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RESEARCH ARTICLE

Microsaccades and preparatory set: a comparison between delayed and immediate, exogenous and endogenous pro- and anti-saccades

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Abstract When we fixate an object, our eyes are not entirely still, but undergo small displacements such as microsaccades. Here, we investigate whether these microsaccades are sensitive to the preparatory processes involved in programming a saccade. We show that the frequency of microsaccades depends in a specific manner on the intention where to move the eyes (towards a target location or away from it), when to move (immediately after the onset of the target or after a delay), and what type of cue is followed (a peripheral onset or a centrally presented symbolic cue). In particular, in the preparatory interval before and early after target onset, more microsaccades were found when a delayed saccade *towards* a peripheral target was prepared than when a saccade *away* was programmed. However, no such difference in the frequency of microsaccades was observed when saccades were initiated immediately after the onset of the target or when the saccades were programmed on the basis of a centrally presented arrow cue. The results are discussed in the context of the neural correlates of response preparation, known as preparatory set.

Keywords Eye movements · Preparatory set · Microsaccades · Anti-saccades

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Introduction

As we navigate around in our world, our eyes shift towards objects in the scene that attract our attention by means of fast eye movements, called saccades. Such overt shifts of gaze and attention are separated by periods of visual fixation, during which the eyes remain relatively still. However, even during visual fixation, small eye movements can be observed, which have been classified into different categories according to their amplitude and velocity profiles (Martinez-Conde 2006). Here, the focus will be on the largest of these fixational eye movements, called microsaccades (Ditchburn and Ginsborg 1953; Steinman et al. 1973), which are high-velocity binocular displacements that occur at a rate of about 1–2 times per second.

Several studies have examined the link between properties of microsaccades, such as their frequency and their directional bias, and brain processes. For example, it has been suggested that the direction of microsaccades provides an indication of covert attention, showing where attention is allocated while the participant maintains fixation (Engbert and Kliegl 2003; Galfano et al. 2004; Hafed and Clark 2002; Laubrock et al. 2005; Laubrock et al. 2007; Rolfs et al. 2004, 2005) (however see Horowitz et al. 2007; Tse et al. 2004). Moreover, a relationship has been proposed between microsaccade rate and the underlying activity in the rostral pole of the superior colliculus (SC) (Rolfs et al. 2008a), consistent with the finding that neurons in this area code for small amplitude saccades (Gandhi and Keller 1999; Krauzlis et al. 1997; Munoz and Wurtz 1993a, b). Evidence for such a connection was obtained in a recent neurophysiological study (Hafed et al. 2009), demonstrating a causal link between microsaccades and activity of cells in the rostral pole of the SC. In particular, neurons in this region of the SC were shown

to increase their firing rate prior to the occurrence of microsaccades. Moreover, reversible inactivation of these neurons decreased microsaccade frequency, strongly suggesting that the rostral area of the SC is responsible for microsaccade generation.

'Preparatory set'

The intention to make a particular type of response, for example a saccade towards a visual target (a pro-saccade) rather than a saccade away from it (an anti-saccade), and the readiness to respond have been termed preparatory set (Hebb 1972; Evarts et al. 1984). Such preparatory processes are assumed to take place even before the presentation of the target stimulus, which is somewhat surprising because, at this stage, the identity of the target is not yet known, and therefore many aspects of the response (e.g., saccade amplitude and direction) cannot be prepared. Evidence for such early preparatory processes has been obtained in studies comparing neural activity prior to pro- and anti-saccades. In particular, single-cell recordings in primates show higher firing rates in the rostral pole of the SC and in parts of the frontal eye fields (FEFs) before anti-saccades than before pro-saccades (Everling et al. 1999; Everling and Munoz 2000; Schlag-Rey et al. 1997) and in humans, fMRI recordings reveal higher levels of cerebral blood flow in several regions of the frontal and parietal lobe prior to anti-saccades than to pro-saccades (Connolly et al. 2002; DeSouza et al. 2003). Besides differences in neural activity due to the direction of the response (comparing pro-saccades and anti-saccades; Connolly et al. 2002; Everling et al. 1999; Everling and Munoz 2000), differential effects were also found of the stimulus probability (Basso 1998; Basso and Wurtz 1998), the number of possible targets (Basso and Wurtz 1997), and the nature of the previous trial (Fecteau and Munoz 2003; Slagter et al. 2006).

The present study aims to determine whether the preparation to make a pro- or anti-saccade modulates the occurrence of microsaccades during the response preparation period. Because of the differences in neural activity in the rostral pole of the SC prior to pro- and anti-saccades (Connolly et al. 2002; Everling et al. 1999; Everling and Munoz 2000) and because of the proposed link between microsaccades and activity in the rostral pole of the SC (Hafed et al. 2009; Rolfs et al. 2008a), a behavioral correlate of response preparation may be obtained in the frequency of microsaccades in the preparatory interval. In Experiment 1, participants were instructed to make pro- or anti-saccades following a peripheral target onset while eye movements were recorded. Two types of instructions were used as to when to make the response. In Experiment 1A, participants delayed their saccades until the offset of the fixation symbol, which acted as a go-signal. This delay between the onset of the target and the signal to make the saccade allowed for an investigation of response preparation effects on microsaccades both before and after target onset. Often, however, preparatory set effects are investigated using an immediate response to the target (DeSouza et al. 2003; Everling et al. 1999; Everling and Munoz 2000) (but see Connolly et al. 2002, who also used a memory-guided saccade paradigm). This led us to Experiment 1B, which was performed to investigate microsaccade rates when participants were asked to respond immediately with a saccade towards or away from the peripheral target rather than delay their response until the offset of the fixation symbol. If microsaccades are directly related to rostral pole activity as recorded from the monkey superior colliculus (Everling et al. 1999; Everling and Munoz 2000), we expect microsaccades to be more frequent before anti-saccades than before pro-saccades in the interval before target onset, due to the increased requirement to maintain fixation in the anti-saccade task. This is predicted to hold both for delayed (Experiment 1A) and immediate (Experiment 1B) responses to the target. For the delay period after target onset in Experiment 1A, we can only base our predictions on Connolly et al.'s (2002) findings in their memory-guided saccade paradigm in which frontal eye field, rather than superior colliculus activity was measured. Following their results, assuming that patterns of activity are similar in both brain areas (which might be a reasonable assumption considering that Munoz and Everling 2004, show similar patterns of pro- and anti-saccade related activity in the two areas), we might expect that in the delay period microsaccades remain more frequent for anti-saccades than for pro-saccades.

Preparatory set with endogenous cues

Peripheral onsets are thought to induce the automatic programming of a response to the new stimulus (e.g. Theeuwes et al. 1999). To investigate whether any differences in microsaccade rates prior to pro- and anti-saccades are related to the suppression of such an automatic response to the target, a second experiment introduced a variation of the task in which participants made pro- and anti-saccades on the basis of a centrally presented (endogenous) arrow cue, for which no automatic response preparations are thought to happen. If microsaccades are, in some way, related to the suppression of an automatic response, the peripheral target condition of Experiment 1 should modulate microsaccade frequency depending on the task (pro- vs. anti-saccade), whereas no such modulation is expected for saccades on the basis of a central cue (Experiment 2).

Experiment 1A: delayed pro- and anti-saccades with peripheral targets

In this experiment, participants performed two tasks: on half of the blocks, they prepared and generated an eye movement towards a suddenly appearing target (pro-saccades), whereas in the other half of blocks, they were required to make a saccade away from the target (anti-saccades) to a place-holder at the opposite position. Participants were asked to delay their response until the offset of the fixation symbol, allowing for an investigation of preparatory set effects on microsaccades before and after target onset.

Methods

Participants

Sixteen participants (age range 18–41 years) took part in the study. The participants were two of the authors (F.H. and R.W.), three colleagues in the department, and 11 students at Royal Holloway, University of London. The students were paid £10 for their participation. All participants reported normal or corrected-to-normal vision. Before the experiment, they gave their informed consent. The procedures were approved by the local ethics committee.

Apparatus

Stimuli were presented on a 21-in. CRT screen controlled by an AMD Athlon 2400+ PC using the Experimental Builder software package (SR Research Osgood, ON, Canada). The refresh rate of the screen was set to 100 Hz. Participants viewed the VDU screen from a distance of 57 cm. Head position was kept stable and viewing distance was maintained by means of a chin rest. Binocular eye movements were recorded by means of the Eyelink II video-based eye-tracker (SR Research Osgood, ON, Canada) at a rate of 500 Hz and stored by a Pentium 4 PC. The spatial resolution of this system is <0.01°, and the accuracy <0.5°.

Stimuli

The stimulus sequence is illustrated in Fig. 1a. Participants were asked to fixate a central fixation stimulus, flanked horizontally by two circles acting as place-holders. The fixation stimulus was 1 cm (1°) in height and width, whereas the two circles measured 7 mm (0.7°) in diameter. The distance between the fixation center and the circles was 8.5 cm (8.5°). The letter 'x' was used as the target, which just fitted inside the place-holder circles. The fixation symbol and the place-holders were presented in white on a gray background. The target was presented in black.

Design

The experiment was run in blocks of 40 trials each. The task was varied between blocks: in half of the blocks participants were asked to make pro-saccades, in the other half they were required to make anti-saccades. The position of the target was randomized across trials within each block, with 50% of the targets appearing on the left and 50% on the right. The order of the tasks (pro- vs. anti-saccade) was counterbalanced for each participant to counteract effects of practice and fatigue in the average data. Five participants (the authors and the colleagues in the department) each performed 16 blocks, which were presented in different sessions of either eight blocks in a total of 50 min (for three participants) or in four blocks with a total of 25 min (two participants) each. The remaining 11 participants completed eight blocks in one session of 50 min.

Procedure

Before each experimental block, a calibration procedure was carried out in which participants fixated a series of ten targets presented on the screen, followed by a drift correction. On each trial, a sequence of a fixation symbol with two peripheral place-holders (circles), and a peripheral target was presented (see Fig. 1a). The target was presented inside one of the peripheral marker circles following a fixation interval of 1,000 ms. After a delay of 1,500 ms, the central fixation stimulus was removed, which signaled that a saccade should be made. The response period of 1,000 ms was followed by a blank screen presented for 1,000 ms before the next trial. Participants were instructed to maintain fixation until the offset of the fixation symbol after which they either had to look towards the target (on pro-saccade trials) or away from the target to the place-holder at the mirror-symmetric location opposite to the target (on anti-saccade trials). The instruction as to which type of movement (pro- vs. anti-saccade) to make, was given at the beginning of each block.

Data analysis

Trials with response times less than 100 ms and longer than 1,000 ms (thresholds based on a visual inspection of the response time distributions) were removed from the analysis, together with trials in which the first saccadic response after fixation offset was in the wrong direction or in which the saccade was of insufficient amplitude (less than 100 pixels or 3.78°), resulting in 12.1% of the trials to be removed. Outcomes of t tests indicate the result of a two-tailed test, unless specified otherwise.

The interval of visual fixation before the cue to make the saccade was analyzed for microsaccades using an algorithm

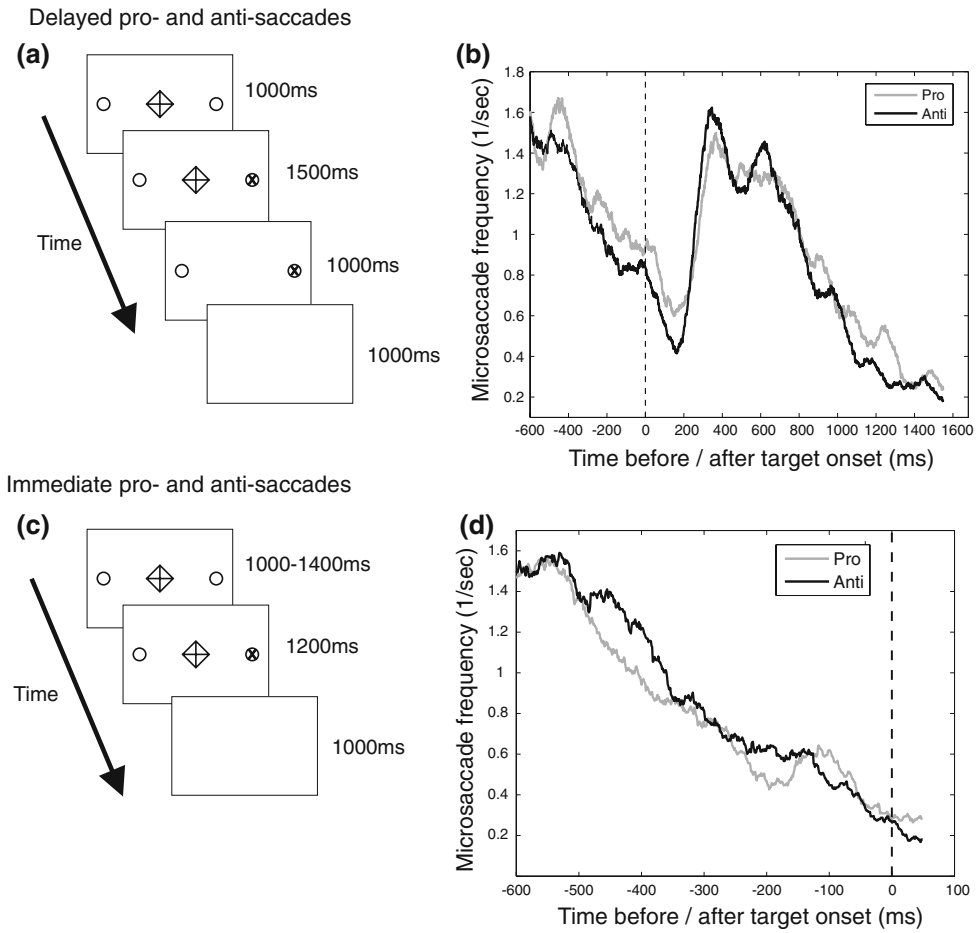

Fig. 1 **a** Illustration of the stimulus sequence used in Experiment 1A. A fixation symbol and two place-holders were presented for 1,000 ms. After this, a peripheral target (the letter 'x') appeared inside one of the two place-holders. Participants were asked to maintain fixation until the offset of the fixation symbol, after which a saccade towards ('pro-saccade') or away from ('anti-saccade') had to be made. For the purpose of illustration, all stimuli are shown in black on a white background (instead of white and black on a gray background as in the experiment). **b** Average microsaccade rate across 16 participants as a function of the time before (negative numbers) and after (positive numbers) target onset for pro-saccades (*gray line*) and anti-saccades (*black line*), obtained by sliding a window of 100 ms across the time-line. The *dashed vertical line* indicates the onset of the target. **c** Stimulus sequence in Experiment 1B. A fixation symbol was presented for a random duration between 1,000 and 1,400 ms, followed by the target for 1,200 ms. A blank screen, presented for 1,000 ms, separated the individual trials. **d** The average microsaccade frequency across the ten participants of Experiment 1B before (negative numbers on the horizontal axis) and immediately after (positive numbers) target onset

by Engbert and colleagues (Engbert and Kliegl 2003; Engbert and Mergenthaler 2006). In this algorithm, microsaccades are defined as those eye movements for which the angular velocity exceeds a criterion based on the overall noise in the data (a relative threshold of 6 standard deviations was used; for an illustration of the procedure, see Fig. S1 in the online supplementary material). Only binocular microsaccades that lasted at least 6 ms (3 samples) and did not exceed 30 pixels (1.13°) in amplitude were included in the analysis. The microsaccades detected with this algorithm had a mean amplitude of 0.3873° (SD = 0.2695) and followed the main sequence (see Fig. S2, and also Zuber et al. 1965). The microsaccade rate on each moment after cue onset was determined separately for each participant by sliding a window spanning 100 ms across the time line in time steps of 1 ms counting the number of microsaccades of which the midpoint (the average of the microsaccade start and end time) fell inside the window, resulting in a smoothed version of the histogram. An average histogram across participants was obtained by averaging the rate of all participants for each point in the curve.

Results

Figure 1b shows the mean microsaccade rate (vertical axis) as a function of the time before (negative values along the horizontal axis) or after (positive values) the onset of the peripheral target, across the 16 participants. A typical microsaccade 'signature' is observed (e.g. Engbert and Kliegl 2003; Laubrock et al., 2005), in which the microsaccade

rate drops after target onset, followed by a later 'rebound' above baseline. In addition, a reduction in the microsaccade rate was found both before target onset and before the cue to make the saccade. Interestingly, differences in the microsaccade rate between pro-saccade and anti-saccade trials can be observed before and after target onset. Before the presentation of the target, fewer microsaccades are made when participants performed anti-saccades compared to pro-saccades (paired samples two-tailed t test for the average rate between −400 and 0 ms: $t(15) = 2.30$, $p = 0.021$). During the delay period after target onset, the microsaccade rate is more strongly modulated for anti-saccades, with fewer microsaccades at the minimum and more microsaccades at the maximum of the curve. This stronger modulation was confirmed to be statistically significant by a repeated measurement ANOVA, comparing microsaccade rates across the early (0–250 ms) and late (250–500 ms) interval, showing a significant interaction between the task and the interval [$F(1,15) = 5.096$; $p = 0.039$]. In addition, a significant main effect was found for the interval [early vs. late; ($F(1,15) = 16.63$; $p = 0.001$), but not of the task (pro- vs. anti-saccades; $F(1,15) = 0.22$; $p = 0.65$]. Paired comparisons showed that in the early interval, the microsaccade rate was higher for anti-saccades than for pro-saccades [$t(15) = 2.30$, $p = 0.036$]. The difference for the late interval, however, did not reach significance [$t(15) = 1.051$, $p = 0.31$].

The above effects were not the result of differences in response times, as the times needed to initiate the delayed pro-saccades and anti-saccades were not significantly different [$t(15) = 0.90$, $p = 0.38$].

Experiment 1B: immediate pro- and anti-saccades with peripheral targets

Experiment 1A demonstrated a difference in microsaccade rates before target onset for delayed pro- and anti-saccades. In Experiment 1B, we investigated whether this difference is also found when participants are required to immediately respond to the target.

Methods

Ten participants took part in Experiment 1B. Participants were two of the authors (F.H. and R.W.), a PhD student, and seven undergraduate students from Royal Holloway, University of London. The students were paid £5 for their participation. The stimulus sequence is illustrated in Fig. 1c. A fixation symbol is presented together with two place-holders for a random duration between 1,000 and 1,400 ms, after which the target appeared inside one of the place-holders for 1,200 ms. Participants were instructed to make an eye movement towards this target (on pro-saccade trials) or away from this target (on anti-saccade trials) as quickly as possible, but at the same time, trying to avoid errors. Each participant completed four blocks of 80 trials each. Except for these differences, the methods were the same as in Experiment 1A; 13.8% of the trials were removed from the analysis because of too fast or too slow responses or because of errors in the response.

Results

The microsaccade frequency before target onset (negative values along the horizontal axis) and shortly after target onset (positive values; until half the moving window size (50 ms)) is shown in Fig. 1d. Even though a random pre-target interval was used, microsaccade rates decreased before the onset of the target. More importantly, no differences were obtained in the microsaccade frequencies on pro- and anti-saccades trials (no significant difference was found either for the interval tested in Experiment 1A, −400 to 0 ms, $t(9) = 0.65$, $p = 0.53$, or for the interval in which there appears to be a difference, between −500 ms and −300 ms, $t(9) = 1.54$, $p = 0.16$).

In contrast to the delayed pro- and anti-saccades, for which no response time (RT) difference was found, faster RTs were found on immediate pro-saccade trials (mean RT = 245 ms) than on immediate anti-saccade trials (mean RT = 289 ms; $t(9) = 7.93$, $p < 0.0001$). No significant difference in error rates was obtained between the two types of saccades [$t(9) = 1.36$, $p = 0.21$].

Discussion of Experiments 1A and 1B

Experiments 1A and 1B investigated the rate at which microsaccades occurred while participants prepared pro- and anti-saccades following a peripheral target. Only when participants were required to delay their response, higher microsaccade rates were observed before the onset of the target on pro-saccade trials compared to anti-saccade trials. This difference in microsaccade frequency for delayed saccades remained during the preparatory (delay) period until briefly after the dip in the microsaccade signature and was followed by a large rebound in the rate on both pro- and anti-saccade trials.

We hypothesized that a difference in the microsaccade rate before delayed pro- and anti-saccades could relate to the suppression of an automatic response towards the target (Hallett and Adams 1980; Munoz and Everling 2004). For the delayed *pro-saccade* trials, this automatic response has to be put 'on hold', whereas for delayed *anti-saccades*, the saccade needs to be canceled completely and a new saccade has to be programmed. This difference might have led to a stronger inhibition of saccade-related activity in the case of

anti-saccades and, as a consequence, possibly to fewer microsaccades. This explanation could hold, were it not for Experiment 1B, which showed no difference in microsaccade frequency on pro- and anti-saccade trials when participants were required to immediately make their response after the onset of the target. However, because differences were found in the response times on immediate pro- and anti-saccades, the effects of the task could have been obscured by differences in task difficulty or differences in preparedness (Betta and Turatto 2006).

To investigate this issue further, a second experiment was performed in which participants were asked to make delayed and immediate pro- and anti-saccades on the basis of a centrally presented cue. Whereas suddenly appearing stimuli tend to induce the automatic programming of a response, this is not thought to occur following the presentation of an endogenous cue. Therefore, if the difference between the microsaccade rate on delayed pro- and anti-saccade trials is the consequence of a difference in how the automatic response to a peripheral onset is treated, no differences in microsaccade rates between pro- and anti-saccade trials are expected for centrally presented cues.

Experiment 2A: delayed pro- and anti-saccades with a centrally presented endogenous cue

Experiment 2A compared microsaccade rates observed before delayed pro- and anti-saccades following the presentation of a centrally presented arrow cue to test for the involvement of the suppression of an automatic response to the peripheral target in the difference in microsaccade rates for delayed pro- and anti-saccades found in Experiment 1A.

Methods

Eight participants, including two of the authors (F.H. and R.W.) and five undergraduate students (age range 19–40 years), took part in Experiment 2A.

As in Experiment 1, each trial presented a central fixation symbol, flanked, on each side, by two peripheral place-holders (circles) indicating the two possible target locations, as illustrated in Figs. 2 a. After a delay of 1,000 ms, two lines were removed from the fixation symbol, changing it from a diamond shape into an arrow pointing either to the left or to the right (see also Walker et al. 2000). As the signal to initiate a saccade, the arrow was removed from the screen after a 1,500 ms delay. In pro-saccade blocks, participants were asked to look at the place-holder circle that had been indicated by the arrow. In anti-saccade blocks, they had to look at the opposite place-holder.

Participants performed 8 blocks of 40 trials. The order of the task (pro- vs. anti-saccade) was counterbalanced for each participant. Data analysis was the same as in Experiment 1. Filtering of the data, according to the criteria listed for Experiment 1, resulted in the exclusion of 7.4% the data.

Results

Microsaccade frequency is shown in Fig. 2b revealing a similar overall pattern or 'signature' as observed in Experiment 1A with a decrease in the microsaccade frequency followed by a later rebound. In contrast to the findings of Experiment 1A, there is no evidence of the microsaccade rate being influenced by the task requirement (pro- or anti-saccade). This is the case for the preparatory interval before the onset of the cue (between −400 and 0 ms; $t < 1$, n.s.), as well as for the interval after onset. In this latter interval, no significant interaction between the part of the signature (the early dip, at 0–250 ms vs. the late peak, at 250–500 ms) and the task (pro-saccade and anti-saccade) was found ($F < 1$; n.s.). A main effect of the interval was found (early/late; $F(1,7) = 23.49$; $p = 0.002$), but no main effect of the task ($F < 1$, n.s.).

As in Experiment 1A, reaction times were not significantly different for the delayed pro- and anti-saccades following the centrally presented arrow cue [$t(7) = 0.79$; $p = 0.46$].

Experiment 2B: immediate pro- and anti-saccades with a centrally presented endogenous cue

In Experiment 2B, microsaccades before immediate pro- and anti-saccades following an arrow cue are investigated.

Methods

Ten participants took part in Experiment 2B. These participants were two authors (F.H. and R.W.), a PhD student, and seven undergraduate students from Royal Holloway, University of London. The students received £5 for their participation. The stimulus sequence is shown in Fig. 2c. For a random interval between 1,000 and 1,400 ms, a fixation symbol and two place-holders was shown, after which two lines were removed from the fixation symbol, turning it into an arrow, which was presented for 1,200 ms. Participants were asked to follow this arrow towards the indicated place-holder (on pro-saccade trials) or to make a saccade to the opposite place-holders (on anti-saccade trials), starting their response as quickly as possible while trying to avoid errors. Participants each performed four blocks of 80 trials. Otherwise, the methods were the same as in Experiment 2A. The

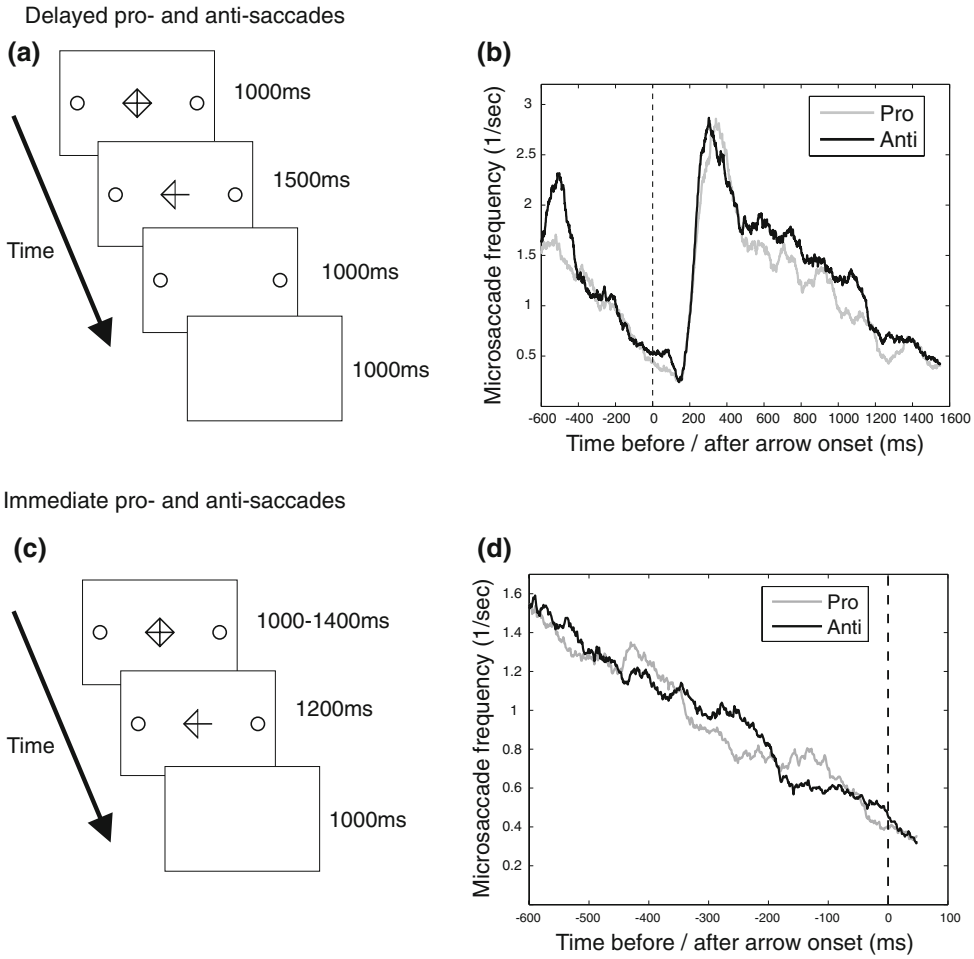

Fig. 2 **a** Illustration of the stimulus sequence in Experiment 2. A fixation symbol was presented for 1,000 ms, after which two lines were taken away turning it into an arrow. After another 1,500 ms, the arrow was removed from the screen and participants then had to look at the peripheral place-holder in the direction indicated by the arrow cue (pro-saccades) or to the place-holder in the opposite direction to the arrow (anti-saccades), depending on the instruction before the block. For the purpose of illustration, black stimuli on a white background are shown (instead of white stimuli on a gray background as used in the experiment). **b** Microsaccade rate averaged for eight participants, obtained by counting the number of microsaccades within a sliding window (width 100 ms) moved across the time-line. The *gray curve* shows the microsaccade frequency for pro-saccade trials, the *black curve* that for anti-saccade trials. The *vertical dashed line* indicates the onset of the arrow cue. **c** The stimulus sequence of Experiment 2B, in which participants immediately made a saccade in the direction of the arrow (pro-saccades) or to the opposite place-holder (anti-saccades). **d** Average microsaccade rate before (negative values on the horizontal axis) and immediately after (positive values) target onset across 10 participants of Experiment 2B

exclusion criteria on response times and saccade amplitude and direction led to the exclusion of 12.3% of the trials.

Results

Figure 2d shows the microsaccade frequency before (negative values along the horizontal axis) and shortly after (positive values) the onset of the arrow cue for pro- and anti-sacccades that immediately followed the cue onset. As for the immediate pro- and anti-saccades on the basis of a peripheral target (Experiment 1B) and the delayed pro- and anti-saccades on the basis of an arrow cue (Experiment 2A), no difference in microsaccade frequency prior to cue onset was found (for the interval used in the previous experiments, −400 to 0 ms, no significant difference was found, $t(9) = 0.33$, $p = 0.75$. Also, for the interval between −300 and −200 ms, where the microsaccade rate appears to be higher for anti-saccades, the difference was not significant: $t(9) = 1.40$, $p = 0.20$).

Response times for the immediate pro-saccades (RT = 285 ms) were significantly shorter than those for immediate anti-saccades (RT = 299 ms; $t(9) = 1.94$, $p = 0.041$; one-tailed). No difference in error rates was found [$t(9) = 1.66$, $p = 0.13$]).

Discussion of Experiments 2A and 2B

Whereas the peripheral target in Experiment 1A resulted in a difference between delayed pro-saccade and anti-saccade related microsaccade rates, no such difference was

found for the centrally presented arrow cue of Experiment 2A. This suggests that the difference in microsaccade rates found in Experiment 1A were, in some way, related to the requirement to completely cancel the automatically generated saccade program on anti-saccade trials (Hallett and Adams 1980; Munoz and Everling 2004). In comparison, on delayed pro-saccades, the automatically generated program only needs to be put 'on hold'. The involvement of the suppression of a saccade program is consistent with the findings of Experiment 2, in which no differences in microsaccade rates prior to delayed pro- and anti-saccades on the basis of an arrow cue were found. Because no difference in microsaccade rates were found for the delayed pro- and anti-saccades following the arrow cue, it was no surprise that also the immediate responses to these cues did not result in a difference in the pre-target rate.

In our experiments, a relatively large fixation symbol was used and it could be argued that this could have led to small saccades scanning the fixation symbol, and, for Experiment 2A, to small gaze shifts towards the center of gravity of the arrow as soon as two lines were removed from the fixation symbol. However, several observations argue against such an interpretation of the observed microsaccade signatures. First, both peripheral targets and arrow cues show a similar microsaccade signature with a decrease in the number of microsaccades after target onset followed by a strong increase. This pattern has been found in many studies, which often used smaller fixation stimuli (e.g. Laubrock et al. 2005) or sometimes stimuli in other modalities (Rolfs et al. 2005). This suggests that the signature is a consequence of the occurrence of a stimulus, rather than reflecting scanning saccades of the fixation stimulus. Moreover, if the signature would have been the consequence of scanning movements of the fixation symbol, it is not clear why the rate would go *down* after a change in the display, rather than going up. Second, only for Experiment 2, a change at fixation was presented, whereas for Experiment 1, the change occurred in the periphery. If microsaccades reflect the scanning of the stimulus at fixation, we would expect an increase in their frequency immediately after the fixation symbol changed into an arrow (Experiment 2), but not when the peripheral target was presented (Experiment 1). However, for both types of changes a similar microsaccade signature was found. Third, if microsaccades are eye movements intended to scan the fixation symbol, it is not clear why their frequency should depend on the task, as was found in Experiment 1A. Across the two tasks, the stimulus sequence was identical and only the instruction to the participant (to make a pro- or an anti-saccade) was varied.

General discussion

The present study investigated the effects of preparatory set on microsaccades, by examining their frequency while participants prepared to make either a pro- or anti-saccade. For delayed saccades towards or away from peripheral target onsets, we found a higher microsaccade frequency before target onset, as well as early after target onset, on pro-saccade trials compared to anti-saccade trials, suggesting that microsaccades are sensitive to the intention where to move the eyes (towards or away from the target). However, no such difference was obtained when participants were required to immediately respond to the peripheral onset, suggesting that both spatial and temporal aspects play a role. Moreover, no differences in pre-target microsaccade rates were found for pro- and anti-saccades following a centrally presented arrow cue, suggesting an involvement of the suppression of a reflexive pro-saccade to peripheral targets in the difference for delayed pro- and anti-saccades following a peripheral onset.

The microsaccade 'signature'

For both delayed saccade tasks (Experiments 1A and 2A), the typical microsaccade 'signature' (Engbert and Kliegl 2003) was found, with a reduction in the microsaccade rate after target onset followed by a later 'rebound' to a value above baseline. In addition, microsaccade rates were found to decrease before target onset and before the cue to make the saccade (i.e., the offset of the fixation symbol). It might be suspected that this gradual decrease in the microsaccade rate reflects an anticipation of the onset of the target or the offset of the fixation symbol, because in the delayed saccade tasks, the offset always occurred at the same interval from the onset of the fixation symbol and was therefore predictable. Such an interpretation would agree with earlier findings, generally showing a decreasing rate when a fixed pre-target interval was used (e.g. Betta and Turatto 2006; Valsecchi et al. 2007), whereas experiments with random stimulus presentation durations (Engbert and Kliegl 2003; Laubrock et al. 2005) more often report a constant rate (but, see Galfano et al. 2004). The decrease in the microsaccade rate before the anticipated signal to make a saccade could reflect a cognitive strategy, as microsaccades could delay a saccadic response (Rolfs et al. 2006, 2008b). With this in mind, participants could have tried to suppress their microsaccades in anticipation of a cue to make a saccade (see Bridgeman and Palca 1980; Steinman et al. 1967, for examples of voluntary microsaccade suppression). However, our results for the immediate responses (Experiments 1B and 2B) make such an interpretation less likely, as they show that even with a random pre-target interval a decreasing

microsaccade rate can be found. Some anticipation effects cannot be excluded however, because a uniform distribution was used for the pre-target interval, which has an increasing 'hazard rate', meaning that the likelihood of the appearance of the target, given that it has not yet appeared, increases over time. The use of an exponentially distributed pre-target interval could have avoided such anticipation effects (as for this distribution the hazard rate is constant). However, an exponential distribution has the disadvantage that extremely long intervals of required fixation can occur.

'Preparatory set'

Experiment 1A demonstrated that microsaccades can be influenced by the intention to make a pro- or anti-saccade towards or away from a peripheral target. However, the exact mechanisms underlying such preparatory effects are not clear. The difference in microsaccade rates was only obtained before delayed, but not before immediate saccades. Why the delay has such a profound effect on the pre-target rates is yet unknown. Possibly other preparatory effects, such as the readiness to respond, often reflected in the response times, could be involved (Betta and Turatto 2006). Furthermore, the pattern of results differed from what would have been predicted on the basis of studies showing differences in neural activity prior to pro- and anti-saccades. In these studies, neurons in the rostral pole of the SC and fixation-related neurons in the frontal eye fields were found to be more active before anti-saccades than before pro-saccades (Everling and Munoz 2000). As the rostral pole is thought to be involved in the generation of small amplitude saccades, including microsaccades (Hafed et al. 2009), this led to the prediction that before target onset more microsaccades might be found on anti-saccade trials. We found exactly the opposite: microsaccade rates were lower before anti-saccades than before pro-saccades.

To conclude, microsaccades rates are sensitive to preparatory set. However, the exact influence of preparatory processes depends on factors such as when to make the sacccade, and whether exogenous or endogenous saccades are made. Our findings extend earlier observations showing that microsaccades are sensitive to whether a response is to be made and, in the case of a response, whether the participant is ready (Betta and Turatto 2006).

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