieri · C. Galletti
Dipartimento di Fisiologia Umana e Generale,
Università di Bologna, Piazza Porta San Donato 2,
40127 Bologna, Italy
e-mail: kutz@biocfarm.unibo.it
Tel.: +39-051-2091748
Fax: +39-051-251731

Introduction

Many primates explore their environment by redirecting their gaze to objects of interest using a pattern of saccades and periods of fixation. Neurones, which discharge in relation to saccades and fixation are present in many areas of the primate brain. Command structures for saccade generation are, for instance, the eye fields in the frontal cortex (Tehovnik et al. 2000), the superior colliculus (Sparks 1986), and the reticular formation in the brain stem (Moschovakis and Highstein 1994); association structures for saccadic movements are the cerebellum (Sparks and Barton 1993), the pulvinar (Robinson 1993), and the posterior parietal cortex (Colby and Duhamel 1996; Snyder et al. 2000a). It is well accepted that the first group of structures sends commands to execute saccades, and copies of these motor signals to the association structures in the form of efference copy or corollary discharge. The second group would use these motor signals for different purposes, such as sequence learning, saccade adaptation, attentional shifts or co-ordinate transformations in visuomotor tasks.

The posterior parietal cortex is often considered to be involved in processes of co-ordinate transformations and attentional shifts. In the caudal-most part of the superior parietal lobule lies area V6A (Galletti et al. 1999b), which contains a large number of neurones related to the oculomotor behaviour (Galletti et al. 1995, 1996). Most of these neurones show eye-position-related activity, and some of them saccade-related activity starting before or after the saccade onset (Galletti et al. 1991). Recent reports have confirmed the existence of postsaccadic activity in area V6A (Nakamura et al. 1999) and in parietal reach region (PRR) (Snyder et al. 2000b), an area

located in the caudal part of the exposed surface of superior parietal lobule, possibly including also the dorsal-most part of V6A; the presaccadic activity was rarely observed, and only in area PRR.

The aim of this work was to analyse the dynamic characteristics of saccade-related activity in V6A to examine the presence of both pre- and post-saccadic activity. Present data confirm the presence of two distinct groups of saccade-related activity in V6A, an early-responding group, active before and during the saccade, and a late-responding group, active after the saccade-onset.

Preliminary results were published in abstract form (Kutz et al. 2001).

Methods

Experiments were carried out in compliance with 'Principles of laboratory animal care' (National Institutes of Health, NIH publication No. 86-23, revision of 1985) and with National and European laws on care and use of laboratory animals and were approved by the Bioethical Committee of the University of Bologna.

Six monkeys (*Macaca fascicularis*; 3.1–7.1 kg) were used in our study. A detailed description of training, surgical and recording procedures, as well as of visual stimulation, anatomical reconstruction of recording sites and animal care are reported elsewhere (Galletti et al. 1995, 1999b). Briefly, the animals underwent a preliminary training for fixation with the head free to move. When well trained in the task, a head-holder and a recording chamber were implanted on the skull. Before surgery, the animals were sedated with ketamine hydrochloride (12 mg/kg i.m.). All surgical procedures were performed under aseptic conditions with the animals under sodium thiopental anaesthesia (15 mg/kg i.v initially, followed by 8 mg/kg per h). The animals received analgesics for 2 days and were given free access to water, fruit and chow for 2 weeks. After recovery from surgery, animals were re-trained for fixation with the head restrained, and finally they were trained for the visually guided saccade task.

During recording sessions, animals performed fixation and saccade tasks with the head restrained while single neurones from the cortex of the posterior pole of superior parietal lobule were extracellularly recorded using home-made glass-coated 'Elgiloy' microelectrodes (Suzuki and Azuma 1976). Animals sat in a primate chair facing a large (80° × 80°) tangent screen. They were trained to look at a fixation spot, rear-projected from an optical bench on to the screen, for 2–6 s without reacting to any other visual stimulus presented on the same screen during this period. Positions of the fixation spot and of visual stimulus were controlled independently using two pairs of mirror galvanometers. Eye positions were recorded using an infrared oculometer (Dr. Bouis, Karlsruhe, Germany; Bach et al. 1983). The sample rate for action potentials was 1 kHz and that for eye position 100 Hz.

A standard protocol was used for testing the visual responsiveness of cells recorded. Cells were first tested with simple visual stimuli as light/dark borders, light or dark spots and bars of different size, orientation, direction and speed of movement. Unresponsive neurones were further tested with more complex visual stimulation as light/dark corners and gratings of different orientation, spatial frequency, direction and speed of movement.

As the visual response of many V6A cells was strongly modulated by the direction of gaze (Galletti et al. 1995), the possibility existed that a cell was not responsive to visual stimulation because the animal was looking towards a non-preferred direction. For this reason, simple and complex visual stimulations were repeated while the animal gazed at different screen locations. In cases where we were not able to activate the cell with the battery of simple and complex visual stimuli described above, we classified that cell as non-visual.

At the end of recording sessions, electrode tracks and the approximate location of each recording site were reconstructed on parasagittal sections of the brain on the basis of marking lesions and several other cues (e.g. the co-ordinates of penetrations within the recording chamber, the kind of cortical areas passed through before reaching the anterior bank of the parieto-occipital sulcus, the location of boundaries between white and grey matter, and the distance of the recording site from the surface of the hemisphere). A detailed description of anatomical reconstruction of recording penetrations is given in Galletti et al. 1999b.

Visually guided saccade task

Monkeys depressed a lever when a 0.2° spot of green light appeared on the screen. They were required to follow the spot with the eyes in case it changed position, and to release the lever after the spot changed in colour from green to red (go-signal). Correct responses were rewarded with water. Animals fixated the spot for 0.75–1 s, then the spot was displaced to a different screen position at a velocity of about 1000°/s: due to the very high speed of motion, the spot seemed to suddenly disappear and re-appear in a new position on the screen. Monkeys followed the fixation spot by making a saccadic eye movement, and maintained fixation at the new position for 0.5–1.5 s; after this time, the spot returned to its initial position and monkeys followed it with a saccade in the opposite direction, maintaining fixation until the spot changed in colour.

In the standard sequence, the fixation point first appeared in the straight-ahead position, at the centre of the screen, in darkness. Then, it jumped to a peripheral position 20° far from the centre and came back to the straight-ahead position at the end of trial. The colour change of the fixation point could happen either when it was at the centre of the screen or in the peripheral position, according to a random sequence. We tried to obtain the best darkness, using very small fixation spot of light adjusted in brightness so as to be barely visible to the experimenter. A great number of trials were performed in order to have at least seven trials with the complete to-and-from sequence of saccades. After a block of trials testing one peripheral position, another block of trials was started to test a different position. Typically, four or eight peripheral positions were tested, 20° apart from the central position and radially arranged around it, at 90° or 45° intervals, respectively. The sequence of positions was randomly chosen.

In some non-standard sequences we varied the initial position of fixation point, the amplitude of fixation-point displacement, or the visual background (light instead of darkness).

Statistical analysis

The onset of saccade was defined as the first eye-position sample where the velocity of eye movement was greater than 300°/s. This allowed detection of the onset of eye movements in saccades as small as 5° (Fuchs 1967). The saccade onset was searched in a time window spanning from target-onset up to 600 ms after it.

This paper focuses on the analysis of the latency of neural response to the saccade onset. Given the high variability of neural latencies, and in order to achieve a reliable result in statistical terms, we followed the suggestions of Snedecor and Cochran (1989) and estimated the minimal sample size using the equation:

$$N \geq Z^2 \frac{\sigma^2}{L^2}$$

with N = number of repetitions, Z = standard normal deviate, σ = standard deviation of the system, and L = acceptance limit. As the phenomenon we studied was highly variable, a value of the ratio $\sigma^2/L^2 \approx 1$ was considered to be acceptable. We also decided to use a high value of Z (Z=2.326, α=1%), so the equation yields $N \geq 6.6$. In practical terms, we quantitatively analysed only those cells with at least seven repetitions of to-and-from saccades.

Change-point algorithm

In order to analyse the relationship between neural response and saccade onset, we first detected the onset and offset of the response in each single trial using a change-point algorithm (Commenges et al. 1986). The change-point algorithm is based on the hypothesis that the spike process is an Erlang process, that is a stochastic point process with independent intervals having gamma distributions of probability density functions. Abrupt changes are indicative of a specific intervention of the neurone in information processing, whereas progressive changes are considered as fluctuations in the overall state of the animal. According to this view, if the neurone is involved in the information processing there is an abrupt change in the spike discharge following the stimulus and, after a certain time of excited or inhibited activity, the neurone reverts to its previous state. The algorithm given by Commenges et al. (1986) allows us to estimate the beginning and end of an excitation or inhibition in the neural activity, as follows.

Given:

$$ISI = \text{array of inter} - \text{spike intervals of length } n$$

$$r_1, r_2 = \text{indexes of ISI with} \quad 1 \le r_1 < r_2 \le n$$

the estimated indices of change are the values of r_1 and r_2, which maximise likelihood-function $L(r_1, r_2)$ as follows:

$$L(r_1, r_2) = \frac{1}{(\bar{x}_0)^{|R_0|}} * \frac{1}{(\bar{x}_1)^{|R_1|}}, \quad \text{with}$$

$$\bar{x}_0 = \text{mean of interval} \quad [ISI(1 \to r_1), ISI(r_2 + 1 \to n)],$$

$$\bar{x}_1 = \text{mean of interval} \quad ISI(r_1 + 1 \to r_2),$$

$$|R_0| = n - r_2 + r_1,$$

$$|R_1| = r_2 - r_1.$$

For a more precise estimation in each trial, the following constraints are added:

- We can specify whether we are looking for an excitation or an inhibition. In the case of excitation we must have $\bar{x}_1 < \bar{x}_0$.
- The change of interest must occur after the stimulus, thus $t > 0$. It is easy to maximise $L(r_1, r_2)$ with these constraints.

If no maximum satisfies the constraints, the trial is eliminated (Commenges et al. 1986).

Note that in case of a transient-tonic response, the algorithm detects the transient part of the response discarding the tonic one. A transient-tonic response was typically evoked in a cell the activity of which depended on both saccadic eye movement and direction of gaze, the transient part being related to the saccade and the tonic one to the gaze. As in this work we study saccade-related activity, we will refer in the following only to the transient part of the response detected by the algorithm.

We used the value of response-onset given by the change-point algorithm to define the intervals target-onset/response-onset and response-onset/saccade-onset. Different directions and/or amplitudes were tested for the same cell. We considered acceptable for the analysis only those neurones in which the change-point was detectable in blocks of at least seven trials for each direction or amplitude.

Kepner-Randles test

In order to determine whether the response was correlated to target-onset or saccade-onset, we analysed the variances of the duration of the intervals target-onset to response-onset and response-onset to saccade-onset, using the Kepner-Randles test (Kepner and Randles 1982, see also Commenges and Seal 1985). The Kepner-Randles test is a distribution-free test that uses the null hypothesis of bivariate symmetry to detect unequal marginal scales in bivariate populations. It is insensitive to unequal marginal locations, when this is due to data treatment. Because of its simplicity and power, this statistical test is highly effective. It is based on a Kendall's tau statistic: given two arrays X_1 and X_2, with X_{1i} and X_{2i} intervals target-onset to response-onset and response-onset to saccade-onset of the i-th trial, respectively, the algorithm is as follows (Commenges and Seal 1985).

Kendall's tau is given by

$$\pi_n = \binom{n}{2}^{-1} \sum_{i<j} \Psi\left[\left(Y_{1j} - Y_{1i}\right)\left(Y_{2j} - Y_{2i}\right)\right] \quad with$$

$$Y_1 = X_1 - X_2,$$

$$Y_2 = X_1 + X_2,$$

$$\Psi(t) = 1, 0 \quad \text{as} \quad t >, \le 0$$

and can be transformed to a normal deviate by

$$Pn = \frac{\sqrt{n}(\pi_n - 0.5)}{\hat{\sigma}_n} \quad with$$

$$\hat{\sigma}_n^2 = \frac{4(n-2)}{n(n-1)} \sum_{i=1}^{n} \left\{h_{1n}(Y_i) - \bar{h}_{1n}\right\}^2 + \frac{1}{2(n-1)},$$

$$\bar{h}_{1n} = n^{-1} \sum_{i=1}^{n} h_{1n}(Y_i),$$

$$h_{1n}(Y_i) = (n-1)^{-1} \sum_{\substack{j=1 \\ j \ne i}}^{n} \psi\left\{\left(Y_{1j} - Y_{1i}\right)\left(Y_{2j} - Y_{2i}\right)\right\}$$

The neural response is correlated to target-onset when the test value of the Kepner-Randles statistic is $Pn \le -1.96$, and is correlated to saccade-onset when $Pn \ge 1.96$ ($\alpha = 5\%$, two-tailed test). We classified a neurone as being correlated to one of the two events when (1) the highest response in a certain experimental condition fulfilled the Kepner-Randles statistic, and (2) in no other condition with strong response was there found a contradictory Kepner-Randles value. Neurones with contradictory Kepner-Randles statistics were excluded from further analyses. In the experimental conditions in which the neurone was weakly or not at all modulated, the Kepner-Randles statistic was not considered. In cases where several conditions showed nearly the same response, we classified the preferred response of the cell as the one with the highest value in the Kepner-Randles test, i.e., that with the most significant correlation to one of the events, target-onset or saccade-onset. Among the 66 V6A cells modulated by saccadic eye movement, 28 fulfilled all these criteria.

Results

In this paper, we describe responses of V6A neurones correlated to visually guided saccades. A total of 597 V6A cells underwent the visually guided saccade test, and many of them were found to discharge in relation to saccadic eye movements. As area V6A contains many visual neurones (61%, see Galletti et al. 1999b), the possibility exists that cellular discharges correlated to visually guided saccades were actually responses to visual stimulations self-evoked by the saccadic eye movement. To exclude this possibility, we classified a discharge occurring around saccade time as saccade-related activity only when the neurone was insensitive to visual stimulation (or to stimulations that mimic those specifically evoked by saccadic eye movement), or when the discharge started before saccade onset. According to

Table 1 Visual backgrounds used in the saccade test. Data are the number of neurones

Type of cell	Background		
	Dark	Dark and light	Light
Non-visual	20	8	3
Visual	16	3	7
Visual unknown	7		2
Total	43	11	12

Table 2 Response behaviour of saccade-related neurones. Data are the number of neurones

Type of cell	Type of neural response		
	Excitation	Inhibition	Inhibition and excitation
Non-visual	27	2	2
Visual	25	1	
Visual unknown	7	2	
Total	59	5	2

these rules, 66 of 597 neurones (11%) showed saccade-related activity.

Figure 1 shows the distribution, within area V6A, of the 597 cells studied during the visually guided saccade task, as well as the distribution of saccade-related cells within this area. Despite some inhomogenities, saccade-related cells were present all over area V6A.

Most of the neurones with saccade-related activity were studied in the dark condition, some of them in dark and light, some others in ambient light only (see Table 1). Thirty-one of 66 saccade-related neurones were insensitive to visual stimulation, 26 were sensitive to visual stimulation, and 9 were not visually tested. As reported in Table 2, most neurones with saccade-related activity responded with excitation to saccade execution, some with inhibition and a few with both excitation and inhibition.

Figure 2 shows different types of saccade-related activity observed in V6A. Cells in Fig. 2A, B show transient responses related to the saccade and weak or no activity during maintained fixation before and after the eye movement. The saccade-related activity starts with the eye movement in the cell of Fig. 2A and after the saccade in the cell of Fig. 2B. The neurone in Fig. 2C shows a more complex behaviour, with a prolonged transient component, largely variable in duration and in time-onset with respect to saccade-onset. The cell in Fig. 2D shows a clear biphasic (transient/tonic) response related to the saccade. The transient component of the response is a post-saccadic discharge similar to that shown in Fig. 2B; the tonic component, which continues without any appreciable decrease after the transient one, is a signal likely to be related to the position of the eye in the orbit. A similar, though less sharp, phenomenon can also be observed in the unit of Fig. 2C.

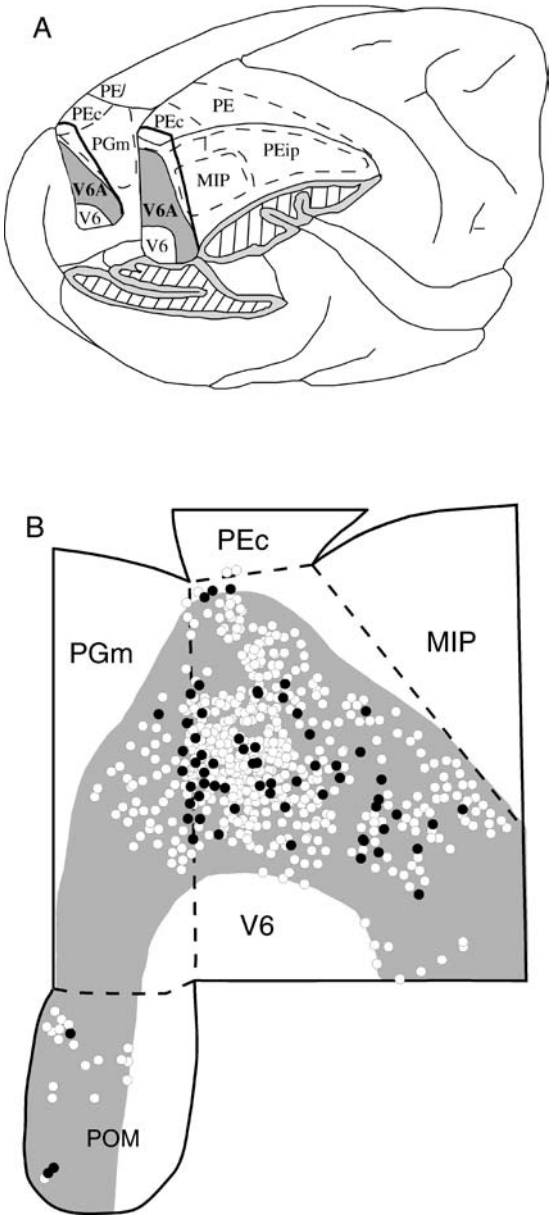

Fig. 1A,B Recording site. **A** Postero-lateral view of the macaque brain. The occipital pole and the inferior parietal lobule of the right hemisphere were partially cut away to show the cortical areas located in the medial bank of intra-parietal sulcus and the anterior bank of parieto-occipital sulcus. Location and extent of areas PE, PEc and PGm are according to Pandya and Seltzer 1982; area MIP is according to Colby et al. 1988; area PEip is according to Matelli et al. 1998; area V6 is according to Galletti et al. 1999a. Area V6A is shown as a *grey area* located in the anterior bank of parieto-occipital sulcus and the mesial surface of the hemisphere. **B** Two-dimensional map of the caudal part of the superior parietal lobule (the anterior limits are represented by *thick lines* in **A**) showing the overall recording site of the six cases we studied. Each circle represents the location on the map of a cell tested with the saccade task; *open circles* cells insensitive to saccade, *filled circles* saccade-related cells. *Dashed lines* in the map represent the lines of reflection of the cortex in the brain. Note that the region of the map indicated as *POM* is the dorsal bank of the medial parieto-occipital sulcus, which is located in the mesial surface of the hemisphere

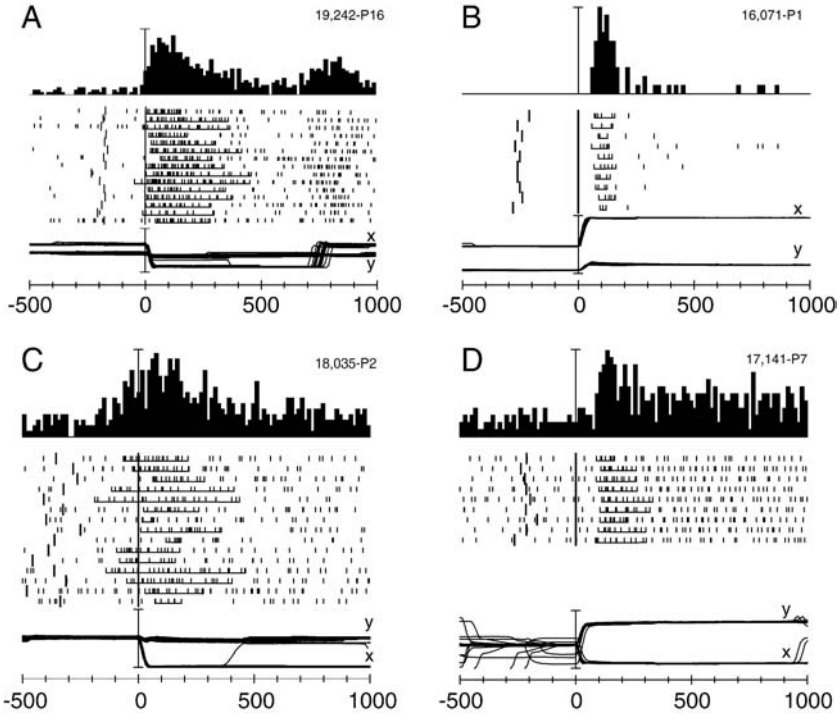

Fig. 2A–D Saccade-related responses in V6A. From top to bottom of each panel, peri-saccade time histogram, raster-plot, and eye-traces are aligned to saccade-onset (t=0 ms). *Small vertical ticks* in the raster-plots indicate the time occurrence of a spike; *longer vertical ticks* before saccade-onset indicate the appearance of the target on the screen. In eye traces, *x* and *y* indicate recording of horizontal and vertical eye displacements, respectively. *Horizontal lines* below the spike ticks in the raster-plots indicate the duration of the transient part of neural response to saccade calculated by the change-point algorithm (see Methods). **A** Saccade-related activity starting with the eye movement, and **B** after the saccade. **C** More complex behaviour, with a prolonged transient component, largely variable in duration and in time-onset with respect to saccade-onset. **D** Biphasic (transient/tonic) response related to the saccade. *Scale bars*: in eye-traces 60°; in histograms **A** 125 impulses/s, **B** 60 impulses/s, **C** 70 impulses/s, **D** 105 impulses/s

Although we discarded from the database cells for which discharges were clearly due to visual stimulations evoked by eye movements, we took into account the possibility that even the saccade-related activity of cells that we found insensitive to visual stimulation could be actually visual in nature. Under our experimental conditions, visual responses could be evoked by fixation-point displacements or by the visual self-stimulation evoked by saccadic eye movements. This latter phenomenon, well present in light, could be present also in a darkened room due to physical displacement of the fixation point on the screen, as well as displacement of the image of fixation point across the retina during the saccade.

In any case, in order to decide whether a neural discharge occurring around a saccadic eye movement was due to the oculomotor event per se, or to the visual stimulations evoked by the saccade, we used the Kepner-Randles test (Kepner and Randles 1982; see Methods) that checks for the impreciseness of neural discharge with respect to stimulus-onset (appearance of saccade target) and saccade-onset, respectively, and assigns the discharge to one of the two events best correlated with it. Figure 3 shows two examples of application of the Kepner-Randles test. Data from the same cell are aligned to the stimulus-onset in the *upper panels* and to the saccade-onset in the *lower panels*. The cell of Fig. 3A shows a response best correlated to the stimulus-onset ($Pn<-1.96$). The latency to stimulus-onset (displacement of the fixation point) was 146.2 ms with a neural impreciseness of 5.7 ms, whereas that to saccade-onset was –80.3 ms with a neural impreciseness of 26.3 ms. Thus, the response of this neurone was considered visual in nature, although in this cell no visual receptive field was found. The cell reported in Fig. 3B, on the contrary, showed a response best correlated to saccade-onset ($Pn>1.96$). The latencies were 185.4 ms to stimulus-onset (with an impreciseness of 67.2 ms) and –44.6 ms to saccade-onset (with an impreciseness of 26.9 ms). A visual receptive field was not found for this cell either.

Twenty-eight of 66 neurones fulfilled the statistical criteria of seven trials with successful saccade and change-point detection (see Methods). The analysis of correlation between response-onset and target-onset, and between response-onset and saccade-onset, showed that 24 of 28 tested neurones were significantly correlated to the saccade-onset (Kepner-Randles test, $Pn>1.96$). In two cells, the response was correlated to the target appearance and in two other neurones the response was not assignable to either of the two events. These four neurones were discarded from the database.

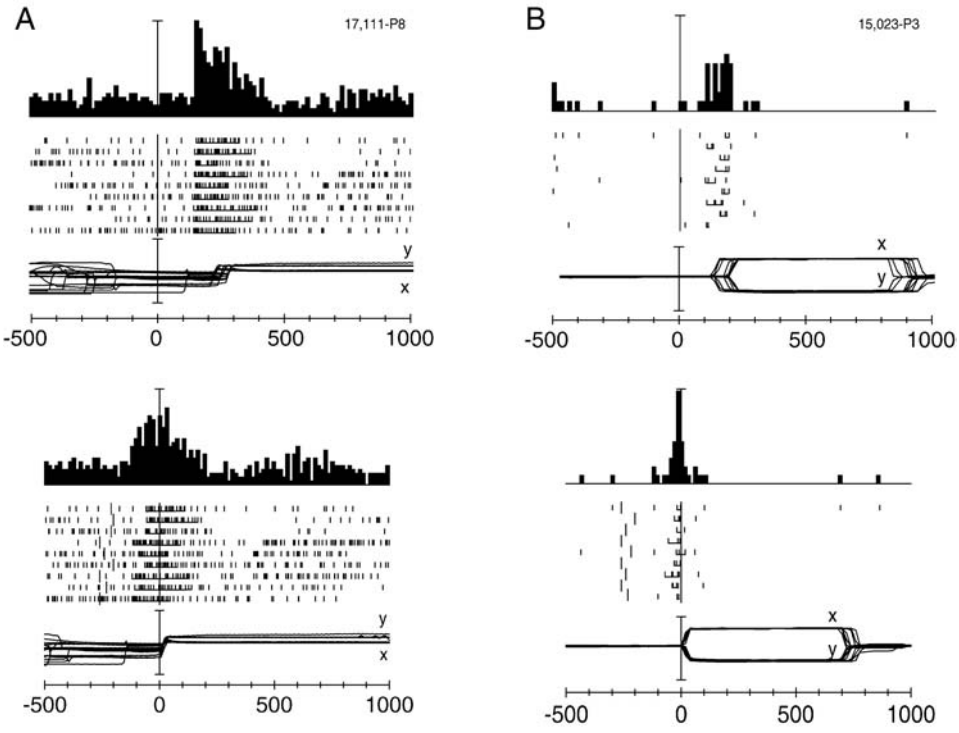

Fig. 3A,B Neural responses correlated to stimulus-onset or saccade-onset. **A** Neurone with response correlated to stimulus-onset, **B** Neurone with response correlated to saccade-onset. From top to bottom of each panel, peri-saccade time histogram, raster-plot, and eye-traces are aligned to stimulus-onset (*upper panels*, t=0 ms) or to saccade-onset (*lower panels*, t=0 ms). Other details as in Fig. 2. *Scale-bars*: in eye-traces 60°; in histograms **A** 190 impulses/s, **B** 75 impulses/s

Latency and variability of saccadic responses

The saccade-related neurones showed a mean latency ranging from 200 ms before saccade-onset to 150 ms after it, with a quite normal distribution (Fig. 4A). Some neurones showed a high variation of the response onset from trial to trial, whereas others responded precisely to the saccade. We considered the standard deviation of the inter-trial variance in response-onset as a measure of the impreciseness of neural response. An example of a neurone with a high inter-trial variance of the response is shown in Fig. 2C. In some trials the neurone started to respond about 200 ms before saccade-onset, whereas in others the response onset was delayed up to 100 ms after saccade-onset. The mean latency of this neurone was –40.5 ms, with a neural impreciseness of 92.6 ms. Figure 2B, conversely, shows an example of a neurone responding precisely to the saccade. This neurone had a mean latency of 76.2 ms with a neural impreciseness of 12.2.

Figure 4B shows the distribution of the neural impreciseness for all studied V6A saccade-related cells. This distribution shows a clear bimodal form, with one class in the range of 10–50 ms and a second one in a range of 60–110 ms.

Figure 5 shows the correlation between the two variables described in Fig. 4, that is the mean response latency and the impreciseness of saccade-related activity. As shown in Fig. 5A, cells with high inter-trial variance showed negative response latency (responses starting before the saccade-onset; *t*=0 ms). The response latency was much more constant (low neural impreciseness) in

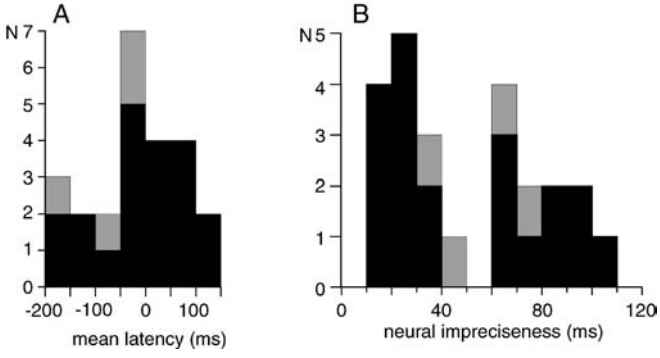

Fig. 4A,B Response latency and response impreciseness of saccade-related neurones. **A** Distribution of mean saccadic response latencies; bin width 50 ms; *x*-axis is mean latency to saccade-onset (t=0 ms); *y*-axis is cell count (N_{Σ}=24). **B** Distribution of response impreciseness; bin width 10 ms; *x*-axis is response impreciseness measured as standard deviation of inter-trial variance of response latency; *y*-axis is cell count (N_{Σ}=24). **A**, **B** *Dark bars* represent counts of non-visual neurones, *grey bars* counts of visual neurones or neurones with unknown visual characteristics

cells with positive response latency (response starting after the saccade onset).

Together with cells with saccade-related activity, in Fig. 5A we also reported the latency of visual responses in 15 V6A cells not belonging to the saccade-related group (see symbols + and × in the Fig. 5). The behaviour of two of these cells is shown in Fig. 6. Figure 6A shows the visual responses evoked by the appearance of an optimal visual stimulus within the receptive field; Fig. 6B shows the responses of another cell in which the receptive field was self-stimulated by the screen borders during saccadic

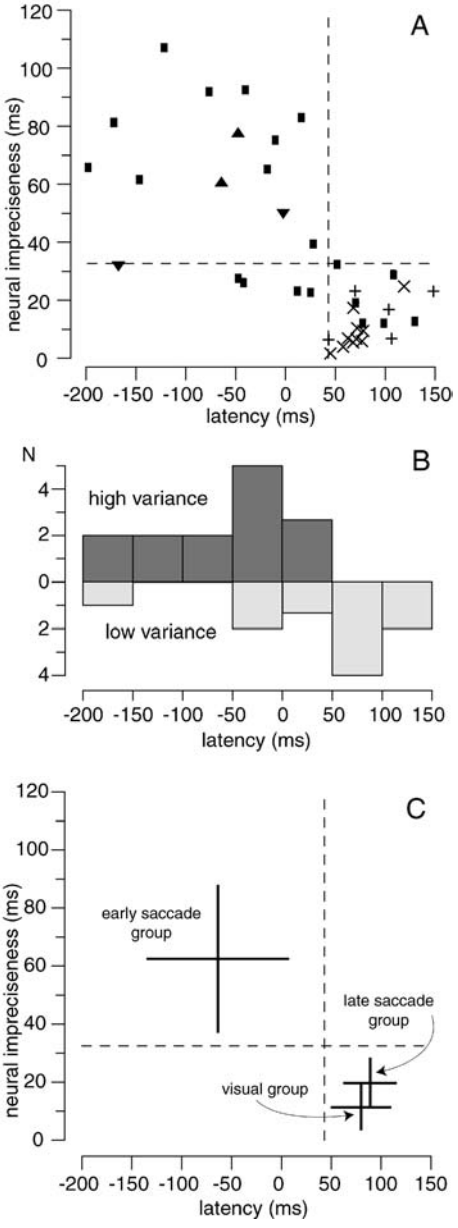

Fig. 5A–C Correlation between response latency and response impreciseness. **A** Scatter plot of response latency vs response impreciseness of saccadic (*filled symbols*) and visual (×, +) neurones; latency *t*=0 ms represents saccade-onset for saccadic neurones and stimulation onset for visual neurones. *Squares* saccade-related response of non-visual neurones (N_{Σ}=20), *inverted triangles* saccade-related response of visual neurones (N_{Σ}=2), *triangles* saccade-related response of neurones with unknown visual characteristics (N_{Σ}=2), × visual responses evoked by stationary stimulation of the receptive field with the optimal visual stimulus (N_{Σ}=10), + visual responses self-evoked by the sweep of screen-border images across the receptive field each time the animal made a saccade in light (N_{Σ}=5); *vertical dashed line* earliest visual latency (+43 ms), *horizontal dashed line* upper 99.5% confidence band of the variation of visual response (32.5 ms). **B** Latency distribution of saccadic responses with high (*upper histogram in dark grey*) and low (*lower histogram in light grey*) variance. Bin width 50 ms. **C** Mean response latency and response impreciseness of the early and late saccade groups of cells, and of visual cells

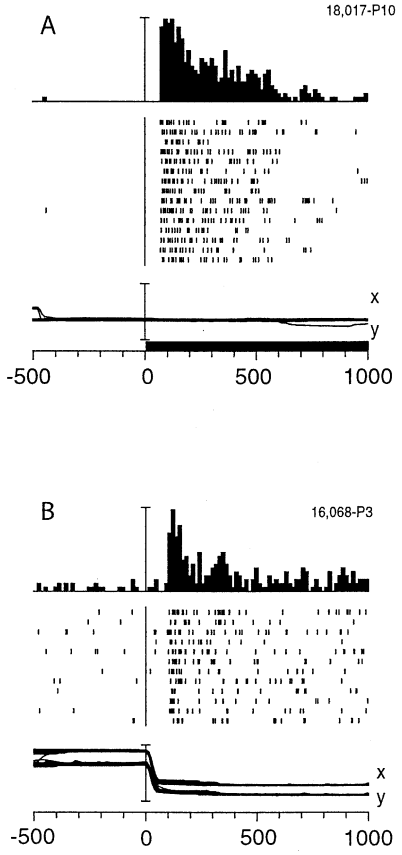

Fig. 6A,B Visual responses in area V6A. **A** Response to stationary stimulation. **B** Response evoked by auto-stimulation during saccade in light. From top to bottom of each panel, peri-saccade time histogram, raster-plot, and eye-traces of single neurone recordings aligned to stimulus-onset in **A** (t=0 ms) or saccade-onset in **B** (t=0 ms). Other details as in Fig. 2. In **A**, *thick horizontal line* above time axis indicates duration of visual stimulation. *Scale bars*: in eye-traces 60°; in histograms **A** 95 Imp/s, **B** 95 Imp/s

eye movements performed in light. The visual responses showed different mean latencies in the two cells (76.2 ms and 106.3 ms, respectively) but very low and similar neural impreciseness (5.9 ms and 6.8 ms, respectively), as expected for neural responses evoked by constant sensory stimulations. Figure 5A shows that the latency and variance of visual responses were very similar to those of saccadic responses in cells with post-saccadic activity.

Figure 5B shows the distribution of cells with high and low latency variance in the saccadic response, according to the mean latencies of responses. High and low variance groups were arbitrarily settled upon by the upper 99.5% confidence band of variance of visual responses (*horizontal dashed line* in Fig. 5A,C). In other words, we considered as high a variance greater than the highest shown by the visual responses. The high variance group responded before or during the saccade, with a peak at the interval −50 ms to 0 ms and a median at −47.6 ms. The low variance group was more distributed, with a peak at the interval 50–100 ms and a median at 51.7 ms.

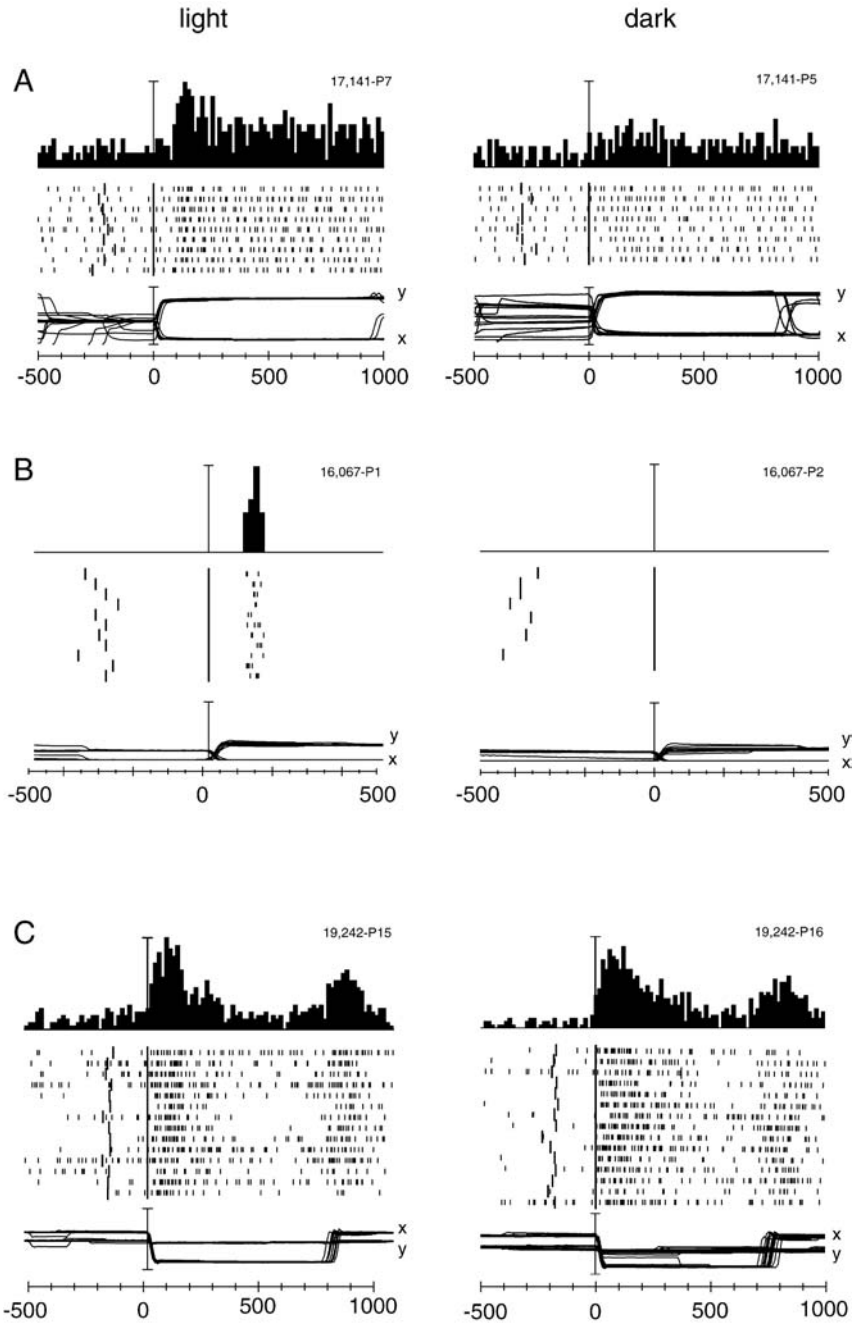

Fig. 7A–C Ambient light modulation of saccade-related activity in three V6A neurones. From top to bottom of each panel, peri-saccade time histogram, raster-plot, and eye-traces are aligned to saccade-onset (t=0 ms). Other details as in Fig. 2. Left panels (*light*) show neural activity under ambient light; right panels (*dark*) show neural activity under dark condition. *Scale bars*: in eye-traces 60°; in histograms **A** 80 impulses/s, **B** 105 impulses/s, **C** 125 impulses/s. Cell in **A** is the same as in Fig. 2D, cell in **B** is the same as in Fig. 2B, cell in C is the same as in Fig. 2A

Figure 5C summarises data on the correlation between response-latency and response-variance. The group of early-responding cells, containing all neurones with a mean latency shorter than the earliest visual activity in V6A (*vertical dashed line* in Fig. 5A,C), had a mean latency of –59.7±70.3 ms and a neural impreciseness of 60.4±25.8 ms. Late-responding saccade-related cells had a mean latency of 88.9±25.6 ms and a neural impreciseness of 19.7±8.3 ms; the responses of visual cells to visual stimulations, grouping together responses to passive and self-stimulations, showed a mean latency of 79.8±29.0 ms and a neural impreciseness of 11.3±7.5 ms.

Effect of ambient illumination

Eleven cells were studied under both dark and light conditions. Although only three of them were sensitive to visual stimulation (see Table 1), in 7 of 11 ambient illumination enhanced the saccade-related response. The phenomenon was very strong in the late-responding group of cells (Fig. 7A, B). The cell reported in Fig. 7A showed a poor response to saccade in the dark and a good response in the light. The cell reported in Fig. 7B was completely silent during and after saccades performed in darkness, whereas it was briskly activated by rapid eye movements performed in the light. Note that for both cells

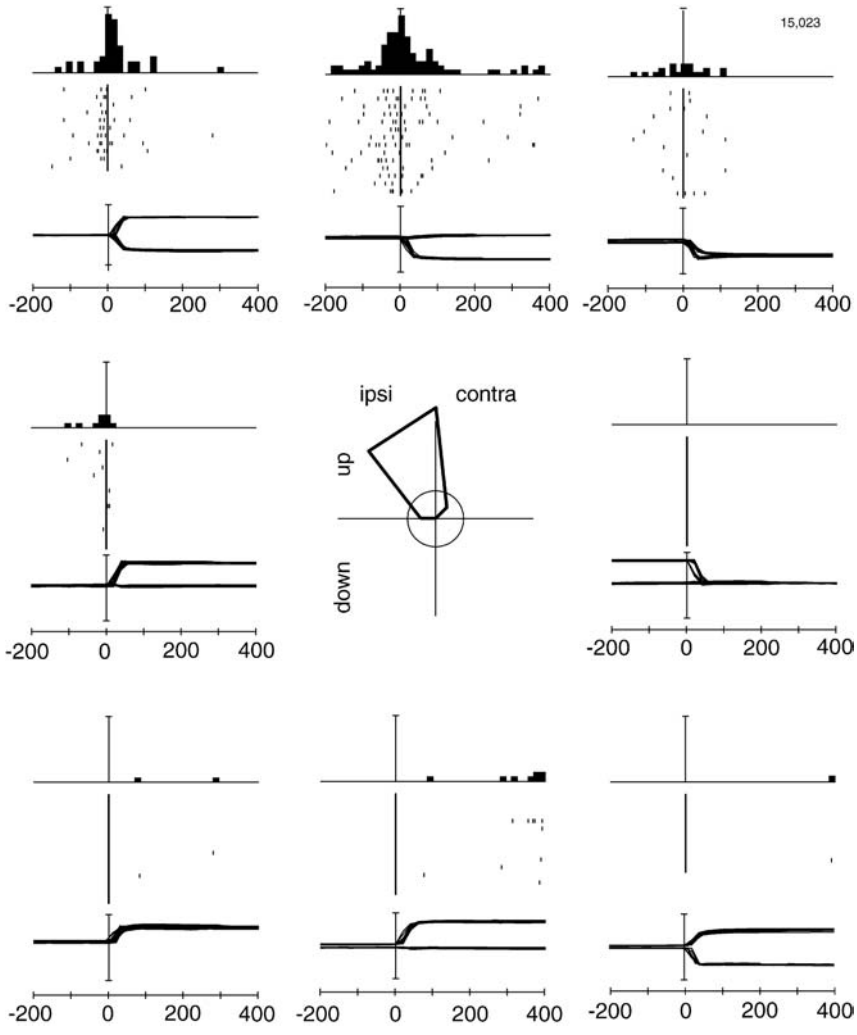

Fig. 8 Sensitivity of a V6A saccade-related cell to the direction of saccade. Each of the eight figures surrounding the central polar plot shows, from top to bottom, the peri-saccade time histogram raster-plot, and eye-traces aligned to saccade-onset (t=0 ms, bin width 15 ms). Each figure is located in the same position as the peripheral target of saccade with respect to the central starting position (see Methods). Central figure is polar plot of the direction sensitivity (mean activity for each direction); *ipsi* ipsilateral direction of saccadic movement, *contra* contralateral direction, *up* upward direction, *down* downward direction. The data are from the same cell as shown in Fig. 3B. Other details as in Fig. 2. *Scale bars*: in histograms 75 impulses/s; in eye-traces 60°; *Scale circle* in polar plot indicates activity of 15 impulses/s

we were not able to map any visual receptive field. All late-responding cells tested showed a significant decrease rate of their discharge in the dark (*t*-test, $P<0.05$). The mean decrease was around 80%.

The fact that cells insensitive to visual stimulation did not show saccade-related activity in darkness but did show such activity in the light (as in the example shown in Fig. 7B) means that we underestimated the incidence of cells with saccade-related activity because in most cases we looked for this activity in darkness.

None of the tested early-responding cells showed a significant decrease of their discharge in the dark (*t*-test, $P<0.05$). The mean decrease was of about 13%. Figure 7C shows the behaviour of one of the cells, where no differences in activity were observed between light and dark conditions.

Sensitivity to the direction and amplitude of saccade

Most V6A neurones with saccade-related activity showed a strong modulation of their activity depending on eye movement direction, whereas a minority were responsive to all directions of movement. Figure 7C shows a cell weakly modulated by the direction of movement, whereas the cell in Fig. 8 shows an example of strong direction-sensitivity. The neurone shown in Fig. 8 preferred upward and ipsilateral directions, and did not respond at all to downward and contralateral directions. For each cell tested in this way, we calculated a direction index (DI) as the ratio of the discharge for movements in the preferred direction, minus the discharge in the opposite direction over their sum. As shown in Fig. 9A, 13 of 24 neurones with saccade-related activity were strongly direction-sensitive ($DI\geq0.7$); four neurones showed a moderate directionality ($0.3\leq DI<0.7$) and seven were less or not at all modulated ($DI<0.3$). We found no correlation between the direction index of the cell response and the mean latency, or the impreciseness. Figure 9B shows the distribution of preferred directions of saccade-related cells. Most of the preferred directions were towards the contralateral lower field or the ipsilateral upper field. Even though data seem to be clustered along this oblique axis, there is no statistical evidence for it (Rayleigh test, $P<0.05$).

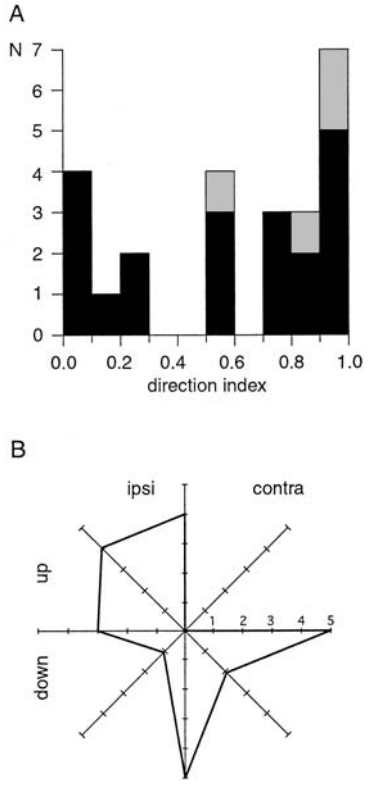

Fig. 9A,B Distribution of direction index and preferred directions in V6A saccade-related cells. **A** Distribution of direction index (bin width 0.1, *x-axis* direction index, *y-axis* cell count, N_{Σ}=24). *Dark bars* represent counts of non-visual neurones, *grey bars* counts of visual neurones or neurones with unknown visual characteristics. **B** Distribution of preferred directions; each *tick* along axis is equal to one cell count (N_{Σ}=24); *ipsi* ipsilateral direction of saccadic movement, *contra* contralateral direction, *up* upward direction, *down* downward direction

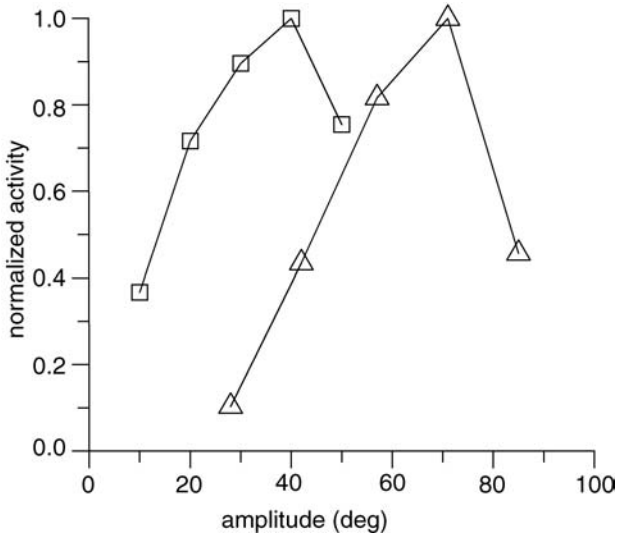

Fig. 10 Cell sensitivity to saccade amplitude. The activity of two different cells with different preferred amplitudes has been normalised. Saccades were performed in the direction preferred by the cell (*x-axis* saccade amplitude in degrees; *y-axis* normalized activity)

V6A saccade-related cells were sensitive to the amplitude of saccades. In some cells we were able to test the effect of a large set of saccadic amplitudes performed in the direction preferred by the cell. Figure 10 shows the behaviour of two of these cells. Both neurones showed a large change in activity when the amplitude of the visually guided saccades was varied. One cell preferred saccades with an amplitude of about 40°, the other had preference for saccades of about 70° but the cell did not discharge for saccades smaller than 20–30°. The range between worst and best responses was about 40° for both units.

Discussion

It is known that area V6A is involved in encoding spatial co-ordinates of visual objects (Galletti et al. 1993, 1995), as well as arm reaching movements aimed to visual objects (Fattori et al. 2001). In this work we studied the involvement of V6A neurones in encoding visually guided saccades that are normally used in real life to visually 'grasp' objects with the eye.

About 10% of V6A cells discharge before, during or after visually guided saccades performed in darkness. On the basis of the dynamic response characteristics of visual cells in area V6A, the saccade-related cells have been divided into two groups, one containing cells responding earlier than the earliest visual activity and one responding after it. Early-responding cells are active before and/or during the saccade, late-responding cells are active after the saccade. These results, in agreement with previous data from our laboratory (Galletti et al. 1991), are partially in contrast with the results of other groups that have recently recorded in the caudal-most part of the superior parietal lobule (Nakamura et al. 1999; Snyder et al. 2000b). These authors reported only post-saccadic activity (Nakamura et al. 1999), or peri-/post-saccadic activity and seldom the pre-saccadic one (see Snyder et al. 2000b). We have found that about 58% of the tested cells (14 of 24) responding pre-saccadically. We believe that the disagreement is due to differences in recording sites. Nakamura's group recorded mainly in parietal area PEc (compare Fig. 2 of Nakamura et al. 1999 with Fig. 1 of Pandya and Seltzer 1982). Area V6A, where we recorded from in this study, occupies the anterior bank of the parieto-occipital sulcus (see Fig. 1 of the present paper and Galletti et al. 1999b). If Nakamura's group did record cells from area V6A, their samples were restricted to the dorsal-most part of this area. The recording site of Snyder's group was in the medial bank of the intraparietal sulcus (see Calton et al. 2002). If Snyder's group did record cells from area V6A, they recorded only from the most lateral part of this area (compare Fig. 3 of Calton et al. 2002 with Fig. 1 of this paper). In conclusion, neither Nakamura's nor Snyder's results can be considered representative of V6A data.

Oculomotor activity or visual responses?

Late-responding cells

The similarities between dynamics of visual responses and saccade-related activities in the late-responding group (see Fig. 5A, C) suggest that the saccade-related activity of the late-responding group could be visual in nature, evoked by the motion on the retina of the screen-border images self-induced by the eye movement. Against this view is the fact that all the cells of this group were insensitive to any kind of passive visual stimulation, including light/dark borders moved on the screen to simulate the motion of the screen border images during saccadic eye movements. In favour is the fact that the saccade-related activity of these cells strongly decreases, or completely disappears, in darkness (see Fig. 7A, B). We advance the hypothesis that these cells are visually activated only by *active* visual stimulation self-induced by the movements of the eyes in a lightened environment. If this were the case, passive visual stimulations would be ineffective on the cell's discharge, as they actually are in the cells of the late-responding group, and eye movements would be effective in the light and poorly effective (if at all) in the dark, as they are too in this type of cells.

Early-responding cells

A possibility exists that the discharges of early-responding cells could be evoked by the appearance of the fixation spot in the new target position, possibly within the visual receptive field of the cell. Against this view is the fact that when we simulated the displacement of the fixation spot in a new target position by displacing a small spot of light, these cells did not respond, no matter where the spot was displaced on the screen. It is, anyway, possible that these neurones responded to a visual stimulus only when the stimulus had a functional meaning, e.g. when it is the target for the next saccade. If this were the case, the response latency with respect to the displacement of fixation spot and the impreciseness of response would be in the order of visual responses, like those shown in Figs. 3A and 6. Figure 11 demonstrates that this was not the case. Response latencies to fixation spot displacements in the early-responding group were higher and much less precise than the visual responses. Note that the earliest response of the saccade group is as late as the latest response of the visual group, and that the neural impreciseness of the saccade group was always higher than the 99.5% confidence band of the visual group. Both differences are highly significant in statistical terms (*t*-test, $P<0.005$). In conclusion, we believe that the saccade-related activity of the early-responding group are not visual responses.

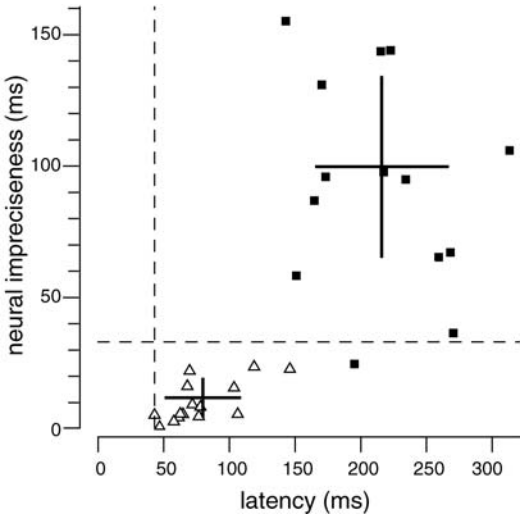

Fig. 11 Correlation between response latency and neural impreciseness in respect to stimulus-onset. Scatter plot of response latency vs response impreciseness of non-visual early-responding saccadic neurones (*filled squares*, $N_{\Sigma}=14$) and visual neurones (*open triangles*, $N_{\Sigma}=15$); t=0: stimulus-onset for all neurones. *Vertical dashed line* earliest visual latency (+43 ms); *horizontal dashed line* upper 99.5% confidence band of the variation of visual response (32.5 ms). *Thick lines* indicate the group means of latency and neural impreciseness for the two groups, respectively. Non-visual early-responding saccadic neurones: latency 214.5±49.3 ms, neural impreciseness 93.7±38.6 ms. Visual neurones: latency 79.8±29.0 ms, neural impreciseness 11.3±7.5 ms

Functional role of V6A saccade-related cells

Late-responding cells

As the late-responding cells show saccade-related responses in light and very weak responses, if any, in dark, we believe that these cells do not receive efference copy of the oculomotor signal that evokes saccades. Conversely, though we were not able to map any visual receptive field, we believe that these cells do receive visual information, which is responsible for their saccade-related activity in light. In support of this view is the fact that we frequently observed multi-unit activity in V6A, strongly modulated by saccadic eye movements in light but silent in dark (C. Galletti et al., unpublished observation). In addition, V6A contains neurones sensitive to visual stimulation of low spatial frequency and high velocity (Galletti et al. 1996) that are activated by the fast movement of the screen-border images on the retina. Cells with similar behaviour, activated by jerking movements of the structured visual field, were also observed in the pretectum (Schweigart and Hoffmann 1992) and the Lateral posterior-Pulvinar complex (Sudkamp and Schmidt 2000) of cat, in the pretectum of wallaby (Ibbotson and Mark 1994; Ibbotson et al. 1994; Price and Ibbotson 2001), and in the monkey pulvinar (Robinson et al. 1986), which in turns is known to project to the parietal cortex (Robinson and McClurkin 1989). These cells, as well as late-responding cells of V6A, are

able to signal the executed saccade by intrinsic visual information. This type of signal could be used in a number of visuomotor transformations, though its actual role in V6A remains at present to be determined.

Early-responding cells

Early-responding cells discharge before the saccadic eye movement, but the onset of the discharge is poorly time-locked to the oculomotor event, and neither is it time-locked to the sensory visual stimulation. A cell directly responding to a sensory input or directly contacting motoneurones should respond with high precision and short latency to stimulus or movement onset. As this is not the case, we suggest that the early-responding saccade-related cells we encountered are not involved either in early steps of visual information processing or late steps of oculomotor programming. Further support for the view that the activity of early-responding saccade-related cells is not a command signal comes from preliminary experiments carried out in our laboratory showing that electrical microstimulation of V6A does not evoke saccadic eye movements (C. Galletti et al., unpublished results). The large variance of the latency of their neural discharge in relation to saccade onset is consistent with the idea that early-responding cells receive a copy of efferent saccade-related signals from cortical and/or subcortical association structures (Moschovakis et al. 1996; Sommer and Wurtz 1998; Ferraina et al. 2002). Recent work reporting direct inputs to V6A from the lateral intraparietal area, frontal eye field, and the pulvinar support this view (Shipp et al. 1998).

It is also possible that the pre-saccadic activity in V6A is an internally driven signal, and somehow correlated with the saccadic event but not directly due to it. Such a type of activity has been described in area PRR (Snyder et al. 2000a). Early-responding saccade-related cells could represent the intended eye movement or the shift of attention towards another part of the visual space that generally accompanies the shift of gaze. According to this view, the signals of these cells could be used to orient the eyes towards objects to be reached and grasped by the hands, or to dictate the shift of attention towards them. New experiments with delayed-saccade tasks are needed to confirm this hypothesis.

Acknowledgements This work was supported by grants from Ministero dell'Istruzione, dell'Universita' e della Ricerca (MIUR), Italy.

References

Bach M, Bouis D, Fischer B (1983) An accurate and linear infrared oculometer. J Neurosci Methods 9:9–14

Calton JL, Dickinson AR, Snyder LH (2002) Non-spatial, motor-specific activation in posterior parietal cortex. Nat Neurosci 5:580–588

Colby C, Duhamel J (1996) Spatial representations for action in parietal cortex. Brain Res Cogn Brain Res 5:105–115

Colby CL, Gattass R, Olson CR, Gross CG (1988) Topographical organization of cortical afferents to extrastriate visual area PO in the macaque: a dual tracer study. J Comp Neurol 269:392–413

Commenges D, Seal J (1985) The analysis of neuronal discharge sequences: change-point estimation and comparison of variances. Stat Med 4:91–104

Commenges D, Pinatel F, Seal J (1986) A program for analysing single neuron activity by methods based on estimation of a change-point. Comput Methods Programs Biomed 23:123–132

Fattori P, Gamberini M, Kutz DF, Galletti C (2001) 'Arm-reaching' neurons in the parietal area V6A of the macaque monkey. Eur J Neurosci 13:2309–2313

Ferraina S, Pare M, Wurtz RH (2002) Comparison of cortico-cortical and cortico-collicular signals for the generation of saccadic eye movements. J Neurophysiol 87:845–858

Fuchs AF (1967) Saccadic and smooth pursuit eye movements in the monkey. J Physiol 191:609–631

Galletti C, Battaglini PP, Fattori P (1991) Functional properties of neurons in the anterior bank of the parieto-occipital sulcus of the macaque monkey. Eur J Neurosci 3:452–461

Galletti C, Battaglini PP, Fattori P (1993) Parietal neurons encoding spatial locations in craniotopic coordinates. Exp Brain Res 96:221–229

Galletti C, Battaglini PP, Fattori P (1995) Eye position influence on the parieto-occipital area PO (V6) of the macaque monkey. Eur J Neurosci 7:2486–2501

Galletti C, Fattori P, Battaglini PP, Shipp S, Zeki S (1996) Functional demarcation of a border between areas V6 and V6A in the superior parietal gyrus of the macaque monkey. Eur J Neurosci 8:30–52

Galletti C, Fattori P, Gamberini M, Kutz DF (1999a) The cortical visual area V6: brain location and visual topography. Eur J Neurosci 11:3922–3936

Galletti C, Fattori P, Kutz DF, Gamberini M (1999b) Brain location and visual topography of cortical area V6A in the macaque monkey. Eur J Neurosci 11:575–582

Ibbotson MR, Mark RF (1994) Wide-field nondirectional visual units in the pretectum: do they suppress ocular following of saccade-induced visual stimulation. J Neurophysiol 72:1448–1450

Ibbotson MR, Mark RF, Maddess TL (1994) Spatiotemporal response properties of direction-selective neurons in the nucleus of the optic tract and dorsal terminal nucleus of the wallaby, Macropus eugenii. J Neurophysiol 72:2927–2943

Kepner J, Randles RH (1982) Detecting unequal marginal scales in a bivariate population. J Am Stat Assoc 77:475–482

Kutz DF, Fattori P, Gamberini M, Breveglieri R, Galletti C (2001) Saccade-related activity in area V6A of the macaque. Abstr Soc Neurosci 27:58.13

Matelli M, Govoni P, Galletti C, Kutz DF, Luppino G (1998) Superior area 6 afferents from the superior parietal lobule in the macaque monkey. J Comp Neurol 402:327–352

Moschovakis AK, Highstein SM (1994) The anatomy and physiology of primate neurons that control rapid eye movements. Annu Rev Neurosci 17:465–488

Moschovakis AK, Scudder CA, Highstein SM (1996) The microscopic anatomy and physiology of the mammalian saccadic system. Prog Neurobiol 50:133–254

Nakamura K, Chung HH, Graziano MSA, Gross CG (1999) Dynamic representation of eye position in the parieto-occipital sulcus. J Neurophysiol 81:2374–2385

Pandya DN, Seltzer B (1982) Intrinsic connections and architectonics of posterior parietal cortex in the rhesus monkey. J Comp Neurol 204:196–210

Price NS, Ibbotson MR (2001) Pretectal neurons optimized for the detection of saccade-like movements of the visual image. J Neurophysiol 85:1512–1521

Robinson D (1993) Functional contributions of the primate pulvinar. Prog Brain Res 95:371–380

Robinson DL, McClurkin JW (1989) The visual superior colliculus and pulvinar. In: Wurtz RH, Goldberg ME (eds) The neurobiology of saccadic eye movements, vol 3. Elsevier, Amsterdam, pp 337–360

Robinson DL, Petersen SE, Keys W (1986) Saccade-related and visual activities in the pulvinar nuclei of the behaving rhesus monkey. Exp Brain Res 62:625–634.

Schweigart G, Hoffmann KP (1992) Pretectal jerk neuron activity during saccadic eye movements and visual stimulations in the cat. Exp Brain Res 91:273–283

Shipp S, Blanton M, Zeki S (1998) A visuo-somatomotor pathway through superior parietal cortex in the macaque monkey: cortical connections of areas V6 and V6A. Eur J Neurosci 10:3171–3193.

Snedecor GW, Cochran WG (1989) Statistical Methods, 8th edn. Iowa State University Press, Ames

Snyder LH, Batista AP, Andersen RA (2000a) Intention-related activity in the posterior parietal cortex: a review. Vision Res 40:1433–1441

Snyder LH, Batista AP, Andersen RA (2000b) Saccade-related activity in the parietal reach region. J Neurophysiol 83:1099–1102

Sommer MA, Wurtz RH (1998) Frontal eye field neurons orthodromically activated from the superior colliculus. J Neurophysiol 80:3331–3335

Sparks DL (1986) Translation of sensory signals into commands for control of saccadic eye movements: role of primate superior colliculus. Physiol Rev 66:118–171

Sparks DL, Barton EJ (1993) Neural control of saccadic eye movements. Curr Opin Neurobiol 3:966–972

Sudkamp S, Schmidt M (2000) Response characteristics of neurons in the pulvinar of awake cats to saccades and to visual stimulation. Exp Brain Res 133:209–218

Suzuki H, Azuma M (1976) A glass-insulated “Elgiloy” microelectrode for recording unit activity in chronic monkey experiments. Electroencephalogr Clin Neurophysiol 41:93–95

Tehovnik EJ, Sommer MA, Chou IH, Slocum WM, Schiller PH (2000) Eye fields in the frontal lobes of primates. Brain Res Rev 32:413–448