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Microsaccade and drift dynamics reflect mental fatigue

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Abstract

Our eyes are always in motion. Even during periods of relative fixation we produce so-called ‘fixational eye movements’, which include microsaccades, drift and tremor. Mental fatigue can modulate saccade dynamics, but its effects on microsaccades and drift are unknown. Here we asked human subjects to perform a prolonged and demanding visual search task (a simplified air traffic control task), with two difficulty levels, under both free-viewing and fixation conditions. Saccadic and microsaccadic velocity decreased with time-on-task whereas drift velocity increased, suggesting that ocular instability increases with mental fatigue. Task difficulty did not influence eye movements despite affecting reaction times, performance errors and subjective complexity ratings. We propose that variations in eye movement dynamics with time-on-task are consistent with the activation of the brain’s sleep centers in correlation with mental fatigue. Covariation of saccadic and microsaccadic parameters moreover supports the hypothesis of a common generator for microsaccades and saccades. We conclude that changes in fixational and saccadic dynamics can indicate mental fatigue due to time-on-task, irrespective of task complexity. These findings suggest that fixational eye movement dynamics have the potential to signal the nervous system’s activation state.

Introduction

Our eyes are always in motion. Even during the periods between saccades, smooth pursuit and reflexive eye movements we produce so called ‘fixational eye movements’, which include microsaccades, drift and tremor (Martinez-Conde *et al.*, 2004). The superior colliculus is critical to triggering microsaccades and saccades (Rolfs *et al.*, 2008; Hafed *et al.*, 2009; Martinez-Conde *et al.*, 2009, 2013; Otero-Millan *et al.*, 2011) and for the control of selective attention, even without eye movements (Lovejoy & Krauzlis, 2010). Accordingly, studies have reported an influence of attention on saccades and microsaccades (Hafed & Clark, 2002; Engbert & Kliegl, 2003).

Few studies, however, have addressed the potential effects of mental fatigue, i.e. the mental tiredness generated by time-on-task (TOT) and task complexity (TC), on microsaccade production (Hafed, 2003; Chen *et al.*, 2008; Otero-Millan *et al.*, 2008; Pastukhov & Braun, 2010; Benedetto *et al.*, 2011). Indeed, only three studies to date have manipulated TC parametrically and measured the effects on microsaccade rate, with varied results (Chen *et al.*, 2008; Pastukhov & Braun, 2010; Benedetto *et al.*, 2011). A solitary preliminary report has addressed the effects of TOT on microsaccade rate (Hafed, 2003). No study has investigated how TOT and/or TC affect microsaccade velocity, or any drift parameters. Thus, the question of whether TC and TOT modulate the characteristics of fixational eye movements remains open.

Conversely, a large body of literature indicates that increased TOT decreases saccadic velocities, both in humans (Dodge, 1917; Hirvonen *et al.*, 2010; Di Stasi *et al.*, 2012, 2013) and in primates (Prsa *et al.*, 2010). The effect of TC on large saccades is less clear (Galley & Andres, 1996; Di Stasi *et al.*, 2010a,b; Di Stasi *et al.*, 2011).

Here we asked whether increased TOT and TC might affect microsaccades and drift. If so, there could be valuable applications in naturalistic scenarios, especially because humans fixate 80% of the time during visual exploration (Otero-Millan *et al.*, 2008; McCamy *et al.*, 2013b). Air traffic control (ATC) operators perform demanding visual search tasks, in which the consequences of impaired performance are severe (Di Stasi *et al.*, 2010a). Thus, we simulated an ATC task to investigate the effects of TC and TOT on saccadic and fixational eye movements.

We tracked the eye movements of human subjects as they performed a simulated ATC task with two levels of TC for 2 h. Microsaccadic and saccadic peak velocity decreased with TOT, consistent

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with previous findings concerning large saccades (Hirvonen *et al.*, 2010; Di Stasi *et al.*, 2012). Drift velocity increased linearly with increased TOT, suggesting that ocular instability increases with mental fatigue. TC did not affect the dynamics of microsaccades, saccades or drift. Because microsaccades, saccades and drift were sensitive to TOT but insensitive to TC, our findings have the potential to help establish an index of mental fatigue. Currently, most physiological measures used to asses mental fatigue (i.e. cardiorespiratory indices) fail to produce reliable results because they lack specificity or are hypersensitive or hyposensitive to subjective and environmental factors (Roscoe, 1992).

Materials and methods

Ethical approval

We conducted the study in conformity with the Code of Ethics of the World Medical Association (Declaration of Helsinki) (World Medical Association [W.M.A.], 1964). The experiments were carried out under the guidelines of the Barrow Neurological Institute's Institutional Review Board (IRB approval number 10BN142). Written informed consent was obtained from each participant prior to the study.

Main experiment

Participants

Twelve participants (two females, 10 males; 10 naive plus two authors: LLDS and MBM; mean ± SD age 30 ± 3.8 years) took part in one experimental session. All participants had normal or corrected-to-normal vision, were right-handed and had no prior ATC experience. Participants were non-smokers and abstained from alcohol (for 24 h) and caffeinated drinks (for 12 h) prior to the session. They reported a habitual 7–9 h of sleep per night, and slept at least 7 h (mean 7.75 h) before the session. All experimental sessions were conducted between 09.00 and 12.00 h (noon) to avoid the potential influence of circadian rhythm or diurnal variation. Written informed consent was obtained from each participant, and naive participants received $45.

Experimental design

The experiment simulated an ATC operator's job and allowed us to measure the effects of TOT vs. TC. Two levels of TC (high and low) and two viewing conditions (free-viewing and fixation) resulted in four ATC conditions: two (TC) × two (viewing conditions). We ran four blocks (one block per ATC condition); each block was approximately 30 min long and contained 41 trials (i.e. a sequence of radar displays; see ATC task section below). Block order was controlled by a semi-Latin-square design, as follows:

Viewing condition order was blocked for all participants: half of the participants ($n = 6$) performed the fixation condition during the first two blocks and the free-viewing condition during the last two blocks. The other half ($n = 6$) started with free-viewing and finished with fixation. For each viewing condition, we balanced TC across subjects (i.e. half the subjects started with the high TC condition and the other half with the low TC condition). This design minimised the effects of potential confounding factors, including learning or series effects and task-switching costs (i.e. the costs associated with going from a complex to an easy task). We ran the following four experimental sequences:

(1) Free-viewing high TC, free-viewing low TC, fixation high TC, fixation low TC.
(2) Free-viewing low TC, free-viewing high TC, fixation low TC, fixation high TC.
(3) Fixation high TC, fixation low TC, free-viewing high TC, free-viewing low TC.
(4) Fixation low TC, fixation high TC, free-viewing low TC, free-viewing high TC.

Our analyses showed no effect of the experimental series, indicating that sequence order did not influence our main results significantly (Supporting Information Tables S1 and S2).

To determine the effects of mental fatigue we analysed the data according to the TOT factor determined by four sequential 30-min blocks of TOT (i.e. TOT 1, TOT 2, TOT 3 and TOT 4). Hereafter we will use the terms TOT and mental fatigue interchangeably.

ATC task. Participants carried out a simplified ATC task. This task contained many of the dynamic elements experienced by actual air traffic controllers, and was realistic enough to be ecologically valid but not so complex that naive participants could not perform it.

In the free-viewing condition we presented a radar display consisting of five grey concentric circles (nodes), representing the distance from the airport, on a black background (Fig. 1). Two degrees (°) of visual angle separated adjacent nodes, and the largest node had a 10° radius. A Cartesian-coordinate axis divided the radar display into four quadrants. The lines forming the nodes and coordinate axes had a thickness of 0.0125°, and their intensity level was chosen to minimise afterimages and viewing discomfort. A small fixation spot consisting of three concentric circles [radius of smallest (red) circle = 0.05°; radius of middle (black) circle = 0.25°; radius of largest (white) circle = 0.5°] was at the origin of the radar display's coordinate axes (i.e. on the center of the monitor). This fixation spot represented the airport, and the rest of the radar display was the airspace. Each radar display contained several colored equilateral triangles (i.e. planes) of side length 1.15°, where color represented aircraft altitude. Every aircraft was rendered either directly on a node or half-way between nodes, and no aircraft could be within the smallest node. Two aircraft were considered in conflict if they had the same color and were on the same node (Fig. 1B). There was never a conflict between aircraft that were lying between nodes. The aircraft parameters (altitude, quadrant location, distance, angular position within the quadrant, and state of conflict) were randomly generated to satisfy the following criteria: equal likelihood of 1–4 conflicts per trial, all colors equally likely to be in conflict, all nodes equally likely to be in conflict, at most one conflict per radar display, 1/3 probability of each aircraft positioned between nodes, 1.16° minimum distance between the center of any two planes, at most one plane per node in each quadrant, equal likelihood of angular position within the quadrant, and equal likelihood for each node in each quadrant to contain a plane. The number of conflicts was kept low in each trial (randomly chosen from one to four) to simulate actual ATC conditions. The conflict angle (i.e. the angle between the conflicted planes) and the airport and the traffic dispersion (i.e. average distance between each plane and the airport) distributions were equivalent in the high- and low-complexity conditions. We used custom code and the Psychophysics Toolbox to create and display the visual stimuli (Brainard, 1997).

In the high-complexity condition we presented four planes in each quadrant (for a total of 16 planes per radar display). Here, each

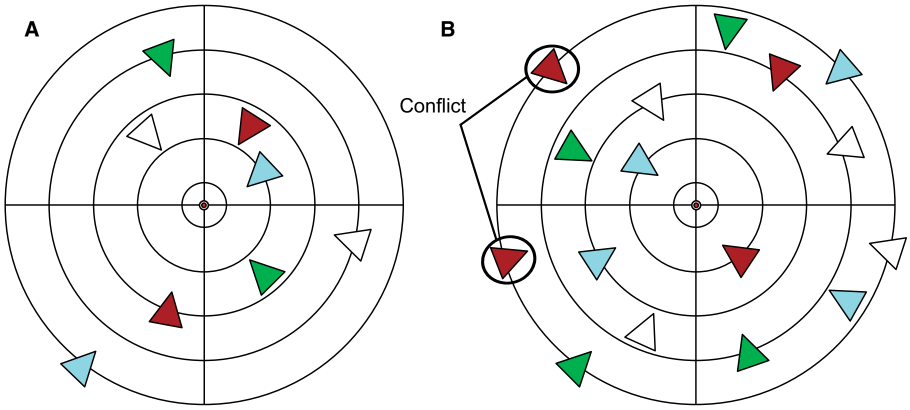

Fig. 1. Visual stimuli. Schematic examples of radar displays in the free-viewing condition. (A) Low-complexity condition; no conflict. (B) High-complexity condition; conflict present. (A and B) Each triangle represents a plane and the central target represents the airport. A conflict occurs when two planes of the same color lie on the same radar node.

quadrant contained at most two planes of the same color. In the low-complexity condition we presented two different colored planes in each quadrant (for a total of eight planes per radar display). In both complexity conditions the colors were balanced in every radar display; that is, each color appeared on the radar display twice for the low-complexity task and four times for the high-complexity task.

We ran twenty 45-s-long trials per block, with seven different radar displays per trial, in which each radar display was displayed for 5 s. Thus, we created 140 radar displays per TC condition. All radar displays were viewed by all participants.

Participants were instructed to explore each radar display and to report, using a gamepad, the presence or absence of a conflict as quickly as possible (conflict present, right trigger; conflict absent, left trigger). In the event of a conflict, participants also had to indicate its color, again via gamepad (the four colors were represented on the gamepad as: blue = Y, green = B, red = A and white = X; the color mapping was displayed on the question screen after every radar display). The purpose of this question was to focus the subjects' attention and heighten their motivation (the subject's answers to the color question were not analysed). Fig. 2 illustrates the experimental timeline.

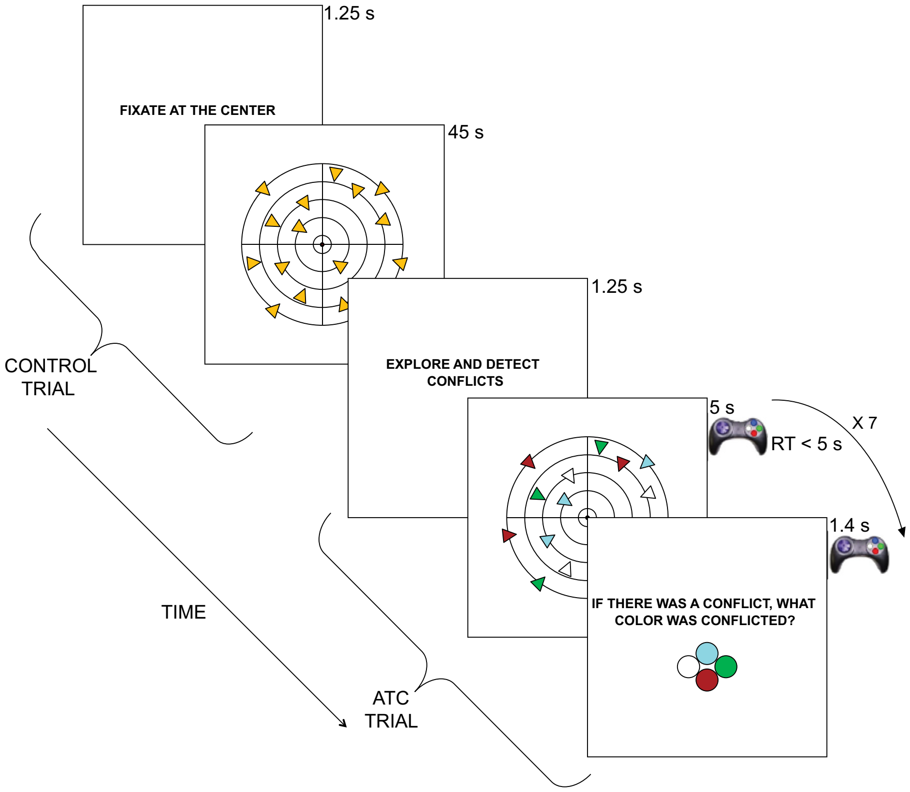

Fig. 2. Schematic representation of experimental timeline. Each trial began with an instructions screen, and each control trial was followed by an ATC trial. The figure shows a free-viewing control trial followed by a high-complexity free-viewing ATC trial.

In all conditions, we calculated the percentage of correct answers and their corresponding reaction times (RTs; Tables 2 and 3). We calculated RT as the latency from the radar display's presentation to trigger press, as long as it was contained within the 5-s period in which the radar display was visible (Fig. 2). We disregarded trigger presses produced after 5 s.

In the fixation condition, participants were asked to keep their gaze on the central fixation dot (the airport). Visual stimuli and other experimental details were as in the free-viewing condition except that the radar display's properties (space between nodes, line widths, plane sizes, radii of nodes, and planes) were scaled to account for the decline in visual acuity from fovea to periphery (Anstis, 1974).

Control tasks. TC analyses were conducted with data from the ATC tasks only (free-viewing and fixation conditions). To assess oculomotor function without the influence of TC, and produce similar oculomotor behavior across participants, we ran one of three 45-second control trials before each ATC trial: a fixation trial, a free-viewing trial and a guided saccade trial. In the fixation and free-viewing control trials, participants viewed a radar display in which all the planes (eight or 16 depending on the TC condition) had the same color (gold). In the fixation trial, participants were asked to fixate on the center of the radar display (Fig. 2). In the free-viewing trials, participants were instructed to explore the radar display at will. In the guided saccade trial (modified from Di Stasi *et al.* (2012), participants were instructed to follow a fixation spot on a black screen. Participants made saccades starting from four randomly-selected locations (each of the four corners of a square centered on the middle of the monitor with 20° side length) of five randomly-selected sizes (measured from the starting location; 10°, 12.5°, 15°, 17.5° or 20°) and in three randomly-selected directions (vertical, horizontal or diagonal). Diagonal saccades could be up left, up right, down left or down right. There were thus 60 ($4 \times 5 \times 3$) possible guided saccades. The same guided saccade trials were performed in each of the four blocks. Thus, the cued saccades had the same magnitude distributions across blocks. Participants conducted each control task seven times (with the order of the control trials being random) during each block.

TOT analyses were conducted with data from the fixation and guided saccade control trials. The free-viewing trials were included to minimise participant discomfort from prolonged fixation during the ATC fixation trials; data from this task were considered only when calculating the r^2 values for each participant (Table 1; see (*Micro)saccadic slope analysis* section).

TABLE 1. Values of r^2 (goodness of fit)

	Microsaccades Duration	MV	PV	Saccades Duration	MV	PV
Linear	0.59	0.70	0.80	0.73	0.76	0.52
Log	0.70	0.72	0.81	0.87	0.92	0.93

The first row gives r^2 for the linear fit of the (micro)saccadic raw data for duration, mean velocity (MV) and peak velocity (PV) against magnitude (MAG). For example, $PV = m MAG + b$. The second row gives r^2 for the linear fit of the log-transformed data (e.g. $\ln(PV) = m \ln(MAG) + b$); this assumes a power law $PV = e^{b} MAG^{m}$. In both cases, b is the y-intercept and m is the slope. We calculated the r^2 values for each subject using all the experimental data for that subject. We display the average r^2 across participants ($n = 12$).

Procedure

In a darkened and quiet room, participants rested their forehead and chin on the EyeLink 1000 (SR Research, Ontario, Canada) head/chin support, 57 cm away from a linearised video monitor (Barco Reference Calibrator V, 75 Hz refresh rate). A 5-min training session was followed by four 30-min experimental blocks. Stimuli and timing parameters in the training session were equivalent to those in the actual experiment. Setup of the eye tracking system followed the completion of the training session.

Participants did not rest between blocks, except to answer subjective questionnaires (described below). Eye position was calibrated at the beginning of every block and every eleven trials. An instruction screen indicating the type of task to be performed preceded each trial. Participants had no control over the pace of the experiment; thus block duration was the same for all participants. Each block consisted of 20 ATC trials and 21 control trials (i.e. a total of 41 trials and approximately 30 min per block; Fig. 2). The 21 control trials corresponded to seven trials for each of the three control tasks per block. The entire experiment had a total of 164 trials and lasted for approximately 2 h.

Questionnaires

Each subject's quality of sleep was subjectively measured with the Groningen Sleep Quality Scale (Meijman, De Vries-Griever, De Vries, & Kampman, 1988) before the training session, for screening purposes: no participants scored > 3 (had they done so they would have been excluded from further testing). At the beginning of the first block and at the end of each subsequent block, participants filled the Stanford Sleepiness Scale (SSS; Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973) and an adapted version of the Borg rating scale of perceived exertion (i.e. fatigue; Borg, 1998). The SSS provides a global measure of sleepiness. In this study, we assumed a linear relationship between TOT and the level of sleepiness and mental fatigue, based on Ahlstrom *et al.* (2013) and Di Stasi *et al.* (2012). In addition, subjects completed the NASA-TLX (Task Load index) questionnaire (Hart & Staveland, 1988), which indicated their perceived degree of TC. All participants filled in the SSS, the Borg scale and the NASA-TLX after each experimental block, in the same order (Tables 2 and 3).

Eye-movement recordings and analyses

Eye movements were sampled binocularly at 500 Hz using the desktop configuration of the EyeLink 1000 eye tracking system with a resolution of 0.01° RMS and a volume of allowable head movement up to $25 \times 25 \times 10$ mm (horizontal × vertical × depth). 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 during which the pupil is never fully occluded (Troncoso *et al.*, 2008; McCamy *et al.*, 2012). We added 200 ms before and after each blink or semi-blink to eliminate the initial and final parts during which the pupil was still partially occluded (Troncoso *et al.*, 2008; McCamy *et al.*, 2012). Saccades were identified with a modified version of the algorithm developed by Engbert and Kliegl (Engbert & Kliegl, 2003; Laubrock *et al.*, 2005; Engbert, 2006; Engbert & Mergenthaler, 2006; 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

TABLE 2. Effect of TOT on subjective and behavioral data

	Pre-experiment	TOT 1	TOT 2	TOT 3	TOT 4
SSS*	1.70 (0.80)	3.10 (1.52)	3.50 (1.71)	4.10 (1.79)	4.90 (1.79)
BORG*	8.40 (2.67)	11.20 (3.82)	10.60 (4.37)	13.80 (3.70)	14.70 (3.59)
% Correct answers	–	83 (10)	87 (11)	79 (19)	84 (12)
RT (ms)	–	342660 (841.24)	342216 (814.75)	348844 (754.99)	343507 (720.14)

Values are mean ± SD. SSS values range between 0 and 7. The scores of the Borg's perceived exertion scale (BORG) range between 6 and 20. For both scales, higher scores indicate more subjective tiredness. Author data were excluded from the SSS and BORG mean and SD calculations. The percentages of correct answers for all participants (n = 12) are indicated, with their corresponding RTs in ms. *P-values < 0.05. Analyses were conducted with data from the fixation and guided saccade control trials only.

TABLE 3. Effect of task complexity and viewing condition on subjective and behavioral data

	Free-viewing Low complexity	Free-viewing High complexity	Fixation Low complexity	Fixation High complexity
NASA-TLX*	48.73 ± 25.93	63.58 ± 25.07	51.83 ± 24.43	57.83 ± 24.43
% Correct answers*	95 ± 03	79 ± 07	90 ± 06	69 ± 16
RT* (ms)	296220 ± 503.29	424016 ± 298.94	271150 ± 52157	385842 ± 36209

Values are mean ± SD. NASA Task Load Index (NASA-TLX) values range between 0 and 100. High values indicate high perceived exertion. Author data were excluded from the NASA-TLX mean and SD calculations. The percentages of correct answers for all participants (n = 12) are indicated, with their corresponding RTs in ms. *P-values < 0.05. Analyses were conducted with data from the ATC conditions only.

only binocular saccades, that is, saccades with a minimum overlap of one data sample in both eyes (Engbert & Kliegl, 2004; Laubrock *et al.*, 2005; Engbert, 2006; Engbert & Mergenthaler, 2006; Rolfs *et al.*, 2006; McCamy *et al.*, 2013a). Additionally, we imposed a minimum intersaccadic interval of 20 ms so that potential overshoot corrections might not be categorised as new saccades (Møller *et al.*, 2002). Microsaccades were defined as saccades with magnitude < 1° in both eyes (Martinez-Conde *et al.*, 2009, 2013). To calculate (micro)saccade properties such as magnitude and peak velocity we averaged the values for the right and left eyes. Supporting Information Table S3 includes the descriptive statistics for microsaccades, saccades and drift. To avoid confounding factors and because (micro)saccades are sensitive to sudden visual and auditory stimuli (Rolfs, 2009), participants performed the experiment surrounded by a dark box while wearing noise-cancelling headphones. For the same reason, subjects received no auditory or visual feedback when their gaze left the fixation dot (i.e. there was no fixation window around the central fixation target). Data from the first second of each 45-s trial were discarded to remove transient effects from the stimulus onset (Otero-Millan *et al.*, 2012; McCamy *et al.*, 2013c).

Drift periods were defined as the eye-position epochs between (micro)saccades, overshoots and blinks. We removed 10 ms from the start and end of each drift period, because of imperfect detection of blinks and (micro)saccades, and we filtered the remaining eye-position data with a low-pass Butterworth filter of order 13 and a cut-off frequency of 30 Hz (Murakami *et al.*, 2006; Cherici *et al.*, 2012). To calculate drift properties (such as mean velocity and duration) we used the filtered data described above and removed an additional 10 ms from the beginning and end of each drift period to reduce edge effects due to the filter. Drifts < 200 ms were discarded. Finally, because drifts are not generally conjugate (Krauskopf *et al.*, 1960; Yarbus, 1967; Martinez-Conde *et al.*, 2004), we used data from both the left and right eyes. Thus, any given drift period had a duration, distance (length of the curve traced out by the drift), peak velocity and mean velocity for each eye. The cumulative distributions in Fig. 4 are the averages across subjects; each subject's distribution is that of the drift mean velocities from both eyes.

(Micro)saccadic slope analysis

We assumed a power-law relationship between (micro)saccade magnitude (MAG) and each of: (i) (micro)saccade duration, (ii) (micro) saccade mean velocity and (iii) (micro)saccade peak velocity (PV). We assumed a power-law relationship rather than a linear one because the r^2 was always higher for the linear fits of the log-transformed data than for linear fits of the raw data (Table 1). Thus, we performed robust linear regressions (using the robustfit function in MATLAB) on the log-transformed data for each subject to obtain the slope for each main sequence relationship. For example, we did a robust linear regression on $\ln(\mathrm{PV}) = m \ln(\mathrm{MAG}) + b$ which assumes the power law $\mathrm{PV} = e^b \mathrm{MAG}^m$. Here and throughout, b is the y-intercept and m is the slope. To study the effects of TC and TOT on (micro)saccades we analysed the slopes of the linear fits of the log-transformed data.

Statistical analyses

We analysed the slopes of the relationship between (micro)saccadic magnitude and (micro)saccadic peak velocity, i.e. the (micro)saccadic main sequence, to investigate the effects of TOT and TC on (micro)saccadic dynamics. To determine the effects of TOT and TC on fixation instability we analysed the mean velocity of ocular drift.

To assess the effects of TOT we conducted separate single-factor repeated-measures ANOVAS (one for each dependent variable) with the four measuring times (TOT 1, TOT 2, TOT 3 and TOT 4) as the within-subject factors. To study the effect of TC we used separate paired-sample t-tests (one for each dependent variable). For violations of the ANOVA assumption of sphericity, P-values were adjusted using the Greenhouse–Geisser correction. The significance level was set at $\alpha = 0.05$. We conducted TC analyses on data from the ATC trials only. To avoid the potential influence of TC on TOT we conducted TOT analyses on data from the control trials only (using the fixation trials for fixational eye movement analyses and the guided saccade trials for saccadic analyses). We did not collapse data across conditions to determine the effects of TC and viewing condition on task performance (% correct answers and RTs) or to determine the effects of TOT on eye movement dynamics. We did collapse the data across TC and viewing condition in each TOT block condition to analyse the effects of TOT on task performance (% correct answers and RTs), as permissible from our balancing procedure (semi-Latin-square design; see Experimental design section for details).

The average signal-to-noise ratio and RMS of the raw velocity signal remained constant throughout the duration of the experiment, indicating that the effects observed were not due to increases in noise with TOT (data not shown).

Head immobilisation experiment

To exclude the possibility that changes in drift velocity with TOT were due to increased head motion, we conducted an additional experiment in which subjects' heads were held in place by means of a dental imprint bite bar (UHCOTech Bite Buddy; TX, USA), mounted on the EyeLink 1000 chin/head rest.

Four naive subjects (one female, three males; no overlap with previous participants) took part in this experiment. The first two subjects ran the complete experiment (i.e. four TOT blocks) but reported extreme tiredness in relationship to using the bite bar for the entire experimental session. Thus, we reduced the session to two TOT blocks for the remaining two subjects. Consequently, data in Fig. 5 are from TOT 1 and TOT 2 only. We ran the following sequences:

1. Free-viewing high TC, free-viewing low TC, fixation high TC, fixation low TC.
2. Fixation low TC, fixation high TC, free-viewing low TC, free-viewing low TC.
3. Free-viewing low TC, free-viewing high TC.
4. Fixation high TC, fixation low TC.

All other details were as in the main experiment.

One subject presented a partial pupil occlusion (from her eyelid) in her right eye so we used data from her left eye only. All eye movement analyses for her data were as described above, except that no binocular criterion was used for saccade detection.

Results

We determined the effects of mental fatigue (i.e. TOT and TC) on fixational and saccadic eye movements during a simulated ATC task. The ATC task required the detection of airplane conflicts in low-complexity (eight planes) and high-complexity (16 planes) radar scenarios, in both free-viewing and fixation conditions. TOT was divided in four 30-min blocks: TOT 1, TOT 2, TOT 3 and TOT 4. Whereas TC analyses used data from the ATC task, TOT analyses used data from non-ATC tasks, i.e. control trials, including a fixation task and a guided saccade task, interleaved with the ATC trials; See Materials and Methods for details.

Effectiveness of TOT and TC manipulations

To examine the effectiveness of the TOT and TC manipulations we analysed performance results (percentage of correct answers and their RTs) and responses to subjective questionnaires (NASA-TLX, SSS and Borg scores). The subjective results indicated the successful manipulation of mental fatigue (i.e. TOT): participants experienced higher levels of fatigue and sleepiness as the experiment progressed (Table 2). TOT did not affect the participants' performance, however: percentage of correct answers and their RTs were stable across the four 30-min blocks (Table 2). Participants may have increased their efforts to maintain an acceptable level of performance to compensate for increasing fatigue (Hockey, 1997). Performance and subjective results, moreover, indicated the correct manipulation of TC: the high-complexity task led to slower RTs and more incorrect answers than the low-complexity task, as well as to higher scores in the subjective scale of TC (Table 3). Subjective ratings were similar for the fixation and free-viewing conditions, although the fixation condition resulted in faster but less accurate answers (Table 3). See Supporting Information for further details.

Effect of TOT on fixational and saccadic eye movements

(Micro)saccadic main sequence

Microsaccadic and saccadic peak velocity–magnitude relationship slopes decreased with increased TOT (Fig. 3; Table 4), indicating, for the first time, an effect of mental fatigue on microsaccadic dynamics. The saccadic results are consistent with those indicated in previous reports (Hirvonen *et al.*, 2010; Di Stasi *et al.*, 2012) and

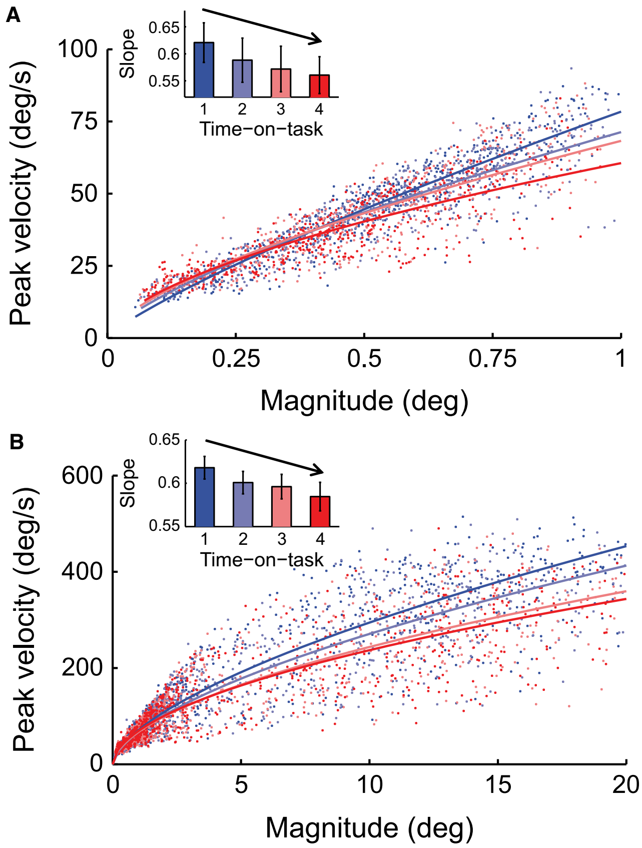

FIG. 3. Effect of TOT on the microsaccadic and saccadic main sequence. (A) Microsaccadic and (B) saccadic main sequences (peak velocity–magnitude relationships) for one participant at four different measuring times: data from the first 30 min of the session (TOT 1) indicated in blue, from the second 30 min (TOT 2) in purple, from the third 30 min (TOT 3) in pink and from the last 30 min (TOT 4) in red. Each dot represents one (micro)saccade. The curves are the power law fits to the data from each block. Insets: average slopes across all participants for each TOT: (A) $F_{3,33} = 4.983$, $P = 0.006$, MSE = 0.002, $\eta_p^2 = 0.312$ and (B) $F_{3,33} = 7.049$, $P = 0.001$, MSE = 0.0001, $\eta_p^2 = 0.391$]. The arrows indicate the significant linear trends of the slopes across TOT: (A) $F_{1,11} = 9.602$, $P = 0.010$, $\eta_p^2 = 0.466$ and (B) $F_{1,11} = 12.579$, $P = 0.005$, $\eta_p^2 = 0.533$. Error bars represent the SEM across participants ($n = 12$). (A) Data from the fixation control trials. (B) Data from the guided saccade control trials.

TABLE 4. (Micro)saccadic main sequence slopes and drift mean velocities for each experimental condition

	Task Complexity		Time-On-Task			
	Low	High	TOT 1	TOT 2	TOT 3	TOT 4
Microsaccadic slope*	0.60 ± 0.11	0.61 ± 0.11	0.62 ± 0.13	0.59 ± 0.14	0.57 ± 0.15	0.56 ± 0.12
Saccadic slope*	0.66 ± 0.05	0.64 ± 0.05	0.62 ± 0.05	0.60 ± 0.04	0.60 ± 0.05	0.58 ± 0.06
Drift mean velocity (°/s)*	1.64 ± 0.34	1.69 ± 0.54	1.43 ± 0.27	1.54 ± 0.24	1.71 ± 0.38	1.88 ± 0.63

Values are mean ± SD. Slopes are calculated as peak velocity/magnitude. Means and SDs of drift were calculated from the mean values of each participant (n = 12 participants). *P-values < 0.05 for the TOT manipulation. TC analyses were conducted with data from the ATC conditions only. TOT analyses were conducted with data from the fixation and guided saccade control trials only.

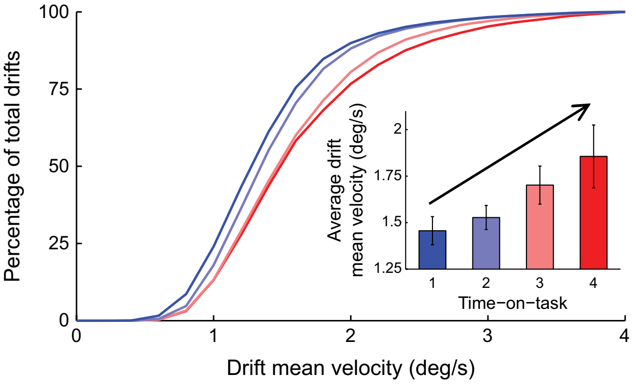

FIG. 4. Effect of TOT on drift velocity. Average drift mean velocity cumulative distributions across the four TOT blocks (n = 12 participants). Colors as in Fig. 4. Inset: average drift mean velocity across participants for each TOT ($F_{3,33}$ = 4.785, P = 0.035, MSE = 0.215, η_p^2 = 0.303). Error bars indicate the SEM across participants. The arrow indicates the significant linear trend of the average drift mean velocity across the TOT ($F_{1,11}$ = 5.868, P = 0.034, η_p^2 = 0.348). Drift peak velocity showed the same behavior (see Supporting Information Table S3). Data from the fixation control trials.

support the hypothesis of a common neural generator for microsaccades and saccades (Zuber *et al.*, 1965; Otero-Millan *et al.*, 2008, 2011; Rolfs *et al.*, 2008). Saccadic durations increased as saccadic velocities decreased, but saccadic gain and latency remained constant across the TOT levels (Supporting Information Table S3), consistent with recent observations on the effects of mental fatigue on primate saccades (Prsa *et al.*, 2010). Supporting Information Table S3 includes additional details about the effects of TOT on other saccadic and microsaccadic parameters.

Drift velocity

The mean velocity of intersaccadic drift increased significantly with increased TOT (Fig. 3; Table 4), suggesting that fixation instability increases with mental fatigue. Drift durations tended to decrease, with increased TOT (although this trend did not reach significance) while the distances covered remained unchanged (Supporting Information Table S3). Few studies have addressed drift behavior (McCamy *et al.*, 2013b), and no previous research has investigated the effects of either TOT or TC on drift parameters. Further, no previous studies of drift have been conducted in ecological or naturalistic situations (McCamy *et al.*, 2013b) such as those employed here. Supporting Information Table S3 contains further details about the effect of TOT on other drift parameters.

To test the possibility that changes in drift velocity with TOT were due to increased head motion, we conducted an additional experiment in which we held the subjects' heads in place with a bite bar (mounted on the chin/head rest used in the main experiment; see Materials and Methods for details).

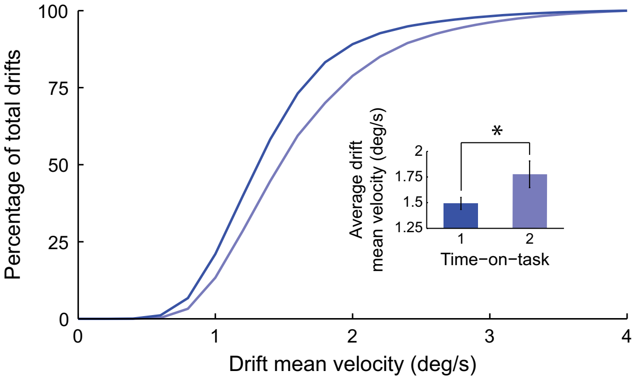

FIG. 5. Effect of TOT on drift velocity in head immobilisation conditions. Average drift mean velocity cumulative distributions across the TOT 1 and TOT 2 blocks (n = 4 participants). Colors as in Fig. 3. Inset: average drift mean velocity across participants for TOT 1 and TOT 2 (one-tailed paired t-test, t_3 = −2.58, P = 0.04). Error bars indicate the SEM across participants. Data from the fixation control trials.

Subjects ran a reduced experimental session including two TOT blocks (i.e. to minimise discomfort from bite bar use; see Materials and Methods for details). Mean drift velocity increased significantly from the first to the second TOT block (Fig. 5), corroborating the results from the main experiment and supporting the hypothesis that increased drift velocity with TOT is not due to increased head motion.

Effect of task complexity on fixational and saccadic eye movements

TC had no significant impact on fixational or saccadic eye movement dynamics (all P-values > 0.05; Table 4). The lack of TC modulation on microsaccades (Supporting Information Table S3) is consistent with the results from a previous study by Chen *et al.* (2008), who found that task difficulty affected area V1′s neuronal responses, but not microsaccadic rates, in the alert primate. The lack of TC modulation on large saccades observed here differs from previously observed increases or decreases in saccadic velocity with increased TC (Galley & Andres, 1996; Di Stasi *et al.*, 2011; see also Discussion). Table S3 contains more details about the effect of TC on other (micro)saccade and drift parameters.

Discussion

We examined the effects of TOT and TC on the dynamics of fixational eye movements and large saccades during a simulated ATC task. Our results show, for the first time, that TOT modulates the dynamics of microsaccades and drift, but with opposing effects: microsaccade velocity decreased and drift velocity increased (suggesting higher fixation instability) with increased TOT. TC did not affect microsaccade or drift parameters; thus, the TOT manipulations were responsible for the effects described above.

The effects of mental fatigue on fixational eye movements

Most of our visual experience happens while fixating (Otero-Millan *et al.*, 2008; McCamy *et al.*, 2013b). Therefore, determining which factors affect the production and characteristics of fixational eye movements, as well as their cognitive and perceptual consequences, is crucial to understanding vision as a whole (McCamy *et al.*, 2013b). Our study provides, for the first time, concrete evidence that mental fatigue modulates fixational eye movements (i.e. microsaccades and drift).

We studied mental fatigue within a temporal window similar to the duration of an actual ATC operator's work period, where the maximum TOT is approximately 2 h before a mandated break. TOT modulated the microsaccadic and saccadic main sequences in a manner consistent with previous observations concerning large saccades (Di Stasi *et al.*, 2013b), thus supporting the hypothesis that microsaccades and saccades share a common generator (Zuber *et al.*, 1965; Otero-Millan *et al.*, 2008, 2011; Rolfs *et al.*, 2008; Engbert, 2012).

No research to date has investigated the effect of attentional variations on drift (McCamy *et al.*, 2013b). Here we found that drift speed increased with increased TOT, a finding that may be linked to, or mediated by, increased sleepiness with increased mental fatigue: Ahlstrom *et al.* (2013) recently showed that high levels of sleepiness correlate with increased ocular instability. Two previous studies, moreover, found that tiredness decreased the gain of smooth pursuit (i.e. the ratio between the mean velocities of eye and target: De Gennaro *et al.*, 2000; Porcu *et al.*, 1998). It is not clear how to link these results to our current observations about drift speed, but it is possible that the low-velocity system that controls smooth pursuit also produces drifts (responding in the former case to a moving target and in the latter case to a stationary target; Nachmias, 1961; Cunitz, 1970). Future research should investigate the effects of mental fatigue on both drift and smooth pursuit in the same experiment.

A physiologically plausible explanation for the effects of mental fatigue on fixational eye movements. Changes in attentional processing (for instance, due to mental fatigue) can affect the strength of excitatory connections from the frontal cortex to the brainstem reticular formation, directly and through the superior colliculus (Munoz & Everling, 2004), thus modifying the characteristics of the main sequence and drift behavior. It follows that mental fatigue may affect eye movement velocity via the inhibitory connections between the sleep-regulating centers and the superior colliculus on the reticular formation and cerebellum. The present experiment was conducted in a darkened and sound-proof environment; thus it is possible that variations in (micro)saccadic and drift velocity were related to the activation of the brain's sleep centers (i.e. nucleus raphe magnus, nucleus raphe dorsalis and locus coeruleus). This possibility is supported by the observation that omnidirectional pause neurons (OPNs), which may modulate arousal in orienting subsystems such as the saccade generator (Optican, 2008), stop discharging during sleep (Henn *et al.*, 1984). Further, OPN inactivation produces slower saccades (Kaneko, 1996). Consistent with this idea, increased TOT led to increased subjective perception of sleepiness (SSS) in the current study (Table 2).

Task complexity

Increased air traffic density is one of the top five factors leading to poor ATC operator performance (Durso & Manning, 2008). Here we manipulated air traffic density to induce different levels of TC. Subjective and behavioral results confirmed our manipulations: higher traffic density (i.e. higher TC) led to slower RTs, more detection errors and higher levels of perceived exertion (Table 3). The above notwithstanding, increased TC did not impact (micro)saccadic or drift dynamics our current experiment.

Previous studies have found that increased TC affects saccadic dynamics (Galley & Andres, 1996; Di Stasi *et al.*, 2010a,b, 2011) and microsaccadic rates (Pastukhov & Braun, 2010; Benedetto *et al.*, 2011), albeit with inconsistent results. The difference between current and former results may be due partly to the presence of one or more secondary tasks (simultaneous to the participants' primary task) in many of the previous experiments (Di Stasi *et al.*, 2010a,b; Benedetto *et al.*, 2011). Whatever the reason for the lack of effects of TC in our study, it is worth noting that it applied to both saccades and microsaccades, thereby lending additional support to the hypothesis that saccades and microsaccades share a common generator (Zuber *et al.*, 1965; Otero-Millan *et al.*, 2008, 2011; Rolfs *et al.*, 2008; Engbert, 2012).

To our knowledge, no previous research has investigated the effect of TC on drift.

Fixational eye movements as a neuroergonomic tool

In our experiment, variations in TOT but not TC modulated fixational and saccadic eye movement parameters. The dissociation of TOT and TC effects is important, as it satisfies several neuroergonomics criteria to establish an ideal measure of attentional state in applied settings (Parasuraman & Rizzo, 2007). Briefly, the main requirements of such an attentional measure (in our case, eye-movement based) are (Luximon & Goonetilleke, 2001): (i) sensitivity: it should detect significant variations in attentional levels; (ii) noninvasiveness: it should not interfere with the primary task; and (iii) selectivity: it should be immune to other variables. The eye movement measures in the present research were: (i) sufficiently sensitive to reveal significant differences between varied levels of mental fatigue; (ii) noninvasive, that is, the eye tracker did not interfere with task performance; and (iii) selective, that is, the measures were sensitive to TOT yet insensitive to TC. To sum up, our results suggest that eye tracking can provide a sensitive, real-time, non-invasive measure of attentional fluctuations due to TOT, without interfering with task performance or compromising safety.

Decreased attentional levels can cause operators to misread or ignore incoming information, with the effect of compromising safety and job performance. Thus, there is a great need to monitor mental state in real-time in complex systems such as ATC towers, where the combination of long duty periods, insufficient sleep, monotonous tasks and high stress leads to physical and mental operator fatigue. Numerous studies have focused on assessing and/or improving ATC work conditions (McKinley *et al.*, 2012), but fatigue-related incidents continue to occur. To address this problem, international agencies have conducted extensive research on ATC operators' fatigue (Eurocontrol, 2013b) and put in place new regulations to increase staff numbers and decrease work hours (FAA, 2012). Here we show that eye movement parameters such as (micro)saccadic and drift velocities can serve as indicators of mental fatigue. These findings are valuable because fixational eye movements occur not only during prolonged fixation but also in the intersaccadic fixation periods during normal visual exploration (Otero-Millan *et al.*, 2008; McCamy *et al.*, 2013b). Thus, it is possible to monitor eye movement indices of mental fatigue while operators are involved in their duty, without the need for currently used artificial oculomotor tests such as the guided saccade task (e.g. Hirvonen *et al.*, 2010; Di Stasi *et al.*, 2012; Ahlstrom *et al.*, 2013). Continuous on-line eye-movement-based evaluation of ATC operators could improve safety and efficiency and reduce operational costs. This effort will require the translation of research findings and methods to ecological and complex environments to enable system designs that maximise human–system interaction.

Concluding remarks

Fixational eye movements (microsaccades and drift) and saccadic parameters can indicate mental fatigue reliably during prolonged visual search, irrespective of task complexity. These findings have potential impacts in the development of neuroergonomic tools to detect fatigue in ecological situations, and moreover suggest that fixational eye movement dynamics have the potential to signal the nervous system's activation state.

Supporting Information

Additional supporting information can be found in the online version of this article:

Data S1. Effectiveness of the Semi-Latin square experimental design.

Data S2. Effectiveness of TOT and TC manipulations.

Table S1. General matrix for the analysis on the effect of the experimental series.

Table S2. Effects of the experimental conditions (p-values) for each for each dependent variable and location in the sequence.

Table S3. Saccadic, microsaccadic, and drift parameters.

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Abbreviations

ATC, air traffic control; RT, reaction time; SSS, Stanford Sleepiness Scale; TC, task complexity; TOT, time-on-task.

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