

Visual oddballs induce prolonged microsaccadic inhibition

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Abstract Eyes never stop moving. Even when asked to maintain the eyes at fixation, the oculomotor system produces small and rapid eye movements called microsaccades, at a frequency of about $1.5\text{--}2\text{ s}^{-1}$. The frequency of microsaccades changes when a stimulus is presented in the visual field, showing a stereotyped response pattern consisting of an early inhibition of microsaccades followed by a rebound, before the baseline is reached again. Although this pattern of response has generally been considered as a sort of oculomotor reflex, directional biases in microsaccades have been recently linked to the orienting of spatial attention. In the present study, we show for the first time that regardless of any spatial bias, the pattern of absolute microsaccadic frequency is different for oddball stimuli compared to that elicited by standard stimuli. In a visual-oddball task, the oddball stimuli caused an initial prolonged inhibition of microsaccades, particularly when oddballs had to be explicitly recognized and remembered. The present findings suggest that high-order cognitive processes, other than spatial attention, can influence the frequency of microsaccades. Finally, we also introduce a new method for exploring the visual system response to oddball stimuli.

Keywords Superior colliculus · Eye movements · Attention · Microsaccades · Visual oddball

Introduction

Microsaccades are fast tiny fixational eye movements, with maximum amplitude of $1^\circ\text{--}2^\circ$ of visual angle, that have recently evoked new interest in visual neurosciences (for a review see Martinez-Conde et al. 2004). Although from the 1970s onward microsaccades have been considered nothing more than “a useless motor habit” (Steinman et al. 1973), single-cell recording and behavioral studies have recently highlighted their role in visual perception (Leopold and Logothetis 1998; Martinez-Conde et al. 2000, 2006). Moreover, behavioral studies have established important links between spatial attention and microsaccades (Hafed and Clark 2002; Engbert and Kliegl 2003; Galfano et al. 2004; Rolfs et al. 2005), as well as between microsaccades and manual motor preparation (Betta and Turatto 2006). In addition to the microsaccadic spatial bias induced by attention shifts, a common finding in these studies is that the absolute frequency of microsaccades first drops shortly after the presentation of a visual or auditory stimulus, and then presents a subsequent rebound. This stereotyped microsaccadic response has been considered as a visuo-motor reflex, possibly generated at the superior colliculus (SC) level (Laubrock et al. 2005; Rolfs et al. 2006). However, recent studies suggest that the SC does not merely have motor functions, but instead would contribute to target selection as well (McPeck and Keller 2004). In addition, as the SC receives direct (Sparks and Nelson 1987) and indirect (Schall 1995) inputs from cortical areas involved in

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object recognition and working memory (e.g., inferior-temporal cortex), one might hypothesize that different cognitive processes, other than spatial attention, could modulate the microsaccade-related activity.

To address this issue, in this study we investigated the microsaccadic response in a visual oddball paradigm, which consists of presenting rare (oddball) stimuli interleaved with more frequent (standard) stimuli. The brain response to oddball stimuli has been studied using different neuroimaging techniques, ranging from event-related potentials (e.g., Mazza et al. 2005; Potts 2004; for a review, see Pazo-Alvarez et al. 2003), to functional magnetic resonance imaging (Stevens et al. 2000; Ardekani et al. 2002). Results from this latter technique indicate that a large set of cerebral areas is active during visual oddball presentation, some of which also project to the SC (Huerta and Harting 1984). Hence, if the SC is the locus where microsaccades mainly originate, one might hypothesize that the microsaccadic response may be different for oddball stimuli compared to standard stimuli.

To ascertain whether the microsaccadic response is modulated by the brain processes involved in oddball detection, we conducted three experiments: a peripheral-oddball experiment (Experiment 1), and a central-oddball experiment (Experiment 2). In Experiment 1, subjects either passively viewed or silently counted the colored oddballs appearing in streams of same-colored stimuli presented to the right or to the left of the fixation point. In Experiment 2, participants were required to silently count the number of oddball stimuli presented centrally around the fixation point. Finally, as compared to Experiments 1 and 2, in Experiment 3 we addressed the microsaccadic response over a longer time interval.

Experiment 1

In the present experiment we sought to address whether the oculomotor system releases a specific response to the occurrence of an oddball stimulus in the temporal domain. In so doing we were motivated by noticing that previous microsaccadic experiments revealed that, regardless of any spatial bias, the absolute microsaccade frequency baseline is modulated by the onset of a stimulus in the visual field or by a sudden change of its luminance (Engbert and Kliegl 2003; Galfano et al. 2004; Laubrock et al. 2005; Rolfs et al. 2006). Hence, we recorded the absolute microsaccade frequency in a standard visual oddball task (e.g., Pazo-Alvarez et al. 2003), in which observers were

either required to passively view the stimuli or to count the occurrence of the oddball among the standards.

Method

Participants

Seventeen subjects participated in Experiment 1 (12 females, mean age = 23.8). All participants were paid volunteers and were naïve as to the purpose of the study. Informed consent to participation was obtained from all subjects, and the experiments were carried out in accordance with the Declaration of Helsinki.

Stimuli

Stimuli were displayed on a CRT 19-inch color monitor, at a viewing distance of 72 cm. The stimuli presented on a black background were red or green disks (2° in diameter) made perceptually equiluminant by a 30-Hz flicker-fusion procedure (Ives 1912). Stimuli appeared at an eccentricity of 5° either to the left or right of a white fixation point (0.73° in diameter) where participants were instructed to maintain their gaze throughout all the experiment (see Fig. 1).

Eye movement recording

Eye position was monitored using an Eye-Link II infrared video-oculographic system (SR-Research, ON, Canada), with a sampling rate of 500 Hz and a spatial resolution of less than 0.05° .

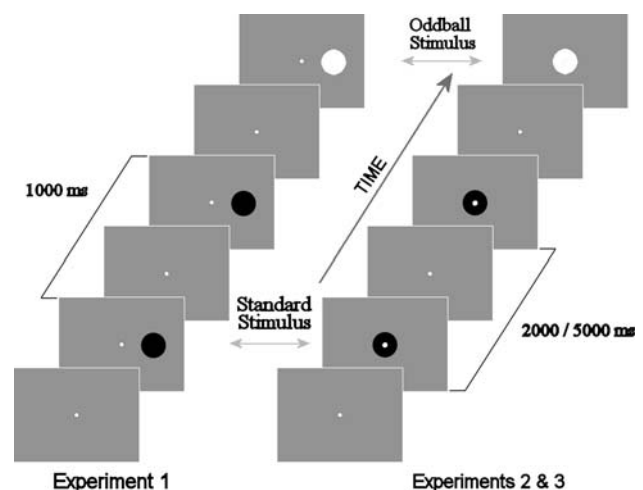


Fig. 1 Example of stimuli used in Experiments 1 (left hand), 2 and 3 (right hand). Stimuli were colored disks presented aside of fixation in Experiment 1 and at fixation in Experiments 2 and 3. The white fixation point was always present throughout the experiment (see Method section for details)

Procedure

Participants sat in a dimly illuminated room in front of the monitor, with their head positioned on a chin-rest (to limit any movements). At the beginning of each experiment, and after any break during the experiment, a standard 9-point calibration was performed.

Each trial consisted of a sequence of ten stimuli. The duration of the stimulus was estimated following the rule described by Bridgeman (1998) and corresponded to 87 ms. Stimulus onset asynchrony was 1 s, while the duration of each trial was 10 s. An automatic drift correction was performed at the beginning of each trial, and its duration was adjusted in order to have equal inter-trial and inter-stimulus intervals, ensuring the perception of a continuous stream of stimuli across trials. If the amplitude of the required drift-correction was larger than 1° the experiment was interrupted and the system recalibrated. Fixation was not monitored during the trial. The oddball stimuli were presented on 80% of trials, whilst the remaining 20% were catch trials and consisted only of standard stimuli (thus, the oddball frequency was below 0.1). To ensure that the oddball stimulus occurred after a minimum number of repeated standard stimuli, we always presented it randomly between the 6th and the 9th position in the sequences.

Each subject underwent 140 trials divided into four sessions of 35 trials each (plus five practice trials before the beginning of each session). Participants were allowed to rest after the 10th, 20th, and 30th trial in the session. The colors of the oddball and standard stimuli were counterbalanced across participants, while being kept constant for each subject. The side (left vs. right) of the presentation of the stimuli was kept constant within each session. Participants were instructed to ignore the stimuli in the first two sessions of the experiment (70 experimental trials plus 10 practice trials) and to silently count them in the second half of the experiment, or vice versa. The order of the conditions was counterbalanced between participants.

Microsaccade detection

Microsaccades were identified using the algorithm described by Engbert and Kliegl (2003), and adapted for the 500 Hz sampling rate (see also Galfano et al. 2004). The algorithm was applied to the recording of the eye position in epochs ranging from 350 ms before the presentation of any given stimulus to 50 ms after the presentation of the next stimulus. Microsaccades were defined as periods in which angular eye velocity (calculated over a moving window of nine samples)

exceeded a combined threshold for the vertical and horizontal components equal to six times the median standard deviation of the velocity within the epoch. Minimum microsaccadic duration was set to 12 ms, while maximum microsaccadic peak velocity was set to 300° s^{-1} . Epochs with eye blinks or saccades exceeding 1.5° in amplitude were discarded from analysis.

All the analyses reported in this study were conducted on epochs of ocular position recordings corresponding to the oddball stimulus and to the standard stimuli preceding the oddball one. The first stimulus of each trial was not analyzed, as the initial 50 ms of the corresponding epoch overlapped with the drift-correction procedure. In addition, in order to avoid confounds due to possible carry-over effects, we did not include the standard stimuli that followed the oddball stimulus in the analysis.

Since the oddball stimulus could only appear between the 6th and the 9th position within a trial, we analyzed the frequency of microsaccades for standard stimuli in positions 2–5 and 6–9 on catch trials to control for possible effects due to stimulus-position based expectancy.

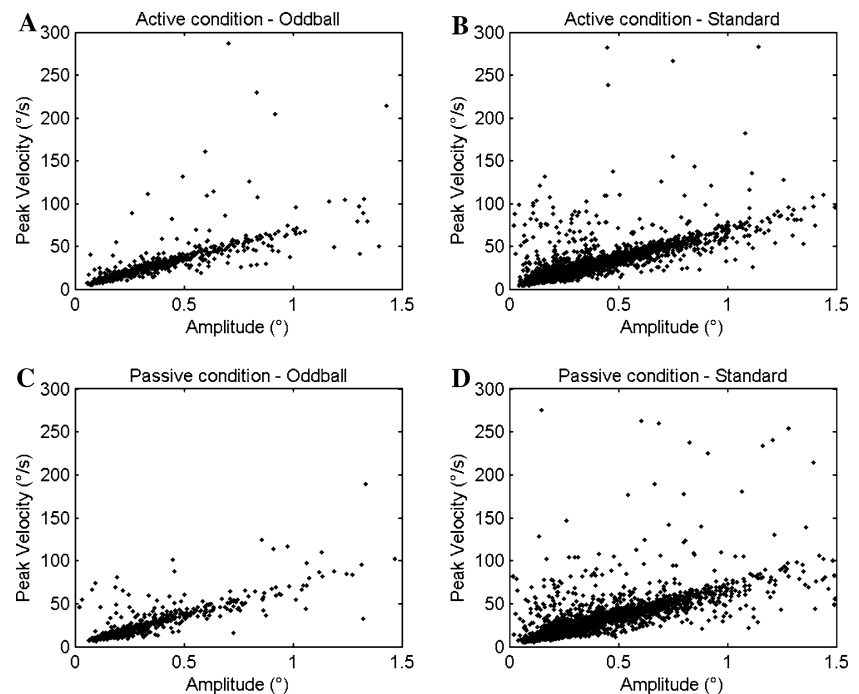
Results

Two out of 17 participants were excluded from the analysis because we could not reconstruct at least 30 artifact-free oddball epochs (i.e., epochs without blinks or saccades larger than 1.5°) in either the passive and/or active conditions. For the remaining 15 participants we collected a minimum of 37 oddball epochs for each condition. On average, in the active condition we analyzed 50.66 oddball epochs and 284.20 standard epochs per subject, while in the passive condition we analyzed 50.06 oddball epochs and 271.6 standard epochs per subject.

The overall frequency of microsaccades was calculated separately for each participant, stimulus type (oddball vs. standard) and condition (active vs. passive), and then averaged across participants. In the oddball epochs the overall frequency of microsaccades was 1.710 and 1.544 s^{-1} in the active and passive condition, respectively. By contrast, in the standard epochs the overall frequency was 1.797 and 1.681 s^{-1} in the active and passive condition, respectively. An ANOVA with stimulus type and condition as factors showed a significant effect of stimulus type ($F_{1,14} = 4.770$, $P = 0.046$), whereas the effect of condition and the interaction between the two factors were not significant (both P s > 0.1).

Microsaccadic peak velocity as a function of microsaccadic amplitude is depicted in Fig. 2. The four

Fig. 2 Peak velocity of microsaccades as a function of their amplitude for both conditions (active vs. passive) and both stimulus types (oddball vs. standard) in Experiment 1. A clear linear relationship is evident in all four cases. The number of plotted microsaccades is 1,396, 8,318, 1,334, 7,782 in panels **a–d**, respectively



panels represent the active-oddball, active-standard, passive-oddball, and passive-standard conditions. A linear relationship is evident in all plots, which is consistent with the findings of Møller et al. (2002), Engbert and Kliegl (2003), and Galfano et al. (2004). This linear relationship, known as the “main sequence”, is typical of saccades and microsaccades (Zuber et al. 1965), and guarantees that the events detected by the algorithm are indeed microsaccades (also see, for example, Engbert and Kliegl 2003).

Absolute microsaccadic frequency, calculated over a 100-ms time window moving in 2 ms steps (the highest temporal resolution allowed by our sampling rate) is depicted in Fig. 3 (panels a and b). A clear microsaccadic inhibition is evident in both the active and passive conditions about 100 ms after stimulus onset, for oddball as well as for standard stimuli. The microsaccadic frequency in the 100-ms time window centered on stimulus onset (0-ms latency) and in the time windows centered on the point of maximum microsaccadic inhibition in response to standard stimuli was entered into a repeated-measure three-way ANOVA with condition (active vs. passive), stimulus type (oddball vs. standard) and time window (0-ms latency vs. maximum inhibition latency) as factors. Latency of the maximum microsaccadic inhibition was established for all participants on the basis of Fig. 3 inspection, and was set to 120 and 110 ms for the active and passive condition, respectively. Only the main effect of time window was significant ($F_{1,14} = 38.639$, $P < 0.001$), whereas the main effects of condition, stimulus type and all the interactions

were not significant with the lowest $P = 0.13$). Mean microsaccadic frequency dropped from 1.420 s^{-1} in the 0-ms latency window to 0.773 s^{-1} in the window where we observed the maximum microsaccadic inhibition. The early microsaccadic inhibition was thus present regardless of stimulus frequency and task.

After the initial depression the onset of standard stimuli elicited the well-known microsaccadic frequency rebound, which reached its peak with a latency of about 320 ms in both the active and passive conditions. In the former this rebound was clearly delayed in response to oddball stimuli, reaching its peak about 760 ms after stimulus onset, while in the passive condition the overall shape of the microsaccadic response was similar for both stimulus types.

To explore any difference in the microsaccadic response between standards and oddball stimuli in both active and passive conditions, we conducted paired t tests for each point in the time axis of the corresponding plot, confronting the estimated values of microsaccadic frequency. Gray areas in Fig. 3 delimit the points where the level of significance survived the procedure described by Benjamini and Yekutieli (2001) to control the false discovery rate (FDR) below 0.05. The procedure we used is a conservative modification of the procedure described by Benjamini and Hochberg (1995), and controls the FDR under any form of dependency. As evident in panel a of Fig. 3, microsaccadic frequency was lower in response to oddball stimuli compared to standard stimuli for the points centered between 166 and 368 ms latency. By contrast,

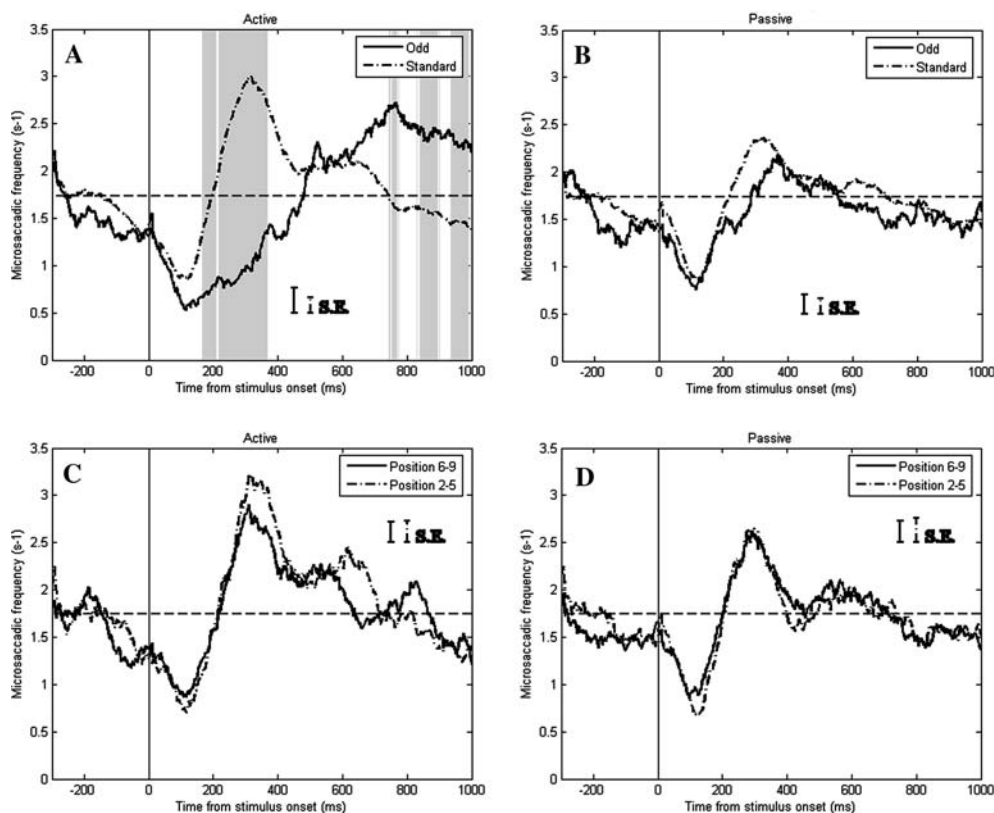


Fig. 3 **a, b** Absolute microsaccadic frequency in response to oddball and standard stimuli in Experiment 1 (active and passive condition, respectively). **c, d** Absolute microsaccadic frequency in response to standard stimuli presented on catch trials in Experiment 1 (active and passive condition, respectively) classified depending on their position in the series. The frequency of microsaccades has been calculated in a window of 100 ms width moving

it was higher in response to standard stimuli compared to oddball stimuli for the points centered between 744 and 1,000 ms latency. When the same procedure was applied to the passive condition data (panel b), we did not detect any significant difference between the microsaccadic frequency in response to oddball and standard stimuli.

The oddball stimulus in our paradigm was always presented between the 6th and 9th position in the sequence of stimuli in a trial. To control effects due to stimulus-position expectancy, we confronted the microsaccadic response to standard stimuli presented between the 6th and 9th position and stimuli presented between the 2nd and 5th position on catch trials. Figure 3 (panels c and d) depicts the microsaccadic frequency in both sets of stimuli in both the active and passive conditions. The FDR control procedure applied to paired *t* tests conducted on each point of both plots did not show any significant difference between the curves, thus ruling out the possibility that the enhanced microsaccadic inhibition in response to

in 2 ms steps. *Gray areas* include the centers of bins where *t* test significance has survived the FDR control procedure (see [Results](#)). *Error bars* represent the average inter-subject standard errors of the mean microsaccadic frequency calculated on each time window of the corresponding plots. The *horizontal dashed line* represents the overall frequency of microsaccades in oddball trials and in catch trials of Experiment 1

oddball stimuli might be due to stimulus-position expectancy.

Furthermore, we analyzed the direction of microsaccades in response to oddball and standard stimuli in both the active and passive conditions. Polar plots of microsaccades direction for both conditions and both stimulus types are depicted in Fig. 4. The direction of microsaccades was mainly horizontal, a finding consistent with the results reported by Laubrock et al. (2005). Given the observed overall directional pattern of microsaccades, we divided microsaccades into two groups as a function of their horizontal component, those directed towards the stimulus hemifield and those in the opposite direction. The directional frequency was calculated in a 100-ms time window moving by 2-ms steps as the difference between the frequency of microsaccades directed towards the target and the frequency of microsaccades in the opposite direction. Figure 5 (panels a–d) shows the directional frequency for both conditions and stimulus types. To address whether the mean directional frequency was

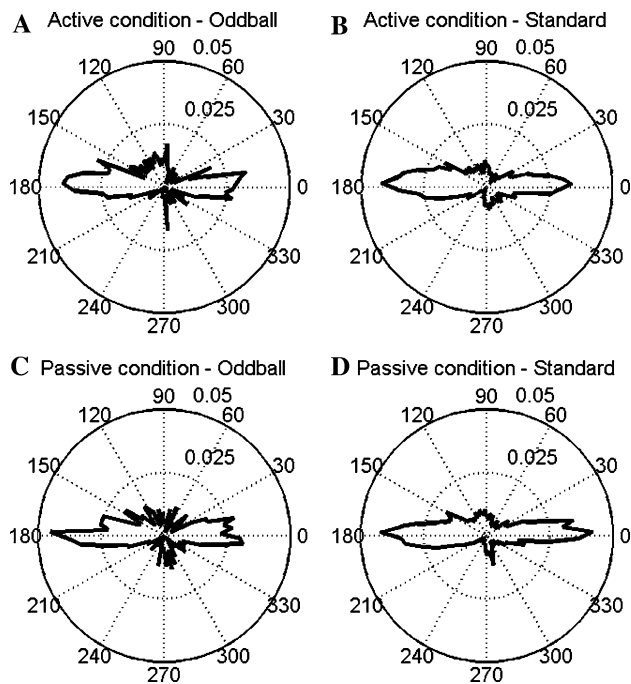


Fig. 4 Polar plots of microsaccade direction probability density calculated over 90 bins for both conditions (active vs. passive) and both stimulus types (oddball vs. standard) in Experiment 1. The distance from the center of the polar plot represents the proportion of all microsaccades contained in each 4° bin. 0° in polar coordinates indexes saccades directed towards the right

significantly different from 0, we conducted one-sample *t* tests on each point of the plots in Fig. 5. The FDR control procedure did not detect any significant directional bias.

Discussion

Despite our impression of being able to keep our eyes still at will, one of the most interesting aspects of the oculomotor system is that the eyes keep moving around the fixation point, producing what are called “fixational eye movements”. Fixational eye movements (tremors, drifts, and microsaccades) seem to play an important role in visual perception, given that if they are counteracted, visual perception fades completely as a result of neural adaptation (Martinez-Conde et al. 2004). In this regard, microsaccades have been shown to modulate stimuli perception by enhancing the corresponding evoked neuronal response in different visual cortex areas (Martinez-Conde et al. 2000) and to counteract the fading of peripherally presented stimuli (Martinez-Conde et al. 2006). Consistently, microsaccades are more frequently produced when the amount of retinal slip is reduced (Engbert and Mergenthaler 2006). In addition to the link between microsaccades

and visual perception, recent studies have shown that the direction of microsaccades can be modulated by shifts of spatial attention (Hafed and Clark 2002; Engbert and Kliegl 2003; Galfano et al. 2004). A common finding of these studies is that when eye movements are not allowed, microsaccades present a stereotyped response pattern upon visual stimulus presentation regardless of their direction. Shortly after (~100 ms) stimulus occurrence microsaccades are strongly inhibited, while a following increment above the microsaccadic baseline takes place after the initial inhibition.

In the present study we explored whether this stereotyped microsaccadic pattern could be modulated by cognitive processes. The result from Experiment 1 showed for the first time that the oculomotor system exhibited a different microsaccadic response when a task-relevant oddball stimulus was encountered among a stream of standard stimuli.

The presentation of standard stimuli produced the well known double-phase effect in the absolute frequency of microsaccades, with an initial inhibition followed by a robust rebound before the frequency returned approximately to the initial value (see Fig. 3). By contrast, a different microsaccadic response emerged in the active-oddball condition of Experiment 1. When stimuli were peripherally presented at a rate of 1 per second the initial inhibition of microsaccades in response to oddball stimuli extended up to 300–400 ms after stimulus onset, whereas the following rebound was delayed in a 750–1,000 ms time window. Interestingly, this effect was not evident in the passive-oddball condition, which suggests that the microsaccadic modulation was not driven by the oddball per se, but by the cognitive processes involved in the active condition, namely when the number of oddballs had to be attended and remembered. Traditionally, the cognitive process thought to be involved in an oddball task are those linked to the updating of information in working memory (e.g., Donchin and Coles 1988).

However, before accepting this conclusion one might note that the prolonged microsaccadic inhibition observed in the active-oddball condition could be related to a shift of spatial attention toward the peripheral oddball stimulus while the eyes were kept at fixation. In addition, the fact that this effect was particularly evident when the oddball was task relevant would be consistent with the contingent attentional capture hypothesis (Folk et al. 1992). However, it is unlikely that the specific microsaccadic inhibition for task-relevant oddballs that we found in Experiment 1 is contingent on a shift of attention. Since shifts in spatial attention modulate the direction of microsaccades (Hafed and Clark 2002; Engbert and Kliegl 2003; Rolfs

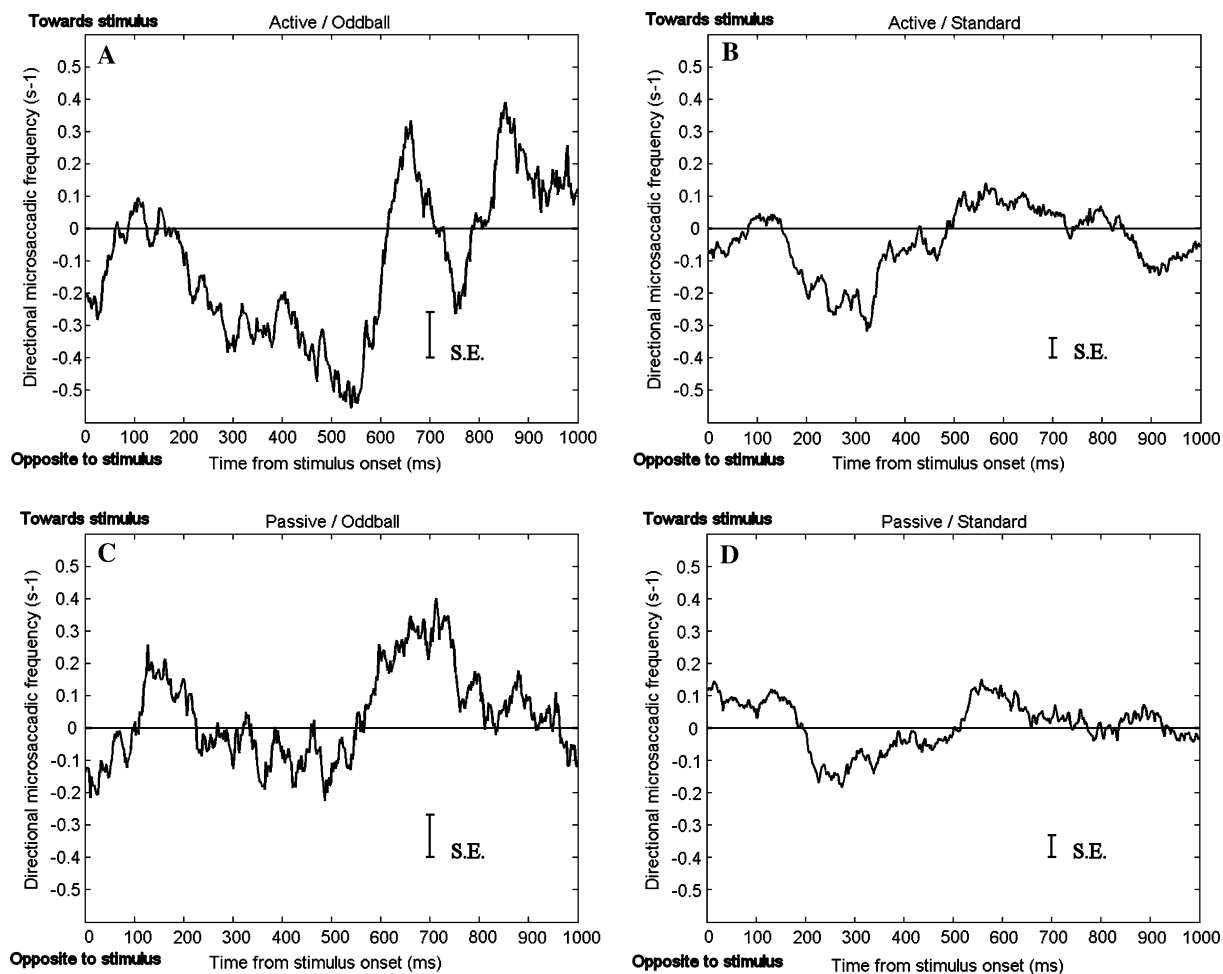


Fig. 5 Directional microsaccadic response to oddball and standard stimuli in the active and passive condition of Experiment 1. The frequency of microsaccades has been calculated in a window of 100 ms width moving in 2 ms steps as the difference between the frequency of microsaccades directed toward the stimulus and

the frequency of microsaccades in the opposite direction. *Error bars* represent the average inter-subject standard errors of the mean microsaccadic frequency calculated on each time window of the corresponding plots

et al. 2004; Galfano et al. 2004), one should predict a bigger microsaccadic directional bias for oddball stimuli compared to standard stimuli. Yet, the fact that we did not find any difference in the directional bias between the two conditions seems to undermine the hypothesis that a shift of spatial attention toward the task-relevant oddball could have induced the prolonged inhibition of the absolute microsaccadic frequency.

Experiment 2

In order to definitely rule out any spatial-shift account for the extended microsaccadic inhibition observed in the active-oddball condition we conducted Experiment 2, in which the stimuli appeared at fixation. If the previous microsaccadic pattern elicited by the oddballs was

due to any attentive and/or oculomotor spatial bias, by presenting stimuli at fixation it should no longer be present. In addition, to achieve a better understanding of the microsaccadic response observed in Experiment 1, in the present experiment we monitored the microsaccadic response to each stimulus in a longer time period (2,000 ms), and we characterized microsaccades binocularly in order to check whether the specific inhibition of microsaccades previously observed was confined to monocular microsaccades only.

Method

Participants

Twenty students from the University of Trento (12 females, mean age = 24.3) participated in Experiment 2. None of them had participated in Experiment 1. All

participants were paid volunteers and were naïve as to the purpose of the study. Informed consent to participation was obtained from all subjects, in accordance with the Declaration of Helsinki.

Stimuli

Stimuli were the same as in Experiment 1, except for the fact that they were presented centrally and the fixation point was superimposed on the colored disks (see Fig. 1).

Eye movement recording

Eye position was monitored using the same equipment as in Experiment 1.

Procedure

The procedure in each trial was the same as in Experiment 1, except for the fact that the stimulus onset asynchrony was 2 s, yielding a total trial duration of 20 s.

In Experiment 2, each subject underwent 80 trials plus five practice trials at the beginning of the experiment. The colors of the oddball and standard stimuli were counterbalanced across participants, while being kept constant for each subject. Participants silently counted the number of oddball stimuli and reported it every ten trials, after which they were allowed to rest.

Microsaccade detection

In Experiment 2, binocular eye-movements were recorded and analyzed. Monocular microsaccades were detected for each eye using the same algorithm as in Experiment 1, whilst binocular microsaccades were individuated on the basis of the temporal overlap between two monocular microsaccades (Engbert and Kliegl 2003). The smallest latency between two overlapping microsaccades in the two eyes was taken as the latency of the binocular microsaccade. Amplitude, peak velocity, horizontal displacement, and vertical

displacement for binocular microsaccades were calculated as the mean of the corresponding parameters in the two overlapping monocular microsaccades. Angular orientation for binocular microsaccades was computed on the basis of the estimated binocular horizontal and vertical displacement.

As in Experiment 1, the analyses reported were conducted on epochs of ocular position recordings corresponding to the oddball stimulus and to the standard stimuli preceding the oddball one. The first stimulus of each trial was not analyzed.

Since the oddball stimulus could only appear between the 6th and the 9th position within a trial, we analyzed the frequency of microsaccades for standard stimuli in positions 2–5 and 6–9 on catch trials to control for possible effects due to stimulus-position based expectancy.

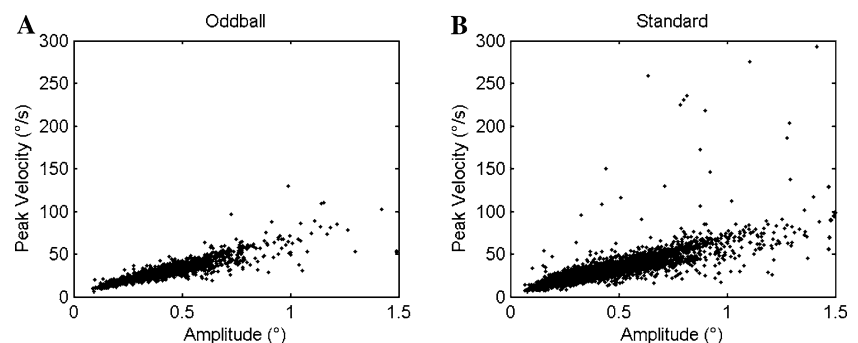
Results

Six out of 20 participants were excluded from the analysis because we could not reconstruct at least 30 artifact-free oddball epochs. For the remaining 14 participants, we collected a minimum of 31 epochs for each condition. On average, we analyzed 47.4 oddball epochs and 276.5 standard epochs per subject. The overall microsaccadic frequency was calculated for each participant and stimulus type (oddball vs. standard) and then averaged across participants. The microsaccadic frequency was 1.269 s^{-1} for oddball epochs and 1.251 s^{-1} for standard epochs in the active and passive condition, respectively. A paired *t*-test showed no statistical difference between the overall microsaccadic frequency for the two stimulus types ($t_{13} = 0.266$, $P < 0.794$).

Microsaccadic peak velocity as a function of microsaccadic amplitude for oddball and standard stimuli is depicted in Fig. 6. As in Experiment 1, a linear relationship is evident in all plots.

Absolute microsaccadic frequency calculated over a 100-ms time window moving in 2-ms steps is depicted in Fig. 7 (panel a). As in Experiment 1, microsaccadic

Fig. 6 Peak velocity of microsaccades as a function of their amplitude for both stimulus types (oddball vs. standard) in Experiment 2. A clear linear relationship is evident in both cases. The number of plotted microsaccades is 1,762 and 10,066 in panels **a** and **b**, respectively



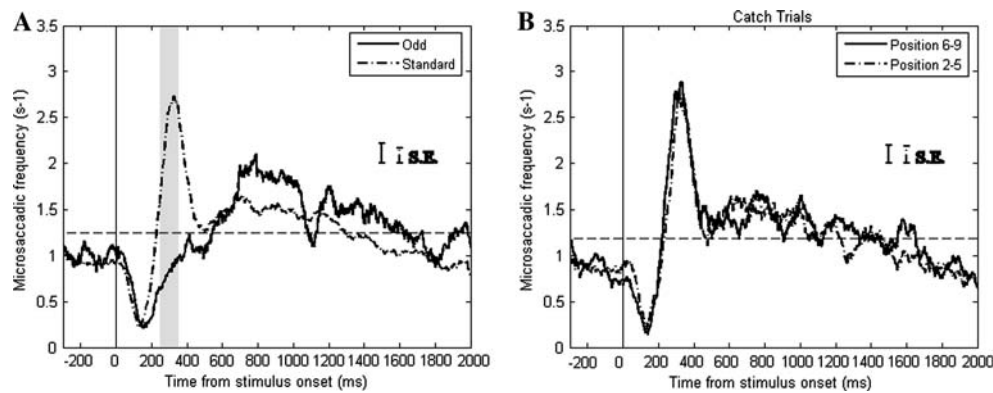


Fig. 7 **a** Absolute microsaccadic frequency in response to oddball and standard stimuli in Experiment 2. **b** Absolute microsaccadic frequency in response to stimuli presented on catch trials in Experiment 2, classified depending on their position in the series. The frequency of microsaccades has been calculated in a window of 100 ms width moving in 2 ms steps. Gray areas include the cen-

ter of bins where *t* test significance has survived the FDR control procedure (see Results). Error bars represent the average inter-subject standard errors of the mean microsaccadic frequency calculated on each time window of the corresponding plots. The horizontal dashed line represents the overall main frequency of microsaccades in Experiment 2

inhibition was evident about 100 ms after stimulus onset for both types of stimuli. The microsaccadic frequency in the 100-ms time window centered on stimulus onset (0-ms latency) and in the time windows centered on the point of maximum microsaccadic inhibition in response to standard stimuli was entered into a repeated-measure two-way ANOVA with stimulus type (oddball vs. standard) and time window (0-ms latency vs. maximum inhibition latency) as factors. The latency of the maximum microsaccadic inhibition was established for all participants on the basis of Fig. 7 (panel a) inspection, and corresponded to a latency of 132 ms. Only the main effect of time window was significant ($F_{1,13} = 27.401$, $P < 0.001$), whilst the main effects of Stimulus type and the interaction between the two factors were not significant (with the lowest $P = 0.447$). Mean microsaccadic frequency dropped from 0.970 s^{-1} in the 0-ms latency window to 0.250 s^{-1} in the window where we observed maximum microsaccadic inhibition. As in Experiment 1 the early microsaccadic inhibition was present regardless of whether the stimulus was an oddball or a standard.

The overall microsaccadic response in Experiment 2 (Fig. 7, panel a) is strikingly similar to the one we observed in the active condition of Experiment 1 (see Fig. 3, panel a). Nonetheless, the FDR control procedure applied to *t* tests performed for each point of the plot revealed significant differences between the microsaccadic response to oddball and standard stimuli only for time windows centered between 250 and 348 ms latency (gray areas). This is the time window in which the microsaccadic rebound in response to standard stimuli reached its peak, whereas the microsaccadic inhibition in response to oddball stimuli was evident as in Experiment 1. No significant differences were found

in the time windows between 600 and 900 ms latency, where the frequency of microsaccades in response to oddball stimuli was higher compared to standard stimuli. This was not due to the fact that the FDR procedure was more conservative, since it was applied to a double number of points compared to Experiment 1. Indeed, when the procedure was applied only to the first 500 points of the plot, we still did not find any significant difference in the time windows where the microsaccadic frequency was higher in response to oddball stimuli.

The same analysis on the microsaccadic response to standard stimuli on catch trials was conducted as in Experiment 1. The mean microsaccadic frequency in response to stimuli presented between the 6th and 9th position, and between the 2nd and 5th position within catch trials is depicted in Fig. 7 (panel b). The two curves are very similar and none of the *t* tests performed on each point of the plots was significant after the application of the FDR control procedure.

To show possible changes in the preferential direction of microsaccades due to performing binocular recording and microsaccade detection and to the use of central stimulation, we drew polar plots of the directional frequency of microsaccades in response to oddball and standard stimuli for all participants (Fig. 8). As in Experiment 1 the microsaccades were mainly horizontally biased.

Discussion

Discussion

The main reason for conducting Experiment 2 was to exclude a possible role of shifts of spatial attention as the cause for the prolonged inhibition in response to oddball stimuli that we observed in the active condition

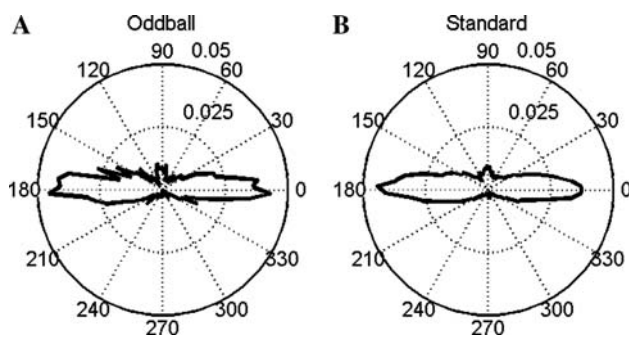


Fig. 8 Polar plots of microsaccade direction probability density calculated over 90 bins for both stimulus types (oddball vs. standard) in Experiment 2. The distance from the center of the polar plots represents the proportion of all microsaccades contained in each 4° bin. 0° in polar coordinates indexes saccades directed towards the right

of Experiment 1. This hypothesis can reasonably be ruled out based on the results we obtained in Experiment 2, in which participants had no reason to move their spatial attention from the fixation point, as the stimuli appeared at fixation. The results of Experiment 2 basically replicated those of the active condition of Experiment 1 by showing a longer inhibition of microsaccadic response for oddballs rather than for standard stimuli. Given that, as stated above, all stimuli were presented at fixation, any spatial bias toward the oddballs (at an attentive or oculomotor level) can no longer account for the results. However, as argued in the [General discussion](#), this does not exclude a possible role of the attention load in the microsaccadic inhibition observed in Experiments 1 and 2.

Experiment 3

Although the crucial effect reported in the present study emerged in the first 300 ms after stimulus' onset, a third experiment was conducted in order to check the microsaccadic response over a longer time window. This was motivated by the fact that in Experiment 2 after 2 s from stimulus' onset the frequency of microsaccades did not seem to reach a stable baseline, but rather it kept decreasing until the end of the epoch and in the pre-stimulus interval. To this purpose in Experiment 3 we presented stimuli at a rate of one every 5 s, which should have been sufficient for the microsaccadic frequency to reach a stable baseline before the next stimulus in the sequence was presented. Note, however, that the usage of such a long stimulus onset asynchrony (SOA) might probably reduce the effect of the oddball stimuli on the microsaccadic response. Indeed, one may note that the longer the SOA, the higher the

possibility that each stimulus is perceived as being a unique event instead of being part of the same sequence, which in turn would reduce the saliency of the odd-colored item in the temporal domain.

Method

Participants

Six participants took part in Experiment 3 (2 females, mean age = 29.1), including two of the authors (M.V. and M.T.). The remaining four participants were naïve as to the purpose of the study. Informed consent to participation was obtained from all subjects, in accordance with the Declaration of Helsinki.

Stimuli, procedure and eye movement recording

As in Experiment 2 (stimuli were presented at fixation, see Fig. 1), except that the SOA was set at 5 s, yielding a total trial duration of 50 s.

In Experiment 3, each participant underwent 5 to 6 sessions composed of 15 trials each, plus 2 to 5 practice trials at the beginning of each session. The colors of the oddball and standard stimuli were counterbalanced across participants, while being kept constant for each participant. Participants silently counted the number of oddball stimuli and reported it every five trials, after which they were allowed to rest.

Microsaccade detection

Given the very high probability of finding at least one eye blink within a 5-s epoch, we slightly modified the criteria for epoch rejection in Experiment 3 in order to avoid rejecting an excessive number of epochs. Instead of discarding the whole epoch containing blinks from the analysis, as we did in Experiments 1 and 2, we excluded only the part of epoch ranging from 200 ms prior to 200 after the blink from the analysis. In addition, we also discarded the microsaccades exceeding the amplitude threshold (1.5°) from the analysis without discarding the whole epoch. Beside this, microsaccades were detected binocularly using the same procedure adopted in Experiment 2.

Results

In the present experiment the number of averaged trials per condition is not the same along the time axis of the epochs, because data from a 400-ms time window centered on eye blinks have been discarded from the epoch (see above in the [Method](#) section). The minimum num-

ber of averaged trials in the oddball epochs for all participants and samples along the time axis was 51.

The frequency of microsaccades in response to oddballs and standard stimuli in Experiment 3 is depicted in Fig. 9. A prolonged microsaccadic inhibition is evident in response to oddball stimuli in a time window comparable to that in which the same effect emerged in Experiment 2. In analyzing data we adopted a different method as compared to that used in previous experiments. This was motivated by the fact that with a 5 s SOA we would have had a huge loss of power due to the excessive number of potentially non significant tests in the last 4 s of the epoch. Instead, we performed two *t*-tests comparing the frequency of microsaccades in response to standard and oddball stimuli in two time windows of interest. The first time window is the one where the highest rebound in microsaccadic frequency was observed in response to standard stimuli (300–400 ms latency), whilst the second time window is the last part of the epoch (4,000–5,000 ms latency). The first time window is the 100-ms time window centered on the sample where the peak of microsaccadic frequency in response to standard stimuli was reached (300–400 ms latency), whilst the second time window is the last part of the epoch (4,000–5,000 ms latency). A significant difference in microsaccadic frequency was observed in the first time window ($t_5 = 3.826$, $P = 0.012$) but not in the second one ($t_5 = 0.966$, $P = 0.378$).

Discussion

The results confirmed the crucial effect observed in previous experiments. Furthermore, we studied the evolution of the microsaccadic response over a longer time span. Although the curves of the frequency in response to oddball and standard stimuli seem to converge in the second half of the epoch, they do not reach a stable baseline, for the frequency continues to decrease until the presentation of the next stimulus, as can be seen in the pre-stimulus interval in the plot. This is probably due to the fact that the regularity in the presentation of the stimuli generates a microsaccadic response which adapts to the SOA. In any case, the differential microsaccadic inhibition in response to oddball stimuli is evident only in the same early time window documented in Experiments 1 and 2.

General discussion

The aim of the present study was to investigate the microsaccadic response to visual oddballs. Since evidence exists that different brain areas, some of which

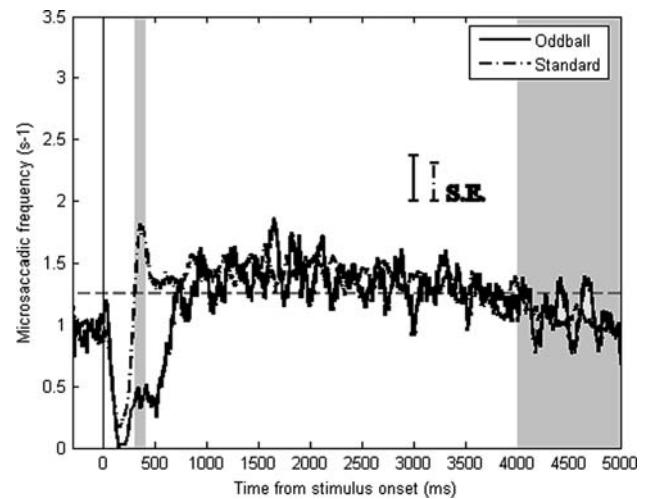


Fig. 9 Absolute microsaccadic frequency in response to oddball and standard stimuli in Experiment 3. The frequency of microsaccades has been calculated in a 100 ms time window moving in 2 ms steps. Gray areas include the 100 ms time window centered on the sample where the maximum peak in microsaccadic frequency is observed in response to standard stimuli, and the time window corresponding to the last 1,000 ms of the epoch. *t*-Tests show a significant difference in the first time window, but not in the latter, indicating a prolonged microsaccadic inhibition in response to oddball stimuli and the reunification of the curves (see Results). The horizontal dashed line represents the overall frequency of microsaccades in Experiment 3

projecting to the SC, are active during visual oddball presentation (Huerta and Harting 1984), and given that the SC is thought to be a likely candidate as the neural substrate from which microsaccades possibly originate, we sought to address whether the occurrence of a visual oddball elicits a specific microsaccadic response. The results confirmed our prediction, by showing a prolonged microsaccadic inhibition when an oddball stimulus is presented instead of a standard one.

The overall result pattern of Experiments 1 and 2 suggests that other processes different from the orienting of spatial attention, and which are involved in oddball tasks, must be responsible for the enhanced inhibition of microsaccades we observed in response to oddball stimuli in the present set of experiments. In our task participants had to count and remember the number of oddballs, thus possible candidates are stimulus evaluation and memory-related processes, which mainly involve the updating of information in working memory (Donchin and Coles 1988; Kok 2001; Potts 2004). Although we are sympathetic with this memory explanation, we acknowledge that attention can still play a role in the microsaccadic inhibition yoked to the occurrence of the oddball stimuli. Indeed, one may observe that the level of attentional resources dedicated to the oddballs might be higher than those

deployed to the standards. In other words, it could be that the microsaccadic inhibition we documented was modulated by the level of attentional load (Lavie 1995). In any case, we believe that the specific oculomotor response caused by the oddball stimuli is not due to a shift of spatial attention, as the effect is evident also when the stimuli are presented at fixation (Experiments 2 and 3). In addition one may note that as the oddball was also a rare stimulus compared to the standards, it could be hypothesized that the specific microsaccadic inhibition we reported might be related to the fact that the visual system produces a specific electrocortical response when a rare stimulus is encountered (for a review, see Pazo-Alvarez et al. 2003).

It has been suggested that microsaccades may be influenced by activity at the level of the SC (Hafed and Clark 2002; Rolfs et al. 2006). Hence, given the widespread connectivity of the SC with other brain areas (e.g., prefrontal cortex and posterior parietal cortex) where high-order cognitive functions are thought to be implemented, it is possible that working-memory related structures may influence microsaccadic behavior. Specifically, the hypothesis that the extended period of microsaccadic inhibition is related to working-memory processes is consistent with the fact that such extended inhibition did not emerge in Experiment 1 when the oddball stimuli were task irrelevant. In this case participants were not required to explicitly recognize and memorize the oddballs to report their total number, and therefore working memory was not involved in the task.

In addition, in Experiment 2 we characterized microsaccades binocularly in order to check whether the specific inhibition of microsaccades previously observed could be confined to monocular microsaccades only. This was clearly not the case, for the prolonged microsaccadic inhibition in response to task-relevant oddball stimuli was observed also for binocular saccades. We also monitored the microsaccadic response to each stimulus in a longer time period (2,000 ms) and again we found that the most striking differences between oddball and standard epochs were within the first 1,000 ms after stimulus onset.

As depicted in Fig. 7a, the peak of microsaccadic frequency was reached later in response to oddball stimuli compared to standard stimuli, though the frequency of microsaccades in response to oddball stimuli was not significantly higher than that observed in response to standard stimuli in any point of the plot. This is consistent with what we found in the active condition of Experiment 1 and supports the hypothesis that the rebound in microsaccadic frequency is related to the clustering of microsaccades at the end of the inhibition phase.

To summarize, our findings demonstrate that the visual system exhibits a specific pattern of oculomotor activity in response to the presentation of an oddball stimulus. In general, microsaccadic frequency is first depressed and then enhanced whenever a stimulus occurs in the visual field. Interestingly, however, amplitude and duration of these phases vary as a function of whether the stimulus is either an oddball or a standard, and as a function of whether the oddball stimulus is task relevant or irrelevant, with an initial magnified microsaccadic inhibition in the former case. Such prolonged microsaccadic inhibition when the oddball needs to be recognized and remembered surprisingly demonstrates the influence of high-order cognitive processes other than the orienting of spatial attention in the modulation of fixational eye movements. Yet, as stated above, the possibility still remains open that the attentional load might have played a role in the effect we document.

Finally, research on oddball stimuli has a long tradition in neuroscience. Starting from the seminal work of Näätänen et al. (1978) the specific brain response to an oddball stimulus either in the auditory or visual modality has been studied by recording the electrocortical event related potentials. More recently, the same issue has been investigated by using the functional magnetic resonance imaging technique, which relies on modifications of the cerebral blood flow in the activated areas of the brain (e.g., Stevens et al. 2000; Ardekani et al. 2002). The present findings show that besides event related potentials and functional magnetic resonance imaging techniques, the visual system response to oddball stimuli can be revealed simply by recording microsaccadic activity.

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