COGNITIVE NEUROSCIENCE

Pupil size reveals preparatory processes in the generation of pro-saccades and anti-saccades

Chin-An Wang, Donald C. Brien and Douglas P. Munoz
Centre for Neuroscience Studies, Queen's University, Botterell Hall, 18 Stuart Street, Kingston, ON K7L 3N6, Canada

Abstract

The ability to generate flexible behaviors to accommodate changing goals in response to identical sensory stimuli is a signature that is inherited in humans and higher-level animals. In the oculomotor system, this function has often been examined with the anti-saccade task, in which subjects are instructed, prior to stimulus appearance, to either automatically look at the peripheral stimulus (pro-saccade) or to suppress the automatic response and voluntarily look in the opposite direction from the stimulus (anti-saccade). Distinct neural preparatory activity between the pro-saccade and anti-saccade conditions has been well documented, particularly in the superior colliculus (SC) and the frontal eye field (FEF), and this has shown higher inhibition-related fixation activity in preparation for anti-saccades than in preparation for pro-saccades. Moreover, the level of preparatory activity related to motor preparation is negatively correlated with reaction times. We hypothesised that preparatory signals may be reflected in pupil size through a link between the SC and the pupil control circuitry. Here, we examined human pupil dynamics during saccade preparation prior to the execution of pro-saccades and anti-saccades. Pupil size was larger in preparation for correct anti-saccades than in preparation for correct pro-saccades and erroneous pro-saccades made in the anti-saccade condition. Furthermore, larger pupil dilation prior to stimulus appearance accompanied saccades with faster reaction times, with a trial-by-trial correlation between dilation size and anti-saccade reaction times. Overall, our results demonstrate that pupil size is modulated by saccade preparation, and neural activity in the SC, together with the FEF, supports these findings, providing unique insights into the neural substrate coordinating cognitive processing and pupil diameter.

Introduction

The ability to act flexibly in response to identical sensory stimuli is a critical cognitive behavior in humans and animals. This flexibility in responding has been attributed to the variations in readiness to respond and the intention to perform a particular act, commonly referred to as preparatory set (Hebb, 1972; Evarts *et al.*, 1984). The anti-saccade task has provided much insight into preparatory set in the oculomotor system (Munoz & Everling, 2004), because subjects in the task are instructed prior to stimulus appearance to generate either a pro-saccade (look at a peripheral stimulus) or an anti-saccade (look in the opposite direction from the stimulus). Unlike the automatic visuomotor response required in the pro-saccade condition, to complete an anti-saccade subjects must suppress the automatic saccade and generate a voluntary response in the opposite direction from the stimulus.

For the successful execution of pro-saccade or anti-saccades, distinct neural preparatory signals are preset after task instruction (e.g. Munoz & Everling, 2004; McDowell *et al.*, 2008). Although many areas have been identified, the superior colliculus (SC) and the frontal eye field (FEF) are particularly important, because they pro-

ject directly to the paramedian pontine reticular formation to provide the necessary input to initiate saccadic eye movements (Schiller *et al.*, 1980). In human functional magnetic resonance imaging (fMRI) studies, there is an increase in FEF activation during preparation for anti-saccades as compared with preparation for pro-saccades (Connolly *et al.*, 2002; DeSouza *et al.*, 2003; Manoach *et al.*, 2007). Additionally, preparatory activity negatively correlates with saccade reaction times (SRTs) (Connolly *et al.*, 2005; Alahyane *et al.*, 2014). Similarly, in single-neuron recordings of the monkey SC and FEF, there is higher fixation-related activity (rostral region) prior to stimulus appearance on correct anti-saccades than on pro-saccades, and the level of pre-saccadic activity (caudal region) negatively correlates with SRTs (Dorris *et al.*, 1997; Dorris & Munoz, 1998; Everling *et al.*, 1998, 1999; Everling & Munoz, 2000).

We hypothesised that these documented preparatory activities should be reflected in pupil size, because the SC has links to the pupil control circuit (C.A. Wang & D.P. Munoz, unpublished data). Microstimulation of both the rostral and the caudal SC evokes transient pupil dilation (Netser *et al.*, 2010; Wang *et al.*, 2012), and modulations of stimulus contrast and modality on SC activity are similar to those modulations on pupil size (Wang & Munoz, 2014; Wang *et al.*, 2014). To examine the relationship between different types of saccade preparation and pupil dynamics, we use an inter-

leaved pro-saccade and anti-saccade paradigm, with a gap of no stimulus inserted between fixation point (FP) disappearance and peripheral stimulus appearance, to investigate preparatory processes for the generation of pro-saccades or anti-saccades. Before stimulus appearance, greater pupil dilation was observed in preparation for correct anti-saccades than in preparation for correct pro-saccades, and pupil dilation was smaller when erroneous pro-saccades were made in the anti-saccade condition. Furthermore, pupil dilation was larger during the generation of faster saccades than during the generation of slower saccades. Together, our results suggest that pupil size is an effective proxy of neural activity related to preparation of pro-sccade and anti-saccade generation.

Materials and methods

Participants

All experimental procedures were reviewed and approved by the Queen's University Human Research Ethics Board in accordance with the Declaration of Helsinki. Nineteen participants, ranging in age between 20 and 23 years, were recruited for this study. All participants had normal or corrected-to-normal vision, and were naïve regarding the purpose of the experiment. They provided informed consent, and were compensated for their participation.

Recording and apparatus

Eye position and pupil size were measured with a video-based eye tracker (Eyelink-1000 monocular-arm; SR Research, Osgoode, ON, Canada) at a rate of 500 Hz with monocular recording (the left eye and pupil were used for consistency with previous studies). Stimulus presentation and data acquisition were controlled by Eyelink Experiment Builder and EYELINK software. Stimuli were presented on a 17-inch LCD monitor at a screen resolution of 1280×1024 pixels (60-Hz refresh rate), subtending a viewing angle of $32 \times 26°$, and the distance from the eyes to the monitor was set at 58 cm (suggested by the Eyelink company). We used the suggested method (Steiner & Barry, 2011; Wang et al., 2012) to transfer output pupil area values recorded from the eye tracker to actual pupil size in diameter (see Wang & Munoz, 2014).

Pro-saccade and anti-saccade task

Participants were seated in a normal illuminated room, and the experiment consisted of 120 trials (Fig. 1A). Each trial began with the appearance of an FP (diameter, 0.5°; 42 cd/m²) on a black background (0.1 cd/m²). The trial condition was revealed via the FP color (pro-saccade, red FP; anti-saccade, green FP; the luminance level of two FP colors was matched). After 1000 ms of central fixation, the FP disappeared for 200 ms (gap) before the peripheral stimulus appeared (diameter, 0.5°; gray dot with luminance 42 cd/m²) to the left or right of the FP (10° eccentricity on the horizontal axis). The gap period between FP disappearance and peripheral stimulus appearance was inserted to examine preparatory processes and induce more directional errors in the anti-saccade condition. In pro-saccade trials, the participants were instructed to look towards the peripheral stimulus as soon as it appeared. In anti-saccade trials, the participants were instructed to look in the opposite direction from the stimulus as soon as it appeared. Trial condition (pro-saccade and anti-saccade) and stimulus location (left and right) were randomly interleaved. Saccades towards either the right or left direction were combined for data analysis.
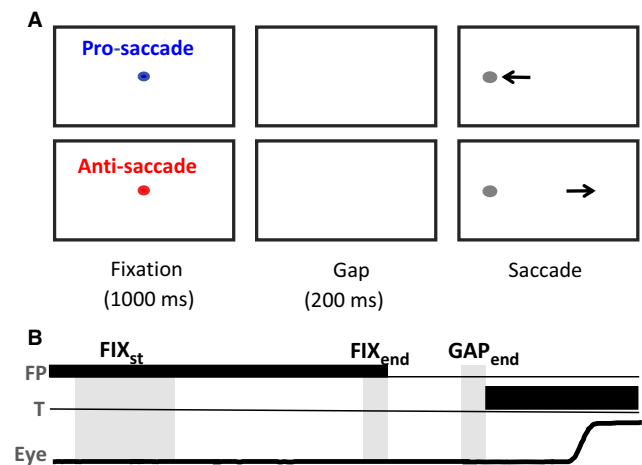

Fig. 1. (A) Each trial started with a central colored fixation point (42 cd/m²: two isoluminant colors for the pro-saccade and anti-saccade conditions, respectively) on a black background. After 1000 ms, a blank screen was presented for 200 ms (gap) before stimulus presentation. Participants were required to move their eyes to the stimulus in the pro-saccade condition, but move to the opposite location in the anti-saccade condition. Note that the FP colors displayed here are only for illustration of the paradigm. (B) Three selected epochs for pupil analyses: FIX_{st} (fixation start), 100–300 ms after fixation onset; FIX_{end} (fixation end), 950–1000 ms after fixation onset; GAP_{end} (gap end), 150–200 ms after gap onset. Eye, eye position; T, target.

Data analysis

SRT was defined as the time from the target appearance to the first saccade away from fixation (eye velocity exceeded 30°/s). Trials were scored as correct if the first saccade after stimulus appearance was in the correct direction (towards the stimulus in the pro-saccade condition; away from the stimulus in the anti-saccade condition). Direction errors were identified as the first saccade in the wrong direction after target appearance (e.g. towards the stimulus in anti-saccade trials). Short-latency stimulus-driven saccades within the first peak of a multimodal distribution of SRTs are traditionally identified as express saccades (Fischer et al., 1997; Munoz et al., 1998). A binomial sign test was used to determine the start and end of the express saccade epoch by measuring when the proportion of anti-saccade error trials (in 10-ms bins) exceeded that of correct anti-saccade trials ($P < 0.1$). Consistent with a previous study (Munoz et al., 1998), saccades with SRTs between 90 and 140 ms were considered to be express saccades in the current study. SRTs longer than express saccades were considered to be regular-latency saccades. The saccades with SRTs of < 90 ms were classified as anticipatory (Munoz et al., 1998), and excluded from analyses.

Trials with blinks or an eye position deviation of > 2° from the central FP during the required period of central fixation (100–1200 ms after the FP onset) were excluded from analysis. There remained > 20 trials for each condition from each participant, except for erroneous and express analyses. It has been found that there are few direction errors for this age group (Munoz et al., 1998; Alahyane et al., 2014). Four participants were excluded from direction-error-related analyses in the current study because they made no errors, and three participants did not make enough express saccades ($N < 4$), and were therefore excluded from this analysis. Because there were few direction errors, the current study mainly focused on the comparison between correct pro-saccade and anti-saccade trials.

Pupil size data can be distorted by eye position, because the size of the pupil depends on the angle of the eyeball in a video-based eye tracker. To maintain an accurate measure of pupil size, the

selected epochs for pupil analysis were either during the central fixation period or before saccade initiation, when eye position was located at the center of the screen. Specifically, three epochs were selected for analysis to examine pupil size under different stages of saccade preparation (Fig. 1B): start of visual fixation epoch (FIX$_{st}$: 100–300 ms after fixation onset), end of visual fixation epoch (FIX$_{end}$: 950–1000 ms after fixation onset), and end of gap epoch (GAP$_{end}$: 150–200 ms after gap appearance).

Following a large body of literature (Bala & Takahashi, 2000; Moresi *et al.*, 2008; Wang *et al.*, 2012), we examined change in pupil diameter by using baseline correction. Two baseline-correction epochs were used to examine pupillary light responses or pupil responses more related to saccade preparation. The first baseline pupil diameter value was determined by averaging pupil size from the first 100–300 ms after fixation onset, and, for each trial, original pupil diameter values were subtracted from this baseline pupil diameter value.

The presentation of the FP (with a higher luminance value relative to background) can change overall illumination to induce the pupillary light reflex (Loewenfeld, 1999), and these pupillary responses were not modulated by task conditions. There were insignificant differences between the pro-saccade and anti-saccade conditions regarding maximum constriction size and time to maximum constriction (Fig. S1). To specifically illustrate pupil dynamics related to saccade preparation, the epoch representing the end of the pupillary light responses was used as a second baseline-correction window. The same baseline-correction procedure was used, except that the second baseline pupil diameter value was determined by averaging pupil size from 800 to 850 ms after FP onset (when the pupil reached its greatest constriction after FP appearance; 843 and 818 ms for pro-saccade and anti-saccade conditions, respectively). With this baseline correction, we differentiated the influence of constriction and dilation components on observed differences in pupil size, and the dilation component of the pupillary responses was more correlated with saccade preparation.

To confirm the validity of the second baseline-correction epoch, an additional baseline-correction epoch (pupil size in FIX$_{end}$ epoch) was performed to reveal changes in pupil size after the gap onset, because the introduction of the gap period between FP disappearance and peripheral stimulus appearance is known to enhance preparatory activity (Dorris *et al.*, 1997; Dorris & Munoz, 1998). These analyses revealed identical modulation with larger pupil dilation in correct anti-saccade trials and in correct pro-saccade or erroneous anti-saccade trials (Fig. S2), and greater pupil dilation for faster SRTs in both correct pro-saccade and anti-saccade trials (Fig. S3).

Note that although the disappearance of the FP in the current study can decrease illumination and therefore increase pupil size, the response latency for these responses during the whole background luminance change was regularly longer than 300 ms (Wang & Munoz, 2014). The duration of the gap (between FP disappearance and peripheral stimulus appearance) was only 200 ms, and it was therefore highly unlikely that the darkness pupillary response induced by FP disappearance could start prior to stimulus presentation (the darkness reflex response latency in the current study should be much longer than the whole background luminance change).

All reported paired *t*-tests are two-sided.

Results

Saccadic responses in pro-saccade and anti-saccade tasks

Saccade behavior in the pro-saccade and anti-saccade tasks is well documented (Everling & Fischer, 1998; Munoz *et al.*, 1998; Munoz & Everling, 2004; Hutton & Ettinger, 2006). Consistently, there were more direction errors in the anti-saccade condition. Error rates were 3.7% and 13.3% for the pro-saccade and anti-saccade conditions, respectively (Fig. 2A; $t_{18} = 3.76$, $P < 0.01$). It took longer to generate correct anti-saccades than pro-saccades: the mean SRTs for pro-saccades and anti-saccades were 175 ms and 230 ms, respectively (Fig. 2B; $t_{18} = 7.31$, $P < 0.01$). Figure 2C and D shows the distribution of correct and erroneous SRTs for pro-saccade and anti-saccade trials, respectively. SRTs for erroneous anti-saccades (mean, 164 ms) were shorter than those for correct anti-saccades (Fig. 2D; $t_{14} = 5.81$, $P < 0.01$).

Pupil dynamics before stimulus appearance

To examine the modulation of pupil dynamics by saccade preparation (pro-saccades and anti-saccades), we analysed pupil size during the instructed fixation period prior to stimulus appearance. Figure 3A shows pupil size baseline-corrected to the diameter at fixation onset (FIX$_{st}$), revealing pupil constriction during the instructed fixation period in both conditions. The initial constriction could be largely attributed to the changes in overall luminance level following the presentation of the luminant FP (typically referred to as the pupillary light reflex). Importantly, the task-dependent pupil size differences between the pro-saccade and anti-conditions began to emerge ~850 ms after instructed fixation onset, with larger pupil size for correct anti-saccade trials than for correct pro-saccade trials. However, these numerical differences were not significant (FIX$_{end}$ epoch – pro-saccade, −0.13 mm, anti-saccade, −0.12 mm, $t_{18} = 1$, $P = 0.32$; GAP$_{end}$ epoch – pro-saccade, −0.1 mm, anti-saccade, −0.08 mm, $t_{18} = 1.5$, $P = 0.15$), which could be the result of concurrent processing for the pupillary light reflex and saccade preparation increasing the level of variability in pupil size.

To reduce this variability, baseline correction was applied to the pupil diameter value when the pupil reached its greatest constriction (800–850 ms after fixation onset) to examine pupil dynamics more specifically related to saccade preparation (for details, see Materials and methods). Figure 3B summarises changes in pupil diameter prior to stimulus appearance, revealing an increase in pupil size in both the pro-saccade and anti-saccade conditions. More importantly, pupil dilation was greater in correct anti-saccade trials than in correct pro-saccade trials, with larger pupil size being observed for correct anti-saccades in the GAP$_{end}$ epoch (50 ms before target presentation) (Fig. 3C; pro-saccades and correct anti-saccades, $t_{18} = 3.01$, $P < 0.01$). These differences in pupil size in the GAP$_{end}$ epoch were observed for the majority (15/19) of participants (Fig. 3D), and these effects in pupil size were observed as early as ~40 ms after the start of the gap period (Fig. 3B; *t*-test in 20-ms bins, $P < 0.05$; black bar above *x*-axis). In addition, pupil dilation was significantly less pronounced when an erroneous pro-saccade was triggered towards the stimulus in the anti-saccade condition (GAP$_{end}$ epoch, Fig. 3C; correct anti-saccade and erroneous anti-saccade, $t_{14} = 2.5$, $P < 0.05$). As mentioned previously, participants in this age group made few errors on anti-saccade trials (only 15 participants were included for this comparison) (Munoz *et al.*, 1998; Alahyane *et al.*, 2014); therefore, anti-saccade error effects should be interpreted with caution.

Larger pupil size in preparation for faster saccades

It has previously been shown that preparatory activity correlates with SRTs in pro-saccade and anti-saccade trials in both humans and monkeys (Dorris *et al.*, 1997; Dorris & Munoz, 1998; Everling
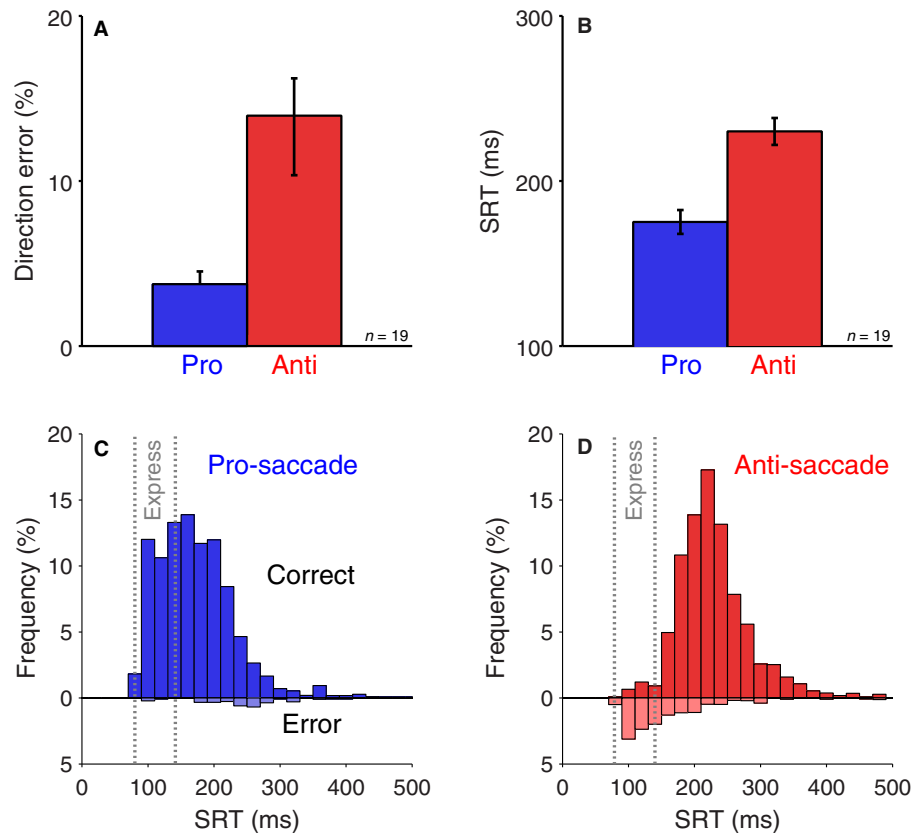
FIG. 2. Saccade behaviors in the pro-saccade and anti-saccade conditions. (A and B) Modulation of direction-error rates by task condition (A) and SRTs (B). (C and D) The distribution of correct or erroneous SRTs for pro-saccades (C) or and anti-saccades (D). The vertical dotted lines represent the range of express saccades. Anti, anti-saccade; Pro, pro-saccade.

et al., 1999; Everling & Munoz, 2000; Connolly et al., 2005). If pupil size reflects the preparatory signal for the generation of pro-saccades and anti-saccades, it should be different between faster and slower saccades. SRTs between 90 and 140 ms were considered to be express saccades (Fig. 2D; see Materials and methods). In pro-saccade trials, the mean SRTs were 116 and 199 ms for express and regular-latency saccades, respectively. Pupil dilation was greater for express saccades prior to stimulus appearance (Fig. 4A). The effects were consistent before saccade initiation, with changes in pupil size of 0.039 and 0.029 mm in the GAP_{end} epoch for express and regular-latency saccades, respectively (Fig. 4B; $t_{15} = 2.1$, $P < 0.05$). However, the correlation coefficients between dilation size in the GAP_{end} epoch and SRTs for each individual subject showed no correlation (a histogram of the correlation coefficients is shown in Fig. 4C), and the mean correlation coefficient was -0.02 (parried t-test of r-values against zeros: $t_{15} = 0.48$, $P > 0.6$). This could be attributable to a reduction in statistical power resulting from small variability in pro-saccade SRTs with fewer express saccades per subject.

To examine these effects in the anti-saccade condition, we separated correct anti-saccade trials according to SRT (including only regular-latency saccades), grouping faster and slower SRTs (median split). The mean SRTs were 200 and 265 ms for short-latency and long-latency anti-saccades, respectively. Figure 4D shows changes in pupil diameter according to stimulus appearance, revealing greater dilation for short-latency than for long-latency correct anti-saccades, with mean diameters of 0.052 and 0.042 mm in the GAP_{end} epoch for short-latency and long-latency saccades, respectively (Fig. 4E;

$t_{18} = 2.2$, $P < 0.05$). Figure 4F shows summary histograms of correlation coefficients for all subjects in anti-saccade trials, revealing a negative correlation between dilation size and SRTs (mean correlation coefficient of -0.11, $t_{18} = 2.9$, $P < 0.05$), suggesting a trial-by-trial correlation between SRTs and pupil size related to anti-saccade preparation. Note that the correlation coefficient found here was only moderate, and this could be partly attributed to a relatively small number of trials per subject (20–50 trials).

Absolute pupil diameter between pro-saccades and anti-saccades

It is well accepted that pupil size is modulated by activity in the locus coeruleus–norepinephrine system, arguably via arousal (Aston-Jones & Cohen, 2005), partly because of a clear correlation between absolute pupil size and neural activity in the locus coeruleus (Rajkowski et al., 1993). To examine the influence of arousal between the pro-saccade and anti-saccade conditions, we also analysed absolute pupil diameter. Absolute pupil diameter between the pro-saccade and anti-saccade conditions was similar between correct pro-saccade and anti-saccade trials. For the pro-saccade and anti-saccade conditions, absolute pupil sizes were 2.78 and 2.77 mm at the start of fixation (FIX_{st} epoch) (Fig. 5A; $t_{18} = 1.44$, $P = 0.16$), 2.65 and 2.65 mm at the end of fixation (FIX_{end} epoch) (Fig. 5B; $t_{18} = 0.31$, $P = 0.76$), and 2.68 and 2.69 mm at the end of a gap period (GAP_{end} epoch) (Fig. 5C; $t_{18} = 0.36$, $P = 0.72$), suggesting that the overall level of arousal between the two conditions was very similar.
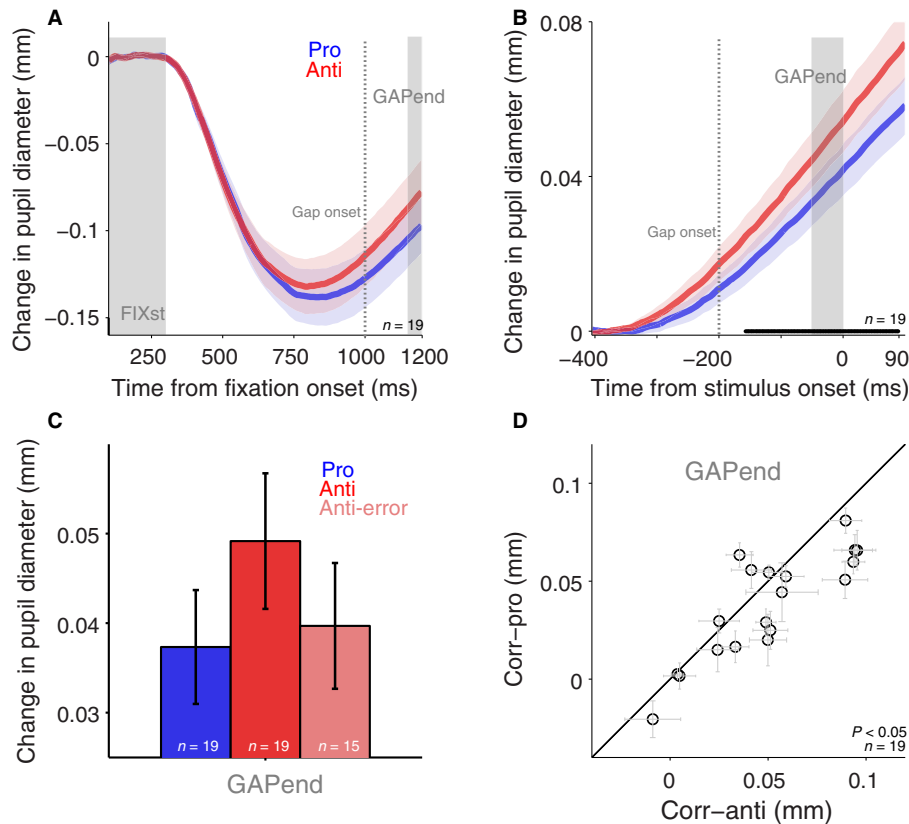
FIG. 3. Pupil size during instructed fixation. (A) Pupil dynamics (baseline-corrected to the FIX$_{st}$ epoch) between the pro-saccade and anti-saccade conditions. (B) Change in pupil diameter (baseline-corrected to 800–850 ms of fixation onset) for the pro-saccade and anti-saccade conditions before stimulus appearance. (C) Pupil dilation extent during the GAP$_{end}$ epoch (50 ms before stimulus presentation) among trials with correct pro-saccades, correct anti-saccades, or erroneous anti-saccades. (D) Pupil dilation extent size for correct pro-saccade and anti-saccade conditions during the GAP$_{end}$ epoch for each individual participant ($n = 19$). In A and B, the shaded colored regions surrounding the pupillary response represent the standard error range (across participants) for different conditions. The gray area represents the epoch selected for pupil analyses. The vertical dotted line indicates the onset of the gap, and the black bar on the *x*-axis indicates the time line at which differences between the pro-saccade and anti-saccade conditions were statistically significant ($P < 0.05$). In C, the error bar represents the standard error across participants. In D, the error bar represents the standard error within participants. Anti, correct anti-saccade trials; Anti-Error, erroneous anti-saccade trials; Pro, correct pro-saccade trials.

Discussion

Pupil size has long been used as an effective indicator of cognitive processing (Hess & Polt, 1964; Kahneman & Beatty, 1966; Kahneman *et al.*, 1967; Beatty, 1982), and has been associated with motor preparation (Richer *et al.*, 1983; Richer & Beatty, 1985; Jainta *et al.*, 2011). In the current study, we specifically examined pupil dynamics during active preparation for the flexible generation of pro-saccades or anti-saccades. We hypothesised that saccade preparatory activity evident in the SC and FEF should be manifested in pupil size in a similar fashion, because of a suggested link between the SC and the pupil control circuit (C.A. Wang & D.P. Munoz, unpublished data). Pupil size was different prior to stimulus appearance in the two conditions: pupil dilation was greater before correct anti-saccades than before correct pro-saccades or erroneous pro-saccades made in anti-saccade trials, reflecting a correlate of top-down inhibitory control. In addition, larger pupil size at the time of stimulus appearance was accompanied by faster SRTs in both the pro-saccade and anti-saccade conditions, reflecting a correlate of saccade preparation. Overall, the results show that pupil size prior to stimulus presentation effectively reflected preparatory set activity related to saccade initiation, and confirmed a direct link between saccade preparation and pupil size.

Two types of preparatory activity during pro-saccade and anti-saccade preparation

In single-neuron recordings, preparatory activity in the monkey SC and FEF is distinctively different prior to stimulus presentation between the generation of pro-saccades and anti-saccades (Everling *et al.*, 1998, 1999; Everling & Munoz, 2000). Two types of preparatory process are preset to generate pro-saccades and anti-saccades (Munoz & Everling, 2004). First, the fixation-related activity (related to top-down inhibition) required to suppress unwanted antomatic responses is increased in correct anti-saccade trials as compared with correct pro-saccade trials during the instructed fixation period before stimulus appearance (Everling *et al.*, 1998, 1999). The failure of sufficient fixation-related activity before stimulus presentation in the anti-saccade condition, as is evident from increased pre-saccadic build-up activity, results in direction errors. Second, the pre-saccadic activity related to motor preparation increases for saccade neurons during the gap period, and SRTs correlate with the level of pre-saccadic activity for both pro-saccades (Dorris *et al.*, 1997; Dorris & Munoz, 1998) and anti-saccades (Everling *et al.*, 1999; Everling & Munoz, 2000).

Similarly, these two types of preparatory signal are evident in human fMRI studies. First, there is a higher level of blood oxygen-level dependent signal in the FEF for anti-saccade preparation than
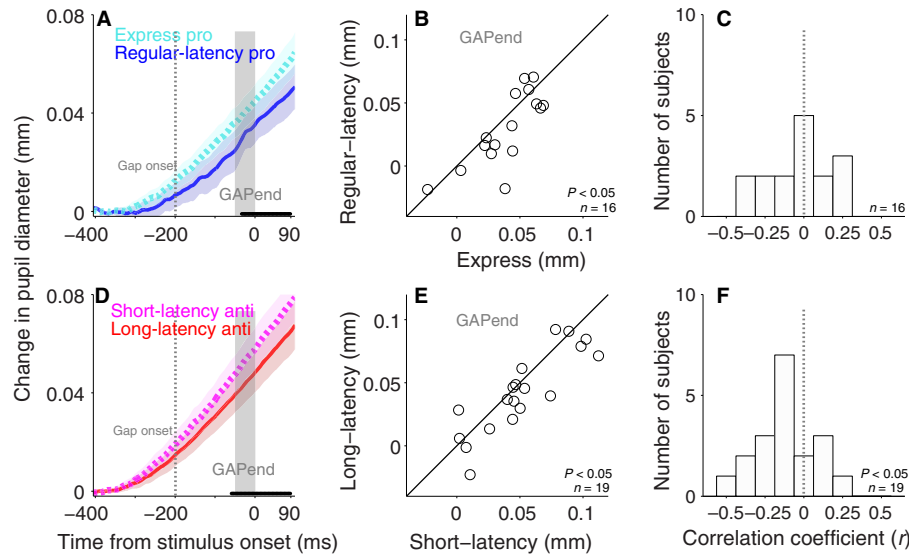
FIG. 4. Pupil size between faster and slower pro-saccades and anti-saccades. (A) Pupil response for express and regular-latency pro-saccades prior to stimulus appearance. (B) Change in pupil size between express and regular-latency pro-saccades in the GAP_{end} epoch for each individual participant ($n = 16$). (C) Distribution of correlation coefficients for the relationship between SRTs and pro-saccade pupil size in the GAP_{end} epoch for all subjects ($n = 16$). (D) Pupil response for short-latency and long-latency anti-saccades prior to stimulus appearance. (E) Change in pupil size between short-latency and long-latency anti-saccades in the GAP_{end} epoch for each individual participant ($n = 19$). (F) Distribution of correlation coefficients for the relationship between SRTs and anti-saccade pupil size in the GAP_{end} epoch for all subjects ($n = 19$). In A and D, the shaded colored regions surrounding the pupillary response represent the standard error range (across participants) for different conditions. The gray area represents the epochs selected for pupil analyses. The black bar on the x-axis indicates the time line at which differences between two conditions are statistically significant ($P < 0.05$). The vertical dotted line represents the onset of the gap. In C and F, the vertical dotted line represents a zero value of the correlation coefficient ($r = 0$). Anti, anti-saccades; Pro, pro-saccades; r, correlation coefficient.
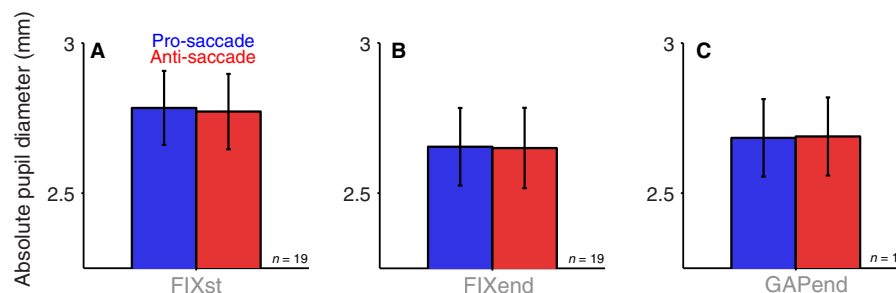

FIG. 5. Absolute pupil diameter between the pro-saccade and anti-saccade conditions in (A) the FIX_{st} epoch (100–300 ms after fixation onset), (B) the FIX_{end} epoch (950–1000 ms after fixation onset), and (C) the GAP_{end} epoch (50 ms before stimulus presentation). In A–C, the error bars represent the standard errors across participants. Pro-saccade, correct pro-saccade trials; Anti-saccade, correct anti-saccade trials.

for pro-saccade preparation (Connolly *et al.*, 2002; DeSouza *et al.*, 2003; Ford *et al.*, 2005; Brown *et al.*, 2007; Manoach *et al.*, 2007), in agreement with the fixation-related activity observed in monkeys. Second, the level of activation related to saccade preparation negatively correlates with SRTs in both pro-saccade and anti-saccade conditions (Connolly *et al.*, 2005; Alahyane *et al.*, 2014), in agreement with the pre-saccadic activity observed in monkeys. The increase in the blood oxygen-level dependent signal in the FEF might reflect these two types of preparatory processing (Alahyane *et al.*, 2014).

The role of the SC in correlating pupil size with saccade preparation

The SC, a midbrain structure known for its causal role in the control of saccadic eye movements and attention (Gandhi & Katnani, 2011; White & Munoz, 2011; Krauzlis *et al.*, 2013), has been hypothesised to encode stimulus salience and relevance to coordinate various components of orienting (Sparks, 1986; Fecteau & Munoz,

2006; Knudsen, 2007; Boehnke & Munoz, 2008; Mysore & Knudsen, 2013; Corneil & Munoz, 2014). The SC has recently been linked to the pupil control circuit, because microstimulation of the SC evoked transient pupil dilation (Netser *et al.*, 2010; Wang *et al.*, 2012), and pupil dilation evoked by SC microstimulation is similar to that evoked by the presentation of salient stimuli (Wang & Munoz, 2014; Wang *et al.*, 2014). Moreover, the effects of stimulus contrast and modality on the pupil response are similar to those observed in the SC. These results extend a central role of the SC in coordinating various components of orienting (Corneil & Munoz, 2014), including not only shifts of gaze and attention, but also pupil dilation (Sokolov, 1963), and suggest that SC activity may be associated with pupil size. Because pupil dilation is evoked by SC microstimulation in the areas associated with both fixation-related (rostral SC) and saccade-related (caudal SC) neurons (Wang *et al.*, 2012), it is possible that pupil size is modulated by both types of preparatory signal described above. Therefore, it is predicted that pupil size should be larger in preparation for correct anti-saccades

than in preparation for correct pro-saccades, and pupil size should be negatively correlated with SRTs.

Greater pupil dilation (Fig. 3B–D) was shown for correct anti-saccades than for correct pro-saccades, consistent with the larger inhibition-related activity observed in the human FEF (Connolly *et al.*, 2002; DeSouza *et al.*, 2003; Ford *et al.*, 2005; Brown *et al.*, 2007; Manoach *et al.*, 2007) and in the monkey SC and FEF (Everling *et al.*, 1999; Everling & Munoz, 2000). The motor preparatory activity negatively correlates with SRTs for both pro-saccades and anti-saccades in the human FEF (Connolly *et al.*, 2005; Alahyane *et al.*, 2014) and in the monkey SC and FEF (Dorris *et al.*, 1997; Dorris & Munoz, 1998; Everling *et al.*, 1998, 1999; Everling & Munoz, 2000). Consistently, there was greater pupil dilation for faster than for slower pro-saccades (Fig. 4A and B) and anti-saccades (Fig. 4D–F). Therefore, our pupil results are in agreement with documented preparatory activities in the anti-saccade task in the human fMRI and monkey neurophysiology literature, suggesting that pupil size can be an effective proxy of neural activity related to saccade preparation. Note that pupil size between pro- and anti-saccade condition, similarly to fMRI FEF activation, does not need to be indifferent even that it reflects two types of preparatory activity (fixation-related and saccade-related), because one activity may be weighted more than another.

Other brain regions are also involved in saccade preparation in the anti-saccade task, such as the supplementary eye field, parietal eye field, dorsolateral prefrontal cortex, anterior cingulate cortex, and basal ganglia (Funahashi *et al.*, 1993; Schlag-Rey *et al.*, 1997; Connolly *et al.*, 2002, 2005; DeSouza *et al.*, 2003; Ford *et al.*, 2005; Johnston & Everling, 2006; Brown *et al.*, 2007; Manoach *et al.*, 2007; Watanabe & Munoz, 2010; Alahyane *et al.*, 2014). However, the preparatory activity observed in these areas is less consistent across different studies, and there is as yet no evidence to clearly link these structures to pupil control. Further research is required to determine the contributions of other structures to modulation of pupil size in the anti-saccade task.

Neural substrate linking pupil size and cognitive processing

Because of the popularity of video-based eye-tracking, pupil recordings are becoming increasingly available in eye movement studies. A growing number of studies have incorporated pupil size measurement to examine the relationship between pupil size and various cognitive processes, such as target detection, covert orienting, subjective perception, decision-making, and rational regulation (Einhauser *et al.*, 2008; Privitera *et al.*, 2010; Gabay *et al.*, 2011; Nassar *et al.*, 2012; Wierda *et al.*, 2012; Eldar *et al.*, 2013; de Gee *et al.*, 2014). Although the locus coeruleus–norepinephrine system is usually implicated as the underlying network for cognition-related pupil responses (Aston-Jones & Cohen, 2005), absolute pupil diameter was similar between the pro-saccade and anti-saccade conditions in our study (Fig. 5A–C), suggesting a similar level of LC activity between pro-saccade and anti-saccade trials. Therefore, an additional neural signal is probably required to coordinate this modulation between the pro-saccade and anti-saccade conditions, arguably via the SC and FEF.

Pupil size related to anti-saccade generation and motor preparation

Pupil dynamics in the anti-saccade task were first examined in a clinical investigation (Karatekin *et al.*, 2010). Unlike here, the preparatory process was not a focus in that study, because participants were required to recognise the peripheral stimulus to identify the trial condition (pro-saccade or anti-saccade), and pro-saccade or

anti-saccade preparation could therefore not begin until after stimulus appearance. Moreover, because their measurement epochs of pupil size were after stimulus presentation, the observed differences in pupil size could also be influenced by stimulus presentation. Although they found greater pupil dilation after stimulus appearance in correct anti-saccade than in erroneous anti-saccade or correct pro-saccade trials, these results could be explained by different types of saccade execution or stimulus presentation (not preparation).

A link between pupil size and motor preparation has been suggested previously, with a slow increase in pupil size prior to a finger movement (Go) as compared with no movement (NoGo) being observed (Richer *et al.*, 1983; Richer & Beatty, 1985). Another study demonstrated greater pupil dilation during an oculomotor task with a gap between FP disappearance and target appearance than in an overlap condition when the FP remained visible (Jainta *et al.*, 2011). However, some factors such as expectation could be involved differently in preparation for Go and NoGo trials, and the presence or absence of a visible FP can have substantial effects on many processes besides motor preparation, including visual processing. Unlike previous studies, the current study contrasted the preparation of pro-saccade and anti-saccade generation, according to well-documented results on preparation from monkey neurophysiology and human fMRI research, to specifically reveal the modulation of pupil size by different types of saccade preparation, providing critical evidence to support a direct link between saccade preparation and pupil size with potential underlying mechanisms.

Other explanations for the observed pupil size differences

Although our study used different FP colors to indicate different conditions, the observed differences in pupil size between the pro-saccade and anti-saccade conditions were unlikely to have resulted from the use of different FP colors, because there were significant differences in pupil dilation size between correct and erroneous anti-saccade trials (same FP color; Fig. 3C), and the modulation of pupil dilation by ensuing SRTs was evident in individual pro-saccade and anti-saccade trials (Fig. 4). Recent studies showed that pupil size was larger during planning of a saccade to a dark area than during planning of a saccade to a bright area (Mathot *et al.*, 2013, 2015). Although participants had to saccade to a brighter region in the pro-saccade condition than in the anti-saccade condition, this was unlikely to be the case, because it is hard to explain the same modulation of pupil dilation by ensuing SRTs in individual correct pro-saccade and anti-saccade conditions (Fig. 4). Specifically, if the preparation for looking at a 'bright' area (pro-saccade condition) makes pupil size smaller, then better preparation, i.e. faster SRTs, should result in smaller pupil size in the pro-saccade condition, but larger pupil size in the anti-saccade condition. However, the same effects of SRTs, i.e. larger pupil dilation observed with faster SRTs, were demonstrated in both pro-saccade and anti-saccade conditions (preparation for looking at 'bright' and 'dark' areas).

Conclusion

Pupil size is an easy-to-measure way of understanding neural processing. The current study showed that pupil size is modulated by active preparation related to the initiation of pro-saccades and anti-saccades, and that pupil size prior to stimulus appearance effectively predicts subsequent saccadic behaviors. The involvement of the SC, together with the FEF, can reconcile the observed results. These findings suggest that pupil size is an effective proxy of neural activity related to saccade preparation, and also implicate a central role

of the SC in coordinating the orienting response, including both saccadic eye movements and pupil dilation, providing an additional neural substrate to coordinate various types of cognitive processing and pupil diameters, given the causal role of the SC in attention (Zenon & Krauzlis, 2012; Krauzlis *et al.*, 2013).

Supporting Information

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

Fig. S1. (A) Magnitude of maximum constriction between correct pro-saccade and anti-saccade trials ($t_{18} = 0.86$, $P = 0.4$). (B) Time to maximum constriction between correct pro-saccade and anti-saccade trials ($t_{18} = 1.1$, $P = 0.29$). The error bar represents standard error across participants ($n = 19$).

Fig. S2. (A) Change in pupil diameter (baseline-corrected to 950–1000 ms of fixation onset) for the pro-saccade and anti-saccade conditions before stimulus appearance. (B) Pupil dilation size during the GAP_{end} epoch (50 ms before stimulus presentation) among trials with correct pro-saccades, correct anti-saccades, or erroneous anti-saccades. (C) Pupil dilation size for correct pro-saccade and anti-saccade conditions during the pre-stimulus epoch for each individual participant ($n = 19$). In A and B, the shaded colored regions surrounding the pupillary response represent the standard error range (across participants) for different conditions. The gray area represents the selected epoch for pupil analyses. The vertical dotted line indicates the onset of the gap, and the black bar on the *x*-axis indicates the time line at which differences between the pro-saccade and anti-saccade conditions were statistically significant ($P < 0.05$). In C, the error bar represents standard error across participants. In D, the error bar represent standard error within participants. Pro, correct pro-saccade trials; Anti, correct anti-saccade trials; Anti-Error, erroneous anti-saccade trials.

Fig. S3. (A) Pupil response for express and regular-latency pro-saccades prior to stimulus appearance. (B) Change in pupil size between express and regular-latency pro-saccades in the GAP_{end} epoch for each individual participant ($n = 16$). (C) Distribution of correlation coefficients for the relationship between SRTs and pro-saccade pupil size in the GAP_{end} epoch for all subjects ($n = 16$). (D) Pupil response for short-latency and long-latency anti-saccades prior to stimulus appearance. (E) Change in pupil size between short-latency and long-latency anti-saccades in the GAP_{end} epoch for each individual participant ($n = 19$). (F) Distribution of correlation coefficients for the relationship between SRTs and anti-saccade pupil size in the GAP_{end} epoch for all subjects ($n = 19$). In A and D, the shaded colored regions surrounding the pupillary response represent the standard error range (across participants) for different conditions. The gray area represents the epochs selected for pupil analyses. The black bar on the *x*-axis indicates the time line at which differences between the two conditions are statistically significant ($P < 0.05$). The vertical dotted line represents the onset of the gap. In C and F, the vertical dotted line represents a zero value of the correlation coefficient ($r = 0$). Anti, anti-saccades; Pro, pro-saccades; *r*, correlation coefficient.

Acknowledgements

Abbreviations

FEF, frontal eye field; FIX_{end}, end of visual fixation epoch (950–1000 ms after fixation onset); FIX_{st}, start of visual fixation epoch (100–300 ms after fixation onset); fMRI, functional magnetic resonance imaging; FP, fixation point; GAP_{end}, end of gap epoch (150–200 ms after gap appearance); SC, superior colliculus; SRT, saccade reaction time.

References

Alahyane, N., Brien, D.C., Coe, B.C., Stroman, P.W. & Munoz, D.P. (2014) Developmental improvements in voluntary control of behavior: effect of preparation in the fronto-parietal network? *NeuroImage*, **98**, 103–117.

Aston-Jones, G. & Cohen, J.D. (2005) An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu. Rev. Neurosci.*, **28**, 403–450.

Bala, A.D. & Takahashi, T.T. (2000) Pupillary dilation response as an indicator of auditory discrimination in the barn owl. *J. Comp. Physiol. A.*, **186**, 425–434.

Beatty, J. (1982) Task-evoked pupillary responses, processing load, and the structure of processing resources. *Psychol. Bull.*, **91**, 276–292.

Boehnke, S.E. & Munoz, D.P. (2008) On the importance of the transient visual response in the superior colliculus. *Curr. Opin. Neurobiol.*, **18**, 544–551.

Brown, M.R., Vilis, T. & Everling, S. (2007) Frontoparietal activation with preparation for antisaccades. *J. Neurophysiol.*, **98**, 1751–1762.

Connolly, J.D., Goodale, M.A., Menon, R.S. & Munoz, D.P. (2002) Human fMRI evidence for the neural correlates of preparatory set. *Nat. Neurosci.*, **5**, 1345–1352.

Connolly, J.D., Goodale, M.A., Goltz, H.C. & Munoz, D.P. (2005) fMRI activation in the human frontal eye field is correlated with saccadic reaction time. *J. Neurophysiol.*, **94**, 605–611.

Corneil, B.D. & Munoz, D.P. (2014) Overt responses during covert orienting. *Neuron*, **82**, 1230–1243.

DeSouza, J.F., Menon, R.S. & Everling, S. (2003) Preparatory set associated with pro-saccades and anti-saccades in humans investigated with event-related FMRI. *J. Neurophysiol.*, **89**, 1016–1023.

Dorris, M.C. & Munoz, D.P. (1998) Saccadic probability influences motor preparation signals and time to saccadic initiation. *J. Neurosci.*, **18**, 7015–7026.

Dorris, M.C., Pare, M. & Munoz, D.P. (1997) Neuronal activity in monkey superior colliculus related to the initiation of saccadic eye movements. *J. Neurosci.*, **17**, 8566–8579.

Einhauser, W., Stout, J., Koch, C. & Carter, O. (2008) Pupil dilation reflects perceptual selection and predicts subsequent stability in perceptual rivalry. *Proc. Natl. Acad. Sci. USA*, **105**, 1704–1709.

Eldar, E., Cohen, J.D. & Niv, Y. (2013) The effects of neural gain on attention and learning. *Nat. Neurosci.*, **16**, 1146–1153.

Evarts, E.V., Shinoda, Y. & Wise, S.P. (1984) *Neurophysiological Approaches to Higher Brain Functions*. Wiley, New York.

Everling, S. & Fischer, B. (1998) The antisaccade: a review of basic research and clinical studies. *Neuropsychologia*, **36**, 885–899.

Everling, S. & Munoz, D.P. (2000) Neuronal correlates for preparatory set associated with pro-saccades and anti-saccades in the primate frontal eye field. *J. Neurosci.*, **20**, 387–400.

Everling, S., Dorris, M.C. & Munoz, D.P. (1998) Reflex suppression in the anti-saccade task is dependent on prestimulus neural processes. *J. Neurophysiol.*, **80**, 1584–1589.

Everling, S., Dorris, M.C., Klein, R.M. & Munoz, D.P. (1999) Role of primate superior colliculus in preparation and execution of anti-saccades and pro-saccades. *J. Neurosci.*, **19**, 2740–2754.

Fecteau, J.H. & Munoz, D.P. (2006) Salience, relevance, and firing: a priority map for target selection. *Trends Cogn. Sci.*, **10**, 382–390.

Fischer, B., Biscaldi, M. & Gezeck, S. (1997) On the development of voluntary and reflexive components in human saccade generation. *Brain Res.*, **754**, 285–297.

Ford, K.A., Goltz, H.C., Brown, M.R. & Everling, S. (2005) Neural processes associated with antisaccade task performance investigated with event-related FMRI. *J. Neurophysiol.*, **94**, 429–440.

Funahashi, S., Chafee, M.V. & Goldman-Rakic, P.S. (1993) Prefrontal neuronal activity in rhesus monkeys performing a delayed anti-saccade task. *Nature*, **365**, 753–756.

Gabay, S., Pertzov, Y. & Henik, A. (2011) Orienting of attention, pupil size, and the norepinephrine system. *Atten. Percept. Psycho.*, **73**, 123–129.

Gandhi, N.J. & Katnani, H.A. (2011) Motor functions of the superior colliculus. *Annu. Rev. Neurosci.*, **34**, 205–231.

de Gee, J.W., Knapen, T. & Donner, T.H. (2014) Decision-related pupil dilation reflects upcoming choice and individual bias. *Proc. Natl. Acad. Sci. USA*, **111**, E618–E625.

Hebb, D.O. (1972) *Textbook of Physiology*. Saunders, Philadelphia, PA, pp. 77–93.

Hess, E.H. & Polt, J.M. (1964) Pupil size in relation to mental activity during simple problem-solving. *Science*, **143**, 1190–1192.

Hutton, S.B. & Ettinger, U. (2006) The antisaccade task as a research tool in psychopathology: a critical review. *Psychophysiology*, **43**, 302–313.

Jainta, S., Vernet, M., Yang, Q. & Kapoula, Z. (2011) The pupil reflects motor preparation for saccades – even before the eye starts to move. *Front. Hum. Neurosci.*, **5**, 97.

Johnston, K. & Everling, S. (2006) Neural activity in monkey prefrontal cortex is modulated by task context and behavioral instruction during delayed-match-to-sample and conditional prosaccade–antisaccade tasks. *J. Cognitive Neurosci.*, **18**, 749–765.

Kahneman, D. & Beatty, J. (1966) Pupil diameter and load on memory. *Science*, **154**, 1583–1585.

Kahneman, D., Beatty, J. & Pollack, I. (1967) Perceptual deficit during a mental task. *Science*, **157**, 218–219.

Karatekin, C., Bingham, C. & White, T. (2010) Oculomotor and pupillometric indices of pro- and antisaccade performance in youth-onset psychosis and attention deficit/hyperactivity disorder. *Schizophrenia Bull.*, **36**, 1167–1186.

Knudsen, E.I. (2007) Fundamental components of attention. *Annu. Rev. Neurosci.*, **30**, 57–78.

Krauzlis, R.J., Lovejoy, L.P. & Zenon, A. (2013) Superior colliculus and visual spatial attention. *Annu. Rev. Neurosci.*, **36**, 165–182.

Loewenfeld, I.E. (1999) *The Pupil: Anatomy, Physiology, and Clinical Applications*. Butterworth-Heinemann, Boston, MA.

Manoach, D.S., Thakkar, K.N., Cain, M.S., Polli, F.E., Edelman, J.A., Fischl, B. & Barton, J.J. (2007) Neural activity is modulated by trial history: a functional magnetic resonance imaging study of the effects of a previous antisaccade. *J. Neurosci.*, **27**, 1791–1798.

Mathot, S., van der Linden, L., Grainger, J. & Vitu, F. (2013) The pupillary light response reveals the focus of covert visual attention. *PLoS One*, **8**, e78168.

Mathot, S., van der Linden, L., Grainger, J. & Vitu, F. (2015) The pupillary light response reflects eye-movement preparation. *J. Exp. Psychol. Human.*, **41**, 28–35.

McDowell, J.E., Dyckman, K.A., Austin, B.P. & Clementz, B.A. (2008) Neurophysiology and neuroanatomy of reflexive and volitional saccades: evidence from studies of humans. *Brain Cognition*, **68**, 255–270.

Moresi, S., Adam, J.J., Rijcken, J. & Van Gerven, P.W. (2008) Cue validity effects in response preparation: a pupillometric study. *Brain Res.*, **1196**, 94–102.

Munoz, D.P. & Everling, S. (2004) Look away: the anti-saccade task and the voluntary control of eye movement. *Nat. Rev. Neurosci.*, **5**, 218–228.

Munoz, D.P., Broughton, J.R., Goldring, J.E. & Armstrong, I.T. (1998) Age-related performance of human subjects on saccadic eye movement tasks. *Exp. Brain Res.*, **121**, 391–400.

Mysore, S.P. & Knudsen, E.I. (2013) A shared inhibitory circuit for both exogenous and endogenous control of stimulus selection. *Nat. Neurosci.*, **16**, 473–478.

Nassar, M.R., Rumsey, K.M., Wilson, R.C., Parikh, K., Heasly, B. & Gold, J.I. (2012) Rational regulation of learning dynamics by pupil-linked arousal systems. *Nat. Neurosci.*, **15**, 1040–1046.

Netser, S., Ohayon, S. & Gutfreund, Y. (2010) Multiple manifestations of microstimulation in the optic tectum: eye movements, pupil dilations, and sensory priming. *J. Neurophysiol.*, **104**, 108–118.

Privitera, C.M., Renninger, L.W., Carney, T., Klein, S. & Aguilar, M. (2010) Pupil dilation during visual target detection. *J. Vision*, **10**, 1–14.

Rajkowski, J., Kubiak, P. & Aston-Jones, G. (1993) Correlations between locus coeruleus (LC) neural activity, pupil diameter and behavior in monkey support a role of LC in attention. *Soc. Neurosci. Abstr.*, **19**, 974.

Richer, F. & Beatty, J. (1985) Pupillary dilations in movement preparation and execution. *Psychophysiology*, **22**, 204–207.

Richer, F., Silverman, C. & Beatty, J. (1983) Response selection and initiation in speeded reactions: a pupillometric analysis. *J. Exp. Psychol. Human.*, **9**, 360–370.

Schiller, P.H., True, S.D. & Conway, J.L. (1980) Deficits in eye movements following frontal eye-field and superior colliculus ablations. *J. Neurophysiol.*, **44**, 1175–1189.

Schlag-Rey, M., Amador, N., Sanchez, H. & Schlag, J. (1997) Antisaccade performance predicted by neuronal activity in the supplementary eye field. *Nature*, **390**, 398–401.

Sokolov, E.N. (1963) Higher nervous functions; the orienting reflex. *Annu. Rev. Physiol.*, **25**, 545–580.

Sparks, D.L. (1986) Translation of sensory signals into commands for control of saccadic eye movements: role of primate superior colliculus. *Physiol. Rev.*, **66**, 118–171.

Steiner, G.Z. & Barry, R.J. (2011) Pupillary responses and event-related potentials as indices of the orienting reflex. *Psychophysiology*, **48**, 1648–1655.

Wang, C.A. & Munoz, D.P. (2014) Modulation of stimulus contrast on the human pupil orienting response. *Eur. J. Neurosci.*, **40**, 2822–2832.

Wang, C.A., Boehnke, S.E., White, B.J. & Munoz, D.P. (2012) Microstimulation of the monkey superior colliculus induces pupil dilation without evoking saccades. *J. Neurosci.*, **32**, 3629–3636.

Wang, C.A., Boehnke, S.E., Itti, L. & Munoz, D.P. (2014) Transient pupil response is modulated by contrast-based saliency. *J. Neurosci.*, **34**, 408–417.

Watanabe, M. & Munoz, D.P. (2010) Presetting basal ganglia for volitional actions. *J. Neurosci.*, **30**, 10144–10157.

White, B.J. & Munoz, D.P. (2011) The superior colliculus. In Liversedge, S., Gilchrist, I. & Everling, S. (Eds), *Oxford Handbook of Eye Movements*. Oxford University Press, Oxford, pp. 195–213.

Wierda, S.M., van Rijn, H., Taatgen, N.A. & Martens, S. (2012) Pupil dilation deconvolution reveals the dynamics of attention at high temporal resolution. *Proc. Natl. Acad. Sci. USA*, **109**, 8456–8460.

Zenon, A. & Krauzlis, R.J. (2012) Attention deficits without cortical neuronal deficits. *Nature*, **489**, 434–437.