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Microsaccadic Response to Visual Events That Are Invisible to the Superior Colliculus

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Even when people think their eyes are still, tiny fixational eye movements, called microsaccades, occur at a rate of ~1 Hz. Whenever a new (and potentially dangerous) event takes place in the visual field, the microsaccadic frequency is at first inhibited and then is followed by a rebound before the frequency returns to baseline. It has been suggested that this inhibition–rebound response is a type of oculomotor reflex mediated by the superior colliculus (SC), a midbrain structure involved in saccade programming. The present study investigated microsaccadic responses to visual events that were invisible to the SC; the authors recorded microsaccadic responses to visual oddballs when the latter were equiluminant with respect to the standard stimuli and when both oddballs and standards were equiluminant with respect to the background. Results showed that microsaccadic responses to oddballs and to standards were virtually identical both when the stimuli were visible to the SC and when they were invisible to it. Although the SC may be the generator of microsaccades, this research suggests that the specific fixational oculomotor activity in response to visual events can be controlled by other brain centers.

Keywords: microsaccades, visual oddball, superior colliculus, microsaccadic inhibition, freezing reflex

When we fixate a stationary stimulus, our eyes are engaged in tiny movements of which we are not aware. Among these fixational eye movements, microsaccades (i.e., saccades smaller than 1.5° of visual angle) are clearly distinguishable from other types of small movements (i.e., tremors and drifts) on the basis of their speed and amplitude (Martinez-Conde, Macknik, & Hubel, 2004).

Microsaccades have been a relevant research topic since the 1950s, when many studies investigated their possible role in visual perception. Cornsweet (1956) initially proposed that microsaccades could serve to correct the fixation error produced by drifts and tremors. Another possible function postulated for microsaccades was that of counteracting image fading due to neural adaptation in the visual system (Ditchburn, Fender, & Mayne, 1959). This topic was debated extensively for 3 decades. Steinman, Haddad, Skavenski, and Wyman (1973) observed that microsaccades are more frequent during prolonged fixation than during normal oculomotor behavior, when large voluntary saccades are usually observed (e.g., during reading). They proposed that microsaccades were possibly executed more frequently during tasks requiring high visual acuity, such as threading a needle or shooting a rifle. By contrast, Winterson and Collewijn (1976) and later Bridgeman and Palca (1980) observed that microsaccades are generally inhibited during high-acuity observational tasks. This inhibition seemed to exclude the possibility that microsaccades were necessary for a correct visual perception. Furthermore, human observers can be trained to inhibit microsaccades for a few seconds, and this does not seem to cause visual fading (Steinman, Cunitz, Timberlake, & Herman, 1967).

On the basis of these observations, Kowler and Steinman (1980) claimed in an influential article that microsaccades had no meaningful function in the visual system. Ditchburn (1980), however, observed that the fact that humans could be trained to voluntarily inhibit their microsaccades did not imply that those movements lacked a function in normal vision. Given the general disagreement on this topic, the debate on the functional role of microsaccades was abandoned at the beginning of the 1980s, which led to a widespread lack of interest in these fixational eye movements.

The last 10 years, however, have seen a renewed interest in microsaccades, which was probably due, in part, to the introduction of more precise and reliable, not to mention less invasive, eye-tracking systems. For example, recent studies have documented that microsaccades enhance perception by counteracting visual fading that is due to neural adaptation (Engbert & Mergenthaler, 2006; Martinez-Conde, Macknik, & Hubel, 2000; Martinez-Conde, Macknik, Troncoso, & Dyar, 2006). Furthermore, microsaccades correlate with ocular (Rolfs, Laubrock, & Kliegl, 2006) and manual (Betta & Turatto, 2006) motor preparation and are modulated by attention and by working memory (Betta, Galfano, & Turatto, 2007; Engbert & Kliegl, 2003; Galfano, Betta, & Turatto, 2004; Rolfs, Engbert, & Kliegl, 2005; Turatto, Valsecchi, Tamè, & Betta, in press; Valsecchi, Betta, & Turatto, 2007). A consistent finding in previous studies on microsaccades and attention is that shortly (~100 ms) after the occurrence of a novel visual (or auditory) event, the absolute frequency of microsaccades is depressed (e.g., Galfano et al., 2004). Next, an above-baseline rebound, peaking at around 300 ms poststimulus,

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takes place before the frequency returns to the baseline of about one microsaccade per s. Given the short latency of the inhibitory response and the fact that it is elicited by both visual and auditory stimuli, researchers have hypothesized that such microsaccadic inhibition is generated at the level of the superior colliculus (SC; Engbert, 2006), an important midbrain center for saccade programming.

Nonetheless, there is clear evidence that the absolute frequency of microsaccades is sensitive to top-down modulations related to the state of the cognitive system (e.g., Betta & Turatto, 2006; Steinman et al., 1967). In his pioneering work on fixational eye movements, Barlow (1952) hypothesized that microsaccades were inhibited during high-load cognitive tasks, such as difficult mathematical operations. Like many of the early studies on microsaccades, his study suffered from methodological flaws. Those methodological shortcomings were likely due to unsystematic data analysis and to the use of highly invasive measurement systems, which resulted in unnatural experimental settings. Thus, despite being suggestive, Barlow's data can only be regarded as anecdotal. His intuition, however, was somewhat confirmed in a recent study by Valsecchi et al. (2007), which showed that the oculomotor system delivers a specific microsaccadic-response pattern when a rare target stimulus is encountered in a visual oddball paradigm. Specifically, as compared with the microsaccadic response to standard stimuli, the occurrence of an oddball that has to be counted induces a longer inhibitory phase, whereas the following rebound is almost abolished. As pointed out by Valsecchi et al. (2007), these results suggest that the absolute frequency of microsaccades is driven not only by reflex-like, bottom-up processes but by cognitive processes involved in oddball detection. This view is consistent with previous findings showing that visual attention can modulate the direction of microsaccades in spatial cuing and in visual search tasks (Engbert & Kliegl, 2003; Turatto et al., in press).

Our main aim in the present study was to determine the extent to which the occurrence of microsaccadic responses to novel visual events is a genuine, reflex-like phenomenon controlled subcortically at the SC level. To this purpose we used an oddball paradigm with equiluminant stimuli, which are known to be indistinguishable to the SC. Indeed, the SC shows weak visual selectivity, as it basically gives the same response to stimuli of different sizes, and to both moving and stationary objects, and it exhibits little to no orientation specificity. Furthermore, the SC does not have color-opponent processing and therefore cannot differentiate between stimuli of different colors that have the same luminance (Marrocco & Li, 1977; Robinson & McClurkin, 1989). Hence, our prediction was straightforward: If the microsaccadic inhibition in response to visual events is produced at the level of the SC, and only on the basis of the SC's direct afferences from the retina, microsaccadic inhibition should not be evident when a stimulus that is equiluminant with the background is presented. On the other hand, if the inhibition can be controlled by other cortical centers that are not color blind, the same microsaccadic pattern should emerge, regardless of whether the stimuli are equiluminant.

Experiment 1

In the present experiment, we recorded microsaccades from participants who were shown a series of colored disks that appeared, one after the other, at fixation. The majority of the disks were of the same color (say green) and were defined as standards, whereas a small proportion of the disks, defined as oddballs, were of a different color (say red). The participants were asked to silently count the number of oddballs (see *Method* section). The colors of oddballs and of standards were matched in terms of luminance. In the luminance-onset condition, the stimuli appeared over a black background, which created luminance changes detectable by the SC. By contrast, in the equiluminant-stimulus condition, oddballs and standards appeared over a gray background of the same luminance and therefore were visual events invisible to the SC.

Method

Participants. A total of 15 healthy volunteers (7 women and 8 men, mean age = 28.6 years) participated in the experiment. All of them gave their informed consent to participate and were treated in conformity with the Declaration of Helsinki. All of the participants had normal or corrected-to-normal vision and were naive as to the purpose of the study.

Stimuli and experimental procedure. Stimuli were displayed on a 19-in. Iiyama CRT monitor (Iiyama Corporation, Nagano-Shi, Japan) controlled by a Radeon 9550 graphics card (ATI, Markham, Ontario, Canada). Color depth was 32 bits, and screen resolution was 1024 × 768 pixels. The frame rate of the monitor was 85 Hz. Participants viewed the stimuli from a distance of 72 cm. Head movements were restricted by a chin rest. Stimuli were either red or green circles, subtending 2° of visual angle in diameter. A white fixation circle (diameter = 0.5°) was superimposed on each stimulus. The luminance of the stimuli was made equal to that of a reference gray color (2.4 cd/m^2), as determined by the flicker fusion technique (frequency of flickering = 21.25 Hz; Ives, 1912). The luminance was adjusted online by the subjects until fusion was reached. In the equiluminant-stimulus condition, the circles were superimposed on a square (side = 3° of visual angle) of the gray reference color; the rest of the screen was black. We estimated stimulus duration to be 98 ms, following the rule introduced by Bridgeman (1998), and stimulus onset asynchrony was 1,000 ms. Only the fixation point remained visible during the interstimulus interval in the luminance-onset condition, whereas the gray square and the fixation point remained visible in the equiluminant-stimulus condition. Stimuli were generated using Matlab (MathWorks, Natick, MA) and the PsychToolbox (Brainard, 1997).

Stimuli were presented in series of 10, and 80% of the series contained 1 rare stimulus (oddball), which we asked the participants to count silently. Altogether, less than 10% of the presented stimuli were oddballs. The oddball stimulus could occupy any position in the series between the 2nd and the 9th stimulus. The interseries interval had the same duration as the interstimulus interval and the same display was shown, so the participants experienced a continuous stream of stimuli between series. Each participant underwent 16 blocks of 10 series each; at the end of each block, the number of oddballs was reported and the participant was allowed to rest. The experimental condition (luminance onset vs. equiluminant stimulus) was alternated between blocks; the condition within the first block and the target color were alternated between participants.

Eye movement recording. Eye movements were sampled binocularly at 500 Hz with the Eyelink II system (SR Research, Ontario, Canada), which has a spatial resolution $< 0.01°$. The eye tracker was interfaced with the stimulus-generation software with the Eyelink Toolbox (Cornelissen, Peters, & Palmer, 2002). A standard nine-point calibration was performed at the beginning of each block. Drifts of up to 1.5° were automatically corrected during the intertrial interval. The position of gaze was monitored during a 400-ms interval starting 100 ms after the presentation of the 10th stimulus, and the calibration plane was subsequently recentered on the mean position during the interval. If the drift exceeded the 1.5° threshold, the experiment was interrupted and the system was recalibrated. We detected microsaccades offline using the algorithm introduced by Engbert and Kliegl (2003), which was adapted for the 500-Hz sampling rate (Valsecchi et al., 2007). The velocity threshold for microsaccade detection was set at six standard deviations; the minimum duration of microsaccades was set at six samples, and the maximum peak velocity allowed was 300°/s. The algorithm was applied to raw eye-position tracks in epochs ranging from 50 ms before the presentation of each stimulus to 50 ms after the presentation of the successive stimulus (1,100 ms). Epochs containing blinks or saccades that exceeded 1.5° in amplitude were discarded from the analysis. The epochs that corresponded to the first and the last stimulus in the series were not analyzed, because they overlapped with the automatic drift correction.

A participant was removed from the pool before data analysis, because it was not possible to obtain a minimum number of 30 artifact-free epochs in each cell of the design. For the remaining 14 participants, on average, 53.7 and 53.1 oddball epochs were analyzed in the luminance-onset condition and in the equiluminant-stimulus condition, respectively. The average number of standard epochs per participant was 381.5 in the luminance-onset condition and 379.7 in the equiluminant-stimulus condition.

Results

The evolution of absolute microsaccadic frequency after the presentation of oddball and of standard stimuli in the luminance-onset condition is represented in Figure 1A. When compared with the microsaccadic baseline frequency occurring at stimulus onset, the microsaccadic response to standards showed a characteristic pattern consisting of an early inhibition phase that peaked at around 150 ms poststimulus, $F(1, 13) = 26.692$, $p < .001$, followed by a rebound that peaked at around 320 ms poststimulus, $F(1, 13) = 46.298$, $p < .001$. A very different microsaccadic response was observed when an oddball appeared, for the initial microsaccadic inhibition was extended, such that the rebound observed in the case of standards was substantially abolished. The difference between the oddball and the standard plots was confirmed by an appropriate statistical analysis. We calculated the frequency of microsaccades for each participant and each stimulus type (oddball vs. standard) in a 100-ms time window, which moved in 2-ms steps from the time of stimulus onset to that of the onset of the following stimulus. Then we performed a paired t test on each of the 500 time points, in which we compared the frequency of microsaccades in oddball and in standard epochs. To control the false discovery rate to under .05 for multiple comparisons, we applied the procedure introduced by Benjamini and Yekutieli (2001) to the obtained p values. As indicated by the vertical gray area in the plot (see Figure 1A), the analysis showed that the frequency of microsaccades was significantly lower in response to oddball stimuli than in response to standards in the time windows centered between 248 and 386 ms after stimulus onset.

We then analyzed the microsaccadic response to oddballs and to standards in the equiluminant-stimulus condition. The results are depicted in Figure 1B. The evolution of microsaccadic frequency in response to both types of stimuli (oddball and standard) was virtually identical to that observed in the luminance-onset condition. The standard stimuli elicited the same early inhibition phase, which peaked at around 150 ms poststimulus, $F(1, 13) = 24.921$, $p < .001$, and was followed by a rebound that peaked at about 340 ms poststimulus, $F(1, 13) = 49.709$, $p < .001$, whereas such a rebound was abolished in the case of oddballs. As indicated by the gray area in Figure 1B, the statistical analysis confirmed the difference between the two curves in the time windows centered between 230 and 382 ms poststimulus onset.

To underline the similarity of the microsaccadic modulation between the luminance-onset and the equiluminant-stimulus conditions, we directly compared the frequency of microsaccades in both conditions (see Figure 2), for standard (Panel A) and for oddball (Panel B) epochs, respectively. We applied the same analysis, involving multiple t tests and correction of the false discovery rate, to the data depicted in these plots in order to detect differences, if any, between conditions. However, in none of the time windows did we find any significant difference between the frequency of microsaccades in the luminance-onset and the equiluminant-stimulus conditions. This finding is in agreement with the hypothesis in the present study, according to which the SC is unlikely to control the microsaccadic response to novel events.

Given that the maximum peak in microsaccadic frequency for standard stimuli was clearly identifiable for each participant, we conducted a paired t test to establish whether its latency was different between conditions (equiluminant stimulus vs. luminance onset). Although the test did not reach significance, $t(13) = 1.771$, $p = .099$, the latency of the rebound in response to standard stimuli in the luminance-onset condition ($M = 324$ ms) was shorter than the latency of the rebound in the equiluminant-stimulus condition ($M = 335$ ms). This finding suggests that the microsaccadic response induced by equiluminant stimuli may have a temporal dynamic similar to the dynamic induced by luminance-onset standards, except that the latency of the response seems to be a bit delayed in the former case. The same analysis could not be performed on the latency of the peak inhibition, because it could not be clearly identified for each participant.

Discussion

The modulation of microsaccadic frequency in response to oddball and to standard stimuli in the luminance-onset condition nicely replicates the findings obtained by Valsecchi et al. (2007). When an oddball stimulus is encountered, the frequency of microsaccades is inhibited for a longer period and the following rebound phase is dramatically reduced compared with the rebound phase after a standard stimulus is encountered. A strikingly similar pattern of results is observed in the equiluminant-stimulus condition. Specifically, the amplitude of the modulation of microsaccadic frequency in response

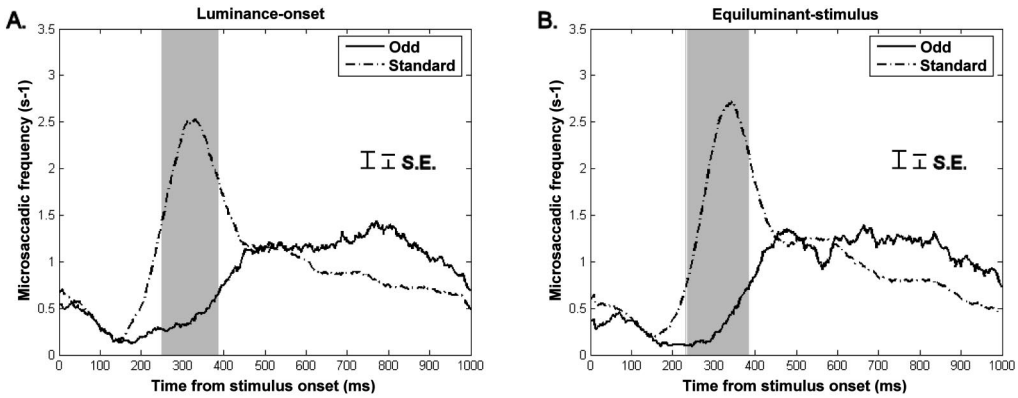

Figure 1. Evolution of absolute microsaccadic frequency after the presentation of oddball and of standard stimuli in (A) the luminance-onset condition and (B) the equiluminant-stimulus condition in Experiment 1. The frequency of microsaccades was calculated in a 100-ms-wide time window, which moves in 2-ms steps for each subject, epoch type, and condition, and was subsequently averaged across participants. Gray areas delimit the centers of the time windows, where paired *t* tests with false discovery rate correction revealed a significant difference between the two curves (see *Results* and *Discussion*). Error bars represent the between-subjects standard error of the mean microsaccadic frequency, which was calculated separately for each time window, stimulus type, and condition and was subsequently averaged across time windows.

to standard stimuli is virtually identical to that observed in response to luminance-onset standard stimuli. This finding could indicate that a visual pathway other than the retino–tectal pathway can support the biphasic inhibition–rebound modulation of microsaccades in response to visual stimuli.

The modulation of microsaccadic frequency appears to be slightly delayed in the equiluminant-stimulus condition, although the latency of the rebound in response to standard stimuli was not statistically longer in the equiluminant-stimulus condition than in the luminance-onset condition. This difference could indicate that the modulation in the former condition is supported by a slower visual pathway and would be consistent with the hypothesis that cortical structures (which are not color blind) can mediate the microsaccadic inhibition–rebound response.

Experiment 2

The application of the flicker fusion technique has been shown to be a difficult task for untrained participants (e.g., Wyszecki & Stiles, 1982). Given that the SC may respond to stimuli that present even small luminance variations from the background (Marrocco & Li, 1977), we decided that it was crucial to replicate the results observed in Experiment 1 using an improved version of the flicker fusion procedure.

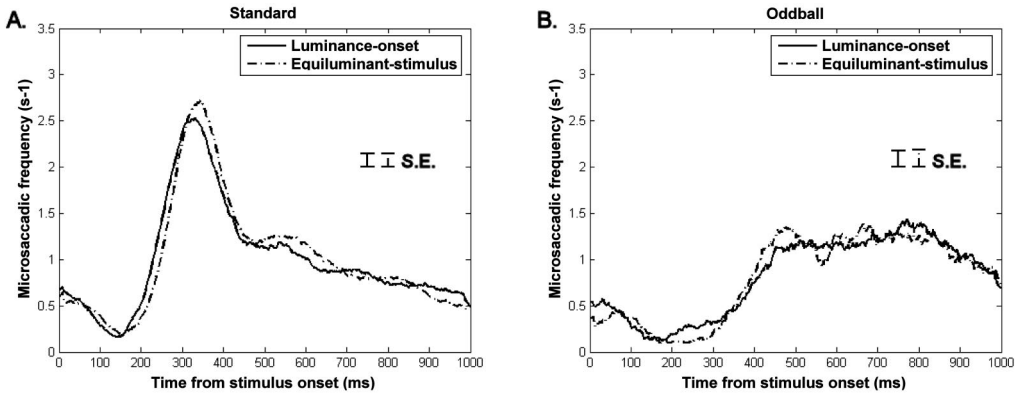

Figure 2. Evolution of absolute microsaccadic frequency in the luminance-onset condition and the equiluminant-stimulus condition after the presentation of (A) standard and of (B) oddball stimuli in Experiment 1. The frequency of microsaccades was calculated in a 100-ms-wide time window, which moves in 2-ms steps for each subject, epoch type, and condition, and was subsequently averaged across participants. Paired *t* tests with false discovery rate correction performed along the time axis did not reveal a significant difference between the two curves (see *Results* and *Discussion*). Error bars represent the between-subjects standard error of the mean microsaccadic frequency, which was calculated separately for each time window, stimulus type, and condition and was subsequently averaged across time windows.

Method

Participants. A total of 6 healthy volunteers (2 women and 4 men, mean age = 32 years) participated in the experiment. Two of the participants were the authors (Matteo Valsecchi and Massimo Turatto), and the remaining participants were staff members from the University of Trento. Participants gave their informed consent to participate and were treated in conformity with the Declaration of Helsinki. All had normal or corrected-to-normal vision and were highly trained psychophysical observers.

Stimuli and experimental procedure. Stimuli and procedure were as in Experiment 1, except for the following changes, which we introduced to make the flicker fusion procedure more reliable. Red and blue circles were used as stimuli, and the color of oddball stimuli was counterbalanced between participants. The luminance of the reference gray color was 6 cd/m^2. We lowered the frequency of flickering to 18.75 Hz by reducing the monitor frame rate to 75 Hz, which yielded an estimated stimulus duration of 97 ms. The flicker fusion adjustment procedure was repeated 20 times for each color (red or blue). On half of the runs, the starting value of luminance was darker than the reference color, whereas on the remaining runs, it was brighter. We used the mean value obtained from the 20 runs during the experiment.

Eye movement recording. Eye movements were sampled as in Experiment 1. On average, 51.3 and 51.1 oddball epochs were analyzed in the luminance-onset condition and in the equiluminant-stimulus condition, respectively. The average number of standard epochs per participant was 339.8 in the luminance-onset condition and 343.6 in the equiluminant-stimulus condition.

Results

The evolution of absolute microsaccadic frequency after the presentation of oddball and of standard stimuli in the luminance-onset condition in Experiment 2 is represented in Figure 3A. When compared with the microsaccadic baseline frequency occurring at stimulus onset, the microsaccadic response to standards showed the characteristic pattern consisting of an early inhibition phase followed by a rebound. As in Experiment 1, the frequency of microsaccades was lower at 150 ms poststimulus onset, $F(1, 5) = 11.587$, $p < .019$, and higher at 320 ms poststimulus onset, $F(1, 5) = 11.254$, $p < .02$, than it was at the time of stimulus onset. The same pattern was observed in the equiluminant-stimulus condition: The frequency of microsaccades was lower in the time window centered at 150 ms poststimulus onset, $F(1, 5) = 9.968$, $p < .025$, and was higher in the time window centered at 320 ms poststimulus onset, $F(1, 5) = 19.492$, $p < .007$.

As expected, in both the equiluminant-stimulus and the luminance-onset conditions, microsaccadic frequency lasted longer (see Figure 3A and 3B) in the oddball epochs. Given the smaller sample size in Experiment 2, we decided to use a less conservative approach in order to evaluate the statistical significance of the microsaccadic inhibition in response to oddball stimuli. Instead of performing multiple *t* tests along the time axis, we performed *t* tests only in the time window where we expected the maximum oddball effect (i.e., the one centered on 320 ms poststimulus onset; see also Valsecchi et al., 2007). In that time window, the frequency of microsaccades was lower in oddball epochs than in standard epochs, $t(5) = 3.89$, $p < .012$. No significant difference was observed between the equiluminant-stimulus and the luminance-onset epochs, $t(5) = .957$, $p = .383$.

As in Experiment 1, we performed a paired *t* test comparing the latency of the peak microsaccadic frequency in response to standard stimuli in the luminance-onset and in the equiluminant-stimulus conditions (see Figure 4A). Unlike in Experiment 1, the peak microsaccadic latency in response to equiluminant standard stimuli ($M = 354$ ms) was reached significantly later than was the peak microsaccadic latency in response to luminance-onset standard stimuli ($M = 320$ ms), $t(5) = 3.317$, $p < .021$.

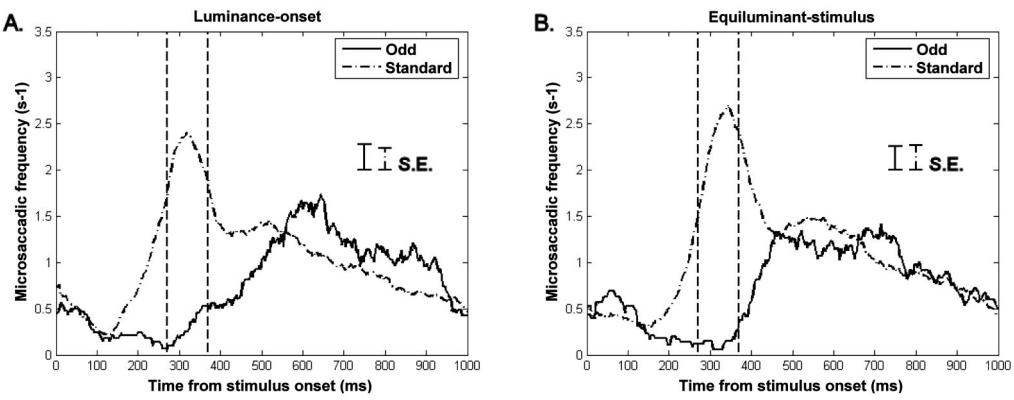

Figure 3. Evolution of absolute microsaccadic frequency after the presentation of oddball and of standard stimuli in (A) the luminance-onset condition and (B) the equiluminant-stimulus condition in Experiment 2. The frequency of microsaccades was calculated in a 100-ms-wide time window, which moves in 2-ms steps for each subject, epoch type, and condition, and was subsequently averaged across participants. Vertical dashed lines delimit the time window, in which paired *t* tests compared the frequency of microsaccades in oddball and in standard epochs (see *Results* and *Discussion*). Error bars represent the between-subjects standard error of the mean microsaccadic frequency, which was calculated separately for each time window, stimulus type, and condition and was subsequently averaged across time windows.

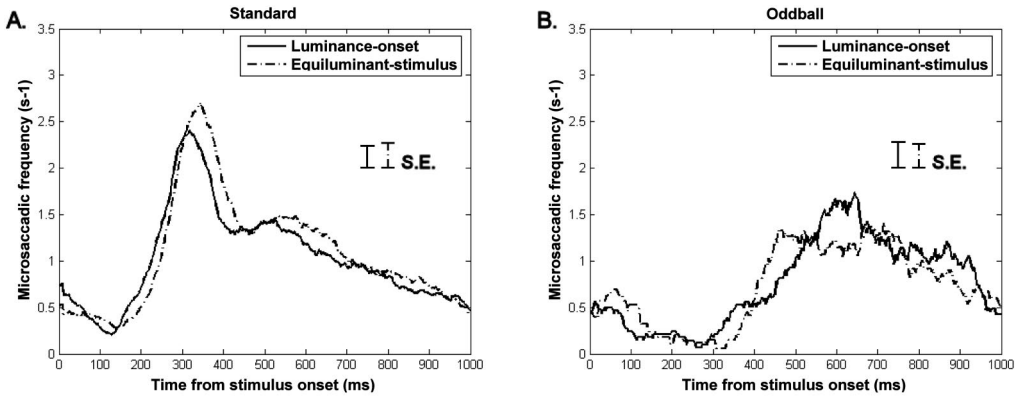

Figure 4. Evolution of absolute microsaccadic frequency in the luminance-onset condition and the equiluminant-stimulus condition after the presentation of (A) standard and of (B) oddball stimuli in Experiment 2. The frequency of microsaccades was calculated in a 100-ms-wide time window, which moves in 2-ms steps for each subject, epoch type, and condition, and was subsequently averaged across participants. Error bars represent the between-subjects standard error of the mean microsaccadic frequency, which was calculated separately for each time window, stimulus type, and condition and was subsequently averaged across time windows.

Discussion

Experiment 2 was conducted with a more reliable and accurate flicker fusion procedure. As in Experiment 1, the double-phase modulation of microsaccadic frequency, with an early inhibition followed by a rebound, was observed in response to equiluminant standard stimuli as well as in response to luminance-onset stimuli. Moreover, in the present experiment we found that the rebound phase of the modulation of microsaccadic frequency in response to stimuli equiluminant with the background had a longer latency compared with the rebound phase observed in response to luminance-onset stimuli. It is worth noticing that this finding, though not significant, was observed in Experiment 1, which indicates that this effect is stable across different groups of observers. Moreover, the improvement of the flicker fusion procedure affected the latency of the rebound in the equiluminant-stimulus condition, which was 19 ms longer than the latency in Experiment 1, whereas it had a more limited effect on the latency of the rebound in the luminance-onset condition, which was only 4 ms shorter than the latency in Experiment 1. The fact that equiluminant stimuli evoke a rebound phase with a longer latency suggests that this response is likely mediated by a slower neural pathway compared with the response elicited by luminance-onset stimuli. The fact that this effect was significant only in Experiment 2 demonstrates the advantage for researchers who use a more accurate flicker fusion procedure to isolate the contribution of the faster subcortical visual pathway in controlling the microsaccadic response.

The results of Experiment 2 also confirmed that the modulation of microsaccadic frequency induced by visual oddballs is markedly different from the modulation induced by standard stimuli, in that it shows an extended inhibitory phase and the absence of the rebound phase (Valsecchi et al., 2007). This effect was observed both for luminance-onset stimuli and for equiluminant stimuli, a result consistent with the fact that the luminance of oddball stimuli and of standard stimuli was the same. In addition, this outcome provides converging evidence that the microsaccadic response to oddballs is unlikely to be controlled subcortically by the SC, given that the SC would not be able to distinguish between oddball and standard stimuli on the sole basis of its subcortical inputs (see also Valsecchi et al., 2007).

General Discussion

Since the seminal work of Näätänen, Gaillard, and Mäntysalo (1978) in the auditory modality, it has been known that the brain exhibits a characteristic (electrocortical) response to novel and rare stimuli (Mazza, Turatto, & Sarlo, 2005; Potts, 2004). However, it has recently been shown that the brain's sensitivity to novel events can also be traced by recording the oculomotor activity during fixation (Valsecchi et al., 2007). Specifically, small fixational eye movements called microsaccades, which we normally produce unconsciously during fixation, are affected by the occurrence of a new event in the visual field. When fixation is required, the first response emitted by the oculomotor system after the appearance of a stimulus is a fast inhibition of the spontaneous microsaccadic activity (about 1–4 Hz). After this inhibition, a rebound usually follows before microsaccadic frequency returns to the baseline, except in the case of novel target stimuli, in which the inhibitory phase is prolonged and the rebound phase is almost absent.

The neural bases that mediate such microsaccadic responses are still unclear. It has been hypothesized that the SC could control the microsaccadic inhibition observed whenever a new stimulus appears in the visual field (Engbert, 2006). However, this hypothesis could be questioned on the basis of recent findings that the initial microsaccadic inhibition persists for a longer period of time and the usual rebound is eliminated when an oddball, equiluminant with respect to the standard stimuli, is encountered (Valsecchi et al., 2007). This specific response to oddballs equiluminant with the standard stimuli provides initial evidence suggesting that the SC, which is color blind and which responds only to luminance variations (Robinson & McClurkin, 1989), might not control such microsaccadic reaction autonomously. The lack of the rebound, which is usually observed 300 ms after stimulus onset, would be consistent with the fact that slower, color-sensitive cortical centers, rather than a faster, color-blind subcortical center, might control

this response. However, because in previous studies oddball and standard stimuli were brighter than the background, therefore introducing luminance variations visible to the SC, the possibility still remained that the initial microsaccadic inhibition was an oculomotor reflex controlled autonomously by the SC (Engbert, 2006).

To directly address the neural basis of the microsaccadic response to novel events and to clarify any possible role of the SC in the initial microsaccadic inhibition induced by the occurrence of a new stimulus in the visual field, we conducted two experiments comparing the microsaccadic response to standard and to oddball stimuli when (a) their appearance was accompanied by a luminance change or (b) they were equiluminant with the background. The latter condition restricts processing to the cortical visual system, which abolishes any relevant contribution from the SC, because it cannot discriminate between stimuli of the same luminance (Marrocco & Li, 1977; Robinson & McClurkin, 1989). The results showed that the amplitude of the microsaccadic response was very similar in the luminance-onset condition and in the equiluminant-stimulus condition, which indicated that neural structures other than the SC are involved in the control of such oculomotor behavior. The findings of Experiment 2, which showed a longer latency in the microsaccadic response to standard stimuli for the equiluminant-stimulus condition, are compatible with the hypothesis that the biphasic microsaccadic modulation is mediated, in this case, by a different pathway, which is slower than the subcortical one.

If one assumes the SC to be the neural structure in which microsaccades are spontaneously and ultimately generated, the data we have reported suggest the possibility that microsaccadic inhibition could be controlled via fast-descending signals from cortical centers that, either directly or indirectly, can discriminate colors. Possible candidates include the frontal eye fields, the lateral intraparietal area, and the occipital visual cortex, for all these neural structures send afferences to the SC (Munoz, 2002). Visual signals related to the appearance of new objects reach the occipital visual cortex, the lateral intra-parietal area, and the frontal eye fields 40, 50, and 70 ms after stimulus onset, respectively (Pouget, Emeric, Stuphorn, Reis, & Schall, 2005; Schmolesky et al., 1998). Hence, such neural response latencies are compatible with the possibility that these structures might influence the generation of microsaccades in the SC with a latency that is only approximately 35 ms longer than the time taken by the direct retino–tectal input pathways.

One may wonder whether the SC is necessary and sufficient for the generation of the microsaccadic response to standard stimuli. As microsaccades have a dynamic profile that looks very similar to the profile of regular saccades (Zuber, Stark, & Cook, 1965), it is commonly assumed that, during fixation, microsaccades reflect spontaneous random activity of SC neurons. Our data neither prove nor disprove this hypothesis, which could be falsified only by the observation of a preserved microsaccadic response after the selective ablation or inactivation of the SC. However, on the ground of the present findings, we can conclude that the SC is not sufficient to generate the microsaccadic response to equiluminant stimuli, as they are invisible to the SC. The response we documented must rely on some cortical structures that are not color blind. As far as the response to luminance-onset standard stimuli is concerned, three possibilities are, in principle, equally viable:

1. The SC itself is sufficient to generate the characteristic response observed when onsets are presented.

2. The same cortical structure (or structures) that controls the microsaccadic response to equiluminant stimuli is involved, but in this case the afferent signals reach this structure via the SC (i.e., via a shorter route). The cortical center can thus send the modulation back to the SC earlier than when equiluminant stimuli are used.

3. Stimuli consisting of luminance variations and equiluminant stimuli reach the cortical structure (or structures) via the same route, but the former are processed faster than the latter, which gives rise to an earlier modulation of the SC activity.

At present, we cannot distinguish among these possibilities, and only the observation of a preserved microsaccadic response to visual onsets after the disconnection of the cortical inputs to the SC could reveal whether this neural structure is sufficient to generate the microsaccadic response we documented.

However, other subcortical structures could participate with the SC in the generation of the response to rare stimuli. In particular, a specific response to rare acoustic targets has been identified in the amygdala and the hippocampus during an oddball task (Halgren et al., 1980). The amygdala, a set of subcortical nuclei anatomically grouped together, is part of the limbic system in the brain and is important for emotional reactions, such as fear and anger. Among the most common reactions the amygdala triggers to potential dangerous events is the "freezing" response (catalepsy), a widespread form of defensive behavior common in nature and found in many animal species (Lang & Davis, 2006). The amygdala plays a special role in detecting environmental threats and in coordinating the appropriate responses to dangerous events.

One may speculate that the inhibition we documented in response to the occurrence of standards and of oddballs is a type of a stereotyped fear–freezing response. Of course, such freezing of the oculomotor activity turns out to be even greater when the stimulus is an oddball (i.e., a rare and surprising event). In this regard, the existence of an extrageniculo–striate (colliculo–thalamo–amygdala) neural pathway involved in the analysis of fear-related visual stimuli is well documented (Morris, Öhman, & Dolan, 1999). Furthermore, it has been shown in the rat that the reentrant pathway from the amygdala to the SC is necessary for the expression of fear-related reflexes (Meloni & Davis, 1999; Zhao & Davis, 2004).

To conclude, the present study confirms recent evidence that the human oculomotor system produces a specific microsaccadic response when a new stimulus is presented at fixation (Valsecchi et al., 2007). This response consists of a reduction of the microsaccadic frequency about 100 ms after the appearance of the stimulus, followed either by a subsequent rebound, if the stimulus is a standard, or by a maintenance of the reduced microsaccadic activity, if the stimulus is an oddball. Crucially, however, we have extended these previous findings by showing that the same characteristic microsaccadic responses can be observed when the stimuli are invisible to the SC, which strongly suggests the possibility that the SC does not autonomously control this response. Although little is known about the functional significance of the microsaccadic response to new events in

the visual field, our data indicate that the reduction of microsaccadic activity is controlled by cortical centers that are not color blind. Finally, a more speculative possibility, which deserves further investigation, is that the inhibition of activity in the oculomotor system might be part of a freezing-like response evoked through the limbic system whenever a new and potentially dangerous visual event occurs in the visual field.

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