Context-Dependent Stimulation Effects on Saccade Initiation in the Presupplementary Motor Area of the Monkey

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Isoda, Masaki. Context-dependent stimulation effects on saccade initiation in the presupplementary motor area of the monkey. J Neurophysiol 93: 3016–3022, 2005. First published February 9, 2005; doi:10.1152/jn.01176.2004. Although evidence suggests that the contribution of the presupplementary motor area (pre-SMA) to voluntary motor control is effector-nonselective, the question of how electrical stimulation of the pre-SMA affects eye movements remains unanswered. To address this issue, stimulus effects of the pre-SMA of monkeys on saccade initiation were investigated during performance of a visually guided saccade task with an instructed delay period. This report describes two major findings. First, when stimuli with currents of ≤80 μA were applied before the presentation of a go signal, the reaction time (RT) of an upcoming saccade shortened with comparable effects on ipsi- and contraversive saccades. Second, stimuli that were delivered after the go signal lengthened the RT; this resulted in greater effects on ipsiversive saccades. In addition, the stimulation yielded a mild impairment of saccade accuracy, particularly when the stimulation was delivered after the go signal. By themselves, however, these stimuli did not directly elicit eye movements. Therefore the stimulus effects appeared only in the context of the behavioral task and were dependent on the phase of the task. These findings provide additional support for the hypothesis that the involvement of the pre-SMA in motor control can be linked to either eye or arm motor system dependent on behavioral context.

INTRODUCTION

The presupplementary motor area (pre-SMA) is a nonprimary motor cortex that resides in the mesial surface of the frontal lobe (Picard and Strick 1996; Tanji 1996). This cortical motor area has been traditionally studied in relation to forelimb motor control, especially by focusing on its higher-order aspects (e.g., Hikosaka et al. 1996; Hoshi and Tanji 2004; Matsuzaka and Tanji 1996; Nachev et al. 2005; Nakamura et al. 1998; Rizzolatti et al. 1990; Shima and Tanji 2000; Shima et al. 1996). On the other hand, accumulating evidence demonstrates that the pre-SMA is also active in tasks that require eye movements as a motor response (Curtis and D’Esposito 2003; Fujii et al. 2002; Isoda and Tanji 2004; Kawashima et al. 1998; Merriam et al. 2001; Yamamoto et al. 2004; Yazawa et al. 2000); this leads to a hypothesis that the pre-SMA contributes to behavioral control regardless of body parts to be employed (effector nonselectivity) (Fujii et al. 2002; Isoda and Tanji 2004; Picard and Strick 2001; Yamamoto et al. 2004; Yazawa et al. 2000). Although this notion seems attractive, there is an apparent discrepancy in effects of electrical stimulation of the pre-SMA; it only elicited forelimb movements but not eye movements (Inase et al. 1999; Isoda and Tanji 2004; Luppino et al. 1991; Matsuzaka et al. 1992; Nakamura et al. 1998; Wang et al. 2001).

The difference in the stimulation effect across the movement effectors could be because the stimulation of the pre-SMA in animal studies has seldom, if ever, applied during the preparation for, or the execution of, eye movements of the animal. Interestingly, Luppino et al. (1991) and Nakamura et al. (1998) reported that at several sites in the pre-SMA, stimulation effects on the forelimb were obtained only during the animal’s movements. In this respect, Matsuzaka et al. (1992) and Inase et al. (1999) also noted that motor effects were variable depending on the animal’s motor behavior; forelimb movements were more elicitable during animal’s reaching movements. Here, to test the hypothesis that the pre-SMA stimulation could affect the ongoing oculomotor process as well, the pre-SMA was electrically microstimulated while the monkey was performing a trained oculomotor task. The data show that electrical microstimulation in the pre-SMA can induce contrasting effects on the initiation of task-related saccades, i.e., shortening and lengthening of saccadic reaction times (RTs), which are critically dependent on task phases during which the stimulation is delivered.

METHODS

Subjects and apparatus

Two male Japanese monkeys (Macaca fuscata, 5–6 kg) participated in this study as subjects and were the same monkeys as used in the previous studies (Isoda and Tanji 2002–2004). During experimental sessions, each monkey was seated in a primate chair while his head was firmly fixed, its arms were gently restrained, and eye movements were monitored using an infrared camera system with sampling at 250 Hz (R-21C-A, RMS, Hirosaki, Japan). All surgical and experimental protocols were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the guidelines for Institutional Animal Care and Use published by the author’s institute.

Behavioral procedures

Monkeys were trained on a delayed visually guided saccade task (Fig. 1). Each trial started with the presentation of a spot of light as a go signal, i.e., a fixation point (FP, 0.2° diam) at the center of a display monitor on which the monkey had to fixate for the duration of 800 ms. Then
monkey was executing natural arm movements as reported previously (Luppi et al. 1991; Matsuzaka et al. 1992). The area that was determined to be the presMA did not overlap the supplementary eye field (SEF), which was also mapped physiologically (Isoda and Tanji 2002, 2003). The stimulation sites were confirmed by magnetic resonance imaging (OPART 3D-System, Toshiba, Tokyo, Japan).

**Data analysis**

Behavioral data were collected and analyzed from all sessions. To compare saccadic RTs between the test and control conditions, RTs were submitted to the Mann-Whitney U test ($\alpha < 0.05$), separately for each ICMS onset delay. To compare the magnitude of RT changes between ipsi- and contraversive saccades at a population level, RTs for each saccade direction were first normalized by subtracting the mean RT under the control condition from the mean RT under the test condition (RT difference), again separately for each ICMS onset delay. RT difference was then submitted to the Wilcoxon signed-rank test ($\alpha < 0.05$).

**RESULTS**

The preceding stimulation procedure produced shortening and lengthening of RTs of task-related saccades depending on the task phases. An example of such stimulus effects is shown in Fig. 2A. When the onset of ICMS was timed around or later than the appearance of the go signal, it delayed the initiation of saccades; the monkey was unable to initiate a saccade during and shortly after the stimulation, especially for saccades to an ipsilateral target. Interestingly, RTs were linearly changed depending on the time of stimulus onset, regardless of the direction of saccades (Fig. 2A). By contrast, when ICMS was applied mainly during the delay period before the go signal was given, RTs were no longer prolonged; instead, contraversive saccades were shortened at this particular site (Fig. 2A, bottom). Stimulation applied during central fixation without targets, or during inter-trial intervals, did not evoke eye movements (not shown). For the majority of sites tested within the pre-SMA, post-go ICMS increased RTs of upcoming saccades, whereas pre-go ICMS decreased RTs (Fig. 2B). In addition to the effects on RTs, stimulation had a mild but significant influence on saccade accuracy especially under the post-go ICMS condition (Table 1), which was caused by stimulation rendering saccades slightly hypometric.

The variability of normal saccadic RTs allowed estimation of the latency of pre-SMA stimulation to affect saccade initiation, by computing the time when, after the onset of stimulation, the occurrence of saccades under the test condition was reduced compared with the saccade occurrence under the control condition. For this estimation, all trials sampled in the sessions in which ICMS significantly increased RTs were used because the accuracy of such estimation depends on large numbers of trials. To compare the saccade occurrence between the test and control conditions, cumulative frequency distributions of RTs for each condition were then constructed and aligned with the onset of ICMS (indicated by “0 ms” in the abscissa in Fig. 3), separately for each ICMS onset delay. Because ICMS was not actually delivered on control trials, those trials were treated as if sham ICMS, or ICMS with a current of 0-μA, was delivered at the same timing as test trials. If ICMS has no effect on the saccade initiation, then, theoretically, the two distributions completely overlap. In fact, the test distribution diverged to the right of the control distribution at

**Stimulation procedures**

Intracortical microstimulation (ICMS) (Asanuma and Sakata 1967) was applied with glass-insulated Eligiloy microelectrodes (Suzuki and Azuma 1976) with an impedance of 0.8–1.5 MΩ at 333 Hz to sites within the pre-SMA while the monkey was performing the saccade task. The parameters of ICMS typically consisted of 50 cathodal pulses of 0.2-ms duration at 333 Hz in the range of 10–80 μA (stimulation trains lasted 150 ms). For each trial, the onset of ICMS was set in order that it was timed either during a delay period between the onset of a saccade target and the offset of the FP (pre-GO ICMS) or during saccadic reaction times (post-GO ICMS; Fig. 1). Specifically, with *monkey 1*, ICMS was delivered at 120, 160, and 200 ms before (pre-GO ICMS) and after (post-GO ICMS) the go signal. In addition, at several sites, ICMS was delivered with a 40-ms step ranging between 200 ms before and 200 ms after the go signal (e.g., Fig. 2A). With *monkey 2*, the onset of ICMS was timed at 140 and 180 ms before and after the go signal. Note that these times represent the time at which the electrical stimulation began. Trials with (test condition) and without (control condition) stimulation were randomly interleaved. For most electrode penetration, two to four depths were tested, each separated by 1.0 mm. In total, 24 cortical sites were studied in 8 electrode penetrations for *monkey 1*, and 29 sites were studied in 9 electrode penetrations for *monkey 2*; all of these sites were estimated to be within the gray matter, based on the background neuronal activity recorded with the same electrode prior to stimulation.

**Cortical localization**

The pre-SMA was defined in accordance with physiological criteria established previously (Matsuzaka et al. 1992); it was identified just rostrally to the face representation of the SMA, characterized by ample visual responses and a paucity of somatosensory responses. In addition, forelimb movements were occasionally elicited by ICMS with relatively higher currents and longer trains, especially when the
FIG. 2. Effects of presupplementary motor area (pre-SMA) electrical stimulation on saccade initiation. A: an example of stimulus effects on saccadic reaction time (RTs, monkey 1). RTs are plotted against intracortical microstimulation (ICMS) onset delays, which represent the time at which the stimulation began relative to the GO signal (=0 ms), separately for ipsiversive (top) and contraversive (bottom) saccades. Negative values of the ICMS onset delay indicate that the stimulation began before the GO signal (pre-GO ICMS); positive values indicate that the stimulation began after the GO signal (post-GO ICMS). For comparison, RTs on control trials are shown (gray circles). At this site, ICMS was applied at intervals of 40 ms between 200 ms before and 200 ms after the GO signal. The current strength was 80 μA. *P < 0.05, **P < 0.01 (Mann-Whitney U test); black asterisks, significantly longer RTs; gray asterisks, significantly shorter RTs. Saccades with RT of less than the ICMS onset delay plus the latency of stimulation to delay saccade initiation (see text and Fig. 3) are marked by arrows.

B: summary of the stimulation effects. The ordinate indicates the number of sites in which ICMS induced significant effects on RTs on the ICMS onset delays tested routinely (see METHODS; Mann-Whitney U test, P < 0.05, corrected for multiple comparisons). N, the total number of sites tested. The distribution of the stimulus effects was significantly different between pre-GO ICMS and post-GO ICMS for both monkeys (P < 0.001, χ² test).

C: laterality of the stimulation effects (population data). The mean RT difference is plotted as a function of the ICMS onset delay for ipsiversive (black) and contraversive (gray) saccades. Error bars represent a SE. *P < 0.05, **P < 0.01 (Wilcoxon signed-rank test).

TABLE 1. Saccade accuracy

<table>
<thead>
<tr>
<th>Monkey 1</th>
<th>Test</th>
<th>Control</th>
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<tr>
<td>ICMS onset (ms)</td>
<td>-200</td>
<td>-160</td>
</tr>
<tr>
<td>Ipsiversive</td>
<td>1.30 ± 0.53</td>
<td>1.29 ± 0.52</td>
</tr>
<tr>
<td>P</td>
<td>0.603</td>
<td>0.882</td>
</tr>
<tr>
<td>Contraversive</td>
<td>1.12 ± 0.45</td>
<td>1.14 ± 0.47</td>
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<tr>
<td>P</td>
<td>0.216</td>
<td>0.624</td>
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</table>

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<tr>
<th>Monkey 2</th>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICMS onset (ms)</td>
<td>-180</td>
<td>-140</td>
</tr>
<tr>
<td>Ipsiversive</td>
<td>1.33 ± 0.55</td>
<td>1.33 ± 0.60</td>
</tr>
<tr>
<td>P</td>
<td>0.160</td>
<td>0.147</td>
</tr>
<tr>
<td>Contraversive</td>
<td>0.78 ± 0.58</td>
<td>0.80 ± 0.53</td>
</tr>
<tr>
<td>P</td>
<td>0.027</td>
<td>0.106</td>
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Values are means ± SD in degree. Saccade accuracy was defined as a distance between the saccade target and the actual endpoint of each saccade. P values are provided for comparison between control and each test condition (Mann-Whitney U test).
a certain point after the onset of ICMS (Fig. 3). The point of divergence was defined at the third bin of five consecutive bins (bin width = 4 ms) in which the difference between the corresponding bin counts in the two cumulative histograms exceeded the mean of the difference during a control period (<0 ms) by >2.56 SD. By computing these divergent points (arrows in Fig. 3), the latency of stimulation to induce the delay of saccade initiation was estimated at 42.0 ms for monkey 1 (42.7 ms for ipsiversive saccades, 41.3 ms for contraversive saccades). For monkey 2 (data not shown), it was 40.0 ms (36.0 ms for ipsiversive saccades, 44.0 ms for contraversive saccades).

To explore the laterality of the stimulation effects at a population level, the RT difference (see METHODS) was compared between ipsi- and contraversive saccades. For the computation of the RT difference, trials on which the RT was less than the ICMS onset delay plus the latency estimated above (e.g., trials indicated by arrows in Fig. 2A) were excluded on the assumption that the saccades in such trials would not have been affected by stimulation. As a population, the increment of RTs by post-GO ICMS was significantly greater for ipsiversive saccades than contraversive ones, whereas the decrement of RTs by pre-GO ICMS was insignificantly different between the two saccade directions (Fig. 2C). When looking at individual sites, however, at approximately half of the sites tested, the shortening of RTs by pre-GO ICMS exhibited spatial preference: ipsiversive saccades were predominantly shortened in 4/15 (monkey 1) and 7/29 sites (monkey 2), and contraversive predominance was found in 3/15 (monkey 1) and 9/29 sites (monkey 2).

To examine whether the two opposing effects are localized to a particular cortical portion within the pre-SMA, the distribution of the RT difference was analyzed. Although the amplitude of RT lengthening by post-GO ICMS in monkey 2 changed depending on the rostrocaudal coordinate ($P < 0.05$ for +140 and +180 ms delays, Kruskal-Wallis test), this was not the case for monkey 1. With both monkeys, no regional preference was observed regarding the strength of RT shortening. The sites inducing the significant lengthening and shortening of RTs substantially overlapped with one another.

DISCUSSION

This study has described the effects of electrical stimulation in the pre-SMA on saccade initiation during performance of a delayed visually guided saccade task. The results indicate that stimulation applied primarily before a go signal for a saccade (pre-GO ICMS) shortened saccade latency, whereas stimulation with the same parameters but delivered after the go signal (post-GO ICMS) lengthened saccade latency. Although both of the effects basically involved bilateral saccades, lengthening of RTs was more drastic for ipsiversive saccades. In addition, by post-GO ICMS, RTs seemed to linearly increase depending on the time of stimulus onset, regardless of saccade direction (Fig. 2, A and C). In the behavioral task such as used in this study, it is generally accepted that two types of eye control mechanisms should be involved: gaze-holding mechanism (such as fixation) and gaze-shifting mechanism (such as saccade). The concept and the model by Reddi and Carpenter (2000) assume that the gaze-holding mechanism is maintained until it is switched to the gaze-shifting mechanism. According to the concept, it can be interpreted that the electrical stimulation of the pre-SMA after the go signal in this study induced suppression of the gaze-shifting mechanism to delay saccade generation, facilitation of the gaze-holding mechanism to maintain fixation, and/or inhibition of a switch from the gaze-holding mechanism to the gaze-shifting mechanism. Because the RT increased linearly, it seems straightforward to interpret that stimulation facilitated the gaze holding mechanism and/or inhibited switching, thereby delaying the saccade onset until the end of stimulation. On the other hand, the possibility that stimulation of the pre-SMA suppressed the gaze-shifting mechanism cannot be discarded entirely; if the magnitude of suppression becomes greater as the gaze shifting mechanism advances, the increment of RTs (“RT difference” in this study) would change depending on the time of stimulus onset. Recording from neural structures that are centrally involved in the gaze-holding and -shifting mechanisms while stimulating the pre-SMA could help better understand how electrical stimulation of the pre-SMA exerts its influence on the two control mechanisms.

The present finding that the stimulation of the pre-SMA after a go signal (post-GO ICMS) lengthened saccade latency is best comparable to that reported by other investigators who stimulated the frontal eye field (FEF) while monkeys were performing visually and memory-guided saccade tasks (Burman and Bruce 1997; Izawa et al. 2004a,b). In these studies, stimulation was applied with the offset of a fixation spot (go signal), which
led to the prolongation of saccadic latency. Burman and Bruce (1997) considered a significant delay in saccade initiation as evidence of “saccade suppression,” and they reported that contraversive saccades were usually more strongly suppressed than ipsiversive ones, especially during a memory-guided saccade task. The stimulation also affected saccade accuracy, rendering saccades less accurate. These suppression effects were typically obtained in the FEF near the arcuate spur, often in and around the fundus. On the other hand, Izawa et al. (2004a,b) recently reported different properties of stimulus effects in the FEF: suppression was induced either exclusively for ipsiversive saccades or nonselectively for bilateral saccades, in both cases visually guided saccades and memory-guided saccades were equally suppressed, and stimulation did not alter the amplitude and direction of saccades. Suppression sites for ipsilateral saccades were widely distributed throughout the FEF, whereas suppression sites for bilateral saccades were relatively localized in the prearcuate gyrus facing the inferior arcuate sulcus. This difference in the stimulus effects between the two studies might be accounted for by the difference in the stimulation site in the FEF and/or by methodological differences such as the stimulus parameter, as extensively discussed by Izawa et al. (2004a). Despite some unsolved discrepancies, these studies provide further support for the notion that the FEF plays a role not only in the generation of purposeful saccades but also in the suppression of various kinds of saccades, thereby maintaining visual fixation (Dias and Segraves 1999; Guitton et al. 1985; Sommer and Tehovnik 1997).

Although the effect of post-go stimulation in the pre-SMA showed some resemblance to that in the FEF, i.e., the prolongation of saccadic RTs (or saccade suppression) and a mild impairment of saccade accuracy (but see Izawa et al. 2004a,b), the directional preference of suppressing saccades is different between the two areas. In the pre-SMA, saccade suppression was usually observed for both directions of saccades with ipsiversive predominance, whereas suppression in the FEF was either predominant in contraversive saccades (Burman and Bruce 1997), exclusive for ipsiversive saccades (Izawa et al. 2004a), or spatially nonselective (Izawa et al. 2004b). In addition, there are apparent differences in stimulus effects that are important to consider differential roles of the FEF and pre-SMA in motor control. First, in the FEF, stimulation with currents of <50 μA can directly evoke saccades (Bruce et al. 1985; Robinson and Fuchs 1969), whereas stimulus effects in the pre-SMA are observable solely when the oculomotor process is in progress. This difference suggests that the FEF can work as a “producer” of purposeful saccades (Bruce and Goldberg 1985), presumably in concert directly with the superior colliculus (SC) (Hanes and Wurtz 2001; Haerta et al. 1986; Schlag-Rey et al. 1992; Stanton et al. 1988), whereas the pre-SMA in itself is incapable of generating saccades yet can work as a “modulator” on ongoing oculomotor processes. Second, in the FEF, stimulus effects are confined to the oculomotor system, whereas stimulation in the pre-SMA can affect the limb-motor system as well (Luppino et al. 1991; Matsuzaka et al. 1992). Therefore the FEF is specialized in controlling eye movements, whereas the involvement of the pre-SMA in motor control is not limited to a particular motor system, i.e., eye or arm, but is effector-nonselective. This notion is in accord with anatomical findings that the pre-SMA is not directly connected with either primary limb-motor regions, such as the primary motor cortex and the spinal cord (Dum and Strick 1991, 1996; He et al. 1995; Lu et al. 1994; Luppino et al. 1993; Matsuzaka et al. 1992; Tokuno and Tanji 1993), or primary oculomotor regions, such as the FEF and the SC (Bates and Goldman-Rakic 1993; Fries 1985; Hartmann-von Monakov et al. 1979; Huerta et al. 1987; Parthasarathy et al. 1992).

The preceding interpretations, in turn, raise an interesting question about possible neural pathways whereby the pre-SMA can exert its influence on the oculomotor system. Considering cortical structures, the FEF is less likely to be involved in that pathway because of the lack of anatomical connections with the pre-SMA (Bates and Goldman-Rakic 1993; Huerta et al. 1987; Parthasarathy et al. 1992). Although the SEF is located in close vicinity of the pre-SMA and the stimulation of the SEF induced the prolongation of saccade latency (Heinen and Anbar 1998), the projection from the pre-SMA to the SEF is uncertain (Luppino et al. 1993). If the pre-SMA can access the SEF, then the SEF becomes more likely a candidate of the responsible pathway involved. At the subcortical level, there are three major candidates that are capable of delaying saccade initiation: omnipause neurons in the nucleus raphe interpositus in the pons (Keller 1974), fixation neurons in the rostral pole of the SC (Munoz and Wurtz 1993a), and neurons in the substantia nigra pars reticulata (SNr) of the basal ganglia (Hikosaka and Wurtz 1983, 1985). If the stimulation of the pre-SMA leads to the activation of these groups of neurons, saccade initiation should be delayed. However, the delay of saccades by the activation of the pontine omnipause region is directionally nonselective (Keller 1974), and activation of the rostral SC preferentially delays contraversive saccades (Munoz and Wurtz 1993b). Thus these two neuronal populations alone are unable to explain the ipsiversive predominance of RT prolongation. On the other hand, recent physiological data have demonstrated that the SNr contains two types of inhibitory neurons that are distinct anatomically and functionally (Hikosaka et al. 2000; Jiang et al. 2003). One group of SNr neurons projects to the ipsilateral SC and disinhibits target SC neurons (Hikosaka and Wurtz 1983, 1985), whereas the other group of SNr neurons projects to the contralateral SC and further inhibits its target SC neurons (Jiang et al. 2003). The finding that pre-SMA stimulation preferentially delays the onset of ipsiversive saccades suggests that the pre-SMA might have indirect access to the contralateral SC through the crossed nigrocollicular pathway. Importantly, the pre-SMA projects to the striatum and subthalamic nucleus (Inase et al. 1999; Parthasarathy et al. 1992). The validity of the basal ganglia hypothesis needs to be tested both anatomically and physiologically.

This study has revealed that electrical microstimulation in the pre-SMA can exert measurable influences on saccade initiation only if it is applied during the performance of a trained oculomotor task. Because eye movements were not elicited when the monkey was not engaged in a behavioral task, stimulation of the pre-SMA appears to affect ongoing oculomotor processes rather than to evoke eye movements de novo. This explanation may be also applicable to the forelimb stimulation effects because forelimb movements are more frequently and sometimes exclusively elicited during animal’s postural changes or movements (Inase et al. 1999; Luppino et al. 1991; Matsuzaka et al. 1992; Nakamura et al. 1998).
present findings, therefore, further substantiate the view that the pre-SMA is involved in voluntary motor control in an effectornonselective manner (Fujii et al. 2002; Isoda and Tanji 2004; Picard and Strick 2001; Yamamoto et al. 2004; Yazawa et al. 2000).

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