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Intersaccadic drift velocity is sensitive to short-term hypobaric hypoxia

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Abstract

Hypoxia, defined as decreased availability of oxygen in the body's tissues, can lead to dyspnea, rapid pulse, syncope, visual dysfunction, mental disturbances such as delirium or euphoria, and even death. It is considered to be one of the most serious hazards during flight. Thus, early and objective detection of the physiological effects of hypoxia is critical to prevent catastrophes in civil and military aviation. The few studies that have addressed the effects of hypoxia on objective oculomotor metrics have had inconsistent results, however. Thus, the question of whether hypoxia modulates eye movement behavior remains open. Here we examined the effects of short-term hypobaric hypoxia on the velocity of saccadic eye movements and intersaccadic drift of Spanish Air Force pilots and flight engineers, compared with a control group that did not experience hypoxia. Saccadic velocity decreased with time-on-duty in both groups, in correlation with subjective fatigue. Intersaccadic drift velocity increased in the hypoxia group only, suggesting that acute hypoxia diminishes eye stability, independently of fatigue. Our results suggest that intersaccadic drift velocity could serve as a biomarker of acute hypoxia. These findings may also contribute to our understanding of the relationship between hypoxia episodes and central nervous system impairments.

Introduction

Human brain function is highly vulnerable to hypoxic insults (Lutz *et al.*, 2003; Papadelis *et al.*, 2007). Hypoxia impairs vision (Tune, 1964; Kobrick & Appleton, 1971), cognition and motor control, and can cause severe incapacitation and death (for a recent review see Petrassi *et al.*, 2012). Reports on the effects of hypoxia on visual function [i.e. dark adaptation, central brightness contrast, color vision and central acuity (Banderet & Shukitt-Hale, 2002)] have been confounded by subjective and environmental factors (i.e. changes in ambient light level and non-compliance by flight crews in accurately reporting physiological disabilities such as color blindness) (Cymerman *et al.*, 2003). The few studies that have addressed the effects of hypoxia on objective oculomotor metrics, such as saccadic velocity, have obtained inconsistent results (Still *et al.*, 2011; Merz *et al.*, 2013). The question of whether hypoxia modulates oculomotor metrics therefore remains open.

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Acute hypoxia, defined as decreased availability of oxygen in the body's tissues that can lead to dyspnea, rapid pulse, syncope, visual dysfunction and mental disturbances such as delirium or euphoria (Miller, 2003; Guerra-Narbona *et al.*, 2013), is one of the most serious single hazards in military and civil aviation (Cable, 2003; Temme *et al.*, 2010). Thus, international organizations such as the US Federal Aviation Administration and the European Aviation Safety Agency recommend hypoxia training [i.e. performance training while reducing oxygen availability to the trainee (Evetts *et al.*, 2005)] as a mandatory part of flight and cabin crew instruction (Smith, 2008). Altitude chamber training – a well-established method to train aircrews to recognize early symptoms and signs of hypoxia – has not eliminated in-flight hypoxic incidents, however. Here we set out to determine if non-invasive eye movement analyses, of the type that could potentially take place continually, unobtrusively and automatically in-flight, could objectively indicate impaired oculomotor function due to hypoxia.

We asked whether acute hypoxia might affect oculomotor dynamics – including saccadic metrics (Di Stasi *et al.*, 2013a) and intersaccadic drift metrics – the involuntary slow movement of the eyes between saccades (Martinez-Conde *et al.*, 2004, 2009, 2013) – while

dissociating the effects of hypoxia from the effects of time-on-duty (TOD) on eye movement parameters, for the first time.

We tracked eye movements in pilots and flight engineers before and after a simulated flight in hypobaric hypoxic conditions, as compared with a control group who did not undergo hypoxia training. Saccadic velocity decreased with TOD in both the hypoxia and the control groups, in correlation with subjective fatigue. Intersaccadic drift velocity increased only in the hypoxia group, however, suggesting that acute hypoxia diminishes stable gaze holding.

Methods

Ethical approval

We conducted the study in conformity with the declaration of Helsinki and the Spanish Defence Medical Inspector General's Office's IRB (approval date: 26 July 2012). Written informed consent was obtained from each participant.

Subjects

Participants attended the Spanish Defence Aero-medical Center (CIMA) for aviation medicine training. Most subjects were members of the Spanish Air Force flight crew (i.e. pilots and flight engineers). All subjects had normal or corrected-to-normal vision and underwent a full physical examination prior to study participation.

Six male subjects, most of them aircrew operating rotary wing aircrafts receiving hypoxia training [mean age, height and weight: 37 years (± 6.4), 176 cm (± 5.1) and 85 kg (± 8.5)], comprised the hypoxia group. Six different male subjects, receiving no hypoxia training [mean age, height and weight: 35 years (± 11), 181 cm (± 3.8) and 83 kg (± 7.3)], comprised the control group.

Experimental design

The study followed a pre/post-test design. Hypoxia training was the between-subjects factor and eye movement metrics, including intersaccadic drift (hereafter drift) velocity, and the saccadic peak velocity–magnitude relationship (and the other main sequence relationships; see Table S1) were the dependent variables. We also recorded the participants' subjective level of fatigue via a standardized questionnaire.

Stimuli and instruments

The CIMA altitude training chamber (Environmental Tectonics Corporation, Southampton, PA, USA) is a computer-controlled, manrated, low-pressure chamber that accommodates ten subjects and one inside safety observer. A vacuum pump removes pressure from the chamber to simulate the pressure of a particular altitude. The CIMA training involves various hypobaric training regimes; in this study we used training Type 1b, which consists of depressurizing the hypobaric chamber to a simulated maximum altitude of 25 000 ft (7620 m), to conduct a demonstration of acute hypoxia (Fig. 1A). Here, each subject experienced hypoxia (equivalent to an altitude of 22 000 ft) for a maximum of 3.25 min without supplemental oxygen. All subjects exhibited cognitive impairment during hypoxia exposure, as indicated by the standard hypoxia demonstration sheet (pencil and paper test) (for an example, see Seedhouse, 2008). Pulse oximetry, measured with a non-invasive pulse oximeter on the subject's non-writing-hand's fingertip, confirmed a final oxyhemoglobin saturation between 62 and 77% (± 5.9) S_pO_2 in each subject.

Before and after the subjects entered the hypoxic chamber, we assessed their oculomotor dynamics via a guided-saccade task (modified from Di Stasi *et al.*, 2012). In a darkened and quiet room, participants sat on a comfortable chair and rested their forehead and chin on an EyeLink 1000 (SR Research, Ontario, Canada) head/chin support, 70 cm from a 21-inch CRT screen (864 × 1152 pixels, refresh rate 100 Hz) in which we displayed visual stimuli. Briefly, participants were instructed to follow a jumping fixation target displayed on the screen (Fig. 1B), and thus produced 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 eight randomly selected sizes (measured from the starting location: 2.5°, 5°, 7.5°, 10°, 12.5°, 15°, 17.5° or 20°) and in three randomly selected directions (vertical, horizontal or diagonal). This resulted in a total of 672 saccades per subject. The saccade directions were balanced across the saccade sizes. Subjects performed the same guided saccade task in each of the two measuring sessions (i.e. before and after hypoxia exposure). Subjects completed the task in ~15 min.

Eye position was sampled binocularly at 500 Hz using the desktop configuration of the EyeLink 1000 eye tracking system (SR Research, Ontario, Canada). We detected and classified eye movements and calculated a linear regression on the log-transformed saccadic peak velocities as a function of their magnitudes for each subject (Di Stasi *et al.*, 2013b,c), where the slope reflected the effect of hypoxia on the saccadic peak velocity–magnitude relationship (see the supplementary section 'Eye movement recordings and analyses' for further details).

Subjective fatigue is a well-known effect of both hypoxia (e.g. Van Liere & Stickney, 1963) and TOD (Di Stasi *et al.*, 2012). TOD is, moreover, known to affect saccadic and drift metrics, perhaps due to variations in fatigue and/or arousal levels (Di Stasi *et al.*, 2013a,b,c). Thus, the present experiment was designed to disambiguate the effects from hypoxia on oculomotor metrics from those of TOD. Participants filled in a self-rating scale of perceived fatigue (Borg, 1998), before each oculomotor test.

Procedure

All subjects received a standard briefing on the effects of simulated altitude and hypoxia on the day preceding the experiment. On the day of the training, aircrews underwent hypoxia training and two measuring sessions (Pre- and Post-Test; Fig. 1B) between 09.00 and 12.30 h (approx. 3 h elapsed between the two sessions). The interval between the return to ground level and the start of the Post-Test session was ~30 min. Control subjects performed their regular office/administrative or workshop (e.g. equipment maintenance) duties between the Pre- and Post-Test sessions.

Statistical analysis

We analysed the oculomotor parameters with a mixed-factorial design. We calculated a separate 2 × 2 repeated-measures ANCOVA for each dependent oculomotor variable. The measuring session served as the within-subjects factor. Thus, the data from each participant before hypobaric oxygen exposure were compared with the data from the same participant after hypobaric oxygen exposure. Group served as the between-subjects factor (i.e. only six participants out of 12 received hypoxia training).

In addition, to control for the potential effects of gaze eccentricity on drift velocity (Fig. 2C and D), we binned drift velocities into six non-overlapping, 5° bins according to the corresponding gaze eccentricities. Gaze eccentricity then served as a new within-subjects

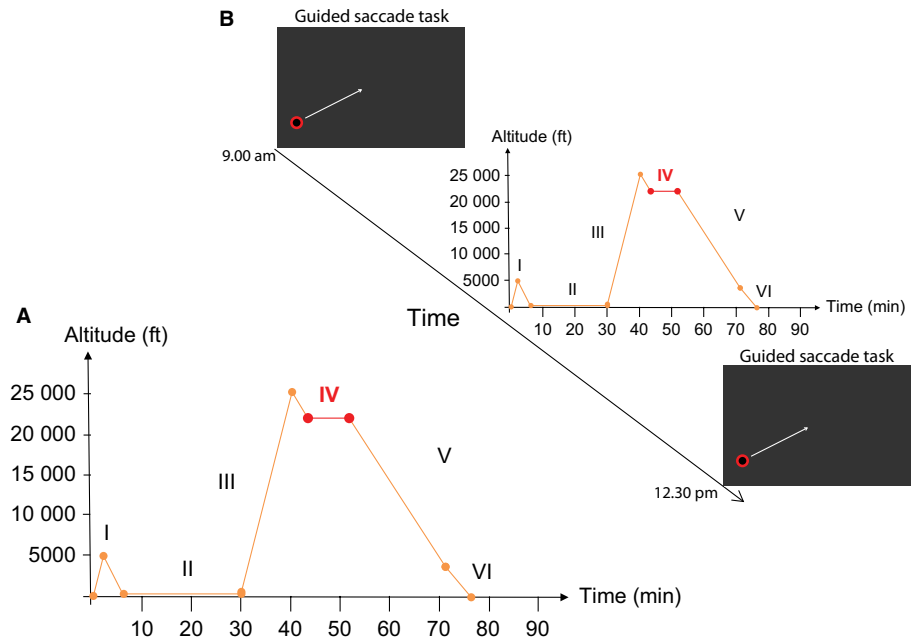

FIG. 1. Experimental design. (A) Simulated-flight altitude as a function of time. (I) An initial ear and sinus check ascent to 5000 ft MSL is followed (II) by a 30-min denitrogenation period at ground level, with the subjects breathing 100% oxygen via a pressure-demand-type oxygen mask. (III) Then, subjects experience an ascent to 25 000 ft, where (IV) hypoxia conditions are administered (~10 min total, with each subject undergoing hypoxia for up to 3.25 min without supplemental oxygen), (V) followed by a simulated descent to ground level. (VI) The total duration of the simulated flight was ~73 min. (B) Experimental timeline. The experiment began (Pre-Test) and finished (Post-Test) with the guided-saccade task. Control subjects carried out their regular duties between the Pre- and Post-Test sessions. All participants filled in a self-rating scale of perceived fatigue before each oculomotor test. See Methods for further details.

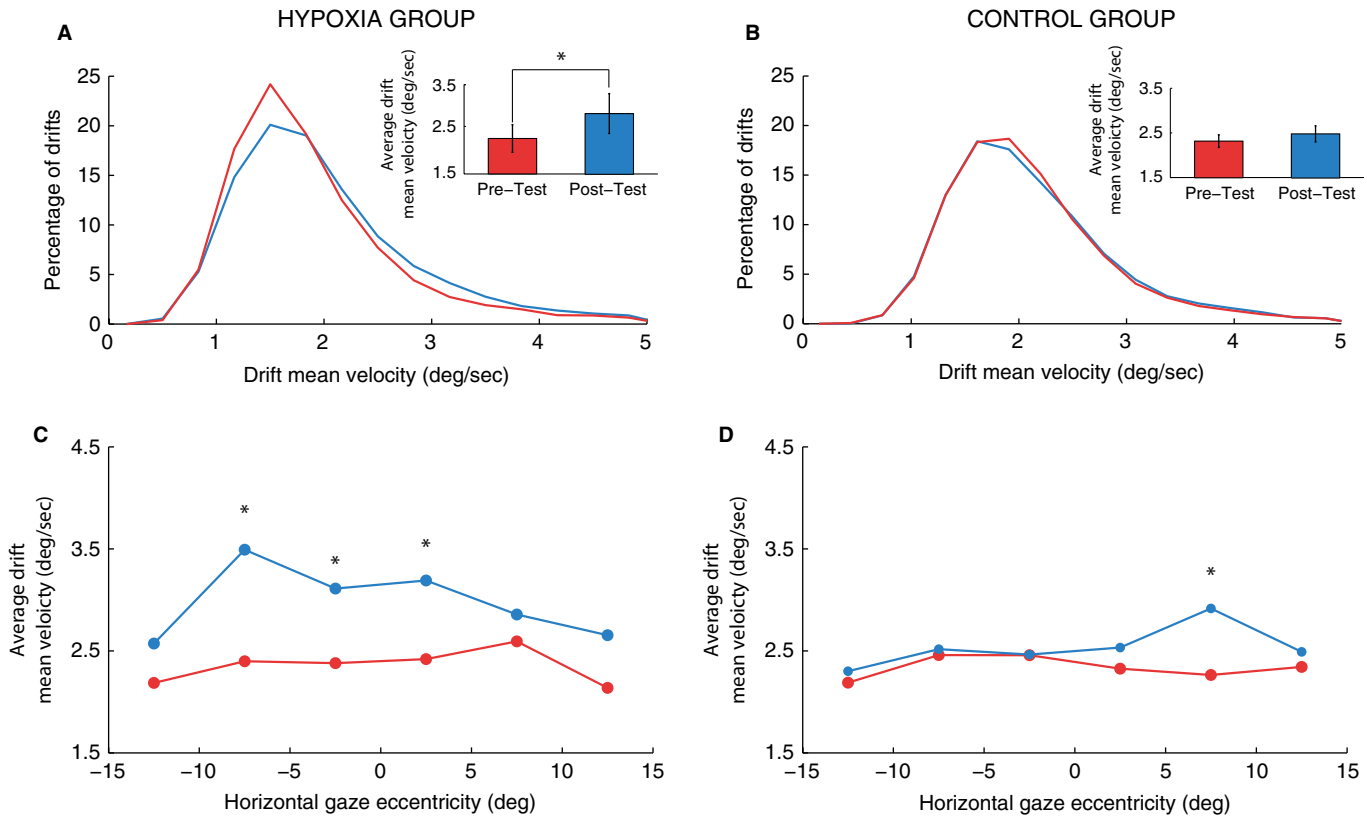

FIG. 2. Effects of hypoxia on intersaccadic drift. (A, B) Drift mean velocity distributions before and after hypoxia training (or equivalent TOD for the control group). Pre-Test indicated in red and Post-Test in blue for all subjects. Insets: average mean drift velocities across all subjects for each of the Pre/Post-Test sessions, in each group. Mean drift velocity increased significantly from the Pre-Test to the Post-Test session in the hypoxia group, but not in the control group. (C, D). Average drift mean velocity as a function of horizontal gaze eccentricity and measuring session. Negative values indicate left eccentricities as seen by the subject. Mean drift velocities were calculated in 5° bins for each subject. There was a larger increase in drift velocities, across eccentricities, for the hypoxia group than for the control group. Asterisks indicate statistical significance.

factor in a separate 2 × 2 × 6 repeated-measures ANCOVA. We used *post-hoc* Holm–Bonferroni corrections to compensate for multiple comparisons across bins.

Subjective scores of perceived fatigue (Borg, 1998) served as covariates in the ANCOVAs above. Thus, we controlled statistically for the influence of TOD on the eye movement variables. For the subjective scores of perceived fatigue we used a 2 × 2 repeated-measures ANOVA with the two measuring sessions (Pre-Test vs. Post-Test) as the within-subjects factor and Group (experimental vs. control group) as the between-subjects factor.

Results

Drift mean velocity

Hypoxia induced an increase in the mean velocity of intersaccadic drift (Fig. 2A and B, and Table 1), suggesting a decrease in visual fixation stability. That is, drift mean velocity was significantly higher in the Post-Test session than in the Pre-Test session for the hypoxia group, but not for the control group, after controlling for the effect of TOD (i.e. by considering the scores of the self-rating scale of perceived fatigue as covariates) (interaction between *measuring session* and *group*: $F_{1,8} = 10.192$, $P < 0.013$; $\eta_p^2 = 0.56$). Drift peak velocity and distance covered by drift were also significantly higher in the Post-Test than in the Pre-Test session for the hypoxia group, but not for the control group, consistent with the hypothesis of higher fixation instability with hypoxia (Table S1). Drift duration was not affected (Table S1). There were no significant main effects of hypoxia versus control groups or of Pre- versus Post-Test sessions (all F-values < 3).

Drift is dependent upon absolute eye position (Eizenman *et al.*, 1990; Bertolini *et al.*, 2013); thus, the subjects' location of fixation in the visual field could affect drift velocity. To control for the potential effects of gaze eccentricity on drift velocity, we conducted an additional analysis in which we sorted the data into left and right gaze positions, and then binned them according to the final absolute eye position to the left and to the right from the center position (i.e. following from Bertolini *et al.*, 2013). Consistent with the previous findings, the results showed a larger increase in drift velocities, across eccentricities, in the hypoxia group than in the control group (interaction between *measuring session* and *group*: $F_{1,8} = 14.385$, $P < 0.006$; $\eta_p^2 = 0.64$). Holm–Bonferroni *post-hoc* comparisons, moreover, showed that three of six bins were higher in the Post-Test than in the Pre-Test in the hypoxia group, versus only one bin being higher in the Post-Test than in the Pre-Test in the control group (maximum adjusted P-value = 0.0125) (Fig. 2C and D, and Table S2). Drift velocities did not differ statistically across eccentricities in either the hypoxia or the control group, Pre- or Post-Test (all F-values < 2), possibly because gaze holding positions were a maximum of 15° from the center of the screen (whereas in Bertolini *et al.*, 2013, the most substantial effects of gaze position on drift velocity occur at eccentricities of 20–40°).

Saccadic main sequence

The slope of the saccadic magnitude/peak velocity relationship decreased from the Pre-Test to the Post-Test session in both the hypoxia and the control groups ($F_{1,10} = 7.32$, $P = 0.02$) (Fig. 3 and Table 1), suggesting that this effect was due to TOD rather than hypoxia. Indeed, when we controlled statistically for the influence of TOD (i.e. by considering the scores of the self-rating scale of perceived fatigue as covariates) we found no significant effects (all F-values < 1) on the saccadic magnitude/peak velocity of either group. Saccadic magnitude/duration and saccadic magnitude/mean velocity relationships showed equivalent behaviors to the saccadic magnitude/peak velocity relationship (Table S1). This is consistent with previous reports of the modulatory effects of TOD on saccade dynamics (for a review see Di Stasi *et al.*, 2013a). Table S1 includes additional details about the effects of measuring session and group on other saccadic parameters.

Borg self-rating scale of perceived fatigue

The degree of perceived fatigue increased from the Pre-Test to the Post-Test session in both groups ($F_{1,10} = 5$; $P = 0.049$; $\eta_p^2 = 0.34$). That is, increased TOD resulted in increased perceived fatigue in both groups. Neither the main effect of the group nor its interaction with the measuring sessions was significant (all F-values < 1) (see Table 1).

Discussion

We examined the effects of short-term hypobaric hypoxia on saccade and drift dynamics, via a standardized oculomotor test. We controlled statistically for fatigue due to TOD by considering the scores of the self-rating scale of perceived fatigue (Borg, 1998) as covariates. Our results show that short-term hypobaric hypoxia gives rise to variations in drift velocity. Hypoxia-triggered increases in drift speed may indicate a decrease in fixation stability, and the rapid compensations of the oculomotor system to correct the ensuing fixation errors.

The few studies that have tested the sensitivity of oculomotor dynamics as a tool to detect acute hypoxic events have produced contradictory results (Van der Post *et al.*, 2002; Cymerman *et al.*, 2003). Van der Post *et al.* (2002) found decreased saccadic peak velocities under moderate normobaric hypoxic conditions and proposed that this

TABLE 1. Subjective, saccadic and intersaccadic drift parameters

	Pre-Test		Post-Test	
	Control Group	Experimental Group	Control Group	Experimental Group
Drift mean velocity (°/s)*	2.31 [2.25] (0.35)	2.30 [2.35] (0.75)	2.50 [2.34] (0.44)	2.85 [2.99] (1.09)
Slope saccadic magnitude/peak velocity (°/s)	0.69 [0. 69] (0.04)	0.68 [0.68] (0.04)	0.69 [0.69] (0.04)	0.67 [0.67] (0.05)
Borg scale	6.67 (2.9)	7.67 (2.0)	8.67 (2.1)	8.67 (2.7)

The scores of the self-rating scale of perceived fatigue (Borg's scale) range between 6 and 20. Higher scores indicate more subjective fatigue. For the eye movement data, we calculated the means and standard deviations (in parentheses) from the mean values of each subject in each group ($n = 6$). The adjusted means (in square brackets) refer to the group means after controlling for the effect of TOD [i.e. by considering the scores of the self-rating scale of perceived fatigue as covariates (ANCOVA-adjusted means)]. * indicates statistical significance.

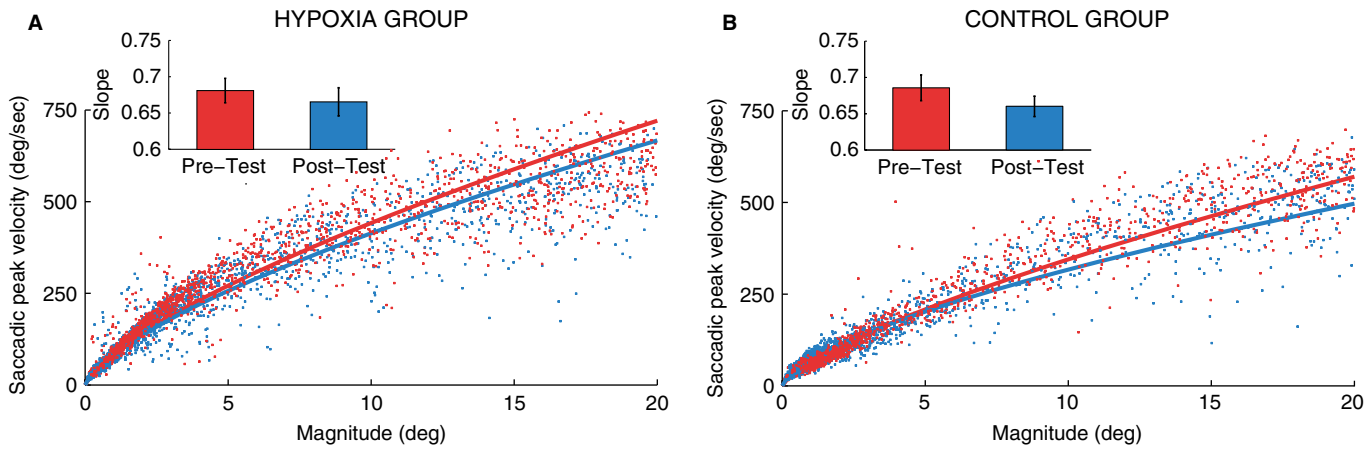

FIG. 3. Saccadic magnitude/peak velocity relationships for one experimental subject (A) and one control subject (B) at two different measuring times: Pre-Test (red) and Post-Test (blue). Each dot represents one saccade. The curves are the power-law fits to the data from each measuring session. Insets – average slopes across all subjects for each of the Pre/Post-Test sessions, in each group. Saccadic peak velocity decreased from the Pre-Test to the Post-Test session for both hypoxia and control groups, but the effect was not statistically significant when controlled for the influence of TOD. In all panels, error bars indicate the SEM across subjects (see Table 1 for the ANCOVA-adjusted means).

impairment resulted from the slowing of brain processes due to decreased oxygen. A different study found no effect of hypobaric hypoxia on saccadic velocities (Cymerman *et al.*, 2003). Neither study accounted for the influence of fatigue due to TOD, however, which is a well-documented modulator of saccadic velocity (Di Stasi *et al.*, 2013a,c). This confounding factor is further complicated by the fact that both hypoxia (Van Liere & Stickney, 1963; Howlett & Hogan, 2007) and TOD (Di Stasi *et al.*, 2012) induce fatigue. The effect of hypoxia on saccadic velocity in the present study was no longer significant after controlling for the influence of fatigue due to TOD. Therefore, the decrease in saccadic peak velocities observed here (and perhaps also in Van der Post *et al.*, 2002) is most parsimoniously explained by TOD, rather than hypoxia. Thus, the current study reconciles previous disparate results.

One may wonder if the present changes in drift velocity might have resulted from increased head motion in the post-acute-hypoxia state. This possibility seems unlikely in light of previous research showing that the same eye-tracking system (EyeLink 1000, SR Research) and forehead/chin rest used here can detect variations in drift velocity independently of head motion (Di Stasi *et al.*, 2013b). Thus, the most parsimonious explanation for the current results is that drift velocity is indeed sensitive to hypobaric hypoxia.

We also note that the drift velocities measured here are higher than in classical studies (Ditchburn & Ginsborg, 1953; Eizenman *et al.*, 1990), but in line with those reported in a number of recent studies (Murakami, 2004, 2010; Khojasteh *et al.*, 2012; Di Stasi *et al.*, 2013b). Drift speeds are highly dependent on the recording system used. For example, McCamy *et al.* (unpublished data) obtained significantly higher drift speeds with EyeLink 1000 (also used here) than with simultaneous recordings conducted with the search coil technique. One additional contributor to higher drift speeds in contemporary than in earlier studies may be differences in the computational tools used (Cherici *et al.*, 2012): (i) drift velocities calculated from the horizontal component of the velocity vector only (i.e. as in many early studies) are smaller than those calculated using the 2D velocity modulus (i.e. as in most recent studies, including this one); (ii) classical studies tended to neglect the curvilinear component of drift motion (due to lacking the necessary computational tools to consider it), which may have led to underestimated drift velocities (i.e. because drift tends to change direction frequently). Furthermore, drift velocities may be higher during visual exploration (i.e. as in the present guided-saccade task) than during sustained fixation (i.e. as in Eizenman *et al.*, 1990) (Poletti *et al.*, 2010). It is important to weigh these considerations in the context of distinguishing drift from end-point nystagmus (Eizenman *et al.*, 1990).

Critically, all drift velocity measurements reported here were accomplished within a single system, and so any changes across experimental conditions (i.e. Pre-Test vs. Post-Test) are relative to that system. Thus, although the present drift velocities may be higher than in classical studies, or studies performed with different eye tracking systems, this fact has no bearing on our study's conclusions.

In summary, we found that short-term hypobaric hypoxia affected drift, but not saccadic, velocities. This dissociation may arise at the level of the neural integrator responsible for gaze holding between saccades. The neural integrator for the vertical oculomotor system is located in the interstitial nucleus of Cajal in the rostral mesencephalon, and the integrator for the horizontal system is located in the nucleus prepositus hypoglossi, an area of the rostral medulla that receives strong projections from the paramedian portion of the pontine reticular formation, the vestibular nuclei and the cerebellum. Accordingly, studies have shown that the cerebellum plays an important role in gaze holding (Leigh & Zee, 2006; Bertolini *et al.*, 2013). Because hypoxia induces alterations in the metabolic system of the cerebellum (Serrano *et al.*, 2003; Atterbury & Wall, 2011), it may increase eye instability by disrupting the cerebellar inputs to the neural integrator (Zee *et al.*, 1980; Eizenman *et al.*, 1990; Aksay *et al.*, 2007).

Concluding remarks

Hypoxia is considered one of the most serious hazards during flight. Thus, early and objective detection of the physiological effects of hypoxia may serve to alert aircrews before they are unable to take corrective action, and help to prevent catastrophes in aviation. The few studies that have addressed the effects of hypoxia on objective oculomotor metrics have obtained inconsistent results, however. Therefore, the question of whether hypoxia modulates eye movement behavior remains open. Here we examined the effects of short-term hypobaric hypoxia on the velocity of saccadic eye movements and intersaccadic drift. Our results showed that intersaccadic drift

velocity increased with hypoxia exposure, suggesting that drift velocity could serve as a biomarker of acute hypoxia. Drift is one of the main types of eye movements that subjects produce whenever they fix their gaze on an object of interest, and therefore an integral part of the oculomotor repertoire of aviators as they carry out their duties in naturalistic scenarios. Thus, our findings have the potential to reduce the gap between basic and applied neuroscience, and to offer medical aviation departments a tool that may assist the development of screening and training programs.

Future studies should determine whether the repeated exposure to hypoxia may lead to an improvement in oculomotor parameters in human subjects. Recent research has found that training in hypoxic environments can improve the performance of some motor and cognitive tasks in mice (López-Ramos *et al.*, 2007; Guerra-Narbona *et al.*, 2013), suggesting that refresher hypoxia training might similarly improve sensorimotor pilot performance. The question of whether refresher hypoxic training may help to improve responses to emergency situations in aviation is currently open (Smith, 2008; Woodrow *et al.*, 2011).

Supporting Information

Additional supporting information can be found in the online version of this article:
Table S1. Saccadic and drift parameters.
Table S2. Intersaccadic drift mean velocity as a function of horizontal *gaze eccentricity* and *measuring session*.

Acknowledgements

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Abbreviations

CIMA, Spanish Defence Aero-medical Center; TOD, time-on-duty.

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