J Neurophysiol 110: 522–535, 2013.
First published May 1, 2013; doi:10.1152/jn.01096.2012.

Fixational saccades reflect volitional action preparation

Masayuki Watanabe,[1,2] Yuka Matsuo,[3] Ling Zha,[3] Douglas P. Munoz,[1] and Yasushi Kobayashi[4,5,6,7]
[1]*Centre for Neuroscience Studies, Queen's University, Kingston, Ontario, Canada;* [2]*Department of Physiology, Kansai Medical University, Osaka, Japan;* [3]*Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan;* [4]*Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan;* [5]*Center for Information and Neural Networks, National Institute of Information and Communications Technology, Osaka, Japan;* [6]*ATR Computational Neuroscience Laboratories, Kyoto, Japan; and* [7]*PRESTO, the Japan Science and Technology Agency, Saitama, Japan*

Submitted 21 December 2012; accepted in final form 24 April 2013

Watanabe M, Matsuo Y, Zha L, Munoz DP, Kobayashi Y. Fixational saccades reflect volitional action preparation. *J Neurophysiol* 110: 522–535, 2013. First published May 1, 2013; doi:10.1152/jn.01096.2012.—Human volitional actions are preceded by preparatory processes, a critical mental process of cognitive control for future behavior. Volitional action preparation is regulated by large-scale neural circuits including the cerebral cortex and the basal ganglia. Because volitional action preparation is a covert process, the network dynamics of such neural circuits have been examined by neuroimaging and recording event-related potentials. Here, we examined whether such covert processes can be measured by the overt responses of fixational saccades (including microsaccades), the largest miniature eye movements that occur during eye fixation. We analyzed fixational saccades while adult humans maintained fixation on a central visual stimulus as they prepared to generate a volitional saccade in response to peripheral stimulus appearance. We used the antisaccade paradigm, in which subjects generate a saccade toward the opposite direction of a peripheral stimulus. Appropriate antisaccade performance requires the following two aspects of volitional control: *1*) facilitation of saccades away from the stimulus and *2*) suppression of inappropriate saccades toward the stimulus. We found that fixational saccades that occurred before stimulus appearance reflected the dual preparatory states of saccade facilitation and suppression and correlated with behavioral outcome (i.e., whether subjects succeeded or failed to cancel inappropriate saccades toward the stimulus). Moreover, fixational saccades explained a large proportion of individual differences in behavioral performance (poor/excellent) across subjects. These results suggest that fixational saccades predict the outcome of future volitional actions and may be used as a potential biomarker to detect people with difficulties in volitional action preparation.

antisaccade; decision making; executive function; fixation; microsaccade

VOLITIONAL ACTIONS ARE PRECEDED by preparatory processes that preset neural circuits based on the knowledge of current situations and/or environment to guide future behavior (Haggard 2008). Without volitional action preparation, behavior would become reactive rather than predictive, which may explain cognitive behavioral deficits observed in a variety of clinical disorders, such as Parkinson's disease (Cameron et al. 2012; Cunnington et al. 1997) and attention deficit hyperactivity disorder (Hakvoort Schwerdtfeger et al. 2013; McLoughlin et al. 2010).

Address for reprint requests and other correspondence: M. Watanabe, New Zealand Brain Research Institute, 66 Stewart St., Christchurch 8011, New Zealand (e-mail: colliculus@gmail.com).

Volitional action preparation is regulated by large-scale networks integrating the cerebral cortex and the basal ganglia (Nachev et al. 2008). The network dynamics of such neural circuits have been studied extensively with saccadic eye movements (Hikosaka et al. 2000; Munoz and Everling 2004; Schall 2004; Sparks 2002; Watanabe and Munoz 2011). In conventional paradigms, subjects maintain their eyes on a central fixation point and generate a saccade in response to peripheral visual stimulus appearance. Cognitive models indicate that saccade behavior is shaped partially by neural processes before stimulus appearance, which corresponds to volitional saccade preparation (Carpenter and Williams 1995; Smith and Ratcliff 2004). Correspondingly, recent studies have shown that neural activity changes gradually before stimulus appearance in accordance with preparation (Amador et al. 2004; Everling et al. 1999; Everling and Desouza 2005; Everling and Munoz 2000; Kunimatsu and Tanaka 2010; Watanabe and Munoz 2010; Yoshida and Tanaka 2009).

Because volitional action preparation is a covert process by definition, its state cannot be measured directly but inferred from cognitive models or neural activity measurements. Here we hypothesize that volitional action preparation can be measured overtly by analyzing miniature eye movements during fixation. This hypothesis is derived from behavioral studies in which fixational saccades, the largest miniature eye movements including microsaccades, are not always generated involuntarily but rather are under volitional control (Bridgeman and Palca 1980; Haddad and Steinman 1973; Ko et al. 2010; Kowler and Steinman 1977; Steinman et al. 1967; Winterson and Collewijn 1976). Fixational saccades also reflect the state of covert spatial attention (Brien et al. 2009; Engbert and Kliegl 2003; Gowen et al. 2007; Hafed et al. 2011; Hafed and Clark 2002; Laubrock et al. 2005; Rolfs et al. 2005). Furthermore, recent studies have shown a linkage between fixational saccade occurrence and macrosaccade initiation (Hafed and Krauzlis 2010; Rolfs 2007; Rolfs et al. 2006; Sinn and Engbert 2011). Accordingly, neural processes preparing volitional actions may influence fixational saccades to optimize behavioral states for upcoming saccades.

We examined whether volitional action preparation is reflected in the pattern of fixational saccades, using a simple behavioral paradigm called an antisaccade in which subjects generate a saccade toward the opposite direction of a peripheral visual stimulus (Hallett 1978). Appropriate antisaccade performance requires the following two aspects of volitional saccade control: *1*) facilitation of saccades away from the stimulus and

2) suppression of inappropriate saccades toward the stimulus. Because an antisaccade instruction is given well before stimulus appearance, it presets neural circuits controlling antisaccades to prepare for both saccade facilitation and suppression (Watanabe and Munoz 2010). We therefore examined whether such dual preparatory signals (saccade facilitation and suppression), which are processed covertly for upcoming antisaccade execution, can be read out overtly from fixational saccades.

METHODS

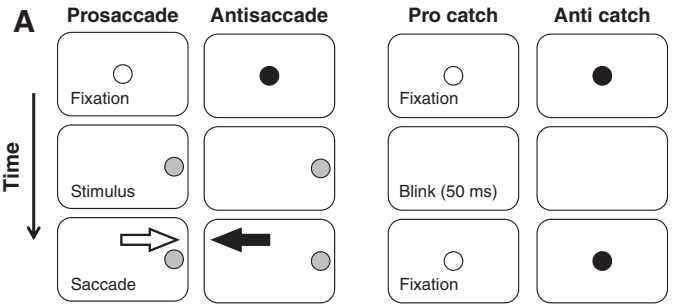
Subjects. Fifty-eight subjects [40 men, 18 women; age: mean ± SD = 21.8 ± 3.0] with normal or corrected to normal vision participated in this study. One of the authors (M. Watanabe) was included because virtually the same statistical results were confirmed with and without this subject. Subjects were paid ¥2,000/h for their participation. They were informed of the nature of the study and consented to be part of the study. This study was approved by the research ethics board of the Osaka University Hospital.

Experimental systems. The control of the behavioral paradigm and the acquisition of eye position data were carried out by the TEMPO/Win computing system (ReflectiveComputing, St. Louis, MO). Left and right eye positions were acquired with a fast video-based eye movement monitor (a dark pupil eye tracking system; iView X Hi-Speed, SensoMotoric Instruments, Teltow, Germany). The temporal and spatial resolutions of the pupil tracking were 500 Hz and 0.01°, respectively. A standard nine-point calibration was conducted to align eye and screen coordinate systems. Drift correction, and additional gain calibration whenever necessary, was performed after every 50 correct trials. Subjects supported their head on a chin/forehead rest that included the support for the camera for eye tracking. A cathode ray tube monitor (60-Hz refresh rate, 1,024 × 768 pixels, 19 in.) was placed at 35 cm from the eyes. A bite-bar was installed for additional support of head fixation during the course of experiments, although our results did not depend on the use of the bite-bar (see below).

Main behavioral paradigm. Forty-two of the fifty-eight subjects performed the following main paradigm (Fig. 1). Each trial was preceded by a 1,000-ms intertrial interval during which the screen was illuminated with a diffuse light to prevent dark adaptation (2 cd/m^2). After removal of the background light, a circular fixation point (size: 0.4°, luminance: 14 cd/m^2) appeared in the center of the screen without background illumination and subjects were required to direct their eyes toward the fixation point within 30 s. After they maintained steady fixation within a computer-controlled window (±2°) for 700–2,300 ms (exponential distribution with constant expectation; average = 1,000 ms) (Oswal et al. 2007), the fixation point disappeared.

On the majority of trials (80%), a peripheral stimulus (size: 0.4°, luminance: 14 cd/m^2, color: yellow) appeared at either 5° left or right from the center of the screen (19° from the border of the monitor) simultaneously with fixation point disappearance. The stimulus remained visible for 1,000–1,500 ms. Subjects generated a saccade either toward the stimulus (prosaccade) or to the opposite direction of the stimulus (antisaccade) based upon fixation point color (red/green) counterbalanced across subjects and maintained fixation on the peripheral stimulus on prosaccade trials or on a blank screen at the mirror position of the peripheral stimulus on antisaccade trials. The sizes of fixation windows for peripheral stimuli were adjusted for each subject to accept relatively inaccurate antisaccade end points [width: 8.3 ± 4.2° (mean ± SD), height: 5.9 ± 2.0°] (Dafoe et al. 2007; Fischer and Weber 1992; Hallett 1978).

For the remaining 20% of trials, the fixation point reappeared 50 ms after its first disappearance (fixation blink) instead of peripheral stimulus appearance, and subjects maintained fixation for additional 1,000–1,500 ms (catch trials). Catch trials were included to evoke fixational saccades and detect fixational saccade readiness under variable behavioral conditions. Another reason for the inclusion of catch trials was to replicate the basic characteristics of fixational saccades in response to abrupt sensory events (see, e.g., Engbert and Kliegl 2003; Rolfs et al. 2005).



B

Paradigm	Fixation duration	Catch
Main (n = 42)	Random	Included (20%)
Secondary (n = 16)	Random / Fixed	Not included

Fig. 1. Behavioral paradigms. *A*: 4 types of trials. On prosaccade trials, subjects fixated on the fixation point and generated a saccade toward a stimulus. On antisaccade trials, subjects generated a saccade to the opposite direction from the stimulus. On pro- and anti-catch trials, subjects fixated on the fixation point throughout the trial while a 50-ms fixation blink was introduced. *B*: differences between main and secondary paradigms. In the main paradigm, fixation durations were randomized from 700 to 2,300 ms with an average of 1,000 ms, and catch trials were included (10% each for pro and anti). In the secondary paradigm, there were 2 separate blocks with fixation duration either randomized or fixed (1,000 ms). Catch trials were not included in this paradigm.

Subjects received auditory feedback at the end of each trial based on their performance. This auditory feedback was generated by a built-in computer speaker (correct: single beep of 2,300 Hz with 150-ms duration; error: 2 beeps of 1,950 Hz with 50-ms duration and 50-ms interval). All task conditions described above were randomly interleaved in a block of trials. Subjects performed this paradigm until they achieved at least 400 correct trials [including all 4 types of trials (pro/anti × saccade/catch)], while they had short breaks every after 100 correct trials. There was no explicit requirement of fixational saccades for performing this paradigm.

Secondary behavioral paradigm. The remaining 16 subjects performed a secondary paradigm designed specifically to examine the influence of temporal expectation of peripheral stimulus appearance on fixational saccades as well as pro- and antisaccades. This paradigm was the same as the above main paradigm with the following exceptions (Fig. 1*B*). Subjects performed two separate blocks of trials, each of which had fixation duration either randomized (700–2,300 ms, exponential distribution with constant expectation, 1,000 ms on average) or fixed to 1,000 ms. The sequence of the blocks (randomized first or fixed first) was counterbalanced equally across subjects. Catch trials were not included in this paradigm. Subjects performed each block of this paradigm until they achieved at least 200 correct trials (including both pro and anti).

Saccade detection. Eye position data were first processed by a digital filter (3rd-order Butterworth low-pass filter with cutoff frequency of 200 Hz). The onset and end of pro- and antisaccades larger than 2° were identified by radial eye velocity criteria (threshold: 30°/s). Because eye positions were recorded binocularly, the onset and end of each saccade were defined by the earlier onset and the later end of both eyes.

Fixational saccades were detected by an algorithm developed by Engbert and colleagues (Engbert and Kliegl 2003; Engbert and Mergenthaler 2006) (Fig. 2). Briefly, the velocity threshold of fixational saccades was defined flexibly depending on the noise level on each

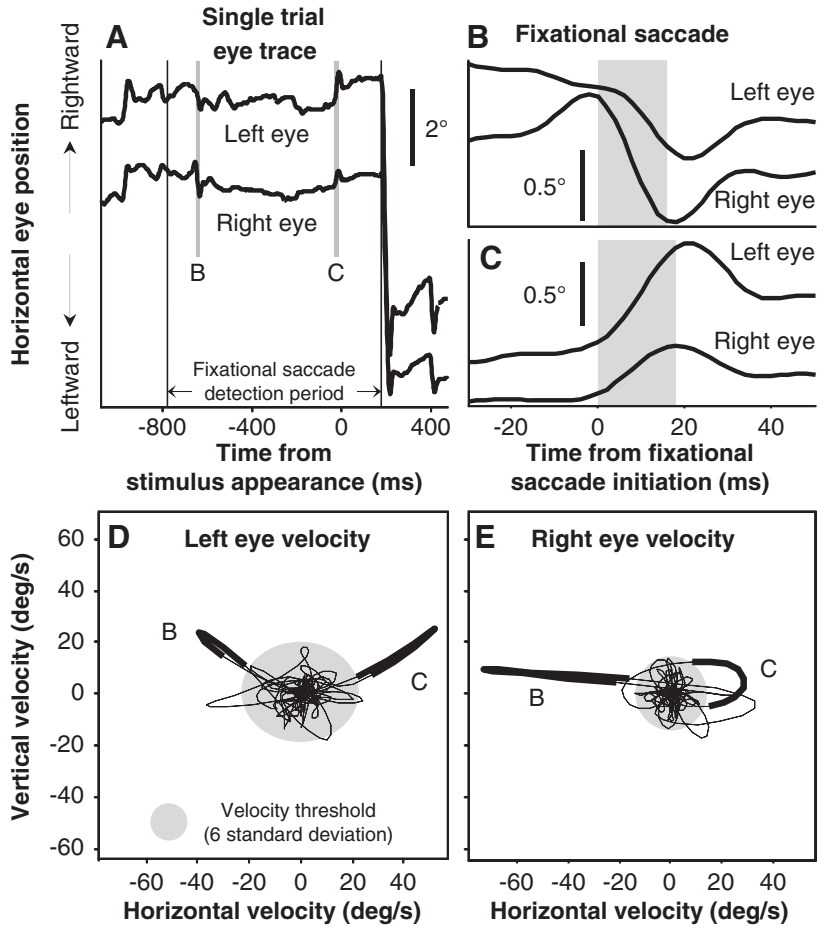


Fig. 2. Fixational saccade detection on single trial. *A*: horizontal eye traces (leftward prosaccade trial). Velocity thresholds for fixational saccade detection were calculated between 200 ms after fixation initiation and prosaccade initiation, indicated by vertical lines. Two fixational saccades (*B* and *C*) were detected on this trial. *B* and *C*: fixational saccades. Gray rectangles indicate fixational saccade durations. *D* and *E*: horizontal and vertical eye velocity in the left (*D*) and right (*E*) eyes during fixational saccade detection period (*A*). Gray ellipses indicate velocity thresholds. Fixational saccades shown in *B* and *C* exceeded the velocity thresholds simultaneously in both eyes.

trial (threshold: 6 SDs) (Fig. 2, *D* and *E*). The minimum duration of fixational saccades that exceeded the velocity threshold was set to 6 ms. This analysis was limited to a temporal period in which eye positions were relatively stable (from 200 ms after the end of a saccade toward the fixation point to the initiation of a pro/antisaccade on saccade trials or to 2nd fixation point disappearance on catch trials) (Fig. 2*A*). We analyzed only fixational saccades that occurred simultaneously in both eyes during at least one data sample (2 ms) to reduce the influence of potential noise on data analyses (Fig. 2, *B* and *C*). This criterion of binocularity set a strong constraint on fixational saccade detection and removed a large number of monocular fixational saccades, or noise detected as monocular fixational saccades. This technical limitation was derived from the limited sampling frequency (500 Hz for binocular recordings), although this is a standard performance of currently available video-based eye trackers. The minimum intersaccade interval was set to 20 ms to avoid defining potential overshoot corrections as new fixational saccades (Moller et al. 2002). The amplitude, direction, and peak velocity of each binocular fixational saccade were analyzed from the right eye. Virtually the same results were confirmed by analyzing the left eye data.

Fixational saccades from an example subject detected by the above criteria are shown in Fig. 3. Consistent with previous studies, the peak velocities of fixational saccades increased linearly with their amplitudes (Fig. 3*A*: main sequence), although our data included fixational saccades larger than microsaccades in the original report (Zuber et al. 1965). The directions of binocular fixational saccades were biased toward the horizontal directions (Fig. 3*B*) (Engbert 2006). The distributions of main sequence slopes, amplitudes, and directions from all subjects are shown in Fig. 4. Pure horizontal (left/right) and vertical (up/down) fixational saccade directions were quantified as 0° and 90°, respectively. The distributions of amplitudes and directions were characterized by calculating 10, 30, 50, 70, and 90 percentiles in each subject, and those values from all subjects are shown cumulatively. The majority of fixational saccade amplitudes were $<1°$, and most fixational saccades had amplitudes $<2°$ (Fig. 4*B*). In the majority of subjects, the medians of fixational saccade directions were $<30°$ from the horizontal meridian (Fig. 3*C*), confirming the horizontal bias of the directions of binocular fixational saccades at the population level.

Because the minimum amplitude threshold of pro- and antisaccades was set to 2° as described above, we adopted the same value for the maximum amplitude threshold of fixational saccades. We excluded trials if saccades larger than this threshold occurred during fixed temporal periods for quantitative analyses (see below). Note that Fig. 4 contains trials with fixational saccades larger than the amplitude criterion because they occurred outside the temporal periods for quantitative analyses (see below). Only $0.9 \pm 2.3\%$ (mean $\pm$ SD) of trials were excluded by this criterion. We confirmed major findings with the threshold of 1°, which has been used for human microsaccade studies with video-based eye trackers (e.g., Engbert and Mergenthaler 2006; Hermens et al. 2010; Rolfs et al. 2006). However, this criterion excluded more trials ($5.2 \pm 6.8\%$) than the 2° criterion. Because there is no specific reason to exclude trials with fixational saccades larger than 1° in the context of our study, we chose the 2° criterion for result presentation.

Fixational saccade quantifications. To quantify the frequency of fixational saccade occurrence while subjects prepared for a pro- or antisaccade before stimulus appearance, we defined a prestimulus period as the 400-ms window ending 70 ms after stimulus appearance. We took into account the 70-ms offset for the minimum delay of visual processing for stimulus appearance before saccade initiation (Fischer and Weber 1993). We chose this temporal period to quantify fixational saccades that occurred before stimulus appearance and

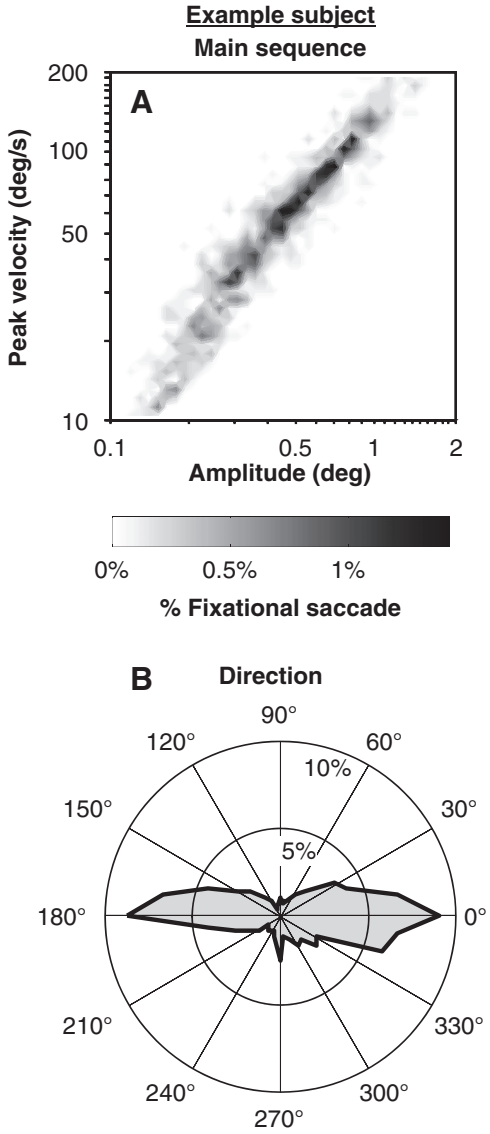


Fig. 3. Fixational saccade characteristics from an example subject. *A*: 2-dimensional histogram of fixational saccade main sequence (slope ± confidence interval = 73.8 ± 0.8). *B*: fixational saccade directions. The percentages of fixational saccades were counted within a 10° window shifted by 10°. Example eye traces from the same subject are shown in Fig. 2.

correlate them with pro- and antisaccade behavior that occurred after stimulus appearance because the neural processes of pro- and antisaccade preparation are initiated well before stimulus appearance and such prestimulus preparatory activity is correlated with behavior (see, e.g., Watanabe and Munoz 2010).

To analyze fixational saccades evoked by a fixation blink on catch trials, we defined a postblink period as the 400-ms window starting at 200 ms after the first fixation point disappearance. We incorporated the 200-ms delay because fixational saccades were suppressed strongly at ~200 ms before they were evoked by fixation blinks (see RESULTS). The suppression and facilitation of fixational saccade generation after an abrupt sensory event are consistent with previous studies (e.g., Engbert and Kliegl 2003; Rolfs et al. 2005).

Within-subject analyses. The occurrence of fixational saccades and the timing and performance of pro- and antisaccades depended on multiple factors. To disentangle the influence of each factor within the behavioral paradigms, we carried out the following regression analyses in individual subjects who performed the main paradigm:

$$y = a_0 + a_1 \times [\text{fixation duration}] + a_2 \times [\text{task instruction}] \quad (1)$$

where "fixation duration" indicates a time period from fixation initiation (eyes entered into the fixation window) to stimulus appearance and "task instruction" indicates a prosaccade (−1) or an antisaccade (+1) instruction. Fixation durations were normalized by their mean

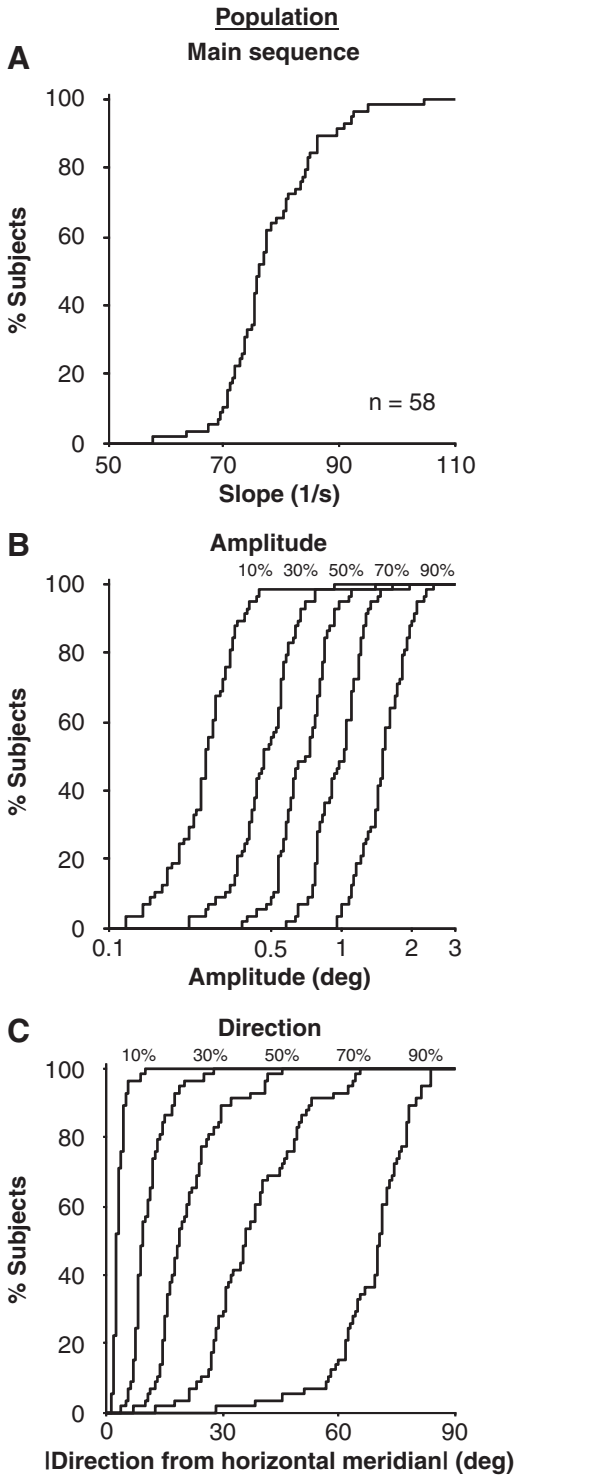


Fig. 4. Fixational saccade characteristics from all subjects. *A*: main sequence. *B*: amplitude. *C*: direction. "0" and "90" indicate horizontal (left/right) and vertical (up/down) fixational saccades, respectively. Each line in *B* and *C* indicates the cumulative distribution of a specific percentile of fixational saccades from each subject.

and SD before applying this equation. To analyze the frequency of fixational saccades, we adopted Poisson regressions with the log link function rather than simple linear regressions because fixational saccade occurrence during the prestimulus period was quantified as a nonnegative integer value on each trial (i.e., $n = 0, 1, 2,\ldots$). Accordingly, y corresponds to the log of the expected value of fixational saccade counts during the prestimulus period [i.e., $y = \log(\mu)$, where μ indicates expected value of fixational saccade counts]. The log link function is connected to the probability density function of Poisson distribution [i.e., $P(n) = e^{-\mu}\mu^n/n!$, where n indicates a fixational saccade count]. For the direction error rates of antisaccades, we adopted the logistic regressions for the binary behavior [i.e., correct or error; $y = \log\{p/(1 - p)\}$, where p indicates a direction error rate]. For the reaction times of pro- and antisaccades, we applied the same equation to linear regressions.

For data collected during the secondary paradigm, we carried out the following regression analyses for all 16 subjects:

$$y = y_1 + y_2 \quad (2)$$

$$y_1 = \sum_n (b_{0n} + b_{1n} \times [\text{fixation duration}] \times b_{2n} \times [\text{task instruction}]) \times [n\text{th subject}]$$

$$y_2 = c_1 \times [\text{random/fixed}] + c_2 \times [\text{1st and 2nd blocks}]$$

where y_1 is the same as *Eq. 1*, except that it calculates the three coefficients (b_{0n}, b_{1n}, and b_{2n}) in individual subjects using an interaction with "nth subject," a set of dummy variables specifying each subject [i.e., "ith subject" = 1 for ith subject and 0 for all others]. In contrast, y_2 contains the following two factors that can be dissociated only by between-subject comparison: "random/fixed" indicates a block of fixation durations randomized (0) or fixed (+1), and "1st/2nd block" indicates the first (0) or second (+1) block. y corresponds to one of the following three values: *1*) the log of the expected value of fixational saccade counts during the prestimulus period; *2*) the log odds of antisaccade direction errors; or *3*) correct pro- and antisaccade reaction times. The fittings of these regression models were successful [*1*) $\chi^2_{(9364)} = 9{,}239$, $P > 0.8$ (Pearson's χ^2-test); *2*) $H_{(8)} = 5.21$, $P > 0.7$ (Hosmer-Lemeshow test); *3*) $R^2 = 0.39$, $F_{(49,9364)} = 122$, $P < 0.0001$]. We describe regression coefficients in y_2 along with those in *Eq. 1* by setting "random/fixed" and "1st/2nd block" to zero to simulate the same condition as the main paradigm (i.e., random fixation durations in 1st block).

Between-subject analyses. Because both fixational saccade characteristics and pro- and antisaccade behavior (reaction times and direction errors) varied significantly across subjects, we analyzed their relationships with linear regressions. Fixational saccade frequencies were characterized by the above Poisson regressions [i.e., constant (a_0, b_{0n}), fixation duration (a_1, b_{1n}), and task instruction (a_2, b_{2n}) in *Eqs. 1* and *2*].

We also quantified the average amplitude of fixational saccades generated during the prestimulus period as follows. We collapsed all conditions in the main paradigm to increase the number of trials with fixational saccades generated during the prestimulus period (the last fixational saccade was analyzed when multiple fixational saccades occurred during the prestimulus period). This enabled us to include the majority of subjects who performed the main paradigm in this analysis (only 2 were excluded). This method was justified because the amplitudes of fixational saccades did not depend on fixation duration [mean ± SD = $(-0.13 \pm 6.64) \times 10^{-2}$; $t_{(30)} = -0.11$, $P > 0.9$ (t-test)] or task instruction [$(-1.48 \pm 6.46) \times 10^{-2}$; $t_{(30)} = -1.28$, $P > 0.2$], which was analyzed by the above linear regression (*Eq. 1*; this analysis was limited to 31 subjects who had enough trials with fixational saccades during the prestimulus period). In the secondary paradigm, we estimated the average amplitudes of fixational saccades from the resultant regression coefficients (*Eq. 2*).

To analyze subjects from the main and secondary paradigms together, we created an additional factor of paradigm (0: main, +1: secondary). We utilized five parameters (constant, fixation duration, task instruction, amplitude, and paradigm) to identify the optimal linear model to explain pro- and antisaccade behavior (reaction times and direction errors) with a stepwise regression method [term selection criterion: summed square error (sse); P values for the entrance and removal of a term: 0.05 and 0.10, respectively]. The model started from a constant and could develop to include linear, interaction, and squared terms.

Remaining methods of data analyses. Trials with opposite saccade directions were collapsed because fixational saccade frequency was not different between them prior to stimulus appearance. Because foveation of the fixation point was usually acquired by saccades, fixation durations were recalculated from the end of the saccade to acquire the fixation point until the time of stimulus appearance during off-line analysis. Ten trials at least were required in each specific condition (e.g., pro-random-1st block) for data analyses. Only correct trials were analyzed in all analyses, except for those focusing on direction errors on antisaccade trials. We utilized similar regression models with several modifications for variable circumstances, which we describe in RESULTS. We collected data with the bite-bar in 16 of the 42 subjects in the main paradigm and all 16 subjects in the secondary paradigm. We did not find any influences of the use of the bite-bar on our results of fixational saccades, except for a very minor effect, which did not influence our conclusion at all (see RESULTS). All data analyses were carried out with MATLAB (MathWorks).

RESULTS

Pro- and antisaccade behavior. The pro- and antisaccade behavior of our subjects was consistent with previous reports (Dafoe et al. 2007; Fischer and Weber 1992; Hallett 1978). The same example subject shown in Figs. 2 and 3 who performed the main paradigm had average ± SD of prosaccade reaction times of 177 ± 26 ms (Fig. 5*A*). There were very few direction errors on prosaccade trials (1.6%). In contrast, on antisaccade trials (Fig. 5*B*), reaction times were longer than prosaccades (250 ± 23 ms) and there were more direction errors (34.2%). In the population of subjects, antisaccade reaction times were longer than prosaccade reaction times (Fig. 5*C*) [main: $t_{(41)} = -17.2$, $P < 0.0001$ (paired t-test); secondary: $t_{(15)} = -6.48$, $P < 0.0001$]. Direction error rates were higher on antisaccade trials than prosaccade trials (Fig. 5*D*) [main: $t_{(41)} = -5.56$, $P < 0.0001$; secondary: $t_{(15)} = -3.80$, $P < 0.005$].

Fixational saccade behavior. The temporal dynamics of fixational saccade occurrence is shown in Fig. 6. The raster and density functions of fixational saccade onset times (Fig. 6, *A* and *B*) were obtained from the same example subject shown in Fig. 2, Fig. 3, and Fig. 5, *A* and *B*. The population averages of fixational saccade density functions from subjects who performed the main paradigm are shown in Fig. 6, *C* and *D*. We first replicated the well-established phenomenon of suppression and facilitation of fixational saccades by abrupt visual events on catch trials (Fig. 6, *B* and *D*) (e.g., Engbert and Kliegl 2003; Rolfs et al. 2005). More importantly, here we found the following two characteristics of fixational saccade occurrence before subjects generated pro- and antisaccades (Fig. 6, *A* and *C*; note that the ranges of y-axes are different between saccade and catch trials to capture the different ranges of fixational saccade frequencies): *1*) the frequency of fixational saccades decreased with increasing time before stimulus appearance, and *2*) such reduction of fixational saccade frequency was more significant on antisaccade trials than prosaccade trials.

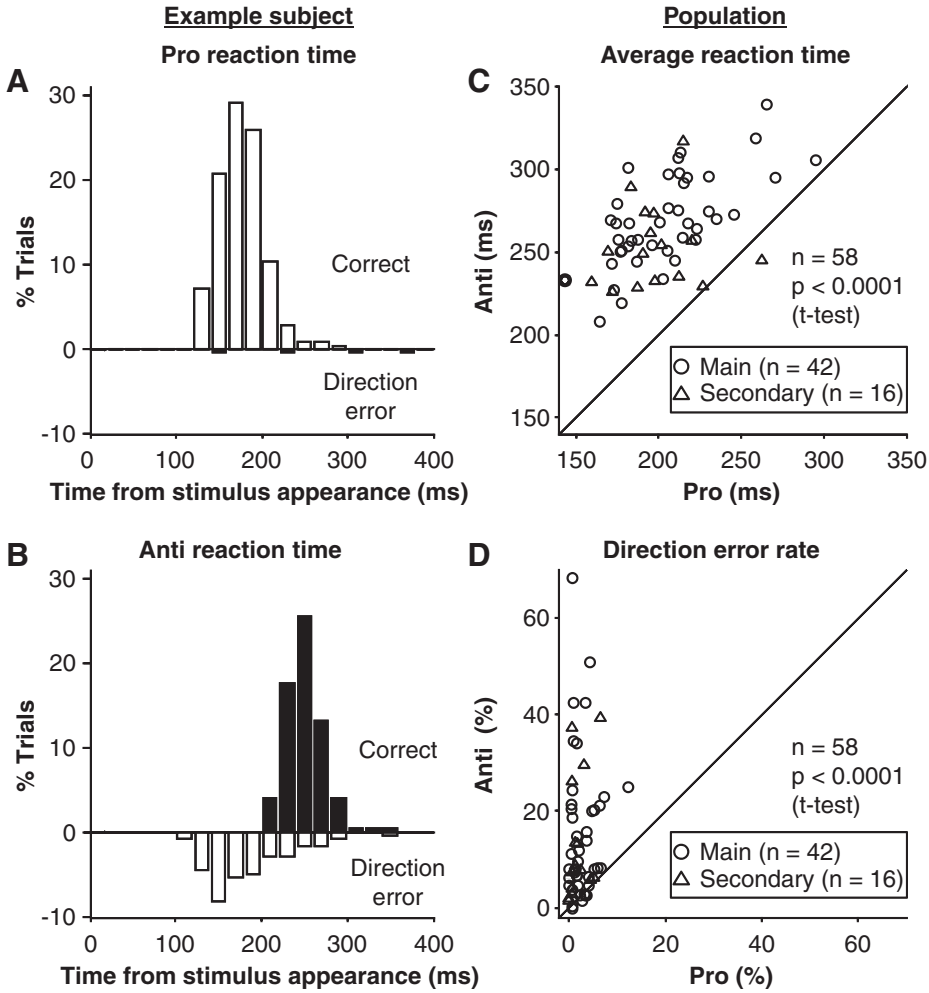


Fig. 5. Prossaccade and antisaccade characteristics. *A*: distribution of prosaccade reaction times from example subject shown in Figs. 2 and 3. *B*: distribution of antisaccade reaction times from the same example subject. *C*: summary of average reaction times of pro- and antisaccades. *D*: summary of direction error rates of pro- and antisaccades. Circles and triangles indicate individual subjects who performed the main and secondary paradigms, respectively. Direction error rates in the secondary paradigm were calculated directly in blocks with fixed fixation duration instead of using regression coefficients because the regression analysis was unsuccessful because of the limited number of prosaccade trials with direction errors.

In the following sections, we first establish a relationship between fixational saccade occurrence and pro- and antisaccade initiation and then address the above two qualitative observations in turn.

Fixational saccades preceded delayed initiation of pro- and antisaccades. Recent studies have shown that fixational saccade occurrence before stimulus appearance precedes delayed initiation of macrosaccades in response to stimulus appearance (Hafed and Krauzlis 2010; Rolfs 2007; Rolfs et al. 2006; Sinn and Engbert 2011). We quantified the impact of fixational saccade occurrence during the prestimulus period (400-ms window ending 70 ms after stimulus appearance, indicated by black horizontal bar above *x*-axis in Fig. 6*C*) on the reaction times of pro- and antisaccades, using multiple linear regressions with an additional factor of "fixational saccade count" ($n = 0, 1, 2\ldots$) (added to *Eq. 1* and y_1 in *Eq. 2*). The distribution of regression coefficients for fixational saccade count calculated in individual subjects was biased toward positive values (Fig. 7) [main: $t_{(41)} = 8.41$, $P < 0.0001$ (*t*-test); secondary: $t_{(15)} = 4.26$, $P < 0.001$], indicating that reaction times were prolonged after fixational saccade occurrence before stimulus appearance.

On the basis of the above observations, we propose the following hypothesis: preparatory mechanisms that facilitate pro- and antisaccade initiation reduce the frequency of fixational saccades before stimulus appearance. We describe evidence supporting this hypothesis in the following section.

Fixational saccade reduction with temporal expectation of stimulus appearance. It has been shown previously that saccade reaction times are shortened with increasing elapsed time from fixation initiation, which reflects the temporal expectation of stimulus appearance (Oswal et al. 2007; Pare and Munoz 1996). Although fixation durations were randomized to generate a constant expectation of stimulus appearance in the main paradigm, we still found this phenomenon [coefficient for fixation duration: main: mean ± SD = −2.35 ± 5.17, $t_{(41)} = -2.94$, $P < 0.001$ (*t*-test); secondary: −2.05 ± 3.16, $t_{(15)} = -2.59$, $P < 0.05$]. This might be explained by residual expectation of stimulus appearance due to incomplete randomization of fixation duration (maximum = 2,300 ms). We addressed this issue more directly by using the secondary paradigm in which temporal expectation of stimulus appearance was manipulated differently in two separate blocks of trials with fixation duration either randomized or fixed (Fig. 1*B*). As expected, we found that reaction times were shortened in blocks with fixed fixation duration compared with those with randomized fixation duration (Table 1; a negative coefficient for macro reaction time and random/fixed). This indicates that enhanced temporal expectation of stimulus appearance facilitated pro- and antisaccade initiation.

If fixational saccades reflect preparatory signals for pro- and antisaccades that underlie the above behavioral phenomena, they are also expected to change with elapsed time from fix-

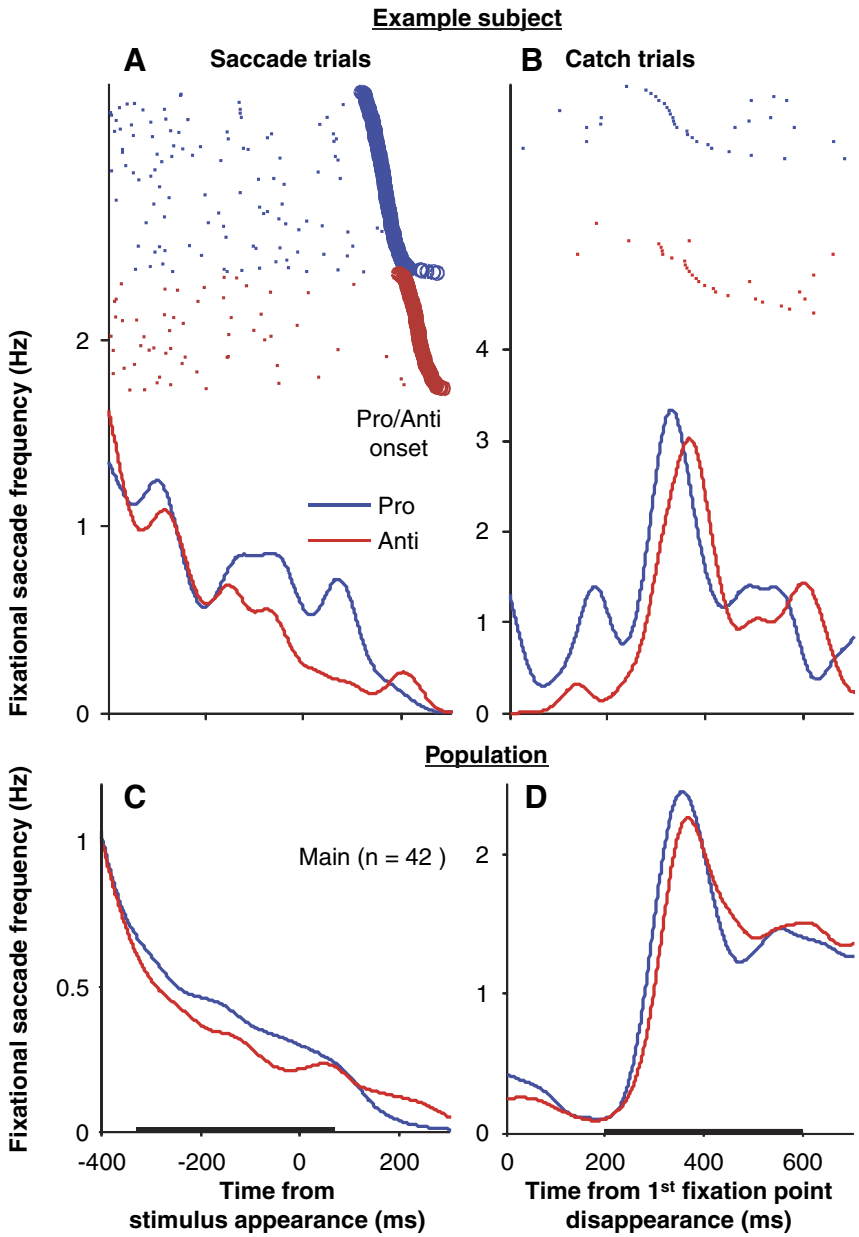


Fig. 6. Time courses of fixational saccade occurrence in the main paradigm. *A*: raster and density functions of fixational saccade onset times on saccade trials from example subject shown in Figs. 2, 3, and 5, *A* and *B*. Trials are sorted by pro- and antisaccade reaction times, indicated by circles. *B*: catch trials from the same example subject. Trials are sorted by the latencies of fixational saccades evoked by fixation blinks. *C* and *D*: average density functions from subjects who performed the main paradigm on saccade (*C*) and catch (*D*) trials, respectively. Horizontal bars on *x*-axes indicate the prestimulus period (*C*) and the postblink period (*D*), respectively. Blue and red indicate trials with pro- and antisaccade instructions, respectively. Density functions were calculated by the convolution of a Gaussian function with SD of 30 ms. The ranges of *y*-axes are different between saccade (*A* and *C*) and catch (*B* and *D*) trials to capture the different ranges of fixational saccade frequencies.

ation initiation as well as temporal expectation of stimulus appearance. We quantified the effects of elapsed time from fixation initiation on the frequency of fixational saccades during the prestimulus period by Poisson regressions. The distribution of regression coefficients for fixation duration was biased toward negative values (Fig. 8*A*) [main: $t_{(41)} = -5.67$, $P < 0.0001$ (*t*-test); secondary: $t_{(15)} = -3.95$, $P < 0.005$; see figure legend for a minor effect of use of the bite-bar]. This indicates the reduced frequency of fixational saccades with fixation duration. Furthermore, during the secondary paradigm, the frequency of fixational saccades was decreased in blocks with fixed fixation duration compared with those with randomized fixation duration (Table 1; a negative coefficient for fixational frequency and random/fixed).

These results suggest that temporal expectation of stimulus appearance facilitates pro- and antisaccade initiation as well as reducing the frequency of fixational saccades before stimulus appearance.

Fixational saccade reduction by antisaccade instruction. The hypothesis that preparatory mechanisms facilitating pro- and antisaccade initiation also reduce the frequency of fixational saccades predicts that fixational saccades are reduced more strongly before prosaccades than antisaccades because prosaccade reaction times are shorter than antisaccade reaction times (Fig. 5, *A–C*). This prediction is supported further by the fact that neurons in the rostral superior colliculus (SC) that are involved in fixational saccade generation (Hafed et al. 2009; Hafed and Krauzlis 2012) have weaker activity during fixation on prosaccade trials than antisaccade trials (Everling et al. 1999).

Despite the above prediction supported by neurophysiological findings, we observed that fixational saccades were reduced more strongly before antisaccades than prosaccades (Fig. 6, *A* and *C*). We analyzed this phenomenon quantitatively by applying the same Poisson regressions described above to the frequency of fixational saccades during the prestimulus period. The distribution of regression coefficients for task in-

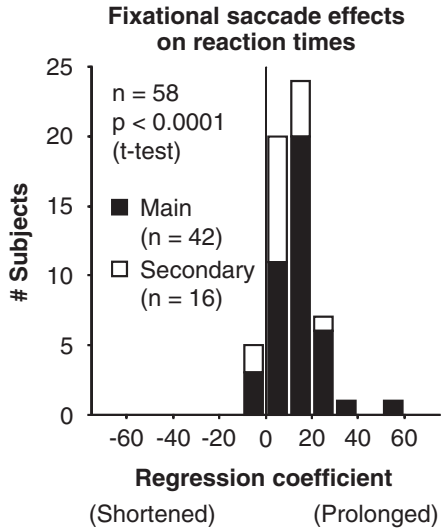


Fig. 7. Influences of fixational saccade occurrence on reaction times. Pro- and antisaccade reaction times were analyzed by linear regressions (*Eqs. 1* and *2*) with an additional factor of "fixational saccade count" ($n = 0, 1, 2,\ldots$) during the prestimulus period. Filled and open bars indicate subjects who performed the main and secondary paradigms, respectively.

struction was biased toward negative values (Fig. 8*B*) [main: $t_{(41)} = -4.68$, $P < 0.0001$ (*t*-test); secondary: $t_{(15)} = -3.28$, $P < 0.01$]. This indicates that fixational saccades were reduced more strongly on antisaccade trials than on prosaccade trials.

The above results suggest that antisaccade preparation reduced the frequency of fixational saccades. We speculate that this reflects the preparation of saccade suppression for inappropriate direction errors programmed automatically in response to stimulus appearance on antisaccade trials.

Correlation between fixational saccades and antisaccade performance. If fixational saccades reflect preparatory signals that suppress direction errors on antisaccade trials, the reduction of fixational saccade occurrence by an antisaccade instruction should be diminished when subjects failed to suppress direction errors. In the following analysis, we focused on a subset of subjects who produced enough antisaccade trials with direction errors (at least 10 trials in each block of trials) during the main ($n = 32$) and secondary ($n = 9$) paradigms.

We confirmed the above prediction qualitatively in the population density function derived from subjects who performed the main paradigm; fixational saccade frequency was lower on antisaccade trials with correct responses than on those with direction errors (Fig. 9*A*). We first analyzed this observation simply by counting fixational saccades during the prestimulus period for subjects who performed the main paradigm. This analysis confirmed the lower frequency of fixational saccades on correct trials than direction error trials [circles in Fig. 9*B*; $t_{(31)} = 2.13$, $P < 0.05$ (paired *t*-test); see figure legend for results of regression analysis]. For those who performed the secondary paradigm, we estimated fixational saccade frequencies from the results of the same Poisson regressions adopted above (*Eq. 2*) with the only exception being the replacement of task instruction (pro/anti) to antisaccade performance (+1: correct; −1: direction error). The lower frequencies of fixational saccades on correct trials were also confirmed in these subjects [triangles in Fig. 9*B*; $t_{(8)} = -2.47$, $P < 0.05$].

The above findings are supported further by the following observation in the secondary paradigm: the frequency of fixational saccade occurrence was decreased significantly when subjects performed antisaccades better in the 2nd block compared with the 1st block (Table 1; negative regression coefficients for fixational frequency and 1st/2nd block and for macro direction error and 1st/2nd block).

The reduced frequency of fixational saccades only before correct antisaccade performance (Fig. 9) and correlated changes in fixational saccade occurrence and antisaccade performance between consecutive blocks of trials (Table 1) support the hypothesis that fixational saccades reflect the preparation of saccade suppression for direction errors on antisaccade trials.

Fixational saccade readiness probed by fixation blink. We used catch trials in the main paradigm to probe the readiness of fixational saccades by triggering them with a fixation blink (transient disappearance of fixation point; Fig. 6, *B* and *D*). This design was inspired by neurophysiological studies in behaving monkeys that applied electrical microstimulation to the oculomotor structures, such as the SC and frontal eye field, to probe the readiness of larger saccades (Gold and Shadlen 2000; Kustov and Robinson 1996). If the reduction of fixational saccade frequencies that occurred with longer elapsed time from fixation initiation (Fig. 8*A*) and an antisaccade instruction (Fig. 8*B*) were caused by mechanisms that suppress fixational saccade occurrence, the latencies of fixational saccades evoked by a fixation blink were expected to be prolonged by such suppression.

This prediction was confirmed for task instruction (Fig. 10*B*). This analysis was focused on a subset of subjects ($n = 38$) who had enough trials with fixational saccades (at least 10 trials for each pro- and antisaccade instruction) evoked during the postblink period (400-ms window starting 200 ms after 1st fixation point disappearance, indicated by horizontal bar in Fig. 6*D*). We analyzed the latencies of evoked fixational saccades with

Table 1. *Summary of between-subject analysis in secondary paradigm*

Coefficient	Fixational Frequency	Macro Reaction Time	Macro Anti Error
Random/fixed	−0.20 (−0.30, −0.11)*	−21.3 (−23.2, −19.3)*	−0.00 (−0.18, 0.17)
1st/2nd block	−0.23 (−0.32, −0.13)*	2.21 (0.26, 4.17)*	−0.42 (−0.60, −0.25)*

Multiple regressions (*Eq. 2*) were applied to the frequency of fixational saccade occurrence during the prestimulus period on correct trials (fixational frequency), correct pro- and antisaccade reaction times (macro reaction time), and the probability of direction errors on antisaccade trials (macro anti error). Each number in this table indicates a resultant regression coefficient (with 95% confidence interval in parentheses). Fixational frequency had significant negative values in both coefficients, each of which indicates that *1*) fixational saccade frequencies were decreased in blocks with fixed fixation duration compared with those with randomized fixation duration (random/fixed) and *2*) they were decreased in the 2nd blocks compared with the 1st blocks (1st/2nd block). The same polarities of changes were applied to macro reaction time [e.g., shortened reaction times in blocks with fixed fixation duration compared with those with randomized fixation duration (random/fixed)] as well as macro anti error [e.g., reduced probability of direction errors in the 2nd blocks compared with the 1st blocks (1st/2nd block)]. *$P < 0.05$.

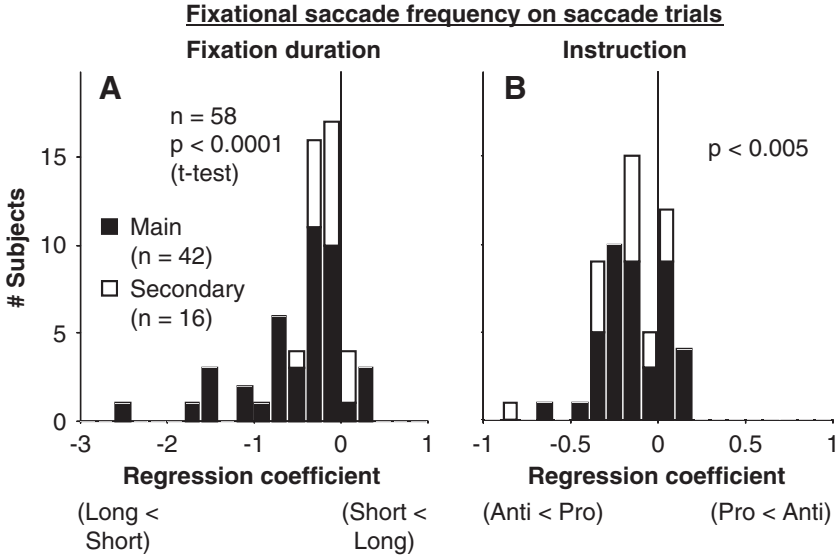


Fig. 8. Summary of Poisson regressions for fixational saccade occurrence. *A*: distribution of coefficients for fixation duration. Positive and negative values indicate increased and decreased frequencies of fixational saccades with fixation duration, respectively. *B*: distribution of coefficients for task instruction. Positive and negative values indicate higher and lower frequencies of fixational saccades before antisaccades than prosaccades, respectively. Fixational saccade frequencies were calculated during the prestimulus period. Filled and open bars indicate subjects who performed the main and secondary paradigms, respectively. Use of the bite-bar had a minor effect on coefficients for fixation duration in the main paradigm (*A*). We analyzed the coefficients with a univariate linear regression that includes a constant and a factor of use of the bite-bar [unused (0), used (+1)]. The constant had a negative value (−0.45) deviating significantly from zero [$t_{(40)} = -5.23$, $P < 0.0001$]. This indicates that the distribution of coefficients for fixation duration was biased toward negative values, even after we eliminated the influence of the use of the bite-bar [$t_{(40)} = 2.05$, $P < 0.05$]. This was the only result that reached statistical significance for use of the bite-bar in the analyses of fixational saccades in this study.

the same linear regression adopted above (*Eq. 1*). The distribution of regression coefficients for task instruction was biased toward positive values [$t_{(37)} = 2.80$, $P < 0.01$ (*t*-test)], indicating longer latencies on trials with an antisaccade instruction. This suggests that the initiation of fixational saccades was suppressed by the antisaccade instruction.

The above hypothesis also predicts the prolonged latencies of fixational saccades with increasing fixation duration. However, we found shorter latencies on trials with longer fixation duration, indicated by the negative bias of regression coefficients for fixation duration [Fig. 10*A*; $t_{(37)} = -3.75$, $P < 0.001$]. This might be explained by more efficient visual processing with lower frequency of fixational saccades after longer fixation duration (Fig. 8*A*) because fixational saccades impede visual processing (see, e.g., Zuber and Stark 1966). However, the facilitation effects remained even after we took into account the frequency of fixational saccades generated during the prestimulus period [regression coefficient for fixation duration: mean ± SD = −10.0 ± 13.8, $t_{(34)} = -4.26$, $P < 0.0005$; 3 subjects were excluded because fixational saccades were not generated at all during the prestimulus period on catch trials] or during a temporal period that combined the prestimulus period and the 130-ms interval between the prestimulus and postblink periods [−10.1 ± 14.0, $t_{(34)} = -4.25$, $P < 0.0005$].

The reduction of fixational saccades before stimulus appearance by an antisaccade instruction (Fig. 8*B*) was likely mediated by mechanisms that suppress fixational saccade occurrence (Fig. 10*B*). However, the effects of time elapsed from fixation initiation (Figs. 8*A* and 10*A*) were difficult to interpret intuitively because mechanisms that facilitate fixational saccade initiation (Fig. 10*A*) might also reduce the frequency of fixational saccades before stimulus appearance (Fig. 8*A*). Nevertheless, this analysis revealed that the effects of task instruction and time elapsed from fixation initiation on fixational saccades were mediated presumably by different mechanisms.

Individual differences in fixational saccades and antisaccade performance. The results described so far focus mainly on behavioral phenomena observed within individual subjects. However, antisaccade performance varied significantly between individual subjects (Fig. 5*D*). If such variation is attributed, at least in part, to differences in preparatory states for antisaccades, it might be reflected in individual differences in fixational saccades across subjects. Indeed, we identified relationships between direction error rates on antisaccade trials and the frequencies (Fig. 11*A*) and amplitudes (Fig. 11*B*) of fixational saccades generated before stimulus appearance. We identified these relationships by a stepwise regression method (see METHODS). The resultant regression model had the following simple form:

$$[\text{direction error rate}] = d_0 + d_1 \times [\text{frequency}] + d_2 \times [\text{amplitude}] \quad (3)$$

where frequency was obtained from constant [i.e., frequency = 2.5 × exp(constant); 2.5 was multiplied to convert from a count within 400 ms (prestimulus period) to frequency in a second (Hz)]. The regression coefficients ± 95% confidence intervals were as follows: $d_0 = -0.07 \pm 0.10$, $d_1 = 0.25 \pm 0.10$, $d_2 = 0.21 \pm 0.16$. The fitting was successful [$R^2 = 0.43$, $F_{(2,53)} = 20.2$, $P < 0.0001$]. The individual relationships between direction error rates and the frequencies (Fig. 11*A*) and amplitudes (Fig. 11*B*) of fixational saccades are summarized as follows: frequency-main: Pearson's $r = 0.58$, $P < 0.0001$, $n = 42$ (2 subjects excluded from the above regression analysis were included); frequency-secondary: $r = 0.56$, $P < 0.05$, $n = 16$; amplitude-main: $r = 0.38$, $P < 0.05$, $n = 40$; amplitude-secondary: $r = 0.56$, $P < 0.05$, $n = 16$. These results indicate that subjects who generated larger fixational saccades more frequently before stimulus appearance had poorer antisaccade performance.

We also performed the same stepwise regression analysis for prosaccade direction errors, prosaccade reaction times, and antisaccade reaction times, but the models remained constant and never added any terms.

DISCUSSION

Our findings are summarized in the following two points. First, pro- and antisaccade initiation was facilitated with elapsed time from fixation initiation and temporal expectation of stimulus appearance. Such saccade facilitation was also reflected in fixational saccades as their reduced frequency

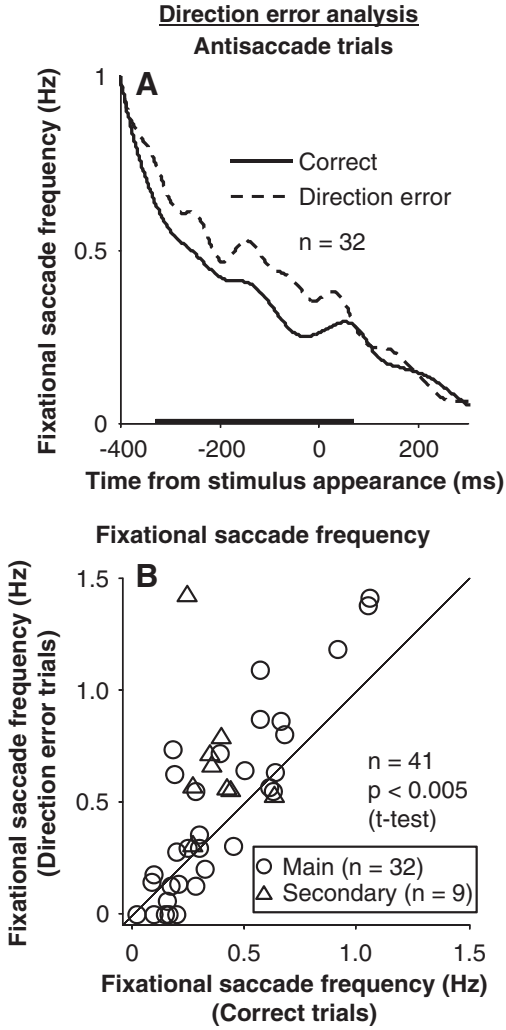


Fig. 9. Fixational saccade occurrence before direction errors on antisaccade trials. *A*: average density functions from 32 subjects who performed the main paradigm and generated enough direction errors (at least 10 trials). *B*: comparison between fixational saccade frequencies on correct trials and those on direction error trials. For subjects who performed the main paradigm ($n = 32$; circles), fixational saccade frequencies were calculated directly during the prestimulus period (indicated by horizontal bar on *x*-axis in *A*). For those who performed the secondary paradigm ($n = 9$; triangles), fixational saccade frequencies were calculated with the regression model (*Eq. 2*). Similar results were confirmed in subjects who performed the main paradigm with the regression model (*Eq. 1*) with marginal statistical significance [average ± SD = −0.10 ± 0.26; *t*-test, $t_{(26)} = -1.98$, $P = 0.06$; 5 subjects were excluded because the model did not converge because of the complete separation of fixational saccade occurrence between correct and direction error trials]. We confirmed similar results shown in Figs. 7 and 8 in these subjects [regression fixational saccade count: main: mean ± SD = 14.2 ± 11.2, $t_{(31)} = 7.21$, $P < 0.0001$ (*t*-test), secondary: 11.2 ± 8.5, $t_{(8)} = 3.97$, $P < 0.005$; regression fixation duration: main: −0.38 ± 0.47, $t_{(31)} = -4.62$, $P < 0.0001$, secondary: −0.14 ± 0.12, $t_{(8)} = -3.46$, $P < 0.01$; regression task instruction: main: −0.17 ± 0.18, $t_{(31)} = -5.43$, $P < 0.0001$, secondary: −0.21 ± 0.29, $t_{(8)} = -2.16$, $P = 0.06$].

before stimulus appearance (Fig. 8*A* and Table 1). Second, fixational saccades were suppressed by an antisaccade instruction (Figs. 8*B* and 10*B*). Such suppression was diminished when subjects failed to cancel direction errors (Fig. 9). This is supported further by the fact that subjects with higher frequencies and larger amplitudes of fixational saccades had poorer antisaccade performance (Fig. 11). On the basis of these results, we suggest that dual preparatory signals that are processed covertly for appropriate antisaccade behavior (facilitation of volitional saccade away from a stimulus and suppression of inappropriate saccade toward the stimulus) can be read out overtly by fixational saccades. In the following sections, we first discuss another perspective to account for our results (spatial attention) and then elaborate the above argument further.

Fixational saccades and spatial attention. Recent studies have shown that fixational saccades reflect covert spatial attention (Brien et al. 2009; Engbert and Kliegl 2003; Gowen et al. 2007; Hafed et al. 2011; Hafed and Clark 2002; Laubrock et al. 2005; Rolfs et al. 2005). In this framework, shorter reaction times of prosaccades than antisaccades could be explained by spatial attention (i.e., facilitation of visual processing) distributed peripherally on prosaccade trials while it remains focused on the center on antisaccade trials. Such differences might account for task instruction effects on fixational saccades (Figs. 8*B* and 9). However, this hypothesis assumes that subjects employed different states of spatial attention even though visual processing for pro- and antisaccades (i.e., fixation point color discrimination, fixation point disappearance detection, and stimulus appearance detection) is equivalent. Nevertheless, the significance of fixational saccades on visual processing (see, e.g., Zuber and Stark 1966), which is controlled presumably by spatial attention, should not be undermined to uncover the whole process of visual-motor transformation.

Fixational saccades reflect both saccade facilitation and suppression. Recent studies suggest that fixational saccades are linked to saccade initiation (Hafed and Krauzlis 2010; Rolfs 2007; Rolfs et al. 2006; Sinn and Engbert 2011). Indeed, we have demonstrated that fixational saccade occurrence before stimulus appearance prolonged pro- and antisaccade reaction times (Fig. 7). Fixational saccades presumably delay saccade initiation through the suppression of incoming visual signals (Bosman et al. 2009; Hafed and Krauzlis 2010; Herrington et al. 2009; Leopold and Logothetis 1998; Zuber and Stark 1966) and/or competitive interactions between different saccade commands (Munoz and Istvan 1998; Rolfs and Ohl 2011; Trappenberg et al. 2001). Accordingly, mechanisms that facilitate saccade initiation might also reduce fixational saccade occurrence to optimize the saccade control system for upcoming visual-motor transformation. Such mechanisms might account for reduced fixational saccade frequencies followed by shortened reaction times with elapsed time from fixation initiation (Fig. 8*A*) and enhanced temporal expectation of stimulus appearance (Table 1). If mechanisms that facilitate pro- and antisaccade initiation reduce the frequency of fixational saccades, it should be reduced more strongly before prosaccades than antisaccades because prosaccade reaction times were shorter than antisaccade reaction times (Fig. 5).

However, we found the following two results that do not correspond to the above prediction. First, fixational saccades were decreased more strongly before antisaccades than before prosaccades (Fig. 8*B*). Second, fixational saccade reduction was diminished when subjects failed to cancel direction errors (Fig. 9). Moreover, these findings are partly supported by a study that analyzed fixational saccades during the pro- and antisaccade paradigm in 30 subjects (Rolfs 2007). Although these findings are inconsistent with a previously published report (Hermens et al. 2010), there are a number of method-

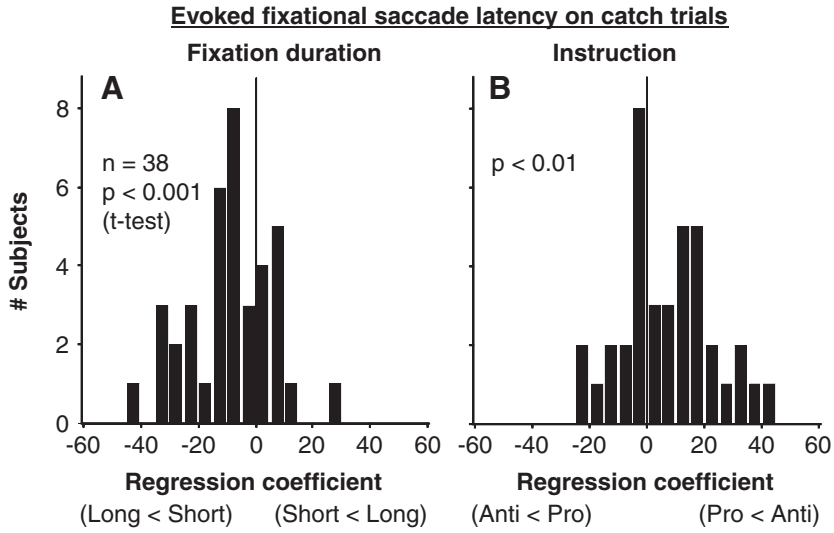


Fig. 10. Summary of evoked fixational saccade latencies on catch trials. *A*: distribution of coefficients for fixation duration. Positive and negative values indicate increased and decreased fixational saccade latencies with fixation duration, respectively. *B*: distribution of coefficients for task instruction. Positive and negative values indicate longer and shorter fixational saccade latencies on trials with an antisaccade instruction than those with a prosaccade instruction, respectively. Fixational saccade latencies were calculated from 1st fixation point disappearance. First fixational saccades evoked during the postblink period were analyzed. This analysis was focused on 38 subjects who had enough catch trials with evoked fixational saccades (at least 10 trials). We confirmed the results of fixational saccades shown in Figs. 7 and 8 in these subjects [regression fixational saccade count: mean ± SD = 14.8 ± 10.9, *t*-test, $t_{(37)} = 8.36$, $P < 0.0001$; regression fixation duration: −0.49 ± 0.59, $t_{(37)} = -5.08$, $P < 0.0001$; regression task instruction: −0.13 ± 0.19, $t_{(37)} = -4.38$, $P < 0.0001$].

ological differences [e.g., the number of our subjects ($n = 58$ in total) was approximately 6 times larger than in the previous study ($n = 10$ in their immediate task); pro- and antisaccade trials were randomly interleaved in our paradigms, while they were blocked in the previous study], which will have to be addressed in the future. Nevertheless, because fixational saccade suppression by antisaccade preparation cannot be explained by the above mechanisms that facilitate saccade initiation, it should reflect other mechanisms that are specifically involved in suppression of inappropriate saccades.

We therefore conclude that fixational saccades reflect two aspects of volitional action preparation required for appropriate saccade behavior: *1*) facilitation of saccade initiation and *2*) suppression of inappropriate saccades.

Do fixational saccades reflect preparatory states in superior colliculus? The two aspects of volitional saccade preparation (facilitation and suppression) reflected in fixational saccades might be originated from the rostral SC because it is critical for fixational saccade generation (Hafed et al. 2009; Hafed and Krauzlis 2012). However, our results are inconsistent with the following fact: rostral SC neurons have higher activity on antisaccade trials than on prosaccade trials during fixation (Everling et al. 1999), while fixational saccades were suppressed more strongly before antisaccades than before prosaccades (Figs. 8*B* and 10*B*).

Neurons in the caudal SC encode larger saccades (Sparks 2002), but recent behavioral studies suggest their potential involvement in fixational saccades (Brien et al. 2009; Engbert and Kliegl 2003; Gowen et al. 2007; Hafed et al. 2011; Hafed and Clark 2002; Laubrock et al. 2005; Rolfs et al. 2005). However, this idea has the following inconsistency: the preparatory activity of caudal SC neurons is higher when reaction times are shorter (Everling et al. 1999), while fixational saccade frequencies were lower when reaction times were shorter (Fig. 7).

Recently developed SC models that integrate the rostral and caudal SC in a continuous motor map could potentially resolve the above inconsistencies (Engbert 2012; Hafed et al. 2009; Rolfs et al. 2008). On the basis of the models and physiological findings (Everling et al. 1999), we suggest the following hypothesis. The spatial distribution of neural activity is focused on the rostral SC on antisaccade trials, while it is more spread to the caudal SC on prosaccade trials. If fixational saccades are triggered when the whole distribution is shifted off the center, they should be more frequent before prosaccades than before antisaccades (Fig. 8*B*) because there are more active neurons

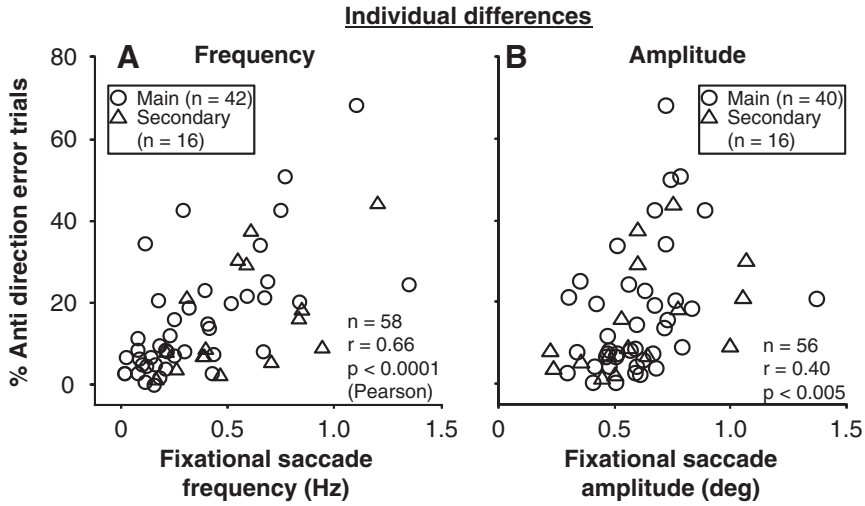


Fig. 11. Individual differences in direction error rates on antisaccade trials and fixational saccades. *A*: correlation between direction error rates and the frequencies of fixational saccades before stimulus appearance. *B*: correlation between direction error rates and the amplitudes of fixational saccades before stimulus appearance. Circles and triangles indicate subjects who performed main and secondary paradigms, respectively.

prone to random noise. This predicts that those subjects with higher frequency and larger amplitude of fixational saccades also had wider distributions of SC neural activity. This fits nicely with their higher direction error rates on antisaccade trials (Fig. 11), because higher preparatory activity in caudal SC neurons is prone to trigger a direction error saccade in response to stimulus appearance (Everling et al. 1998).

The above model might also explain the paradoxical effects of elapsed time from fixation initiation on the frequency (Fig. 8*A*) and latency (Fig. 10*A*) of fixational saccades by the following mechanism. The left and right caudal SCs receive preparatory signals that reflect the equal probabilities of upcoming pro- or antisaccade directions (i.e., 50% for both leftward and rightward) (Dorris and Munoz 1998). Such preparatory signals balance the SC map and reduce the frequency of fixational saccades before stimulus appearance (Fig. 8*A*). Furthermore, the enhanced activity in the caudal SC facilitates the initiation of fixational saccades evoked by a fixation blink (Fig. 10*A*). However, this hypothesis requires noise reduction not to trigger fixational saccades with high preparatory activity.

The model provides a promising framework to account for fixational saccade as well as pro- and antisaccade behavior correctively, although it will have to be tested quantitatively (e.g., Engbert 2012). It is also important to address how the SC map is read out by the brain stem premotor circuitry for fixational saccade control (Otero-Millan et al. 2011; Rolfs et al. 2008; Van Gisbergen et al. 1981; Van Horn and Cullen 2012). Nevertheless, the model helps us seek a potential source of the dual preparatory signals of saccade facilitation and suppression reflected in fixational saccades, which we discuss in the following section.

Do fixational saccades reflect preparatory states in basal ganglia? The spatial-temporal activity on the SC map is controlled by inhibitory output signals from the basal ganglia. The output signals are then controlled by the caudate nucleus, a major input stage of the basal ganglia that integrates a variety of cortical and thalamic signals (Hikosaka et al. 2000; Mink 1996; Watanabe and Munoz 2011). We have reported previously the preparatory activity of putative projection neurons in monkey caudate nucleus (Watanabe and Munoz 2010). Interestingly, the caudate preparatory activity has the following four characteristics that are the mirror image of fixational saccade characteristics. *1*) Caudate neurons have higher preparatory activity before pro- and antisaccades with shorter reaction times (for corresponding fixational saccades in this study, see Fig. 7). *2*) The caudate preparatory activity increases with temporal expectation of stimulus appearance (see also Fig. 8*A* and Table 1). *3*) An antisaccade instruction enhances the preparatory activity of a subset of caudate neurons encoding volitional saccades (see also Fig. 8*B*). *4*) Such enhancement of preparatory activity is absent when direction errors are generated instead of correct antisaccades (see also Fig. 9).

The striking similarity between the preparatory activity of caudate neurons and fixational saccade characteristics suggests that saccade facilitation and suppression reflected in fixational saccades may originate from the basal ganglia. Indeed, this is in line with their anatomical connections: the caudate nucleus gives rise to two pathways that facilitate and suppress saccade initiation, respectively (Hikosaka et al. 2000; Mink 1996; Watanabe and Munoz 2011). Accordingly, we propose a new hypothesis that saccade facilitation and suppression reflected in fixational saccades are mediated by the facilitation and suppression pathways (also known as direct and indirect pathways, respectively) in the basal ganglia. To test this hypothesis, it will be critical to disentangle the individual contributions of the facilitation and suppression pathways to understand how signals carried by these pathways converge on the SC map and influence fixational saccades.

Future direction of fixational saccade analysis for basal ganglia disorders. Recent neuroimaging studies have shown that antisaccade deficits (longer reaction times and higher direction error rates) in Parkinson's disease and attention deficit hyperactivity disorder, both of which induce basal ganglia dysfunctions (Giedd et al. 2001; Obeso et al. 2000), are explained by inappropriate volitional saccade preparation (Cameron et al. 2012; Hakvoort Schwerdtfeger et al. 2013). Furthermore, unstable visual fixation by frequent fixational saccades has been reported in the same disorders (Gould et al. 2001; Shaikh et al. 2011). Our findings bridge these two lines of research and suggest that fixational saccades may be used as a potential biomarker to detect patients with basal ganglia disorders that induce difficulties in volitional action preparation.

Inappropriate antisaccade preparation (Cameron et al. 2012; Hakvoort Schwerdtfeger et al. 2013) might be probed as the lack of fixational saccade suppression by an antisaccade instruction (Figs. 8*B* and 10*B*). Abnormal time perception in basal ganglia disorders (Buhusi and Meck 2005; Castellanos and Tannock 2002) might be detected by fixational saccades with their time dependence (Figs. 8*A* and 10*A*; Table 1). Saccade impulsivity (Everling and Fischer 1998; Leigh and Kennard 2004; Munoz and Everling 2004) might be inferred well from the frequency and amplitude of fixational saccades (Fig. 11) without the use of cognitively demanding paradigms, such as antisaccades, whose instruction might be difficult to comprehend in some patients.

Multiple parameters extracted from fixational saccades can be integrated with the conventional parameters of larger saccades (e.g., reaction times) by sophisticated statistical methods, such as machine learning (Benson et al. 2012; Lagun et al. 2011; Tseng et al. 2013). Such a new approach may utilize fixational saccades as parts of quantitative clinical diagnoses based on oculomotor behavior in the future.

ACKNOWLEDGMENTS

We thank Dr. Y. T. Kitamura, S. Asahara, and F. Tanaka for technical support and members of the Munoz lab for comments on this manuscript.

GRANTS

This work was supported by Nakayama Foundation for Human Science, Adaptable and Seamless Technology Transfer Program (ASTEP) through target-driven R&D (AS231Z03528F), Precursory Research for Embryonic Science and Technology (PRESTO) from the Japan Science and Technology Agency, Grants-in-Aid for scientific research from the Japan Society for the Promotion of Science (24120511), and Osaka University Global COE program Human Behavior and Socioeconomic Dynamics.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.W. and Y.K. conception and design of research; M.W., Y.M., and L.Z. performed experiments; M.W. analyzed data; M.W.,

D.P.M., and Y.K. interpreted results of experiments; M.W. and Y.K. prepared figures; M.W. drafted manuscript; M.W., D.P.M., and Y.K. edited and revised manuscript; M.W., Y.M., L.Z., D.P.M., and Y.K. approved final version of manuscript.

REFERENCES

Amador N, Schlag-Rey M, Schlag J. Primate antisaccade. II. Supplementary eye field neuronal activity predicts correct performance. *J Neurophysiol* 91: 1672–1689, 2004.

Benson PJ, Beedie SA, Shephard E, Giegling I, Rujescu D, St Clair D. Simple viewing tests can detect eye movement abnormalities that distinguish schizophrenia cases from controls with exceptional accuracy. *Biol Psychiatry* 72: 716–724, 2012.

Bosman CA, Womelsdorf T, Desimone R, Fries P. A microsaccadic rhythm modulates gamma-band synchronization and behavior. *J Neurosci* 29: 9471–9480, 2009.

Bridgeman B, Palca J. The role of microsaccades in high acuity observational tasks. *Vision Res* 20: 813–817, 1980.

Brien DC, Corneil BD, Fecteau JH, Bell AH, Munoz DP. The behavioural and neurophysiological modulation of microsaccades in monkeys. *J Eye Mov Res* 3: 1–12, 2009.

Buhusi CV, Meck WH. What makes us tick? Functional and neural mechanisms of interval timing. *Nat Rev Neurosci* 6: 755–765, 2005.

Cameron IG, Pari G, Alahyane N, Brien DC, Coe BC, Stroman PW, Munoz DP. Impaired executive function signals in motor brain regions in parkinson's disease. *Neuroimage* 60: 1156–1170, 2012.

Carpenter RH, Williams ML. Neural computation of log likelihood in control of saccadic eye movements. *Nature* 377: 59–62, 1995.

Castellanos FX, Tannock R. Neuroscience of attention-deficit/hyperactivity disorder: The search for endophenotypes. *Nat Rev Neurosci* 3: 617–628, 2002.

Cunnington R, Iansek R, Johnson KA, Bradshaw JL. Movement-related potentials in Parkinson's disease. Motor imagery and movement preparation. *Brain* 120: 1339–1353, 1997.

Dafoe JM, Armstrong IT, Munoz DP. The influence of stimulus direction and eccentricity on pro- and anti-saccades in humans. *Exp Brain Res* 179: 563–570, 2007.

Dorris MC, Munoz DP. Saccadic probability influences motor preparation signals and time to saccadic initiation. *J Neurosci* 18: 7015–7026, 1998.

Engbert R. Computational modeling of collicular integration of perceptual responses and attention in microsaccades. *J Neurosci* 32: 8035–8039, 2012.

Engbert R. Microsaccades: a microcosm for research on oculomotor control, attention, and visual perception. *Prog Brain Res* 154: 177–192, 2006.

Engbert R, Mergenthaler K. Microsaccades are triggered by low retinal image slip. *Proc Natl Acad Sci USA* 103: 7192–7197, 2006.

Engbert R, Kliegl R. Microsaccades uncover the orientation of covert attention. *Vision Res* 43: 1035–1045, 2003.

Everling S, Desouza JF. Rule-dependent activity for prosaccades and antisaccades in the primate prefrontal cortex. *J Cogn Neurosci* 17: 1483–1496, 2005.

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

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

Everling S, Dorris MC, Munoz DP. Reflex suppression in the anti-saccade task is dependent on prestimulus neural processes. *J Neurophysiol* 80: 1584–1589, 1998.

Everling S, Dorris MC, Klein RM, Munoz DP. Role of primate superior colliculus in preparation and execution of anti-saccades and pro-saccades. *J Neurosci* 19: 2740–2754, 1999.

Fischer B, Weber H. Express saccades and visual attention. *Behav Brain Sci* 16: 553–610, 1993.

Fischer B, Weber H. Characteristics of "anti" saccades in man. *Exp Brain Res* 89: 415–424, 1992.

Giedd JN, Blumenthal J, Molloy E, Castellanos FX. Brain imaging of attention deficit/hyperactivity disorder. *Ann NY Acad Sci* 931: 33–49, 2001.

Gold JI, Shadlen MN. Representation of a perceptual decision in developing oculomotor commands. *Nature* 404: 390–394, 2000.

Gould TD, Bastain TM, Israel ME, Hommer DW, Castellanos FX. Altered performance on an ocular fixation task in attention-deficit/hyperactivity disorder. *Biol Psychiatry* 50: 633–635, 2001.

Gowen E, Abadi RV, Poliakoff E, Hansen PC, Miall RC. Modulation of saccadic intrusions by exogenous and endogenous attention. *Brain Res* 1141: 154–167, 2007.

Haddad GM, Steinman RM. The smallest voluntary saccade: implications for fixation. *Vision Res* 13: 1075–1086, 1973.

Hafed ZM, Krauzlis RJ. Similarity of superior colliculus involvement in microsaccade and saccade generation. *J Neurophysiol* 107: 1904–1916, 2012.

Hafed ZM, Krauzlis RJ. Microsaccadic suppression of visual bursts in the primate superior colliculus. *J Neurosci* 30: 9542–9547, 2010.

Hafed ZM, Clark JJ. Microsaccades as an overt measure of covert attention shifts. *Vision Res* 42: 2533–2545, 2002.

Hafed ZM, Lovejoy LP, Krauzlis RJ. Modulation of microsaccades in monkey during a covert visual attention task. *J Neurosci* 31: 15219–15230, 2011.

Hafed ZM, Goffart L, Krauzlis RJ. A neural mechanism for microsaccade generation in the primate superior colliculus. *Science* 323: 940–943, 2009.

Haggard P. Human volition: towards a neuroscience of will. *Nat Rev Neurosci* 9: 934–946, 2008.

Hakvoort Schwerdtfeger RM, Alahyane N, Brien DC, Coe BC, Stroman PW, Munoz DP. Preparatory neural networks are impaired in adults with attention-deficit/hyperactivity disorder during the antisaccade task. *Neuroimage* 2: 63–78, 2013.

Hallett PE. Primary and secondary saccades to goals defined by instructions. *Vision Res* 18: 1279–1296, 1978.

Hermens F, Zanker JM, Walker R. Microsaccades and preparatory set: a comparison between delayed and immediate, exogenous and endogenous pro- and anti-saccades. *Exp Brain Res* 201: 489–498, 2010.

Herrington TM, Masse NY, Hachmeh KJ, Smith JE, Assad JA, Cook EP. The effect of microsaccades on the correlation between neural activity and behavior in middle temporal, ventral intraparietal, and lateral intraparietal areas. *J Neurosci* 29: 5793–5805, 2009.

Hikosaka O, Takikawa Y, Kawagoe R. Role of the basal ganglia in the control of purposive saccadic eye movements. *Physiol Rev* 80: 953–978, 2000.

Ko HK, Poletti M, Rucci M. Microsaccades precisely relocate gaze in a high visual acuity task. *Nat Neurosci* 13: 1549–1553, 2010.

Kowler E, Steinman RM. The role of small saccades in counting. *Vision Res* 17: 141–146, 1977.

Kunimatsu J, Tanaka M. Roles of the primate motor thalamus in the generation of antisaccades. *J Neurosci* 30: 5108–5117, 2010.

Kustov AA, Robinson DL. Shared neural control of attentional shifts and eye movements. *Nature* 384: 74–77, 1996.

Lagun D, Manzanares C, Zola SM, Buffalo EA, Agichtein E. Detecting cognitive impairment by eye movement analysis using automatic classification algorithms. *J Neurosci Methods* 201: 196–203, 2011.

Laubrock J, Engbert R, Kliegl R. Microsaccade dynamics during covert attention. *Vision Res* 45: 721–730, 2005.

Leigh RJ, Kennard C. Using saccades as a research tool in the clinical neurosciences. *Brain* 127: 460–477, 2004.

Leopold DA, Logothetis NK. Microsaccades differentially modulate neural activity in the striate and extrastriate visual cortex. *Exp Brain Res* 123: 341–345, 1998.

McLoughlin G, Albrecht B, Banaschewski T, Rothenberger A, Brandeis D, Asherson P, Kuntsi J. Electrophysiological evidence for abnormal preparatory states and inhibitory processing in adult ADHD. *Behav Brain Funct* 6: 66, 2010.

Mink JW. The basal ganglia: focused selection and inhibition of competing motor programs. *Prog Neurobiol* 50: 381–425, 1996.

Moller F, Laursen ML, Tygesen J, Sjolie AK. Binocular quantification and characterization of microsaccades. *Graefes Arch Clin Exp Ophthalmol* 240: 765–770, 2002.

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

Munoz DP, Istvan PJ. Lateral inhibitory interactions in the intermediate layers of the monkey superior colliculus. *J Neurophysiol* 79: 1193–1209, 1998.

Nachev P, Kennard C, Husain M. Functional role of the supplementary and pre-supplementary motor areas. *Nat Rev Neurosci* 9: 856–869, 2008.

Obeso JA, Rodriguez-Oroz MC, Rodriguez M, Lanciego JL, Artieda J, Gonzalo N, Olanow CW. Pathophysiology of the basal ganglia in Parkinson's disease. *Trends Neurosci* 23: S8–S19, 2000.

Oswal A, Ogden M, Carpenter RH. The time course of stimulus expectation in a saccadic decision task. *J Neurophysiol* 97: 2722–2730, 2007.

Otero-Millan J, Macknik SL, Serra A, Leigh RJ, Martinez-Conde S. Triggering mechanisms in microsaccade and saccade generation: a novel proposal. *Ann NY Acad Sci* 1233: 107–116, 2011.

Pare M, Munoz DP. Saccadic reaction time in the monkey: advanced preparation of oculomotor programs is primarily responsible for express saccade occurrence. *J Neurophysiol* 76: 3666–3681, 1996.

Rolfs M. *In-Between Fixation and Movement: On the Generation of Microsaccades and What They Convey About Saccade Preparation*. (PhD dissertation). Potsdam, Germany: University of Potsdam, 2007 (http://opus.kobv.de/ubp/volltexte/2007/1458/).

Rolfs M, Ohl S. Visual suppression in the superior colliculus around the time of microsaccades. *J Neurophysiol* 105: 1–3, 2011.

Rolfs M, Kliegl R, Engbert R. Toward a model of microsaccade generation: The case of microsaccadic inhibition. *J Vis* 8: 5.1–5.23, 2008.

Rolfs M, Laubrock J, Kliegl R. Shortening and prolongation of saccade latencies following microsaccades. *Exp Brain Res* 169: 369–376, 2006.

Rolfs M, Engbert R, Kliegl R. Crossmodal coupling of oculomotor control and spatial attention in vision and audition. *Exp Brain Res* 166: 427–439, 2005.

Schall JD. On building a bridge between brain and behavior. *Annu Rev Psychol* 55: 23–50, 2004.

Shaikh AG, Xu-Wilson M, Grill S, Zee DS. "Staircase" square-wave jerks in early parkinson's disease. *Br J Ophthalmol* 95: 705–709, 2011.

Sinn P, Engbert R. Saccadic facilitation by modulation of microsaccades in natural backgrounds. *Atten Percept Psychophys* 73: 1029–1033, 2011.

Smith PL, Ratcliff R. Psychology and neurobiology of simple decisions. *Trends Neurosci* 27: 161–168, 2004.

Sparks DL. The brainstem control of saccadic eye movements. *Nat Rev Neurosci* 3: 952–964, 2002.

Steinman RM, Cunitz RJ, Timberlake GT, Herman M. Voluntary control of microsaccades during maintained monocular fixation. *Science* 155: 1577–1579, 1967.

Trappenberg TP, Dorris MC, Munoz DP, Klein RM. A model of saccade initiation based on the competitive integration of exogenous and endogenous signals in the superior colliculus. *J Cogn Neurosci* 13: 256–271, 2001.

Tseng PH, Cameron IG, Pari G, Reynolds JN, Munoz DP, Itti L. High-throughput classification of clinical populations from natural viewing eye movements. *J Neurol* 260: 275–284, 2013.

Van Gisbergen JA, Robinson DA, Gielen S. A quantitative analysis of generation of saccadic eye movements by burst neurons. *J Neurophysiol* 45: 417–442, 1981.

Van Horn MR, Cullen KE. Coding of microsaccades in three-dimensional space by premotor saccadic neurons. *J Neurosci* 32: 1974–1980, 2012.

Watanabe M, Munoz DP. Probing basal ganglia functions by saccade eye movements. *Eur J Neurosci* 33: 2070–2090, 2011.

Watanabe M, Munoz DP. Presetting basal ganglia for volitional actions. *J Neurosci* 30: 10144–10157, 2010.

Winterson BJ, Collewijn H. Microsaccades during finely guided visuomotor tasks. *Vision Res* 16: 1387–1390, 1976.

Yoshida A, Tanaka M. Enhanced modulation of neuronal activity during antisaccades in the primate globus pallidus. *Cereb Cortex* 19: 206–217, 2009.

Zuber BL, Stark L. Saccadic suppression: Elevation of visual threshold associated with saccadic eye movements. *Exp Neurol* 16: 65–79, 1966.

Zuber BL, Stark L, Cook G. Microsaccades and the velocity-amplitude relationship for saccadic eye movements. *Science* 150: 1459–1460, 1965.