

Fixational saccades alter the gap effect

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Keywords: attention, cognitive, executive functions, eye movements, human, saccade

Abstract

The reaction times of saccadic eye movements have been studied extensively as a probe for cognitive behavior controlled by large-scale cortical and subcortical neural networks. Recent studies have shown that the reaction times of targeting saccades toward peripheral visual stimuli are prolonged by fixational saccades, the largest miniature eye movements including microsaccades. We have shown previously that the frequency of fixational saccades is decreased by volitional action preparation controlled internally during the antisaccade paradigm (look away from a stimulus). Instead, here we examined whether fixational saccade modulation induced externally by sensory events could also account for targeting saccade facilitation by the same sensory events. When targeting saccades were facilitated by prior fixation stimulus disappearance (gap effect), fixational saccade occurrence was reduced, which could theoretically facilitate targeting saccades. However, such reduction was followed immediately by the rebound of fixational saccade occurrence in some subjects, which could eliminate potential benefits from the previous fixational saccade reduction. These results do not mean that fixational saccades were unrelated to the gap effect because they indeed altered that effect by delaying targeting saccade initiation on trials without the fixation gap more strongly than trials with it. Such changes might be attributed to the disruption of volitional saccade preparation because the frequency of fixational saccades observed in this study was associated with the ability of volitional control over antisaccade behavior. These results suggest that fixational saccades alter the gap effect on targeting saccade reaction times, presumably by disrupting volitional saccade commands.

Introduction

The reaction times of saccadic eye movements vary significantly even when they are triggered in response to identical sensory events. Such variability has been studied extensively as a probe for the mechanisms underlying cognitive behavioral control (Fischer & Weber, 1993; Carpenter & Williams, 1995; Smith & Ratcliff, 2004; MacDonald *et al.*, 2006). In conventional paradigms, subjects maintain gaze on a central fixation point before generating a targeting saccade toward a peripheral visual stimulus. A variety of studies have manipulated targeting saccade preparation during fixation, and analysed its impact on behavioral outcomes and neural processes preceding it in distributed cortical and subcortical networks (Schall, 2001; Munoz & Everling, 2004; Schiller & Tehovnik, 2005; Gold & Shadlen, 2007).

However, the above experimental techniques influence not only targeting saccades but also miniature eye movements that occur during fixation (Hafed & Clark, 2002; Engbert & Kliegl, 2003;

Rolfs *et al.*, 2005). This is important to consider because fixational saccades, the largest miniature eye movements, including microsaccades, have significant impact on targeting saccades (Rolfs *et al.*, 2006; Hafed & Krauzlis, 2010; Sinn & Engbert, 2011; Watanabe *et al.*, 2013). Fixational saccades could delay targeting saccade initiation by the suppression of incoming visual signals (Zuber & Stark, 1966; Leopold & Logothetis, 1998; Hafed & Krauzlis, 2010) and/or competitive interactions between motor commands for fixational and targeting saccades (Munoz & Istvan, 1998; Trappenberg *et al.*, 2001; Rolfs & Ohl, 2011). Therefore, mechanisms that facilitate targeting saccades should decrease the occurrence of fixational saccades to optimize the saccade control system for upcoming visual-motor transformation.

We have shown previously (Watanabe *et al.*, 2013) that fixational saccades are decreased by volitional action preparation for the anti-saccade paradigm [look away from a stimulus (Hallett, 1978)]. That previous study focused on fixational saccade modulation by factors controlled internally [i.e. task instructions (look toward or away from a stimulus), and temporal expectation of stimulus appearance]. Instead, here we examined whether fixational saccade modulation induced externally by sensory events could account for at least some

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Received 28 August 2013, revised 13 February 2014, accepted 19 February 2014

of the targeting saccade facilitation by the same sensory events. We used the gap paradigm in which targeting saccades are facilitated by fixation point disappearance before peripheral stimulus appearance (gap effect; Saslow, 1967). We hypothesized that the fixation gap decreases fixational saccades, which in turn facilitate targeting saccades. This disagrees with a previous study in which the fixation gap did not influence fixational saccades (Kingstone *et al.*, 1995). However, the number of subjects analysed in the previous study was too limited to take into account the diversity of fixational saccade behavior across subjects (Engbert & Mergenthaler, 2006; Watanabe *et al.*, 2013).

We have overcome the above issue by analysing fixational saccades in a much larger number of subjects than the previous study (Kingstone *et al.*, 1995). This allowed us to reveal the precise temporal dynamics of fixational saccade occurrence and the impact of fixational saccades on targeting saccade reaction times, and to characterize individual differences across subjects.

Methods

Subjects

Forty-five subjects [12 females; age -22.3 ± 3.6 years (mean \pm SD)] with normal or corrected-to-normal vision participated in this study. Subjects were paid ¥2000/h for their participation. They were informed of the nature of the study and gave written and informed consent. This study was approved by the research ethics board of the Osaka University Hospital, and adhered to the principles of the Declaration of Helsinki.

Experimental systems

The details of our procedures have been reported previously (Watanabe *et al.*, 2013). Briefly, the control of the behavioral paradigm and the acquisition of eye position data were carried out by the Tempo/Win computing system (Reflective Computing, St Louis, MO, USA). 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 resolution of the pupil tracking was 500 Hz, and the manufacturer's stated spatial resolution was 0.01° . Detected fixational saccades were much larger than the spatial resolution (normally $> 0.1^\circ$) because of the limited temporal resolution as well as the conservative detection criteria described below (Watanabe *et al.*, 2013). A cathode ray tube monitor (60 Hz refresh rate, 1024×768 pixels, 19-inch) was placed 35 cm from the eyes.

Behavioral paradigm

Each trial was preceded by a 1000-ms inter-trial 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 ; color $-$ red or green, counterbalanced across subjects) 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. The following three conditions were randomly interleaved in a block of trials with equal probabilities (Fig. 1).

On gap trials, subjects maintained fixation for 800 ms, and the fixation point disappeared. They were required to maintain eye position on the blank screen for 200 ms (gap period) until a peripheral stimulus (size -0.4° ; luminance -14 cd/m^2 ; color $-$ yellow)

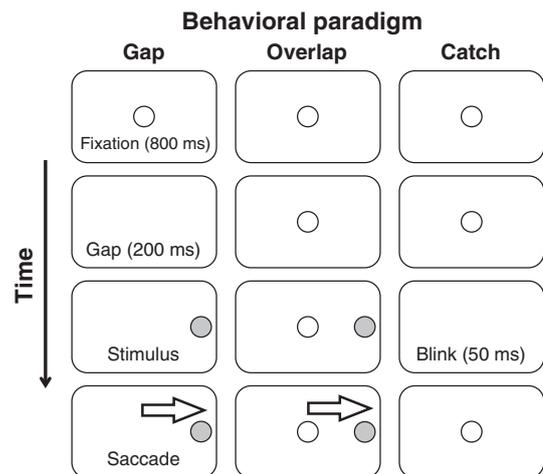


FIG. 1. Behavioral paradigms. On gap trials, subjects fixated on the fixation point, and generated a targeting saccade in response to peripheral stimulus appearance. A temporal gap (200 ms) was introduced between fixation point disappearance and stimulus appearance, during which subjects had to maintain fixation on the blank screen. On overlap trials, the fixation point remained visible after stimulus appearance. On catch trials, subjects fixated on the fixation point throughout the trial, while a 50-ms fixation blink was introduced.

appeared at either 5° left or right from the center of the screen (19° from the border of the monitor). Subjects generated a targeting saccade toward the stimulus, and maintained fixation on it. The stimulus was visible for 1000–1500 ms.

On the overlap trials, everything was the same as the gap trials, except that the fixation point remained visible until the end of the trial. Subjects were required to generate targeting saccades in response to peripheral stimulus appearance.

On catch trials, after 1000 ms fixation followed by brief fixation point disappearance for 50 ms, the fixation point reappeared for 1000–1500 ms instead of a peripheral stimulus. Subjects were required to maintain fixation throughout the trial. Catch trials were included to evoke fixational saccades and detect fixational saccade readiness during this behavioral paradigm. Another reason for the inclusion of catch trials was to replicate the basic characteristics of fixational saccades in response to abrupt sensory events (see, for example, Engbert & Kliegl, 2003; Rolfs *et al.*, 2005).

The inclusion of catch trials might have attenuated the gap effect on targeting saccade reaction times because fixation point disappearance led to stimulus appearance on only 50% of trials when it happened. To reduce this potential effect, we adopted different durations for the fixation blink (50 ms) and the fixation gap (200 ms) to clarify the difference between catch and gap trials.

Subjects performed this paradigm until they achieved at least 150 correct trials (including all three types of trials). There was no explicit requirement of fixational saccades for performing this paradigm. That is, they were irrelevant to the paradigm except for their potential role in fixation maintenance. The majority of subjects ($n = 34$) also performed the antisaccade paradigm [look away from a stimulus (Hallett, 1978)] immediately after they had completed the current behavioral paradigm on the same day. The results of the antisaccade paradigm have been reported previously (Watanabe *et al.*, 2013).

Saccade detection

Eye position data were first processed by a digital filter (third-order Butterworth low-pass filter with a cutoff frequency of 200 Hz). The

onset and end of targeting saccades larger than 2° were identified by radial eye velocity criteria (threshold $-30^\circ/\text{s}$). Because eye positions were recorded binocularly, the onset and end of each targeting saccade was 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 & Kliegl, 2003; Engbert & Mergenthaler, 2006). The velocity threshold of fixational saccades was defined flexibly depending on the noise level on each trial (threshold -6 SD). The minimum duration of fixational saccades that exceeded the velocity threshold was set to 6 ms. This analysis was limited to a temporal period where eye positions were relatively stable (from 200 ms after the end of a saccade toward the fixation point until the initiation of a targeting saccade on gap and overlap trials, or until the disappearance of the second fixation point on catch trials). We analysed 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. The onset and end of each fixational saccade was defined by the earlier onset and the later end from either eye. The minimum inter-saccade 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 was analysed from the right eye. Virtually the same results were confirmed from analysing the left eye data.

Because the minimum amplitude threshold of targeting saccades 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). Only $0.39 \pm 0.97\%$ (mean \pm SD) of trials were excluded by this criterion.

Fixational saccade quantification

As we describe in the Results, fixational saccades had biphasic responses of the reduction and rebound of their frequencies. To capture these dynamic responses, we defined the following two temporal periods for quantitative analyses – pre- and post-stimulus periods.

The pre-stimulus period started 70 ms after fixation point disappearance and ended at stimulus appearance. The total duration of the pre-stimulus period was 130 ms. We took into account 70 ms of delay required for visual input to influence saccades (Fischer & Weber, 1993). We defined the end of the pre-stimulus period at 200 ms after fixation point disappearance because a transient visual event evokes fixational saccades with an approximate latency of 200 ms (see Results; Engbert & Kliegl, 2003; Rolfs *et al.*, 2005; Watanabe *et al.*, 2013).

The post-stimulus period started at stimulus appearance, immediately after the end of the pre-stimulus period. The duration of the post-stimulus period was determined in each subject based on targeting saccade reaction times to maximize its length to capture as many fixational saccades as possible. Instead of determining the post-stimulus period using the shortest reaction time, which would be influenced significantly by outliers, we identified the time at which 2.5% of all targeting saccades on gap and overlap trials were triggered. Because reaction times were shorter on gap trials than on overlap trials, the above criterion corresponded approximately to 5% of gap trials, which was roughly two to three trials among 50 gap trials. The duration of the post-stimulus period was 139 ± 18 ms (mean \pm SD). We confirmed similar results using a temporal window ending at the shortest reaction time (119 ± 27 ms) and that with a fixed duration of 70 ms.

Remaining methods of data analyses

Trials with opposite targeting saccade directions were collapsed because fixational saccade frequency was not different between them. Trials with anticipatory responses [reaction times < 70 ms (Fischer & Weber, 1993)] were excluded from data analyses. Only $0.12 \pm 0.42\%$ (mean \pm SD) of trials were excluded by this criterion. Only correct trials were analysed in all analyses. Data preprocessing and simple statistical analyses were carried out using Matlab (MathWorks, Natick, MA, USA). Fitting multilevel linear models (linear mixed models; West *et al.*, 2006; Gelman & Hill, 2007) to the parameters of targeting saccades was performed by the *lme* function from the *nlme* package (Pinheiro *et al.*, 2010) in the statistical environment R (R Development Core Team, 2012).

Results

Targeting saccade behavior

We confirmed the gap effect on the reaction times of targeting saccades toward peripheral stimuli (Fig. 2). Average reaction times on gap trials [191 ± 27 ms (mean \pm SD)] were shorter than those on overlap trials (235 ± 48 ms; paired *t*-test $-t_{44} = 8.40$, $P < 0.0001$).

Fixational saccade behavior

We also confirmed the following two characteristics of fixational saccade behavior in the population averages of density functions for fixational saccade onset times (Fig. 3). First, fixational saccades decreased and then increased after fixation blink on catch trials (Fig. 3C; Engbert & Kliegl, 2003; Rolfs *et al.*, 2005; Hafed & Ignashchenkova, 2013). Second, the frequency of fixational saccades decreased gradually before stimulus appearance on overlap trials (Fig. 3A; Rolfs *et al.*, 2006; Pastukhov & Braun, 2010; Hafed *et al.*, 2011; Sinn & Engbert, 2011; Watanabe *et al.*, 2013).

We hypothesized that the frequency of fixational saccades decreases during the gap period, which in turn facilitates targeting saccade initiation because fixational saccades delay targeting saccade initiation (Rolfs *et al.*, 2006; Hafed & Krauzlis, 2010; Sinn &

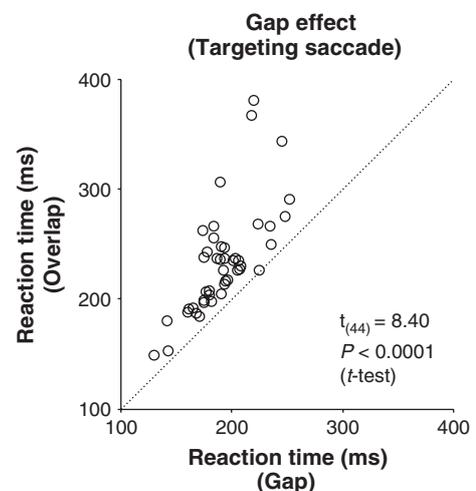


FIG. 2. Gap effects on the reaction times of targeting saccades. Reaction times were shorter on gap trials than on overlap trials, consistent with previous studies. Each data point indicates average reaction times on gap and overlap trials from each subject.

Engbert, 2011; Watanabe *et al.*, 2013). This prediction is consistent with the fact that neurons in the rostral superior colliculus (SC) that are involved in fixational saccade generation (Hafed *et al.*, 2009) decrease activity during the gap period (Dorris & Munoz, 1995; Dorris *et al.*, 1997; Everling *et al.*, 1999).

In line with the above prediction, the frequency of fixational saccades decreased after fixation point disappearance (Fig. 3B). However, such reduction did not last until targeting saccade initiation because fixational saccades rebounded immediately after the gap period. We describe these observations quantitatively in the following sections.

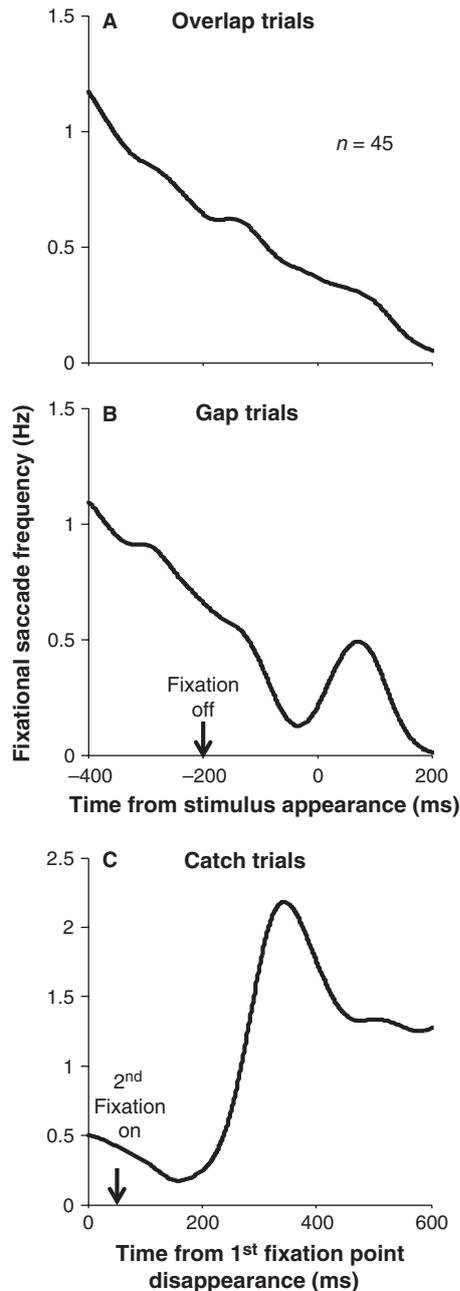


FIG. 3. Population density functions of fixational saccade onset times. (A) Overlap trials. (B) Gap trials. The fixation point disappeared at -200 ms on gap trials, indicated by an arrow. (C) Catch trials. Note that the range of the y-axis in C is different from those in A and B to capture the characteristic response on catch trials. Density functions were calculated by the convolution of a Gaussian function with the standard deviation of 30 ms.

Fixational saccade reduction during the gap period

We examined the impact of the fixation gap on fixational saccades that occurred during the pre-stimulus period (130-ms period ending at stimulus appearance; see Methods for details). The majority of subjects generated fixational saccades more frequently on overlap trials than gap trials (paired t -test – $t_{44} = 3.94$, $P < 0.0005$; Fig. 4A).

Fixational saccade rebound before saccade initiation

We next characterized rebound fixational saccades that occurred immediately after fixational saccade reduction during the gap period. The fixational saccade rebound diminished the difference in fixational saccade frequencies between gap and overlap trials during the post-stimulus period at the population level ($t_{44} = 1.40$, $P > 0.1$; Fig. 4B). However, close inspection of Fig. 4B suggests that the lack of a difference between gap and overlap trials is explained by individual differences in fixational saccade behavior across subjects.

Figure 5 shows the average density functions of fixational saccade onset times for three example subjects marked in Fig. 4B. The subject shown in Fig. 5A (labeled as A in Fig. 4B) generated fixational saccades more frequently on overlap trials than gap trials, consistent with our hypothesis. In contrast, another subject in Fig. 5B (B in Fig. 4B) had significant fixational saccade rebound on gap trials before targeting saccade initiation. The last example subject in Fig. 5C (C in Fig. 4B) did not generate fixational saccades at all after stimulus appearance. Despite the variety of fixational saccade behavior during the post-stimulus period, these subjects generated more fixational saccades on overlap trials than gap trials consistently during the pre-stimulus period (see also Fig. 4A).

Correlation between fixational saccade rebound on gap trials and evoked fixational saccades on catch trials

The diversity of fixational saccade rebound might be explained by different responsiveness to foveal visual events (i.e. fixation point disappearance). Indeed, the temporal dynamics of fixational saccade rebound (Fig. 3B) were very similar to those evoked by fixation blink on catch trials (Fig. 3C). We therefore analysed relationships between rebound fixational saccades on gap trials and evoked fixational saccades on catch trials (Fig. 6).

We first quantified evoked fixational saccades on catch trials as follows (catch index; Fig. 6A). We created the cumulative distribution of evoked fixational saccade latencies from the disappearance of the first fixation point. The longest latency included in this analysis was set to 1000 ms. We then calculated a catch index for each subject as the area under the curve and normalized it to range from zero to one. The catch index would be close to one if fixational saccades were evoked with very short latencies on the majority of trials. In contrast, it would be close to zero if fixational saccades were generated only occasionally with long latencies. When multiple fixational saccades were generated, the earliest latency was selected. The example catch index in Fig. 6A was 0.54.

Figure 6B demonstrates that catch indices were correlated with the frequencies of rebound fixational saccades on gap trials (Pearson's $r = 0.42$, $P < 0.005$, $n = 45$). This suggests that rebound fixational saccades on gap trials were probably evoked by fixation point disappearance. However, other factors were presumably reflected in rebound fixational saccades because there were some subjects who had large catch indices but did not generate rebound fixational saccades at all.

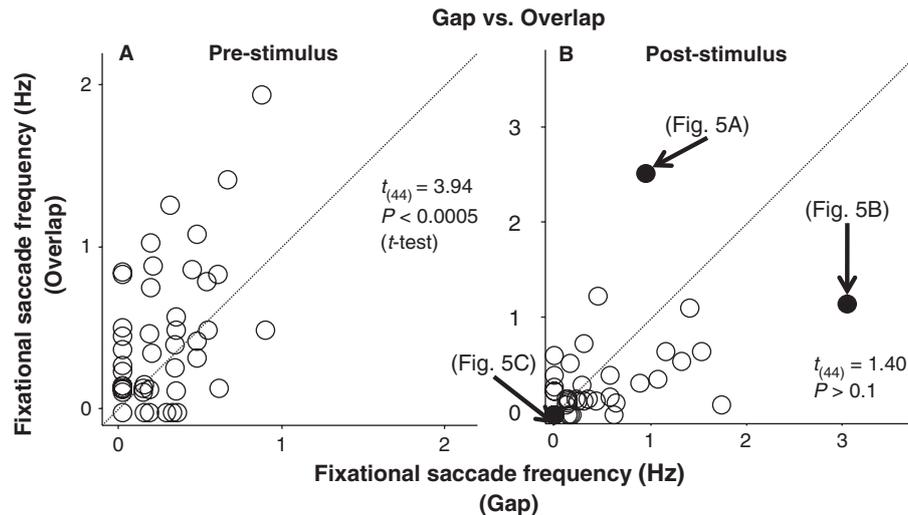


FIG. 4. Comparison of fixational saccade frequency between gap and overlap trials. (A) Pre-stimulus period (between -130 and 0 ms from stimulus appearance). (B) Post-stimulus period [started at stimulus appearance and lasted for 139 ± 18 ms (mean \pm SD); see Methods for the details of the definition]. Three filled markers with arrows and labels in B indicate subjects whose density functions of fixational saccade onset times are shown in Fig. 5. Each data point indicates each subject.

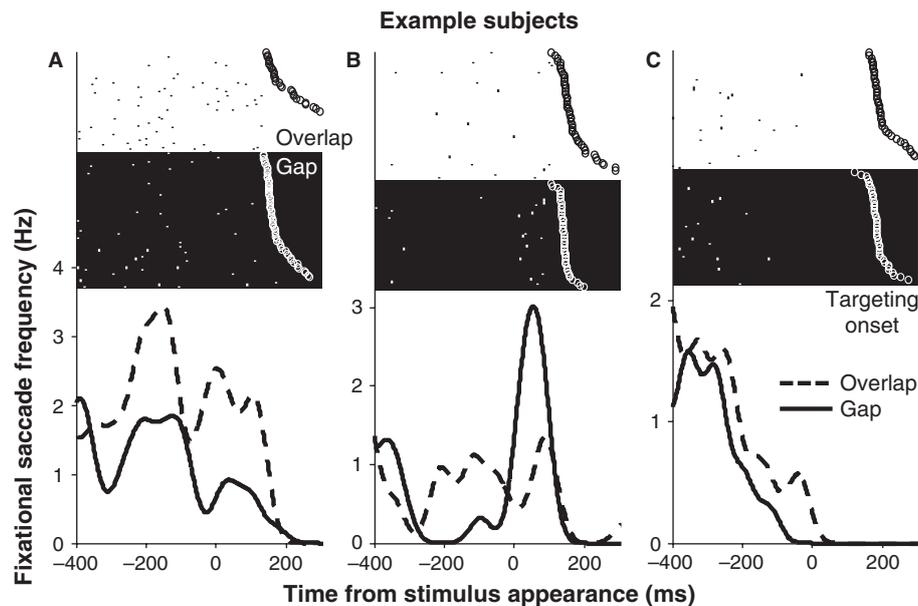


FIG. 5. Raster and density functions of fixational saccade onset times on gap and overlap trials from three example subjects labeled in Fig. 4B. Trials are sorted by targeting saccade reaction times, indicated by circles. Reaction times longer than 300 ms are not marked in this figure. Average reaction times on overlap trials and corresponding gap effects were as follows: (A) 256 ms (overlap trials), 76 ms (gap effect); (B) 181 ms, 40 ms; (C) 214 ms, 21 ms. Density functions were calculated by the convolution of a Gaussian function with the standard deviation of 30 ms.

Impact of fixational saccades on gap effect

It has been shown repeatedly that fixational saccades prolong targeting saccade reaction times (Rolfs *et al.*, 2006; Hafed & Krauzlis, 2010; Sinn & Engbert, 2011; Watanabe *et al.*, 2013). Here, we examined whether fixational saccades also affected the gap effect on targeting saccade reaction times. We used multilevel linear models (linear mixed models) to evaluate the overall effect of fixational saccades on targeting saccade reaction times across subjects while their individual differences are taken into account (West *et al.*, 2006; Gelman & Hill, 2007). More specifically, multilevel models are

extensions of classical regressions in which data are structured in groups and regression coefficients, including intercepts, can vary by group. The following are three hierarchical levels in our data structure in descending order – subject, fixation condition [gap (-1)/overlap ($+1$)] and fixational saccade count. The model is as follows:

$$\begin{aligned} \text{Reaction time} = & \beta_0 + \beta_1 \times [\text{fixational saccade count}] \\ & + \beta_2 \times [\text{fixation condition}] \\ & + \beta_3 \times [\text{fixational saccade count}] \\ & \times [\text{fixation condition}] + u_1 + u_2 + u_3 + \epsilon \end{aligned}$$

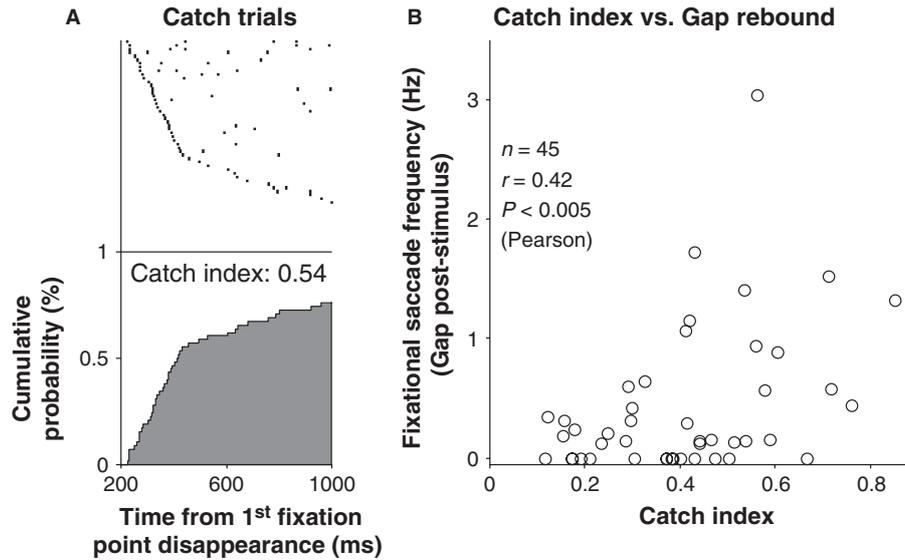


FIG. 6. Correlation between fixational saccade rebound on gap trials and evoked fixational saccades on catch trials. (A) Definition of catch index. We calculated the area under the cumulative distribution of evoked fixational saccade latencies and normalized it to range from zero to one. See the main text for details. (B) Correlation between catch indices and the frequency of rebound fixational saccades during the post-stimulus period. Each data point indicates each subject.

$$\begin{aligned}
 u_1 &\sim N(0, \sigma_{\text{Subject}}^2) \\
 u_2 &\sim N(0, \sigma_{\text{Subject:Fixation condition}}^2) \\
 u_3 &\sim N(0, \sigma_{\text{Subject:Fixation condition:Fixational saccade count}}^2) \\
 \epsilon &\sim N(0, \sigma_{\text{Residual}}^2)
 \end{aligned}$$

where β_0 – β_3 are regression coefficients for fixed effects, and u_1 – u_3 are random effects that follow Gaussian distributions with variance specific to each level (i.e. u_1 – subject, u_2 – fixation condition within subject, u_3 – fixational saccade within fixation condition within subject) and ϵ indicates residuals. The fixed effects (β_0 – β_3) are effects that are common across subjects, while the random effects (u_1 – u_3) account for individual differences at the three hierarchical levels. To estimate the above parameters reliably, we combined the pre- and post-stimulus periods in this analysis to increase the number of trials with fixational saccades.

Table 1 shows the result of the above multilevel model. The regression coefficient for fixational saccade count was +13.0 ms ($P < 0.001$), indicating that fixational saccade occurrence delayed targeting saccade reaction times for 13.0 ms on average. More importantly, fixational saccade occurrence prolonged the gap effect by 7.9 ms on average, as indicated by a significant interaction between fixational saccade count and fixation condition (i.e. additional gap effect by fixational saccade occurrence; $P < 0.01$, Table 1). The prolonged gap effect by fixational saccades was induced by stronger suppression effects on targeting saccades on overlap trials, which was confirmed by modifying the above multilevel model slightly [changing the coding of fixation condition from +1 (overlap)/–1 (gap) to +1 (overlap)/0 (gap)].

Individual differences in fixational saccades and gap effect on targeting saccades

The above analysis revealed that fixational saccades contribute to the gap effect on targeting saccade reaction times at the population

TABLE 1. Multilevel model for targeting saccade reaction times

	Regression coefficient (ms)	SE	d.f.	<i>t</i>	<i>P</i>
Intercept	211.2	5.1	4515	41.5	< 0.001
Fixation condition (Gap: –1, Overlap: +1)	20.3	2.4	44	8.6	< 0.001
Fixational saccade count	13.0	2.8	96	4.6	< 0.001
Interaction (Fixation condition × Fixational saccade count)	7.9	2.8	96	2.8	< 0.01

Standard deviations of random effects and residual are as follows: $\sigma_{\text{Subject}} = 30.2$ ms, $\sigma_{\text{Subject:Fixation condition}} = 20.5$ ms, $\sigma_{\text{Subject:Fixation condition:Fixational saccade count}} = 4.3$ ms, $\sigma_{\text{Residual}} = 55.1$ ms. The results were virtually the same when trials with multiple fixational saccades were excluded (only 0.4% of all trials).

level. However, the gap effect (Fig. 2) as well as the frequency of fixational saccades (Fig. 4) varied substantially across subjects. We therefore examined whether individual differences in fixational saccades account for some of the variability in the gap effect on targeting saccade reaction times.

We quantified the gap effect on targeting saccade reaction times by simple subtraction of average values (i.e. overlap–gap) in individual subjects. We also quantified the difference and average of fixational saccade frequencies between gap and overlap trials in individual subjects. We did not find a correlation between the gap effects on targeting saccade reaction times and the differences or averages of fixational saccade frequencies regardless of pre-, post-, or combined (pre- and post-) stimulus period (Pearson's $|r| < 0.18$, $P > 0.2$, $n = 45$).

The above results suggest that individual differences in the gap effect of targeting saccade reaction times might be explained mainly by factors other than fixational saccades, even though fixational saccades prolonged the gap effect when they occurred (Table 1).

Fixational saccades were associated with impulsive saccade behavior

We have shown previously that fixational saccades explain a large proportion of individual differences in antisaccade performance across subjects (Watanabe *et al.*, 2013). The majority of subjects included in this study ($n = 34$) performed the antisaccade paradigm immediately after they had completed the current behavioral paradigm on the same day. We therefore examined whether fixational saccades that occurred during the current behavioral paradigm were associated with future antisaccade performance.

We calculated the average frequencies of fixational saccades during the current behavioral paradigm using the same temporal period adopted in the previous study (400-ms window ending at 70 ms after stimulus appearance). We also quantified the amplitudes of fixational saccades that occurred during the same temporal period. The last fixational saccade was chosen for the analysis of amplitudes when multiple fixational saccades occurred during the temporal period on the same trial. We limited this analysis to overlap and catch trials because the fixation gap was not used in the previous study. For antisaccade performance, we calculated the rates of direction errors generating inappropriate saccades toward peripheral stimuli.

The following linear equation identified in the previous study was fitted to the above data:

$$[\text{direction error rate}] = d_0 + d_1 \times [\text{fixational saccade frequency}] + d_2 \times [\text{fixational saccade amplitude}] \quad (1)$$

The fitting was successful ($R^2 = 0.28$, $F_{2,31} = 6.12$, $P < 0.01$). The regression coefficients $\pm 95\%$ confidence intervals were as follows – $d_0 = -2.2 \pm 16.5\%$, $d_1 = 14.3 \pm 10.2\%$, $d_2 = 13.5 \pm 21.7\%$. The individual relationships between direction error rates and the frequencies (Fig. 7A) and amplitudes (Fig. 7B) of fixational saccades were summarized as follows (Frequency – Pearson's $r = 0.50$, $P < 0.005$, $n = 34$; Amplitude – $r = 0.31$, $P > 0.05$). Although the relationship between fixational saccade amplitude and direction error rates did not reach statistical significance, the overall fitting result of the regression analysis suggests that fixational saccades during the current behavioral paradigm were associated with future antisaccade performance.

Fixational saccade directions

The direction of fixational saccades also affected targeting saccade reaction times. We quantified the direction of fixational saccades that occurred during the combined pre- and post-stimulus period. We chose the last fixational saccade when multiple fixational saccades occurred on single trials. Using a multilevel model [fixed effects – fixation condition (gap/overlap), cosine of the angle between fixational saccade and stimulus, and interaction between them; random effects – subject, fixation condition within subject, and cosine of the angle between fixational saccade and stimulus within fixation condition within subject], we found that reaction times were longer after fixational saccades directed to the opposite direction of the target (fixed effect coefficient for the cosine = -10.4 ms, standard error = 3.7 ms, $t_{340} = -2.8$, $P < 0.01$). However, such effect was unrelated to fixation condition (fixed effect coefficient for interaction between fixation condition and the cosine of angle = -0.5 ms, standard error = 3.7 ms, $t_{340} = -0.1$, $P > 0.9$).

Discussion

We examined the hypothesis that gap effects on targeting saccade reaction times are at least partially explained by the reduction of fixational saccades. Indeed, we found evidence supporting this hypothesis during the fixation gap (Fig. 4A). However, fixational saccades rebounded immediately in some subjects (Fig. 4B), which diminished the potential benefits of the prior fixational saccade reduction. This does not mean necessarily that fixational saccades are unrelated to the gap effect because, when they occurred, they altered it by prolonging reaction times more strongly on overlap trials than gap trials (Table 1). These results suggest that fixational saccades affect the gap effect on targeting saccades, but not in the way hypothesized originally.

Fixational saccade reduction and the rostral SC

The frequency of fixational saccades decreased during the gap period (Fig. 4A). This reduction started as early as the visual delay of the saccade control system [70 ms (Fischer & Weber, 1993)] (Fig. 3B). It is therefore likely that the reduction of fixational saccades was induced mainly by mechanisms recruited by the external sensory event of fixation point disappearance.

The reduction of fixational saccades by fixation point disappearance is also consistent with the following neurophysiological studies in behaving monkeys. Neurons in the rostral part of the SC, the activity of which is critical for fixational saccades (Hafed *et al.*, 2009), have tonic firing during active fixation (Munoz & Wurtz, 1992, 1993). Furthermore, their firing rates decrease during the fixation gap (Dorris & Munoz, 1995; Dorris *et al.*, 1997; Everling *et al.*, 1999). This immediate reduction of firing rates is presumably induced by the termination of incoming visual input from the fixation point (for another possibility, see Hafed & Ignashchenkova, 2013).

Fixational saccade rebound and the caudal SC

Immediately after the gap period, fixational saccades rebounded before targeting saccade initiation in a subset of subjects (Figs 3B, 4B and 5). This rebound diminished the difference in fixational saccade frequencies between gap and overlap trials at the population level (Fig. 4B). This is inconsistent with the hypothesis that the gap effects on targeting saccade reaction times are explained by the lower frequency of fixational saccades.

We speculate that fixational saccade rebound was a part of responses to fixation point disappearance on gap trials. This is supported partly by the fact that fixational saccade rebound on gap trials was correlated with evoked fixational saccades by fixation blink on catch trials (Fig. 6). However, other mechanisms, such as temporal expectation of stimulus appearance based on the fixed gap period, could also influence fixational saccade rebound. Accordingly, fixational saccade rebound might reflect the resultant interactions between multiple mechanisms, which might account for the diversity of fixational saccades during the post-stimulus period across subjects (Figs 4B and 5).

We suggested the rostral SC as a potential source of fixational saccade reduction during the gap period. However, the rostral SC is unlikely to be responsible for fixational saccade rebound because their activity does not increase after fixation point disappearance (Dorris & Munoz, 1995; Dorris *et al.*, 1997). Instead, we speculate that the caudal SC, where neurons encode targeting saccades, could account for fixational saccade rebound because recent studies have

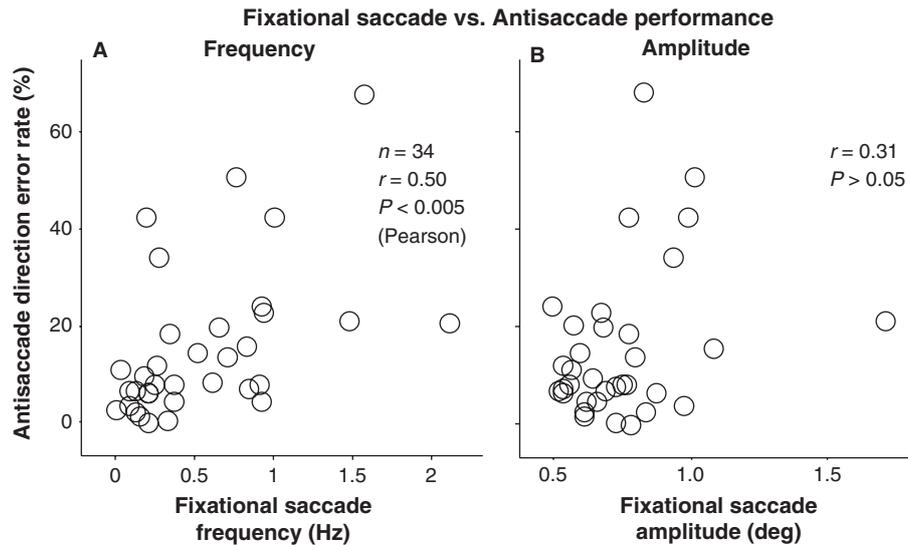


FIG. 7. Relationships between fixational saccades during the current behavioral paradigm and direction error rates during the antisaccade paradigm across subjects. (A) Correlation between direction error rates and the frequencies of fixational saccades. (B) Correlation between direction error rates and the amplitudes of fixational saccades. Fixational saccades were analysed during a temporal window used in the previous study (400-ms duration ending at 70 ms after stimulus appearance; Watanabe *et al.*, 2013). This analysis was limited to overlap and catch trials because the fixation gap was not used in the previous study. Thirty-four subjects performed the antisaccade paradigm immediately after the current behavioral paradigm on the same day.

shown its relationship to fixational saccades (Hafed *et al.*, 2013). However, because of the diversity of fixational saccade behavior across subjects during the post-stimulus period, it might be difficult to infer the mechanisms based on results from the limited number of animals.

Altered gap effect by fixational saccades

Fixational saccades delayed targeting saccade initiation (Table 1), consistent with previous reports (Rolfs *et al.*, 2006; Hafed & Krauzlis, 2010; Sinn & Engbert, 2011; Watanabe *et al.*, 2013). More importantly, we found that fixational saccades prolonged the gap effect on targeting saccade reaction times (regression coefficients for interaction in Table 1). The prolonged gap effect was explained by the stronger suppression effects of fixational saccades on targeting saccade initiation on overlap trials than gap trials.

The asymmetric effect of fixational saccades on targeting saccades on gap and overlap trials might be understood by taking into account the following two theoretical saccade commands – automatic and volitional (Fischer & Weber, 1993; Munoz & Everling, 2004). It has been hypothesized that automatic saccade commands evoked directly by visual input reach the SC earlier than volitional saccade commands programmed based on visual input and task requirements, and such difference in latencies may account for the bimodal distribution of targeting saccade reaction times observed during saccade paradigms with gap and overlap conditions (Fischer & Weber, 1993; Dorris & Munoz, 1995; Dorris *et al.*, 1997). The gap effect on targeting saccade reaction times is presumably explained by different contributions of automatic and volitional saccade commands on gap and overlap trials; the contribution of automatic saccade commands is stronger on gap trials, while the contribution of volitional saccade commands is stronger on overlap trials.

We extend the above hypothesis to account for the asymmetric effect of fixational saccades on gap and overlap trials. We hypothesize that fixational saccades disrupt volitional saccade commands more strongly than automatic saccades. This mechanism would

prolong targeting saccade reaction times on overlap trials more strongly than gap trials because of the dominance of volitional saccade commands on overlap trials.

The above hypothesis is consistent with our result of a correlation between fixational saccades and antisaccade performance (Fig. 7). It has been thought that automatic saccade commands are directed to a peripheral stimulus, while volitional saccade commands are directed to the opposite direction of the stimulus in the antisaccade paradigm (Munoz & Everling, 2004). Because automatic and volitional saccade commands compete with each other, antisaccade error rates reflect presumably the strength of automatic saccade commands relative to volitional saccade commands. Accordingly, if fixational saccades disrupt volitional saccade commands more strongly than automatic saccade commands, antisaccade error rates should be more pronounced in people with higher fixational saccade frequencies. Further studies are needed to test this hypothesis because it is highly speculative.

Independence between gap effects on targeting and fixational saccades

There were significant individual differences in fixational saccade behavior across subjects (Engbert & Mergenthaler, 2006; Watanabe *et al.*, 2013), especially in fixational saccade rebound after the gap period (Fig. 5). The gap effects on targeting saccade reaction times were also variable across subjects (Fig. 2). However, the individual differences in the gap effects on targeting and fixational saccades were independent of each other. A possible explanation for such independence is the limited number of trials with fixational saccade occurrence during the pre- and post-stimulus periods; the gap effects on targeting saccade reaction times might be determined mainly by the majority of trials without fixational saccade occurrence.

Despite the limited number of trials with fixational saccades, their impact on the gap effect should not be dismissed because they prolonged the gap effect when they occurred (Table 1). Although we did not find a relationship between the average frequency of fixational saccades and the gap effects on targeting saccade reaction times

across subjects in our population, different results could emerge in a population with abnormal fixational saccade behavior, such as Parkinson's disease (Gould *et al.*, 2001; Shaikh *et al.*, 2011) and attention deficit hyperactivity disorder (Gould *et al.*, 2001; Munoz *et al.*, 2003). Accordingly, it will be important to take into account fixational saccades to understand the mechanisms underlying saccadic behavior in health and disease in future research.

Acknowledgements

We thank Dr Y. T. Kitamura for technical support, and Dr Daniel Myall for comments on statistical analyses. This work was supported by a researcher exchange program between the Japan Society for the Promotion of Science and the Royal Society of New Zealand, 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, and Strategic Research Program for Brain Sciences, Ministry of Education, Culture, Sports, Science and Technology, Japan.

Abbreviation

SC, superior colliculus.

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