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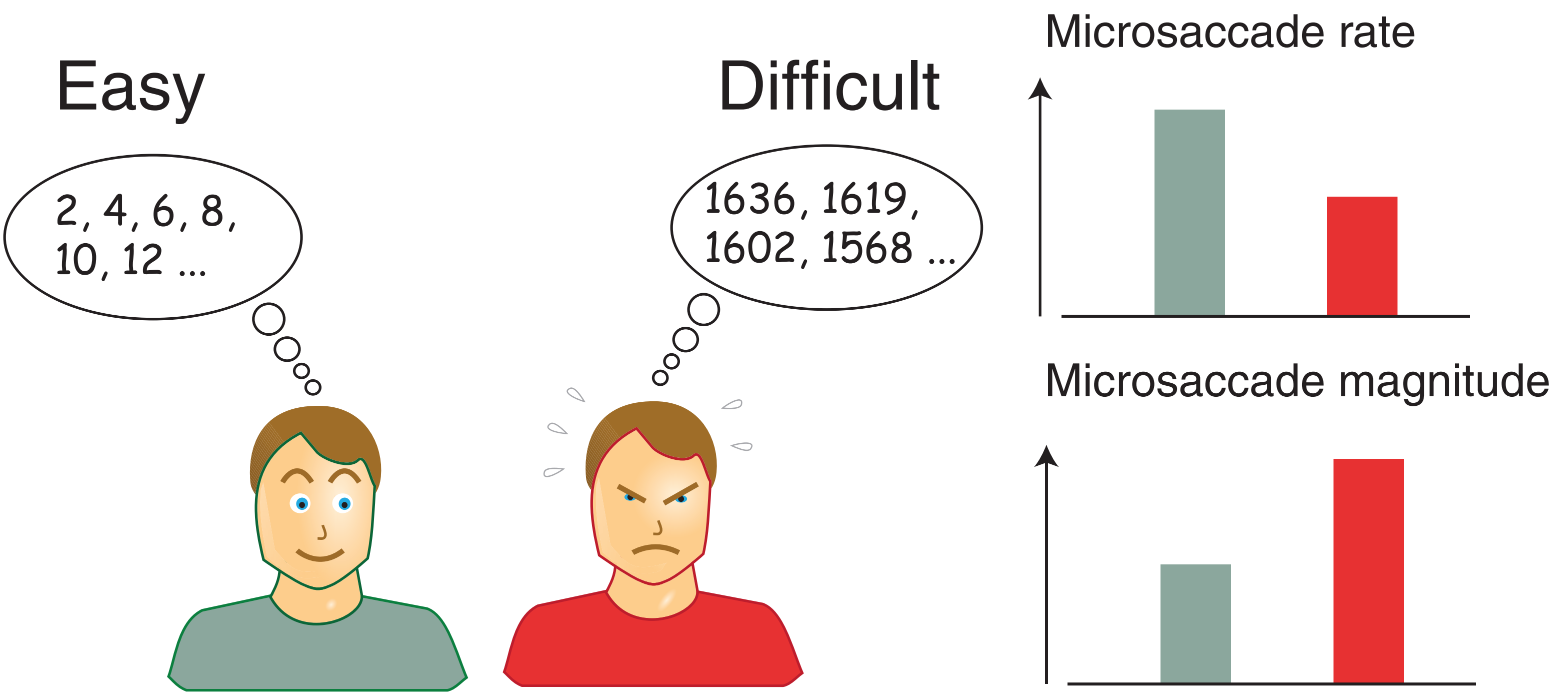

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FEATURED ARTICLE

Vesicular glutamate transporter 2 is required for the respiratory and parasympathetic activation produced by optogenetic stimulation of catecholaminergic neurons in the rostral ventrolateral medulla of mice *in vivo*

S. B. G. Abbott et al.–with commentary by D. Mendelowitz

WILEY Blackwell

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Task difficulty in mental arithmetic affects microsaccadic rates and magnitudes

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Keywords: attention, fixational eye movements, microsaccades, task load

Abstract

Microsaccades are involuntary, small-magnitude saccadic eye movements that occur during attempted visual fixation. Recent research has found that attention can modulate microsaccade dynamics, but few studies have addressed the effects of task difficulty on microsaccade parameters, and those have obtained contradictory results. Further, no study to date has investigated the influence of task difficulty on microsaccade production during the performance of non-visual tasks. Thus, the effects of task difficulty on microsaccades, isolated from sensory modality, remain unclear. Here we investigated the effects of task difficulty on microsaccades during the performance of a non-visual, mental arithmetic task with two levels of complexity. We found that microsaccade rates decreased and microsaccade magnitudes increased with increased task difficulty. We propose that changes in microsaccade rates and magnitudes with task difficulty are mediated by the effects of varying attentional inputs on the rostral superior colliculus activity map.

Introduction

Microsaccades are involuntary, small-magnitude saccadic eye movements that occur during attempted visual fixation (Martinez-Conde *et al.*, 2004, 2009, 2013; Rolfs, 2009). Recent research suggests that microsaccades and saccades share a common neural generator, and that microsaccades may serve as varied functions during fixation as saccades do during exploration (McCamy *et al.*, 2012; Martinez-Conde *et al.*, 2013; Otero-Millan *et al.*, 2013). Several studies have found that microsaccades (as saccades) can be modulated by attention, most likely due to the extensive overlap between the neural system that controls attention and the system that generates saccadic eye movements. For instance, the spatial location indicated by an attentional visual cue can bias microsaccade directions towards or away from the cue (for review, see Martinez-Conde *et al.*, 2013).

Despite the growing body of literature on the attentional modulation of microsaccades, few studies have addressed the effects of task difficulty on microsaccade parameters, with varied results (Chen *et al.*, 2008; Pastukhov & Braun, 2010; Benedetto *et al.*, 2011; Di Stasi *et al.*, 2013a). Pastukhov & Braun (2010) found that microsaccade rates decreased during the performance of high-difficulty visual tasks, but the directions of the remaining microsaccades were highly informative as to the spatial location of the attentional focus. In contrast, Benedetto *et al.* (2011) reported that microsaccade rates increased with task difficulty during a simulated driving task. Di Stasi *et al.* (2013a) found that neither task difficulty nor time-on-task affected microsaccade rates during a simulated air traffic control task (although time-on-task, but not task difficulty, did affect the microsaccadic peak velocity–magnitude relationship). Chen *et al.* (2008) found no effects of task difficulty on primate microsaccade rates.

In this previous research, microsaccade recordings took place during a variety of visual tasks with differing levels of difficulty. The influence of task difficulty on microsaccades therefore remains unclear, especially if isolated from visual processing.

Here we investigated the effects of task difficulty on microsaccade dynamics during the performance of a non-visual, mental arithmetic task. Participants fixated on a small spot while conducting one of two mental arithmetic tasks (Easy: counting forward by two; or Difficult: counting backwards by 17), or no arithmetic task (Control condition). We found that microsaccade rates decreased and microsaccade magnitudes increased with increased task difficulty.

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These results are consistent with the effects of varying attentional inputs to the microsaccade triggering circuit, as a function of task difficulty. Specifically, we propose that task difficulty-induced variations in attentional load modulate microsaccadic rates and magnitudes via changes in the intensity and shape of the rostral superior colliculus (SC) activity map.

Materials and methods

Participants

Eleven participants [five males, six females; average age: 32 years (SD ± 6.01); six native English speakers, five non-native English speakers (two Arabic, one Spanish, one Swedish and one German native speakers); 10 naive, one author (E.S.)] participated in a single experimental session. Author data were not considered in the subjective measurements analyses (see Questionnaires section). All participants were college-educated: five had PhDs and six had MSc degrees. All subjects had normal or corrected-to-normal vision. The Barrow Neurological Institute's Institutional Review Board approved the study (protocol number 10BN142). Experiments conformed with The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the *British Medical Journal* (18 July 1964; WMA, 1964). Written informed consent was obtained from each participant. Subjects were paid $40 for their participation.

Experimental design

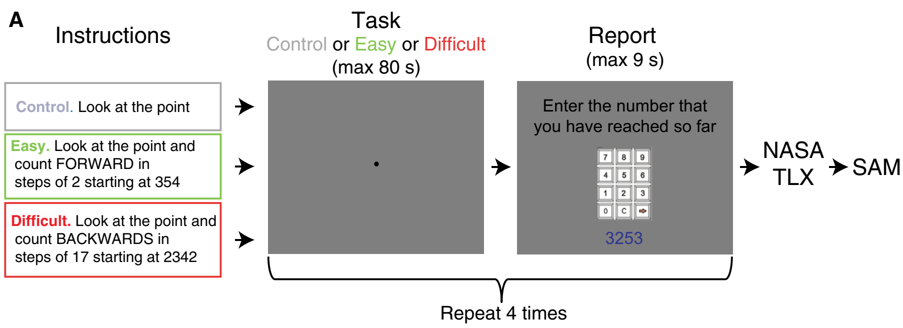
In a dark room, participants rested their forehead and chin on the EyeLink 1000 head/chin support, ~57 cm away from a linearized video monitor (Barco Reference Calibrator V, 75 Hz refresh rate). There were two experimental conditions (an Easy mental arithmetic task, and a Difficult mental arithmetic task) and one Control condition (fixation only). The experiment consisted of one session with six blocks. Each block included three trials (one trial per condition; each trial was 180 s long). Thus, each subject ran six blocks * three trials * 3 min per trial, for a total of 54 min of recorded data. The first trial in each block was always the Control task, and the last two trials corresponded to the Easy and Difficult mental arithmetic tasks. Trial sequence was balanced within each participant and randomized across participants (see Fig. 1B for one example). Participants took short breaks (~2–5 min) after each block. The entire session lasted ~1.45 h.

An instruction screen indicating the task to perform preceded each trial. Participants were instructed to look at the center of a black circular target with a diameter of 0.05 degrees of visual angle (deg) presented at the center of the monitor's screen, on a 50% gray background, in each task (Fig. 1A). A beep sounded whenever the participants' gaze wandered beyond 3 deg from the fixation target, to remind them to keep looking at it.

During the Control task, participants performed no mental arithmetic (i.e. they fixated the central target solely). During the Easy task, participants were instructed to count forwards mentally, as fast and accurately as possible, in steps of two starting at a random three-digit even number (same random numbers for each subject). During the Difficult task, participants were instructed to count mentally backwards, as fast and accurately as possible, in steps of 17 starting at a random four-digit number (same random numbers for each subject). All participants were instructed to count mentally in their native language.

A numeric keypad appeared on the screen and asked the participant to enter a number at three random times during each trial, and then again at the end of the trial (minimum of 15 s and maximum of 80 s between keypad screens; Fig. 1A). Each trial thus provided four numeric answers that served to analyse subject performance. If

A



B

Block	Trials			
1	C	E	D	Break
2	C	D	E	Break
3	C	D	E	Break
4	C	E	D	Break
5	C	E	D	Break
6	C	D	E	Break

FIG. 1. Schematic representation of the experiment. (A) Timeline for one trial. Each trial began with an instruction screen, followed by a fixation screen (fixation target magnified here for clarity). A numeric keypad appeared at three random times during the trial and once again at the end of the trial. Participants filled in subjective questionnaires (NASA-TLX and SAM) after the last keypad. (B) Example of blocking for one experimental session. A session included 6 blocks with the Control condition and the two experimental conditions (Easy and Difficult) in each block. The first trial of each block always corresponded to the Control task, and the last two trials consisted of the Easy and Difficult tasks, presented in random and balanced order. A short break (~2–5 min) followed each block.

no numeric answer was entered within 9 s, the keypad disappeared (this happened five times out of 480 total keypads across all participants). In these cases, we interpolated the number of mental calculation steps using the nearest-neighbor method).

In the Easy and Difficult tasks, participants were instructed to enter the value of their current mental calculation (Fig. 1A). In the Control task, participants were instructed to enter any number they wanted to.

Participants' eye position was calibrated at the beginning of the experimental session, and re-calibrated after each break. We used custom code and the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997; Kleiner *et al.*, 2007) to generate/display visual stimuli.

For one participant, the pupil was lost during the fourth block of the experiment. This amounted to a total of three trials (one Control, one Easy and one Difficult) of 3 min each. For this participant, we replaced the missing microsaccade rate, microsaccade magnitude and microsaccade peak velocity values with the average values from the corresponding conditions in the other five blocks (Roth, 1994).

Performance

In the Easy task, a correct answer was defined as any even number that was higher than the starting number, or the previously entered number on the keypad. In the Difficult task, a correct answer was defined as any number that was smaller than the starting number or the previously entered number on the keypad and divisible by 17 after subtraction from the trial's starting number. If a subject produced an incorrect answer, we reset the starting number to the value of the incorrect answer, so as to assess the correctness of subsequent counting within the same trial. Correct answers and number of iterative calculations during the trial indicated performance in both mental arithmetic tasks. There was a maximum of four correct answers per trial.

We imposed a minimum performance criterion, requiring an average of at least one correct numeric answer per trial in the Difficult task (that is, a minimum of six out of 24 correct answers throughout the experimental session; the Easy task generated virtually no incorrect answers). One participant failed to meet this requirement and was discarded.

Questionnaires

Participants completed a modified NASA Task Load Index (NASA-TLX; Hart & Staveland, 1988; Hart, 2006) for mental, physical and temporal demand, performance success, effort and frustration, as an indicator of perceived task difficulty, and the Self-Assessment-Manikin (SAM, Valence and Arousal scales; Bradley & Lang, 1994).

Each NASA-TLX dimension was presented as a visual analog scale with a title and a bipolar descriptor (very low/very high) at each end. Numerical values were not displayed, but values ranging from 0 to 8 (9 points) were assigned to scale the position during data analysis.

The SAM uses a nine-point scale to rate the perceived valence (i.e. level of happiness) and arousal. Values range between 1 and 9, with higher scores indicating higher valence/arousal.

Eye movement analyses

Eye position was acquired binocularly and non-invasively with a fast video-based eye tracker at 500 Hz (desktop configuration of the EyeLink 1000, SR Research, instrument noise 0.01 deg RMS). First, we discarded the eye position data corresponding to the time periods in which participants entered their answers on the keypad. Then, we identified and removed blink periods as portions of the raw data where pupil information was missing. We also removed portions of data where very fast decreases and increases in pupil area occurred (> 50 units/sample, such periods are probably semi-blinks where the pupil is never fully occluded; Troncoso *et al.*, 2008). We added 200 ms before and after each blink/semi-blink to eliminate the initial and final parts where the pupil was still partially occluded (Troncoso *et al.*, 2008). We identified saccades with a modified version of the algorithm developed by Engbert and Kliegl (2003; Laubrock *et al.*, 2005; Engbert, 2006a; Rolfs *et al.*, 2006) with $\lambda = 6$ (used to obtain the velocity threshold) and a minimum saccadic duration of 6 ms. To reduce the amount of potential noise, we considered only binocular saccades, that is, saccades with a minimum overlap of one data sample in both eyes (Laubrock *et al.*, 2005; Engbert, 2006a,b; Rolfs *et al.*, 2006). Additionally, we imposed a minimum intersaccadic interval of 20 ms so that potential overshoot corrections might not be categorized as new saccades (Møller *et al.*, 2002). Microsaccades were defined as saccades with magnitude < 2 deg in both eyes (Martinez-Conde *et al.*, 2006, Martinez-Conde *et al.*, 2009; Troncoso *et al.*, 2008; McCamy *et al.*, 2013b). To calculate microsaccade properties, such as magnitude and peak velocity, we averaged the values for the right and left eyes (McCamy *et al.*, 2012; Costela *et al.*, 2013). Figure 2 shows the microsaccadic peak velocity–magnitude relationship (main sequence),

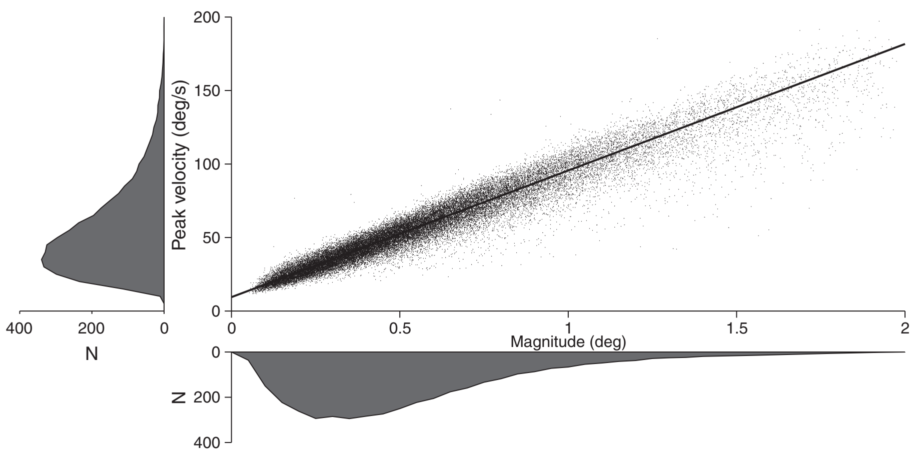


FIG. 2. Microsaccadic peak velocity-magnitude relationship and descriptive statistics. Main panel: Each black dot represents a microsaccade with peak velocity indicated on the *y*-axis and magnitude on the *x*-axis. Bottom and side panels: Microsaccade magnitude and peak-velocity distributions (n = 10 subjects).

and the corresponding microsaccade magnitude and peak velocity distributions.

Fixation breaks

We defined fixations as those time periods during which subjects were not blinking or making saccades larger than 2 deg (Otero-Millan *et al.*, 2008).

Microsaccadic peak velocity–magnitude relationship slope analysis

We assumed a linear relationship between microsaccade magnitude and peak velocity rather than a power law one because the value of r^2 was always higher for the linear fits (r^2: linear 0.908; power law 0.906). Thus, we performed robust linear regressions (using the robustfit function in MATLAB) on the data for each subject to obtain the slope of the microsaccadic peak velocity–magnitude relationship: peak velocity = m·(magnitude) + b. Here, b is the y-intercept and m is the slope. To study the effects of time-on-task and task difficulty on microsaccades, we analysed the slopes of the linear fits of the data from the peak velocity–magnitude relationship slope per block (Di Stasi *et al.*, 2013a,b).

Statistics

Microsaccade rates, microsaccade magnitudes and peak velocity–magnitude relationship slopes met the assumption of normality (Kolmogorov–Smirnov test, all P-values > 0.05). For each of these variables we performed a 2 × 6 repeated-measures ANOVA with the experimental condition (Easy vs. Difficult) and time-on-task (blocks 1–6) as the within-subjects factors.

Microsaccade directions, number of fixation breaks, and blink rates were not normally distributed, so we used non-parametric analyses for these variables (Friedman's test and Wilcoxon's matched paired tests).

Results

We determined the effect of task difficulty during mental arithmetic on microsaccade dynamics. Participants performed one Control task (fixation only) and two types of mental arithmetic tasks (Easy and Difficult) over six consecutive time blocks, during a single experimental session.

Effectiveness of task difficulty manipulation: task performance and subjective data

Task performance and subjective ratings are commonly used to assess task difficulty (Di Stasi *et al.*, 2013a,b; Gao *et al.*, 2013). Here, both task performance (Fig. 3) and subjective ratings (Table 1) data indicated a successful manipulation of task difficulty. The Difficult task generated less correct answers and lower numbers of mental calculation steps than the Easy task (Fig. 3), and the Difficult task led to higher levels of perceived difficulty ($F_{1,8}$ = 19.40, P < 0.001; MSE = 1.98) and lower levels of happiness ($F_{1,8}$ = 6.75, P < 0.05; MSE = 2.41) than the Easy task (Table 1).

Time-on-task affected the number of mental calculation steps. The number of mental calculation steps increased linearly with time-on-task in both mental arithmetic conditions, indicating an improvement in performance throughout the session (Fig. 3, right panel), presumably due to practice. Time-on-task did not affect subjective ratings (all F-values < 3; Table 2).

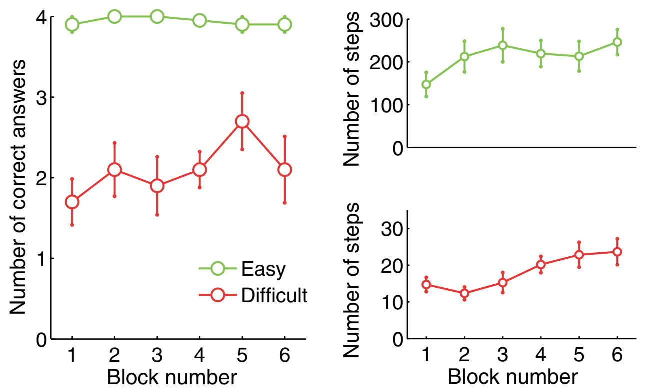


FIG. 3. Effectiveness of the task difficulty manipulation. Average performance across subjects. Left: mean number of correct answers for the Easy and Difficult tasks ($F_{1,9}$ = 188.27, P < 0.001; MSE = 0.54). Time-on-task did not affect performance, and there was no significant interaction between task difficulty and time-on-task (F-values < 1). Right: average number of mental steps per trial ($F_{1,9}$ = 42.08, P < 0.001; MSE = 29438), throughout the session ($F_{5,45}$ = 4.89, P < 0.001; MSE = 1872). The number of mental calculation steps increased linearly with time-on-task (linear trend $F_{1,9}$ = 9.63, P < 0.05). The interaction between task difficulty and time-on-task was significant ($F_{5,45}$ = 3.44, P < 0.05; MSE = 2078). Error bars represent the SEM across participants (n = 10).

TABLE 1. Effect of time-on-task on the dependent variables

Variable	Control task	Easy task	Difficult task
Microsaccade rate (Hz)	1.35 (0.32)	1.53 (0.34)	1.13 (0.38)
Microsaccade magnitude (deg)	0.45 (0.12)	0.57 (0.11)	0.70 (0.17)
Microsaccadic slope	80.05 (8.39)	82.03 (6.99)	82.70 (6.32)
Microsaccade direction*	−0.05 (0.19)	−0.02 (0.14)	−0.02 (0.13)
Fixation breaks (N)	48.37 (36.44)	52.28 (36.22)	67.65 (50.57)
Blink rate (Hz)	0.20 (0.17)	0.23 (0.20)	0.30 (0.26)
No. of correct answers	–	3.96 (0.07)	2.11 (0.41)
No. of mental calculations	–	221.18 (102.50)	17.96 (5.85)
NASA-TLX (0–8)	1.89 (0.63)	2.77 (0.98)	3.96 (0.87)
SAM valence (1–9)	6.94 (1.62)	6.24 (1.67)	5.46 (1.62)
SAM arousal (1–9)	2.70 (1.46)	3.07 (1.10)	3.66 (1.31)

Values are mean ± SD (n = 9 for the subjective data and n = 10 for all other variables). NASA-TLX, NASA Task Load Index; SAM, Self-Assessment-Manikin. *Microsaccade direction ranges between −1 and 1, where 0 indicates no horizontal component, −1 indicates a completely horizontal microsaccade to the left, and 1 indicates a completely horizontal microsaccade to the right.

Effects of task difficulty and time-on-task on microsaccade rate

Microsaccade rate was lower for the Difficult task than for the Easy task (Figs 4A and S1; Table 1), and increased linearly with time-on-task in both conditions. There was no significant interaction between task difficulty and time-on-task (Fig. 4A; Table 2). Microsaccade rates in the Control (i.e. fixation only) condition were consistent with those reported in previous research (Martinez-Conde *et al.*, 2009, 2013).

Effects of task difficulty and time-on-task on microsaccade magnitude

Microsaccade magnitude was higher for the Difficult task than for the Easy task (Fig. 4B; Table 1), and did not change with time-on-task

TABLE 2. Effect of the time-on-task on the dependent variables

Variable	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
Control task						
Microsaccade rate (Hz)	1.29 (0.31)	1.33 (0.29)	1.37 (0.37)	1.35 (0.36)	1.41 (0.34)	1.35 (0.38)
Microsaccade magnitude (deg)	0.46 (0.12)	0.46 (0.14)	0.46 (0.13)	0.45 (0.12)	0.43 (0.11)	0.42 (0.11)
Microsaccadic slope	82.86 (8.75)	80.66 (8.00)	80.15 (9.47)	79.87 (8.97)	79.53 (8.97)	79.93 (8.70)
Microsaccade direction*	−0.07 (0.21)	−0.05 (0.21)	−0.05 (0.20)	−0.06 (0.20)	−0.04 (0.18)	−0.04 (0.20)
Fixation breaks (*N*)	53.80 (38.10)	45.20 (33.01)	49.10 (40.43)	42.30 (32.54)	44.30 (40.84)	55.50 (47.47)
Blink rate (Hz)	0.21 (0.15)	0.18 (0.16)	0.19 (0.18)	0.16 (0.13)	0.20 (0.22)	0.27 (0.27)
No. of correct answers	–	–	–	–	–	–
No. of mental calculations	–	–	–	–	–	–
NASA-TLX (0–8)	1.77 (0.48)	1.72 (0.63	1.94 (0.79)	1.75 (0.90)	2.09 (0.72)	2.09 (0.88)
SAM valence (1–9)	7.11 (1.53)	6.77 (1.78)	6.88 (1.61)	7.11 (1.69)	6.88 (1.69)	6.88 (1.69)
SAM arousal (1–9)	3 (1.65)	2.66 (2)	2.77 (1.92)	2.77 (1.56)	2.33 (1)	2.66 (1.65
Experimental task						
Microsaccade rate (Hz)	1.08 (0.33)	1.25 (0.35)	1.37 (0.41)	1.43 (0.40)	1.45 (0.39)	1.41 (0.35)
Microsaccade magnitude (deg)	0.62 (0.15)	0.63 (0.17)	0.65 (0.14)	0.64 (0.13)	0.63 (0.12)	0.62 (0.11)
Microsaccadic slope	85.64 (5.68)	83.97 (5.85)	81.54 (6.75)	81.19 (7.48)	80.44 (8.10)	81.41 (5.96)
Microsaccade direction*	−0.05 (0.17)	−0.03 (0.12)	−0.02 (0.13)	−0.01 (0.13)	0.00 (0.12)	−0.01 (0.12)
Fixation breaks (*N*)	67.55 (53.94)	58.75 (41.88)	57.75 (38.87)	58.05 (41.70)	59.55 (49.86)	58.15 (42.17)
Blink rate (Hz)	0.28 (0.25)	0.25 (0.21)	0.25 (0.21)	0.24 (0.20)	0.28 (0.29)	0.27 (0.24)
No. of correct answers	2.8 (0.48)	3.05 (0.55)	2.95 (0.59)	3.05 (0.36)	3.25 (0.54)	3.15 (0.66)
No. of mental calculation	80.27 (47.19)	112.31 (62.14)	126.98 (66.65)	141.23 (68.57)	131.06 (63.28)	125.57 (48.41)
NASA-TLX (0–8)	3.50 (0.77)	3.63 (0.94)	3.47 (0.79)	2.96 (1.47)	3.38 (1.06)	3.24 (1.00)
SAM valence (1–9)	5.72 (1.52)	5.77 (1.52)	5.94 (1.60)	5.5 (2.44)	5.83 (1.71)	6.33 (1.39)
SAM arousal (1–9)	3.88 (1.57)	3.5 (1.25)	3.5 (1.34)	2.83 (1.34)	3.27 (1.09)	3.22 (1.17)

Values are mean ± SD (n = 9 for the subjective data and n = 10 for all other variables). NASA-TLX, NASA Task Load Index; SAM, Self-Assessment-Manikin.*Microsaccade direction ranges between −1 and 1, where 0 indicates no horizontal component, −1 indicates a completely horizontal microsaccade to the left, and 1 indicates a completely horizontal microsaccade to the right.

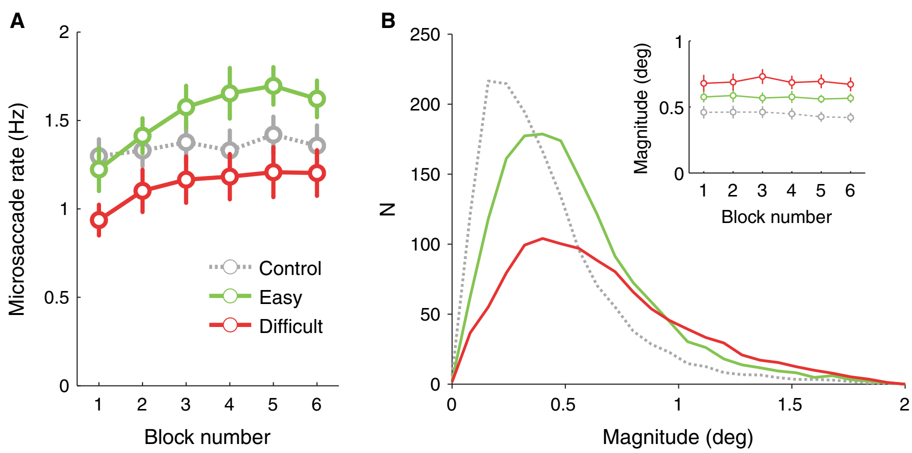


FIG. 4. Task difficulty modulates microsaccade rates and magnitudes. (A) Average microsaccade rate per experimental condition ($F_{1,9}$ = 81.35, $P < 0.001$; MSE = 0.06) and time-on-task ($F_{5,45}$ = 12.21, $P < 0.001$; MSE = 0.03). Microsaccade rate increased linearly with time-on-task ($F_{1,9}$ = 18.54, $P < 0.01$). There was no significant interaction between task difficulty and time-on-task ($F < 2$). (B) Microsaccade magnitude distributions across conditions. Task difficulty affected microsaccade magnitude ($F_{1,9}$ = 16.94, $P < 0.001$; MSE = 0.03). Time-on-task did not affect microsaccade magnitude, and there was no significant interaction between task difficulty and time-on-task (all F-values < 2). Error bars represent the SEM across participants (n = 10).

in either condition. There was no significant interaction between task difficulty and time-on-task (Fig. 4B inset; Table 2). Microsaccade magnitudes in the Control (i.e. fixation only) condition were consistent with those reported in previous studies (Martinez-Conde *et al.*, 2009, 2013).

Effects of task difficulty and time-on-task on the microsaccadic peak velocity–magnitude relationship

Task difficulty did not affect the microsaccadic peak velocity–magnitude relationship, in agreement with Di Stasi *et al.* (2013a; Tables 1 and 2). Time-on-task had a significant effect on the microsaccadic peak velocity–magnitude relationship ($F_{5,45}$ = 7.29, $P < 0.001$; MSE = 11). Slopes decreased with increased time-on-task (linear trend: $F_{1,9}$ = 61.41, $P < 0.001$), also in agreement with Di Stasi *et al.* (2013a,b). The interaction between task difficulty and time-on-task was not significant (F-values < 1).

Effects of task difficulty and time-on-task on microsaccade directions, number of fixation breaks, and blink rates

Blinks and saccades were regarded as breaks in fixation (see Materials and methods for details). There were no significant differences in microsaccade directions, number of fixation breaks or blink rates with either task difficulty or time-on-task (Friedman's test and Wilcoxon's matched paired tests; all *P*-values > 0.05; Tables 1 and 2).

Discussion

We examined the effects of task difficulty in a mental arithmetic task on microsaccade dynamics. Our results show that task difficulty can modulate microsaccade rates and magnitudes in a non-visual task.

The effects of task difficulty and time-on-task on microsaccade rate and magnitude

Microsaccade rates decreased and microsaccade magnitudes increased with higher task difficulty.

Perceived difficulty (NASA-TLX scores) remained stable throughout the session, but microsaccade rates increased and task performance improved (increased number of mental steps) with time-on-task in both Easy and Difficult task conditions, suggesting that participants may have become accustomed to the arithmetic tasks and/or developed strategies and/or increased their efforts over time to compensate for the effects of increasing fatigue (Hockey, 1997; Di Stasi *et al.*, 2013b).

The Control (i.e. fixation only) task produced microsaccade rates in between the Easy and Difficult tasks, and microsaccade magnitudes below both the Easy and Difficult tasks. Participants' cognitive activities during the Control task may have varied: some may have focused more on fixating whereas others may have drifted away mentally. Anecdotally, some participants reported that the Easy task was easier than the Control task. Others said that the Control task was the easiest of all three.

Putative influence of working memory load on microsaccade parameters during easy and difficult tasks

Our finding that microsaccade rate is inversely related to task difficulty is in agreement with the previous report of a similar effect in a visual attention task (Pastukhov & Braun, 2010). This study proposed that participants might suppress microsaccade production during target presentation, so as to avoid potential visual disruptions. Because here we used a non-visual task, however, the suppression of microsaccades had no perceptual cost or benefit. Thus, task difficulty itself (or its associated cognitive workload), rather than the possibility of visual disruption, affected microsaccade rates and magnitudes.

The effects of task difficulty on microsaccade parameters may be mediated by working memory load. Studies indicate a close link between working memory and attention (Awh *et al.*, 1998; Awh & Jonides, 2001), as well as a common attentional substrate underlying eye movement production and the execution of working memory tasks (Theeuwes *et al.*, 2009).

Microsaccade generation could be affected by working memory performance in the present experiment as follows. In the mental arithmetic tasks, the participants' attention is divided between the fixation task and the counting task, increasing the load on working memory. The more difficult the task (i.e. the higher the working memory load), the less well participants will be able to execute the fixation task: thus, they will produce less frequent microsaccades, with poorly controlled (i.e. larger) magnitudes.

Effects of task difficulty on the microsaccade triggering circuit

Fluctuations of SC activity at the rostral poles are thought to give rise to microsaccades during fixation (Rolfs *et al.*, 2008; Hafed *et al.*, 2009; Otero-Millan *et al.*, 2011). Further, the shape of the activity on the two-dimensional SC surface, which represents visual saccadic target space, will influence the distribution of microsaccade magnitudes, so that broad activity will correspond to a broad distribution of microsaccade magnitudes (i.e. larger magnitudes) and high activity will correspond to a high rate of microsaccades (Rolfs *et al.*, 2008).

The shape of the rostral SC activity depends on excitatory inputs from frontal (i.e. frontal eye fields) and parietal cortical areas, and on inhibitory inputs from the basal ganglia. Based on the known relationship of these brain areas with attention (Hikosaka & Sakamoto, 1986; Schall, 2004), varying levels of attention should affect rostral SC activity during fixation, and thus microsaccadic rates and magnitudes (Rolfs *et al.*, 2008).

Increased attention to the mental arithmetic task due to increased task difficulty (Chen *et al.*, 2008) will reduce attention to the fixation task. Thus, increased task difficulty will decrease SC activity in the region corresponding to the fixation location and enhance activity in surrounding areas, thereby broadening the activity profile (Ignashchenkova *et al.*, 2004). Conversely, decreased attention to

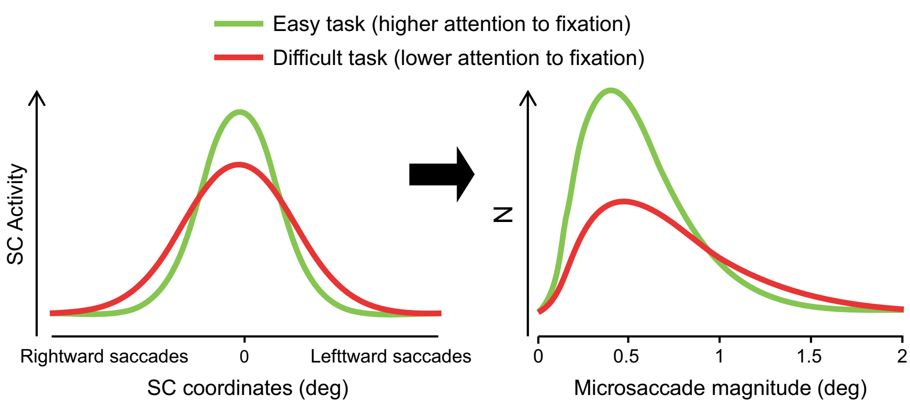


FIG. 5. Schematic of task difficulty influences on an activation-map model of microsaccade generation (after Rolfs *et al.*, 2008). Increased attention to the mental arithmetic task, due to increased task difficulty, will reduce attention to the fixation task (red). Decreased attention to the fixation task will produce a broader and lower SC activity profile, and therefore lower microsaccade rates and higher microsaccade magnitudes. Conversely, reduced attention to the mental arithmetic task, due to decreased task difficulty, will increase attention to the fixation task (green). This will result in a narrower and higher SC activity profile, and therefore higher microsaccade rates and lower microsaccade magnitudes.

the mental arithmetic task due to decreased task difficulty (Chen *et al.*, 2008) will increase attention to the fixation task. Thus, decreased task difficulty will enhance SC activity in the region corresponding to the fixation location and suppress activity in surrounding areas, thus sharpening the activity profile (Fig. 5).

This proposal is consistent with the previous finding that smaller fixation targets result in higher microsaccade rates and narrower microsaccade magnitude distributions (McCamy *et al.*, 2013). A reduction in fixation target size will increase the difficulty of, and enhance attention to, the fixation task. Thus, decreased target size will enhance SC activity at the fixation location and suppress activity in surrounding areas, which will sharpen the activity profile.

The effects of time-on-task on the microsaccadic peak velocity–magnitude relationship

Task difficulty did not affect the microsaccadic peak velocity–magnitude relationship, in agreement with Di Stasi *et al.* (2013a). However, the microsaccadic peak velocity–magnitude relationship slope decreased significantly with time-on-task. Di Stasi *et al.* (2013a) previously found a similar decrease in the microsaccadic peak velocity–magnitude relationship slope with time-on-task during a simulated air traffic control task, and attributed this change to fatigue. In the present study, performance improvement throughout the session could argue against a simple fatigue-based explanation, but we also note that participants may have redoubled their efforts throughout the session, to compensate for the effects of fatigue (Hockey, 1997; Di Stasi *et al.*, 2013b). Future studies should investigate the possibility that the effects of time-on-task on the microsaccadic peak velocity–magnitude relationship are mediated by changes in sympathetic nervous system activation, that is, by variations in physiological arousal (Di Stasi *et al.*, 2013c). It is interesting that time-on-task had an effect on the microsaccadic peak velocity–magnitude slopes (and on microsaccade rates) but not on microsaccade magnitudes. It might be that different microsaccade parameters are differentially susceptible to various types of task modulations: microsaccade magnitude could reflect task difficulty accurately while being insensitive to time-on-task, whereas the microsaccade peak velocity–magnitude relationship could behave in the opposite fashion. Future research should explore this possibility.

Are microsaccades indicative of cognitive workload?

The relationship between task difficulty and microsaccade rate and magnitude points to the potential use of microsaccades as an indicator of cognitive workload, especially in applied settings (Di Stasi *et al.*, 2013d). There is no current reliable psychophysiological measure of cognitive workload. The advantages of such a measure would extend to a variety of domains, ranging from the improvement of working conditions to the optimization of workstation design (Cain, 2007). Future research should further probe the relation between cognitive workload and microsaccades, particularly in ecologically valid scenarios.

Conclusions

We have shown that task difficulty modulates microsaccade rates and magnitudes during the performance of a non-visual task. These results are consistent with the effects of varying attentional inputs on the rostral SC activity map, as a function of task difficulty. The present findings may open up new possibilities concerning the use of microsaccades as an indicator of task difficulty.

Supporting Information

Additional supporting information can be found in the online version of this article:
Fig. S1. Microsaccade rates throughout the experimental session.

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Abbreviations

NASA-TLX, NASA Task Load Index; SAM, Self-Assessment Manikin; SC, superior colliculus.

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