

Working memory load modulates microsaccadic rate

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Microsaccades are tiny eye movements that individuals perform unconsciously during fixation. Despite that the nature and the functions of microsaccades are still lively debated, recent evidence has shown an association between these micro eye movements and higher order cognitive processes. Here, in two experiments, we specifically focused on working memory and addressed whether differential memory load could be reflected in a modulation of microsaccade dynamics. In Experiment 1, participants memorized a numerical sequence composed of either two (low-load condition) or five digits (high-load condition), appearing at fixation. The results showed a reduction in the microsaccadic rate in the high-load compared to the low-load condition. In Experiment 2, five red or green digits were always presented at fixation. Participants either memorized the color (low-load condition) or the five digits (high-load condition). Hence, visual stimuli were exactly the same in both conditions. Consistent with Experiment 1, microsaccadic rate was lower in the high-load than in the low-load condition. Overall, these findings reveal that an engagement of working memory can have an impact on microsaccadic rate, consistent with the view that microsaccade generation is pervious to top-down processes.

these fixational movements are important for vision, during the last fifteen years the study of microsaccades has experienced the most flourishing interest among researchers (see Rolfs, 2009).

Microsaccades are the largest of fixational eye movements ($< 0.5^{\circ}$ – 1° ; see Collewyn & Kowler, 2008; Martinez-Conde, Macknik, Troncoso, & Hubel, 2009; Martinez-Conde, Otero-Millan, & Macknik, 2013; Poletti & Rucci, 2016; see also Nyström, Hansen, Andersson, & Hooge, 2016). Generally, during fixation, the base frequency of microsaccades is about one or two per second (see Martinez-Conde et al., 2013). However, after perceptual transients, they show a peculiar rate signature characterized by an inhibition phase, followed by a rebound phase and a return to the baseline (see Engbert & Kliegl, 2003; Hafed & Ignashchenkova, 2013; Rolfs, 2009). Moreover, as suggested by their name, microsaccades also share a variety of characteristics with saccades (see Martinez-Conde et al., 2009): For instance, microsaccades are generally binocular (Ciuffreda & Tannen, 1995; see also Gautier, Bedell, Siderov, & Waugh, 2016) and characterized by a positive correlation between their amplitude and peak velocity, a pattern known as “main sequence” (Zuber, Stark, & Cook, 1965).

The vast majority of the studies concerning microsaccades attempted to delineate their role both for vision and perception. In this regard, it seems that microsaccades contribute to the maintenance of a precise fixation by correcting displacements due to drifts (e.g., Engbert & Kliegl, 2004) or eye blinks (e.g., Costela et al., 2014), and prevent both foveal and peripheral image fading by keeping retinal image in motion (e.g., Costela, McCamy, Macknik, Otero-

Introduction

Our eyes never stop. Even when we try to hold our eyes as still as possible, tiny, involuntary, and unconscious eye movements take place. These “fixational” eye movements can be classified in three distinct categories: drift, tremor, and microsaccades. Even if all

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Millan, & Martinez-Conde, 2013; Martinez-Conde, Macknik, Troncoso, & Dyar, 2006; McCamy et al., 2012; McCamy, Macknik, & Martinez-Conde, 2014). Furthermore, microsaccades are also involved in visual search within small regions (e.g., Otero-Millan, Troncoso, Macknik, Serrano-Pedraza, & Martinez-Conde, 2008), in tasks that require substantial visual precision (e.g., Ko, Poletti, & Rucci, 2010) and in information acquisition during the scanning of natural scenes (McCamy, Otero-Millan, Di Stasi, Macknik, & Martinez-Conde, 2014). Even if a full consensus about the possible functions of microsaccades has not yet been achieved (e.g., Collewijn & Kowler, 2008; Martinez-Conde et al., 2009; Martinez-Conde et al., 2013; Poletti & Rucci, 2016; Rolfs, 2009), their relevance for our visual system is unquestionable.

Another fascinating stream of studies provided evidence suggesting that microsaccades are associated even with some higher cognitive mechanisms. For instance, in a set of experiments, Engbert and Kliegl (2003) observed that the direction of microsaccades was correlated with the direction of attentional shifts elicited by predictive symbolic cues presented at fixation (see also Pastukhov & Braun, 2010), a finding reported even in response to central social stimuli (i.e., eye-gaze direction; Deaner & Platt, 2003; Yokoyama, Noguchi, & Kita, 2012) and peripheral cues (e.g., Betta, Galfano, & Turatto, 2007; Galfano, Betta, & Turatto, 2004; Hafed & Clark, 2002; Rolfs, Engbert, & Kliegl, 2005). Preparatory processes could also impact on microsaccadic response, as a decrement in their absolute frequency has been reported when participants are asked to provide a manual response to an upcoming signal, as compared with a condition in which no manual response is required (Betta & Turatto, 2006).

Recent research evidence has been reported showing that microsaccades statistics can be modulated by several factors such as the perceptual awareness of the stimuli (White & Rolfs, 2016) and the perceptual properties of the stimuli (e.g., Bonnef, Adini, & Polat, 2015; Rolfs, Kliegl, & Engbert, 2008). More relevant for the present study, a decrement in microsaccadic frequency has been documented when individuals were involved in mental counting and arithmetic (Gao, Yan, & Sun, 2015; Siegenthaler et al., 2014; Valsecchi, Betta, & Turatto, 2007). In more detail, Valsecchi et al. (2007) employed a visual oddball task and observed that microsaccadic rate was lower in response to rare visual targets, but only when participants were required to actively count these occurrences. Interestingly, the decrement of microsaccadic rate was observed only in the rebound phase. On the contrary, when participants were asked to look at the stimuli passively (i.e., without counting), microsaccadic rate did not differ between standard and rare stimuli (for similar results see also Valsecchi, Dimigen, Kliegl, Sommer, & Turatto, 2009;

Valsecchi & Turatto, 2007, 2009). More recently, Siegenthaler et al. (2014) and Gao et al. (2015) found an inverse relation between task difficulty and microsaccadic rate. In more detail, Siegenthaler et al. (2014) asked participants to mentally count forward (low difficulty) or backward (high difficulty) while looking at a fixation spot that was the only visual stimulus employed in the task. On the other hand, Gao et al. (2015) asked participants to sum or to subtract, from a visually presented number, a second one that could be either small (low difficulty) or large (high difficulty). In both studies, when participants were asked to perform difficult arithmetic, an overall decrement in microsaccadic rate was observed (Gao et al., 2015; Siegenthaler et al., 2014). Interestingly, the decrement reported by Gao et al. (2015) emerged in the rebound phase, in line with Valsecchi et al. (2007, 2009).

Crucially, in all the aforementioned studies (Gao et al., 2015; Siegenthaler et al., 2014; Valsecchi et al., 2007, 2009), a latent mechanism could have contributed to the modulation of microsaccadic rate: working memory (e.g., Baddeley, 1992). Indeed, the participants were requested to perform complex mental operations as, for instance, forward versus backward counting (Siegenthaler et al., 2014), or arithmetic verification tasks (Gao et al., 2015) that are known to call into play different arithmetic operations such as carrying and borrowing (e.g., DeStefano & LeFevre, 2004). Whereas there is a well-established, albeit indirect, link between this type of tasks and the involvement of working memory, as shown by the fact that performance in these tasks is impaired by concomitant high-demanding secondary tasks (e.g., Logie, Gilhooly, & Wynn, 1994, also see DeStefano & LeFevre, 2004), arithmetic tasks involve several processing stages and operations that are highly domain-specific (e.g., Dehaene, Molko, Cohen, & Wilson, 2004). Following this reasoning, the evidence of a link between working memory and microsaccade dynamics is still indirect and in need of further investigation.

The present study represents the first attempt to directly assess the potential role of working memory in shaping microsaccadic rate. In two experiments, participants were asked to hold in memory a shorter (low-load condition) or a longer (high-load condition) list of items, applying a standard test of working memory, namely a modified version of the Sternberg task (Sternberg, 1966, 1975). At the same time, they were also asked to maintain fixation on a central spot. In more detail, in Experiment 1, at the beginning of each trial participants were required to memorize a numerical sequence composed of either two digits (low-load condition) or five digits (high-load condition) that appeared at fixation. After this learning phase, the digits disappeared and the retention phase started. Finally, a memory test was administered. Experiment 2

had the same structure, but the numerical sequence was always composed of five digits all colored in either red or green. Participants were asked to memorize either the color (low-load condition) or the digits (high-load condition) of the numerical sequence. In so doing, the same visual stimuli were employed in both conditions. In both experiments, in the retention phase (i.e., when the numerical stimuli were not physically available to the participants) we expected to observe a decrement in microsaccadic rate in the high-load condition as compared to the low-load condition. Based on previous studies (Valsecchi et al., 2007, 2009; see also Gao et al., 2015), we expected to observe this reduction especially during the rebound phase, whereas we had no specific predictions about eventual differences in the postrebound sustained activity.

Methods

Experiment 1

Participants

Participants were 24 undergraduates (Mean age = 22 years, $SD = 1.6$, 13 males, 11 females) with normal or corrected-to-normal vision who took part in exchange of course credits. All the participants were naïve to the purpose of the experiment. The study has been conducted in accordance with the Declaration of Helsinki.

Apparatus

Eye movements were recorded binocularly at 500 Hz by using an EyeLink 1000 Plus (SR Research Ltd., Ottawa, Canada). Participants sat approximately 65 cm away from a 24-in monitor (1280×1024 pixels, 120 Hz). A chinrest was used in order to prevent head movements. A display PC running Experiment Builder (SR Research Ltd.) handled timing and stimuli presentation.

Procedure

Color background was set to gray and stimuli were set to black. A nine-point calibration and a validation procedure were followed by the experimental session. Before each trial, participants were asked to fixate on a centrally placed circle (0.4° in diameter), and then the experimenter initiated the trial through the host PC. This procedure ensured that participants fixated on the center of the screen and allowed us to perform a drift checking. A successful drift checking was accompanied by a brief tone that informed the participants of the imminent start of the trial.

Each trial began with the presentation of a centrally placed circle (0.4° in diameter) that remained on the screen for the whole duration of the trial (Figure 1, Fixation frame). After 500 ms, a numerical sequence (14-point Arial) composed of either two digits (low-load condition; $0.3^\circ \times$ height 0.5° width) or five digits (high-load condition, $0.3^\circ \times$ height 1.3° width) appeared 0.4° above the circle for 1500 ms. In both conditions, digits were randomly selected from the range 1–9, with the constraint that a given digit could only appear once in the sequence. Participants were asked to memorize the digits while maintaining their eyes on the centrally placed circle (Figure 1, Learning frame). After that, the digits disappeared for 2000 ms (Figure 1, Retention frame). Finally, two probe digits reappeared 0.4° above the central circle and remained visible until the participant responded or 3000 ms were elapsed, whichever came first (Figure 1, Test frame). Participants were asked to provide a manual response on a standard keyboard (keys D or K, counterbalanced across participants) to decide whether the two probe digits were presented in the same order as they had been presented in the learning frame (for a similar procedure see, for instance, Dalton, Lavie, & Spence, 2009). Responses had to be as accurate as possible and without speed pressure. After each response, a first visual feedback (500 ms) informed the participants about their performance; then a second visual feedback (1000 ms) invited the participants to blink, if needed.

Participants were instructed to maintain their eyes on the fixation spot and to avoid blinking for the whole trial duration; otherwise in both cases a visual feedback appeared for 1000 ms, and the trial was automatically aborted and appended at the end of the session. This allowed us to collect a reasonable number of blink-free epochs while avoiding an excessive duration of the experiment. In either low- and high-load conditions, 15 practice trials were followed by 80 experimental trials. A short break was allowed every 20 trials. Block order was counterbalanced across participants. The whole experiment lasted about 1 hr.

Results

Working memory task: Data from one participant were removed because his accuracy rate in the high-load condition approached chance level (48.8%), leaving the sample composed of 23 individuals (Mean age = 22 years, $SD = 1.6$, 12 males, nine females).

As for accuracy data, the percentage of missed responses was very low (0.5%) and therefore no further analyses were carried out. The percentage of incorrect responses (9%) was analyzed through a two-tailed paired t test comparing low- and high-load conditions, and the results confirmed the presence of a significant difference, $t(22) = 6.86$, $p < 0.001$, $d > 1$. Furthermore,

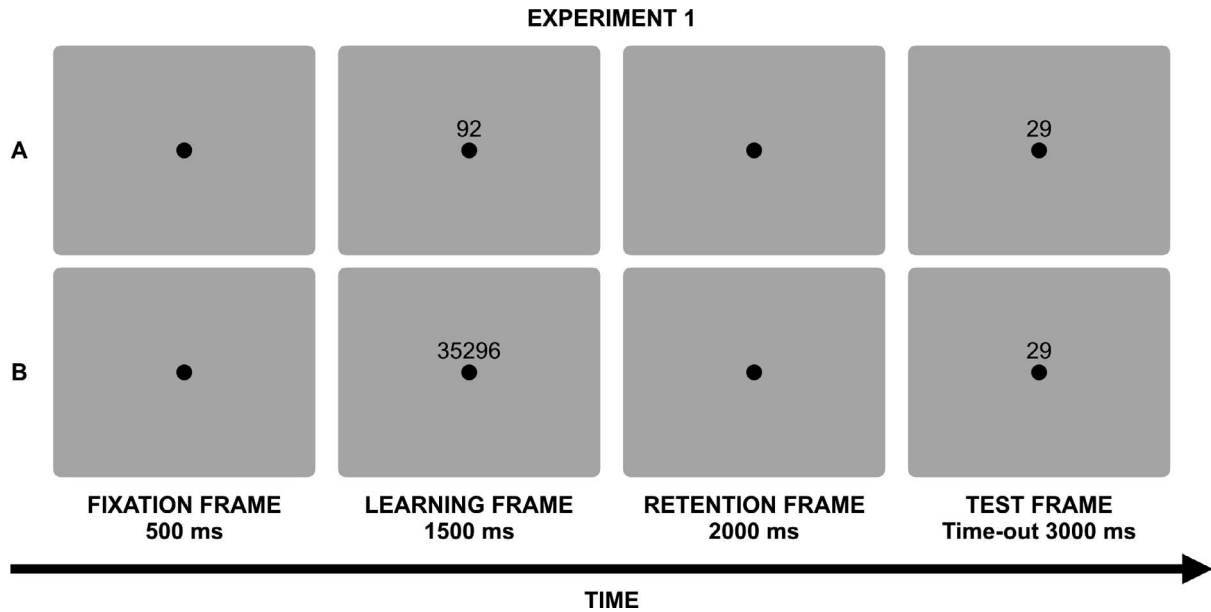


Figure 1. Stimuli (not drawn to scale) and sequence of events for Experiment 1. Participants were asked to fixate on the central spot for the whole duration of the trial, and to provide a manual response in the “Test frame.” Panel A depicts a trial of the low-load condition, in which the probe digits in the “Test frame” were presented in the opposite order as they had been presented in the learning frame. Panel B depicts a trial of the high-load condition, in which the probe digits in the “Test frame” were presented in the same order as they had been presented in the learning frame.

in order to assess which model (i.e., H0 vs. H1) was more likely supported by the current data, the Bayes Factor (BF; e.g., Rouder, Speckman, Sun, Morey, & Iverson, 2009) was also computed. This analysis indicated that, as expected, the model supporting H1 (i.e., low-load condition \neq high-load condition) was preferable over the model supporting H0 (i.e., low-load condition = high-load condition), $BF_{10} > 150$. Specifically, participants committed fewer errors in the low-

load condition ($M = 1.9\%$, $SE = 0.52$) than in the high-load condition ($M = 15.7\%$, $SE = 1.97$; see Figure 2).

As for reaction times (RTs), the latencies of correct responses were analyzed through a two-tailed paired t test between low- and high-load conditions, confirming the presence of a significant difference, $t(22) = 16.08$, $p < 0.001$, $d > 1$. BF analysis indicated that H1 was preferable over H0, $BF_{10} > 150$. RTs were smaller in the low-load condition ($M = 689$ ms, $SE = 24$) than in the high-load condition ($M = 1342$ ms, $SE = 48$).

Overall, these results indicate that the manipulation of working memory load was effective.

Microsaccadic rate: Microsaccades were detected and analyzed by employing a modified version of the algorithm proposed by Engbert and Kliegl (2003), adapted for the 500-Hz sampling frequency used in the present study and implemented in MATLAB. The velocity threshold was set to $\lambda = 4$ whereas the minimum duration threshold was set to four samples (e.g., Gautier et al., 2016). Finally, only binocular microsaccades on correctly responded trials and with a maximum amplitude of 1° were considered (see Martinez-Conde et al., 2009, 2013).

We focused our analyses on 1000-ms epochs centered around the retention period (Figure 1, Retention frame). These epochs started 200 ms before the disappearance of the digits and ended 800 ms after.

Firstly, we tested whether in the present study we detected microsaccades accurately. Since it is well known that most microsaccades, similarly to saccades,

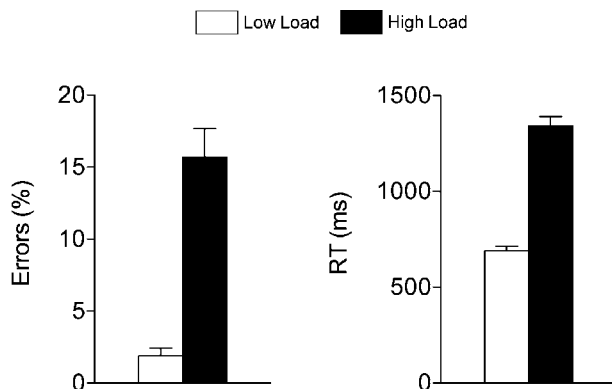


Figure 2. Mean accuracy and reaction times observed in the manual task of Experiment 1. Error bars depict the standard error of the mean. Smaller RTs and fewer errors in the low-load condition—as compared to the high-load condition—confirmed that the manipulation of working memory load was effective.

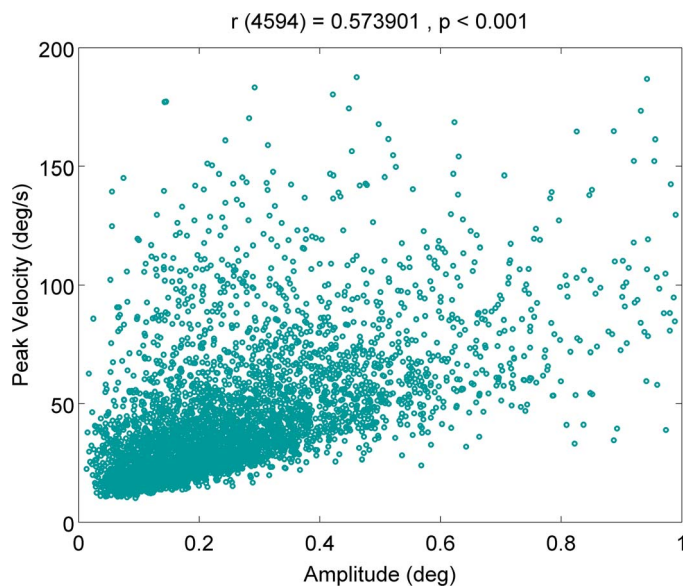


Figure 3. Positive correlation between amplitude and peak velocity in Experiment 1. This “main sequence” (see Zuber et al., 1965) confirmed that microsaccades were correctly identified. The plot is based on 4,594 microsaccades.

show a peculiar positive correlation between amplitude and peak velocity (the “main sequence,” Zuber et al., 1965), we performed a correlation analysis between these two measures. The results confirmed that amplitude and peak velocity were positively correlated, $r(4594) = 0.574$, $p < 0.001$ (see Figure 3). BF analyses confirmed that H1 (amplitude correlates with peak velocity) was preferable over H0 (amplitude does not correlate with peak velocity), $BF_{10} > 150$. Hence, microsaccades were identified correctly.

Secondly, we computed the microsaccadic rate within the 1000-ms epochs. This was achieved by calculating microsaccadic rate separately for each participant and experimental condition, and then averaging these data across participants. As depicted in Figure 4, after the disappearance of the digits ($t = 0$), microsaccadic rate showed a period of inhibition followed by period of rebound. Overall, this pattern of results is fully consistent with previous evidence (see Rolfs, 2009). In order to test our hypothesis, we focused our main analysis in a 200-ms time window roughly centered around the rebound period (i.e., 200–400 ms). Furthermore, other two 200-ms time windows were extracted before (0–200 ms, inhibition period) and after (400–600 ms, baseline period) our critical time window. Two-tailed paired t tests were performed between microsaccades observed in the low-load and in the high-load conditions in each of the three windows. The only significant result emerging from this analysis was that concerning the 200–400 ms time window, $t(22) = 2.02$, $p < 0.05$, $d = 0.4$, due to more microsaccades in the low-load condition ($M = 1.5$ Hz, $SE = 0.16$) than in

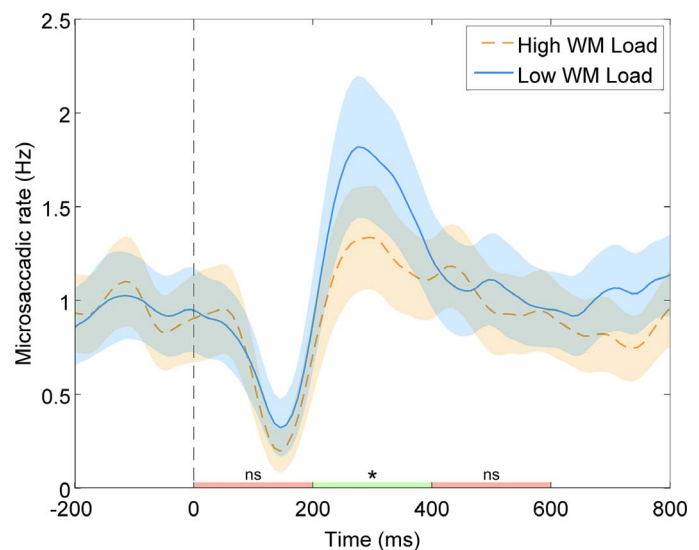


Figure 4. Mean microsaccadic rate calculated within 1000-ms epochs centered around the disappearance of the digits ($t = 0$; learning frame offset) in Experiment 1. Shaded areas indicate the standard error of the mean. Red and green areas above x axis indicate the three time windows (i.e., 0–200 ms, 200–400 ms, 400–600 ms) used for statistical testing. Asterisk denotes a significant difference (i.e., $p < 0.05$) between the two experimental conditions while “ns” means that the difference was nonsignificant. Fewer microsaccades emerged in the high-load condition as compared to the low-load condition, and this was evident only in the time window centered around the rebound phase (i.e., 200–400 ms).

the high-load condition ($M = 1.2$ Hz, $SE = 0.11$). BF analysis indicated that H1 was preferable over H0, $BF_{10} = 1.2$. The comparisons involving the control time windows (0–200 ms and 400–600 ms) did not yield any significant effect ($ts < 1$, $ps > 0.8$, $BF_{10}s < 1$, two-tailed).¹

Additional analyses were also performed to explore whether WM load exerted any effect on microsaccadic rate during the learning frame. These analyses were performed using different a priori-determined time windows (150-ms and 200-ms steps) as well as considering the whole 1500-ms time window. No significant differences emerged.

Experiment 2

Overall, the results of Experiment 1 suggest that working memory load plays a key role in shaping microsaccadic rate. More specifically, during the rebound period, fewer microsaccades were observed in the high-load condition than in the low-load condition. However, it should be noted that during the learning frame participants were exposed to two perceptually different visual stimuli, namely a numerical sequence

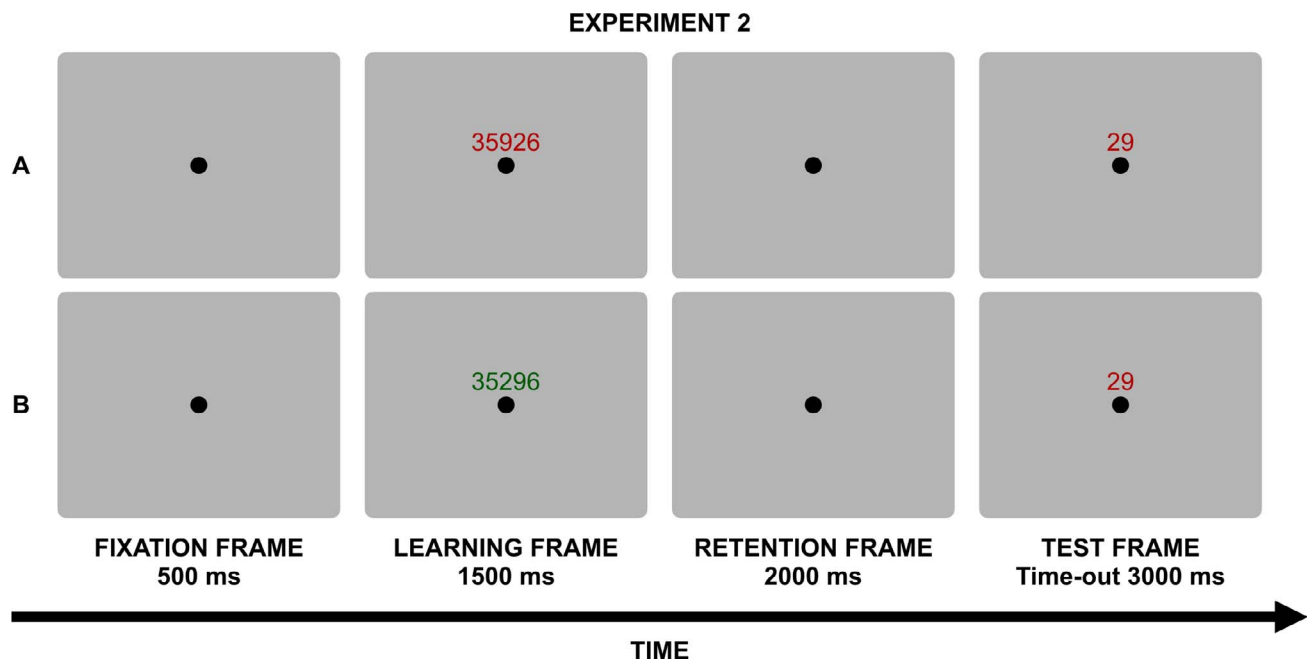


Figure 5. Stimuli (not drawn to scale) and sequence of events for Experiment 2. Participants fixated on the central spot throughout the trial, and provided a manual response in the “Test frame.” Depending on condition, participants had to either remember the color (low-load condition) or the five digits (high-load condition) of the numerical sequence. Panel A depicts a trial in which the probe digits in the “Test frame” were presented in the opposite order as they had been presented in the “Learning frame,” and the color remained unchanged. Panel B depicts a trial in which the probe digits in the “Test frame” were presented in the same order as they had been presented in the “Learning frame,” and the color changed from green to red.

composed of two (low-load condition) or five digits (high-load condition). The aim of Experiment 2 was to conceptually replicate the findings of Experiment 1 by adopting an even more controlled experimental setting. Indeed, participants were here presented with the same visual stimuli in both the high-load and low-load conditions. This was achieved by presenting participants, in the learning frame, always with five digits all colored in either red or green. Depending on the experimental condition, participants were instructed to either remember the color (low-load condition) or the five digits (high-load condition) of the numerical sequence.

Participants

Participants were 20 undergraduates (Mean age = 24 years, $SD = 2.01$, 5 males) with normal or corrected-to-normal vision who took part in exchange of course credits. All the participants were naïve to the purpose of the experiment. None of them had taken part in Experiment 1. The study has been conducted in accordance with the Declaration of Helsinki.

Apparatus

Everything was identical to Experiment 1.

Procedure

Everything was identical to Experiment 1, with the following exceptions: First, in the learning frame, there were always five digits; second, the five digits displayed in the learning frame and the two probe digits displayed in the test frame were all colored in either red or green; finally, participants were asked to either remember the color (low-load condition) or the five digits (high-load condition) of the numerical sequence, depending on condition (see Figure 5).

Results

Working memory task: Manual responses were analyzed in the same manner as in Experiment 1.

As for accuracy, missed responses were not analyzed due to their low percentage of occurrence (0.4%) while the percentage of uncorrected responses (8%) was analyzed through a two-tailed, paired t test between low- and high-load conditions. The analysis confirmed the presence of a significant difference, $t(19) = 5.05$, $p < 0.001$, $d > 1$, and BF analysis indicated that H1 was preferable over H0, $BF_{10} > 150$. Specifically, participants committed fewer errors in the low-load condition ($M = 2.4\%$, $SE = 0.64$) than in the high-load condition ($M = 13.3\%$, $SE = 2.85$; see Figure 6).

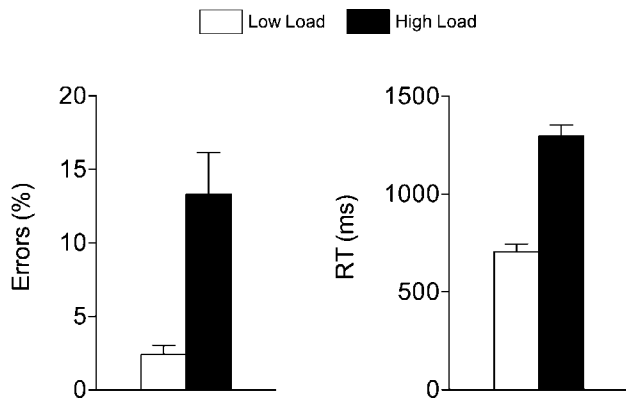


Figure 6. Mean accuracy and reaction times observed in the manual task of Experiment 2. Error bars depict the standard error of the mean. As in Experiment 1, smaller RTs and fewer errors in the low-load condition—as compared to the high-load condition—confirmed that the manipulation of working memory load was effective.

RTs for correct responses were analyzed through a two-tailed, paired t test between low- and high-load conditions. The results confirm that the two conditions were different, $t(19) = 13.93$, $p < 0.001$, $d > 1$. BF analysis indicated that H1 was preferable over H0, $BF_{10} > 150$. Specifically, RTs were smaller in the low-load condition ($M = 706$ ms, $SE = 40$) than in the high-load condition ($M = 1297$ ms, $SE = 57$; see Figure 6).

These results confirm that the manipulation of working memory load was effective.

Microsaccadic rate: Microsaccades were analyzed in the same manner as in Experiment 1. Amplitude and peak

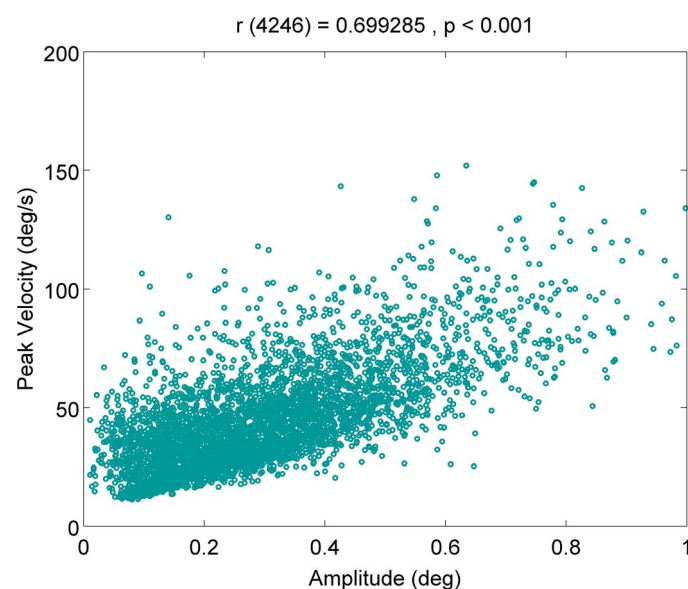


Figure 7. Positive correlation between amplitude and peak velocity in Experiment 2. As in Experiment 1, this “main sequence” (see Zuber et al., 1965) confirmed that microsaccades were correctly identified. The plot is based on 4,246 microsaccades.

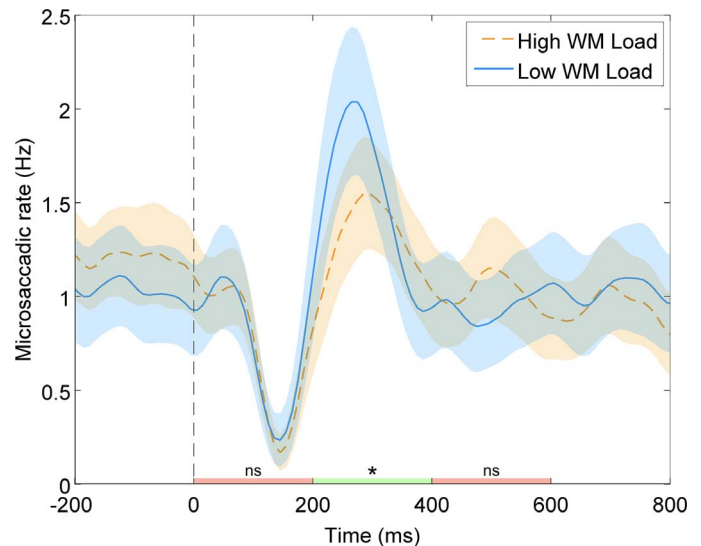


Figure 8. Mean microsaccadic rate calculated within 1000-ms epochs centered around the disappearance of the digits ($t = 0$) in Experiment 2. Shaded areas indicate the standard error of the mean. Red and green areas above x axis indicate the three time windows (i.e., 0–200 ms, 200–400 ms, 400–600 ms) used for statistical testing. Asterisk denotes a significant difference (i.e., $p < 0.05$) between the two experimental conditions while “ns” means that the difference was nonsignificant. As in Experiment 1, fewer microsaccades emerged in the high-load condition as compared to the low-load condition, and this was evident only in the time window centered around the rebound phase (i.e., 200–400 ms).

velocity were positively correlated, $r(4246) = 0.699$, $p < 0.001$ (Figure 7), and BF analyses confirmed that H1 was preferable over H0, $BF_{10} > 150$, confirming that microsaccades were identified correctly.

Moreover, as in Experiment 1, the only significant difference between the low-load condition and the high-load condition emerged in the 200–400 ms time window, $t(19) = 2.25$, $p = 0.037$, $d = 0.5$, (two-tailed), due to more microsaccades in the low-load condition ($M = 1.5$ Hz, $SE = 0.17$) than in the high-load condition ($M = 1.3$ Hz, $SE = 0.12$; see Figure 8). BF analysis indicated that H1 was preferable over H0, $BF_{10} = 2$. The other two comparisons yielded nonsignificant effects ($ts < 1.1$, $ps > 0.3$, $BF_{10s} < 1$, two-tailed).²

As in Experiment 1, the analyses conducted on the data collected during the learning frame did not lead to any significant result.

Discussion

The aim of this study was to provide the first evidence of a direct role of working memory in shaping microsaccade dynamics. Indeed, even if results stem-

ming from previous studies suggested an influence of working memory on the microsaccade generation system (e.g., Gao et al., 2015; Valsecchi et al., 2007), so far a direct evidence supporting this relationship was missing.

Here, in two experiments, participants were administered a modified version of the Sternberg test (Sternberg, 1966, 1975), which required to retain in memory either a short (low-load condition) or a long (high-load condition) list of items. At the same time, they fixated on a centrally-placed spot and eye movements were recorded binocularly. In both experiments, the results revealed that microsaccadic rate was inversely related to working memory load: Fewer microsaccades were performed in the high-load condition as compared to the low-load condition.

The existence of a relationship between working memory and eye movements has been previously documented through several oculomotor measures other than microsaccades. For instance, there is evidence that saccadic latency (Schaeffer, Chi, Krafft, Li, Schwarz, & McDowell, 2015), saccadic suppression (Mitchell, Macrae, & Gilchrist, 2002; Roberts, Hager, & Heron, 1994), saccadic curvature (Theeuwes, Olivers, & Chizk, 2005) and even the pupillary light reflex (Blom, Mathôt, Olivers, & Van der Stigchel, 2016), are influenced by mechanisms related to working memory functioning. A possible explanation for this link calls into question the potential role of the Superior Colliculus (SC). Indeed, it is well known that the SC is highly engaged during the execution of saccadic eye movements (Hafed & Chen, 2016; Sparks, 1988), and there is also evidence supporting a role of the SC in both saccadic curvature (Van der Stigchel, Meeter, & Theeuwes, 2006) and pupil response (Wang, Boehnke, White, & Munoz, 2012). Also for microsaccades, even if the neural pathway that would support these tiny movements is still debated (e.g., Otero-Millan, Macknik, Serra, Leigh, & Martinez-Conde, 2011), converging evidence indicates the rostral pole of the SC as the most likely neural generator of microsaccades (e.g., Hafed, Goffart, & Krauzlis, 2009; see also Martinez-Conde et al., 2013). This notion is also in line with a model of microsaccade generation put forward by Rolfs et al. (2008), which correctly predicted the involvement of the rostral pole of the SC in microsaccades before neurophysiological data were available. At the same time, there is also evidence that some output neurons of the dorsolateral prefrontal cortex, a cortical area that plays a crucial role in working memory (e.g., Gilbert et al., 2006), directly project to the SC during working memory tasks (Johnston & Everling, 2009). Moreover, a recent study (Shen, Bezgin, Selvam, McIntosh, & Ryan, 2016) has proposed the existence of a broad neural network that would mediate the exchange of information between

memory and oculomotor systems. Taken together, these pieces of evidence seem to suggest that ongoing memory tasks can modulate the SC functioning and, in turn, this modulation might be reflected in oculomotor responses.

Another coherent pattern of results emerging from the present experiments is that, whereas the microsaccadic response in the low- and high-load conditions was virtually identical during the inhibition phase, a clear difference emerged in the rebound phase. This difference between the inhibition and the rebound phases is fully consistent with previous studies in which working memory has likely played a role (e.g., Valsecchi et al., 2007, 2009; see also Gao et al., 2015) and, more in general, is in line with the present knowledge concerning microsaccadic generation. Indeed, because the inhibition phase arises very quickly after perceptual transients, it is likely that this behavior might reflect the involvement of a fast subcortical pathway. This could explain why the inhibition phase seems particularly stable and robust across studies. On the other hand, the rebound phase requires, by definition, more time to emerge and several higher-order cognitive processes can therefore take place and influence this stage (see Engbert, 2006; see also Rolfs, 2009). Even if the precise mechanisms shaping this biphasic microsaccadic response are not entirely understood, it seems clear that microsaccades cannot be merely confined to oculomotor responses supporting vision, but rather they might also be considered as a multifaceted index of cognitive processing. Importantly, Gao et al. (2015) observed that sustained cognitive activity was associated with a long-lasting reduced microsaccadic rate for the high-difficulty condition, whereas we observed no differences in the postrebound phase. In this regard, however, it should be noted that participants in Gao et al. (2015) and in the present experiments were likely involved in very different mental operations. Indeed, in the time window in which microsaccades were detected by Gao et al. (2015), participants had to compute additions/subtractions (see also Zhou et al., 2006, for electrophysiological supporting evidence) whereas in the current experiments retention in WM was the only mental operation required. This key difference might explain why the difference in microsaccadic rate between low and high load was much more sustained in the study by Gao et al. (2015) than in the present experiments.

The results of the present experiments are also aligned with a stream of studies that observed a microsaccadic modulation due to mental tasks. Indeed, while microsaccades have been traditionally examined in association with perceptual stimulation, both in the visual (e.g., Engbert & Kliegl, 2003; Ko et al., 2010; McCamy et al., 2012) and in the auditory modalities (e.g., Rolfs, Engbert, & Kliegl, 2005; Rolfs et al., 2008),

only few studies investigated the additional role of higher order cognitive processing, such as mental arithmetic, on microsaccade dynamics (Gao et al., 2015; Siegenthaler et al., 2014). Here, we confirmed that even a pure working memory manipulation is reflected in microsaccadic rate. In this regard, it is important to note that in Experiment 2 the visual displays in both low- and high-load conditions were physically identical (for a similar approach, see also Siegenthaler et al., 2014), unlike Experiment 1 in which the displays in the learning frame were composed of either two (low-load condition) or five (high-load condition) digits. Because in both experiments the results were virtually identical, it is reasonable to conclude that they have been shaped by mental mechanisms related to working memory, rather than by mere perceptual processes.

To conclude, this study suggests the existence of a link between working memory and the microsaccadic generator system and strengthens the idea that even involuntary and unconscious fixational eye movements—such as microsaccades—are pervious to higher level cognitive mechanisms that are not primarily involved in either vision or perceptual mechanisms.

Conclusion

In two experiments, we revealed that working memory load shapes microsaccadic rate. In particular, in the high-load condition a lower microsaccadic rate was observed with respect to the low-load condition. These results confirm that microsaccades are sensitive to higher order cognitive processes and prompt us to consider these microscopic eye movements as a powerful and noninvasive tool that can be used to shed fresh light on the fascinating interplay between vision, eye movements, and cognition.

Keywords: microsaccades, working memory, eye movements

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Footnotes

¹ Because microsaccadic amplitude is debated (see Collewijn & Kowler, 2008; Nyström et al., 2016; Poletti & Rucci, 2016; Rolfs, 2009), the same analyses were also conducted by considering microsaccades with a maximum amplitude of 0.5° (see Poletti & Rucci, 2016). Similar results emerged. Microsaccadic amplitude and peak velocity were positively correlated, $r(4209) = 0.464$, $p < 0.001$, $BF > 150$. As for the microsaccadic rate, the only significant comparison between low- and high-load conditions emerged in the 200–400 ms time window ($M_{\text{low}} = 1.4$ Hz, $SE = 0.15$, $M_{\text{high}} = 0.99$ Hz, $SE = 0.11$, $t(22) = 2.6$, $p = 0.016$, $d = 0.5$, $BF_{10} = 3.3$, two-tailed), while the other two comparisons were both nonsignificant ($ts < 1$, $ps > 0.3$, $BF_{10s} < 1$, two-tailed). Microsaccadic amplitude was analysed as well. Indeed, since there is evidence that microsaccadic amplitudes increase with task difficulty (see Siegenthaler et al., 2014), the same pattern of results could be also expected in a working memory load task. The analyses supported this scenario. Mean microsaccadic amplitude was calculated within the three time windows employed for the microsaccadic rate analyses. Overall, microsaccadic amplitude was smaller in the low-load condition than in the high-load condition, both in the 200–400 ms time window ($M_{\text{low}} = 0.208^\circ$, $SE = 0.012$, $M_{\text{high}} = 0.3^\circ$, $SE = 0.025$, $t(22) = 4.89$, $p < 0.001$, $d > 1$, $BF_{10} > 150$, two-tailed), and in the other two time windows ($ts > 3.1$, $ps < 0.005$, $BF_{10s} > 9$, two-tailed). Similar results emerged also considering microsaccades with a maximum amplitude of 0.5° , both in the 200–400 ms time window ($M_{\text{low}} = 0.196^\circ$, $SE = 0.009$, $M_{\text{high}} = 0.252^\circ$, $SE = 0.014$, $t(22) = 5.12$, $p < 0.001$, $d > 1$, $BF_{10} > 150$, two-tailed) and in the other two time windows ($ts > 2.45$, $ps < 0.023$, $BF_{10s} > 2.5$, two-tailed).

² As in Experiment 1, the same analyses were also performed by considering microsaccades with a maximum amplitude of 0.5° . Similar results emerged. Microsaccadic amplitude and peak velocity were positively correlated, $r(3736) = 0.546$, $p < 0.001$, $BF > 150$. As for the microsaccadic rate, the only significant comparison between low- and high-load conditions emerged in the 200–400 ms time window ($M_{\text{low}} = 1.4$ Hz, $SE = 0.16$, $M_{\text{high}} = 1.01$ Hz, $SE = 0.1$, $t(19) = 3.69$, $p = 0.002$, $d = 1$, $BF = 25$, two-tailed), while the other two comparisons were both nonsignificant ($ts < 1.5$, $ps > 0.2$, $BF_{10s} < 1$, two-tailed). As in Experiment 1,

microsaccadic amplitudes were also analysed. Microsaccadic amplitudes were smaller in the low-load condition than in the high-load condition, both in the 200–400 ms time window ($M_{\text{low}} = 0.247^\circ$, $SE = 0.018$, $M_{\text{high}} = 0.355^\circ$, $SE = 0.022$, $t(19) = 6.09$, $p < 0.001$, $d > 1$, $BF_{10} > 150$, two-tailed) and in the other two time windows ($ts > 2.9$, $ps < 0.01$, $BF_{10s} > 5$, two-tailed). Similar results emerged also considering microsaccades with a maximum amplitude of 0.5° , both in the 200–400 ms time window ($M_{\text{low}} = 0.23^\circ$, $SE = 0.015$, $M_{\text{high}} = 0.293^\circ$, $SE = 0.016$, $t(19) = 4.71$, $p < 0.001$, $d > 1$, $BF_{10} > 150$, two-tailed) and in the other two time windows ($ts > 3.01$, $ps < 0.007$, $BF_{10s} > 6.8$, two-tailed).

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