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Executive control of countermanding saccades by the supplementary eye field

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The supplementary eye field registers the occurrence of conflict, errors and reward in macaque monkeys performing a saccade-countermanding task. Using intracortical microstimulation, we determined whether the supplementary eye field only monitors or can actually influence performance. Weak microstimulation of many sites in the supplementary eye field improved monkeys' performance on a 'stop signal' task by delaying saccade initiation. This effect depended on the context of the task because simple visually guided saccades were not delayed by the same stimulation. These results demonstrate that the supplementary eye field can exert contextual executive control over saccade generation.

Flexible adjustments of behavior require the continuous monitoring and evaluation of the outcome of past choices so that future choices can be adapted on the basis of these feedback signals. Recent work has clearly demonstrated the existence of evaluation signals in the medial frontal cortex of humans[1,2] and has described error- and reward-related signals in the supplementary eye field (SEF)[3] and the anterior cingulate cortex (ACC) of macaque monkeys[4]. It is less clear whether these evaluation signals influence behavior[5–8]. In the case of the oculomotor system, the SEF is uniquely positioned to mediate between the executive and the motor systems. The SEF has strong reciprocal connections with the ACC (refs. 9,10), but unlike the ACC, the SEF can influence saccade generation through strong projections to the frontal eye field[9,11], the oculomotor circuit of the basal ganglia[12], the superior colliculus and the brainstem[13]. These connections are not strong enough for the SEF to initiate saccades directly[14]; however, they might allow the SEF to influence behavior by biasing the activity distribution within the oculomotor system.

To test this hypothesis, we gently modulated activity in the SEF by means of intracortical microstimulation while monkeys performed a countermanding task (**Fig. 1**). This task probes a subject's ability to control the initiation of movements by infrequently presenting a 'stop' signal after a random delay (stop signal delay) in a response-time task[15]. Thus it is well suited to measure changes in the extent of executive control that subjects exert. If microstimulation increases the degree of executive control, subjects should make fewer incorrect movements in the presence of a stop signal. This can be accomplished by increasing the saccade reaction time (RT) on stimulated trials, independent of the occurrence of a stop signal. Further evidence for executive control would be a dependence of this RT increase on the context of the countermanding task. We found that microstimulation influenced the number of errors committed and the RT for generating saccades.

RESULTS

We delivered intracortical microstimulation with currents up to 100 μA in order to locate sites from which saccades could be evoked; then we determined the threshold for evoking saccades. We tested sites within and surrounding the SEF using currents below the threshold to elicit saccades. We delivered subthreshold microstimulation for 200 ms synchronously with the stop signal or, in no stop signal trials, at the time when the stop signal would have occurred. Results were obtained from 106 sites in the medial frontal cortex of 2 monkeys. Of these, 61 sites were in the SEF and provided sufficient statistical power to be analyzed.

SEF microstimulation affects error rate

We analyzed the effects of stimulation on performance using a generalized linear model with four factors—stop signal delay (SSD), saccade direction, delivery of microstimulation and the interaction between saccade direction and microstimulation[16]. Subthreshold microstimulation had no significant ($P > 0.05$) effect on countermanding performance at 19 of 61 sites. At most sites (42 of 61, or 69%), stimulation influenced performance significantly ($P < 0.05$) and these effects were of three kinds. At most sites in the SEF, subthreshold microstimulation resulted in fewer erroneous (that is, non-canceled) saccades, both contraversive and ipsiversive to the stimulated hemisphere (36 of 61, or 59%; monkey N, 31 of 44; monkey F, 9 of 17; **Fig. 2a**). Microstimulation with superthreshold currents always evoked saccades into the contralateral hemifield. The improved performance was revealed as a shift of the inhibition function (error rate as a function of SSD) toward later stop signal delays. For example, on contraversive trials, with a stop signal presented after 268 ms and no stimulation, the monkey failed to cancel the saccade on 52% of trials; with stimulation, he failed on only 19% of trials. A similar effect was present for all other

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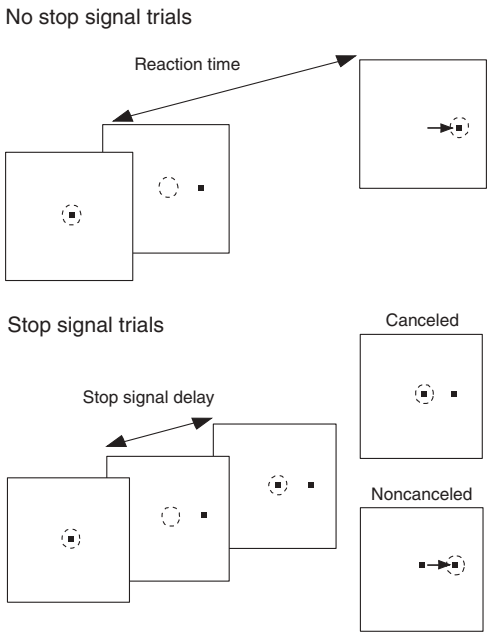

Figure 1 Countermanding task. The dotted circle indicates the focus of gaze at each interval; the arrow indicates the saccade. After monkeys fixated a central spot for a variable interval, a peripheral target appeared at one of two locations in opposite hemifields—one of which was at the endpoint of the evoked saccade—simultaneous with the disappearance of the fixation spot. In trials with no stop signal, monkeys were rewarded for shifting gaze to the target. On a fraction of trials, a short time after the target appeared (the stop signal delay), the fixation point reappeared, instructing the monkeys to withhold the movement. On these stop signal trials, monkeys were rewarded for maintaining fixation on the central spot for a fixed interval (canceled trials). If the monkeys failed to cancel the movement and generated a saccade to the target on these trials, no reward was given (noncanceled trials).

stop signal delays. The general linear model allowed us to estimate the average effect of stimulation on the inhibition function by comparing the stop signal delay at which the monkey failed in 50% of trials for trials without and with stimulation. At the illustrated site (**Fig. 2a**), the influence of stimulation was equivalent to a stop signal presented 102 ms earlier (contraversive, 113 ms; ipsiversive, 92 ms).

Stimulation at other sites in the SEF had lateralized effects (4 of 61, or 7%; monkey N, 2 of 44; monkey F, 2 of 17; **Fig. 2b**). Microstimulation caused more noncanceled contraversive saccades, and this impaired performance resulted in a shift of the inhibition function toward earlier stop signal delays. On the other hand, stimulation also caused fewer noncanceled ipsiversive saccades; this improved performance resulted in a shift of the inhibition function toward later stop signal delays. At the illustrated site (**Fig. 2b**), the effect of microstimulation on contraversive saccades was equivalent to a stop signal presented 21 ms later and that for ipsiversive saccades was equivalent to a stop signal presented 39 ms earlier. At a very few sites, stimulation had the opposite effect (2 of 61, or 3%; monkey N, 1 of 44; monkey F, 1 of 17). At the illustrated site (**Fig. 2c**), the effect of stimulation was equivalent to a stop signal presented 51 ms earlier for contraversive saccades and 6 ms later for ipsiversive saccades.

We took the average of the inhibition function across all sites that gave rise to one of the three classes of effects: all sites at which subthreshold microstimulation improved the monkeys' ability to cancel both the contraversive and the ipsiversive saccades (**Fig. 3a**), those at which it improved the canceling of ipsiversive saccades but impaired that of contraversive saccades (**Fig. 3b**), those at which it did the reverse (**Fig. 3c**), and those at which microstimulation had no significant effect on performance in individual sessions (**Fig. 3d**). Altogether, stimulation of the SEF facilitated the countermanding of contraversive saccades in 88% of sites in which a significant effect was measured, and in 61% of all sites. The mean magnitude of the shift of the inhibition function produced by SEF stimulation across all sites for contraversive and ipsiversive saccades was 63 ms and 61 ms, respectively (**Fig. 4**). It is clear that even the experiments at sites that did not result in a significant shift tended to produce a positive shift. Although the mean shift on these trials was not significantly different from 0.0 (contraversive $t = 1.24$, d.f. $= 16$, $P = 0.22$; ipsiversive $t = 1.23$, d.f. $= 16$, $P = 0.24$), the pooled inhibition function revealed a

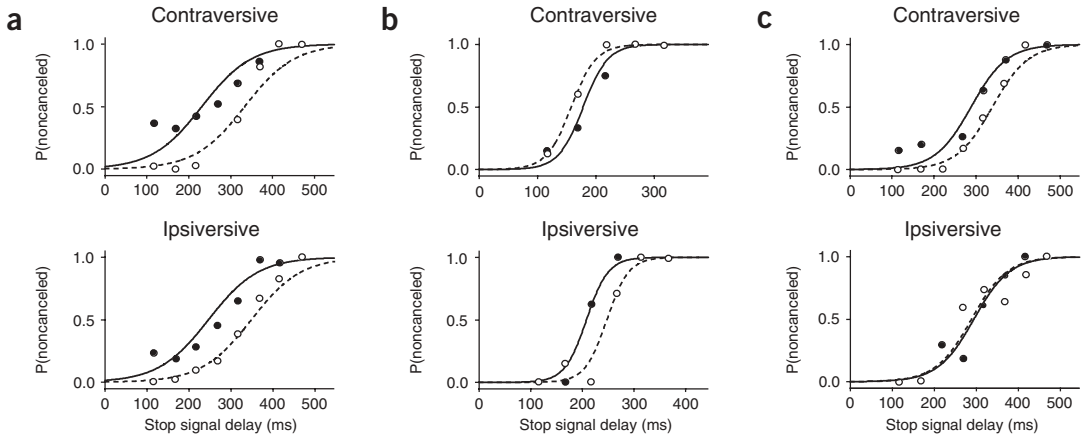

Figure 2 Representative results from three sites in the SEF. The inhibition function plots the proportion of erroneous (that is, noncanceled) saccades as a function of stop signal delay. Closed circles, performance on control trials without stimulation; open circles, performance with stimulation for contraversive and ipsiversive saccades. Best-fitting logistic regression is plotted for control trials (solid) and stimulation data (dashed). (**a**) Representative site at which subthreshold microstimulation improved the canceling of both contraversive and ipsiversive saccades (combined shift = 102 ms; $D = 87.16$; $P < 0.0001$; $R^2 = 0.82$). (**b**) Site at which microstimulation improved the canceling of ipsiversive saccades but impaired that of contraversive saccades (contraversive shift = –21 ms; ipsiversive shift = 39 ms; $D = 8.36$; $P = 0.03$; $R^2 = 0.83$). (**c**) Site at which microstimulation improved the canceling of contraversive saccades and impaired that of ipsiversive saccades (contraversive shift = 51 ms; ipsiversive shift = –6 ms; $D = 50.29$; $P = 0.013$; $R^2 = 0.84$).

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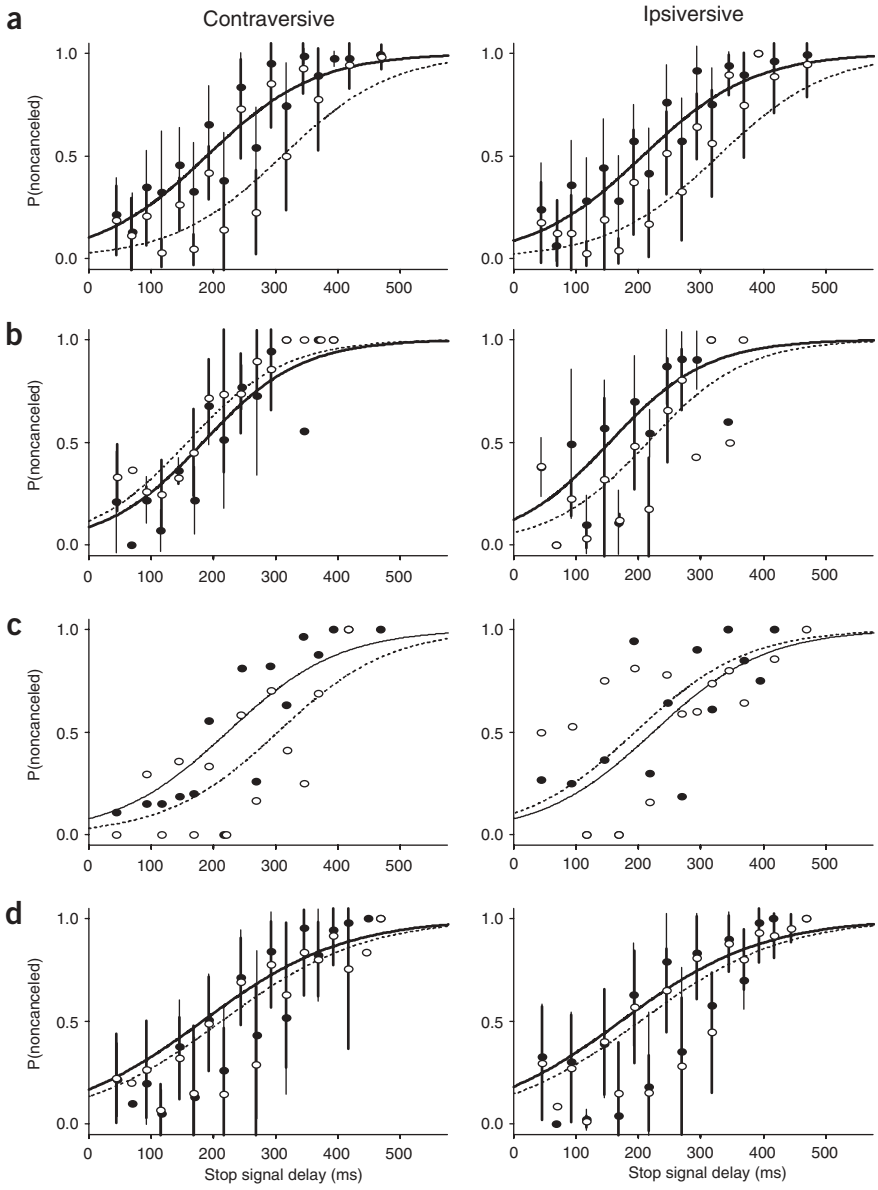

Figure 3 Inhibition functions averaged across all sites with a particular pattern of effects. Conventions are the same as in **Figure 2**. Error bars represent s.d. (**a**) All sites at which subthreshold microstimulation improved the canceling of both contraversive and ipsiversive saccades (combined shift = 120 ms; $D = 5439.3$; $P < 0.0001$; $R^2 = 0.58$). (**b**) All sites at which microstimulation improved the canceling of ipsiversive saccades but impaired that of contraversive saccades (contraversive shift = –26 ms; ipsiversive shift = 61 ms; $D = 322.7$; $P < 0.0001$; $R^2 = 0.56$). (**c**) All sites at which microstimulation improved the canceling of contraversive saccades but impaired that of ipsiversive saccades (contraversive shift = 81 ms; ipsiversive shift = –29 ms; $D = 315.5$; $P < 0.0001$; $R^2 = 0.53$). No error bars are shown because each SSD was sampled only once. (**d**) All sites at which microstimulation had no significant effect on performance in individual sessions. The average inhibition function from these experiments indicated that stimulation weakly but significantly improved the canceling of both contraversive and ipsiversive saccades (combined shift = 31 ms; $D = 3468.3$; $P < 0.0001$; $R^2 = 0.39$).

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significant ($P < 0.0001$) bilateral improvement of 31 ms (**Fig. 3d**). Thus, the experiments without a significant improvement may have simply lacked statistical power.

SEF microstimulation affects saccade reaction time

Performance in the countermanding task can be adjusted through changes in response time (E.E. Emeric, V. Stuphorn & J.D. Schall, *Soc. Neurosci. Abstr.* 211.11, 2004; J.D. Schall & T.L. Taylor, *Soc. Neurosci. Abstr.* 24.172, 1998). To measure the effect of SEF stimulation on saccade latency, in some sessions we delivered microstimulation in a fraction of the no stop signal trials, at the same times as when the stop signal would have been presented (42 of 61 sites, or 69%; monkey N, 25 of 44; monkey F, 17 of 17). We subtracted the mean saccade latency in trials without stimulation from the latencies measured in trials with stimulation, and pooled the results across sessions (separately for the different saccade directions). Subthreshold microstimulation of the SEF during the no stop signal trials most commonly had an effect on saccade latencies that followed the pattern of effects on the inhibition function (**Table 1**). At sites where stimulation decreased the frequency of contraversive and ipsiversive noncanceled saccades (**Figs. 2a** and **3a**), it significantly ($P < 0.001$) increased saccade latencies in both directions. Where stimulation increased the frequency of contraversive noncanceled saccades and decreased the frequency of ipsiversive saccades (**Figs. 2b** and **3b**), it significantly ($P < 0.001$) shortened contraversive saccade latencies and significantly ($P < 0.001$) lengthened ipsiversive saccade latencies. In contrast, where stimulation decreased the frequency of contraversive noncanceled saccades and increased the frequency of ipsiversive saccades (**Figs. 2c** and **3c**), it lengthened contraversive saccade latencies and significantly ($P < 0.001$) shortened ipsiversive saccade latencies. Notably, even at sites where stimulation had no effect on the frequency of noncanceled saccades (**Fig. 3d**), it significantly ($P < 0.001$) lengthened ipsiversive saccade latencies on no stop signal trials.

In contrast, subthreshold microstimulation had a much weaker effect on the latency of noncanceled saccades. The strongest effect was a significant shortening of contraversive saccade latency during the stimulation of sites at which the result was an increased frequency of contraversive noncanceled saccades and a decreased frequency of ipsiversive saccades (**Figs. 2b** and **3b**).

Effects of microstimulation on saccade latency were more common than those on the probability of saccade cancellation. There were significant effects ($P < 0.05$) on saccades in at least one direction in 37 of 42 sites (88%; monkey N, 23 of 25; monkey F, 14 of 17). As outlined above, stimulation affected the frequency of noncanceled saccades in only 42 of 61 experiments (69%). This is consistent with previous observations that natural sequential effects in the stop signal task are more clearly seen in terms of response times than in terms of the inhibition function (E.E. Emeric, V. Stuphorn & J.D. Schall. *Soc.*

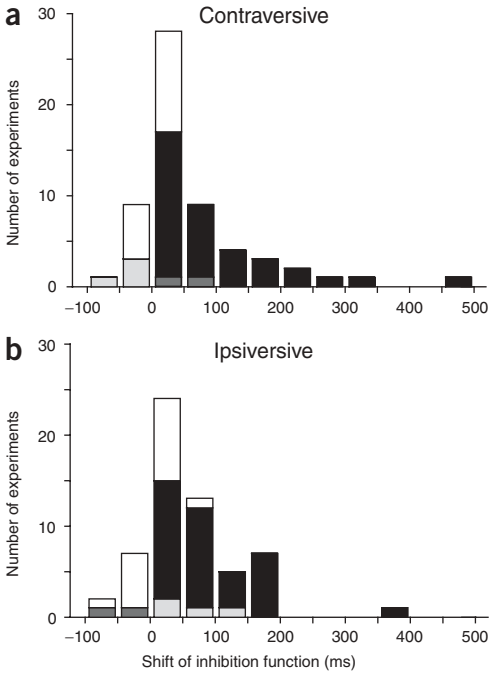

Figure 4 Magnitude of the shift in the inhibition function produced by SEF stimulation. (**a**) For contraversive saccades. (**b**) For ipsiversive saccades. Black bars, significantly improved countermanding of contraversive and ipsiversive saccades (**Figs. 2a** and **3a**). Light gray bars, significantly improved countermanding of ipsiversive saccades but impaired countermanding of contraversive saccades (**Figs. 2b** and **3b**). Dark gray bars, significantly improved countermanding of contraversive saccades but impaired countermanding of ipsiversive saccades (**Figs. 2c** and **3c**). Open bars, remainder of the cases (that is, those in which no significant change was measured (**Fig. 3d**)). In all cases the significance level is $P < 0.05$.

Neurosci. Abstr. 211.11, 2004, and ref. 17). Several reasons may account for this. First, at each delay, there are more trials without than with a stop signal, providing greater statistical power. Second, cancellation probability depends on the interaction of gaze shifting and gaze holding processes; both of these are stochastic and so their interaction will be less sensitive to small changes in either.

Effects of microstimulation are task dependent

To test if the influence of subthreshold stimulation on saccade latency was contingent on performance on the stop signal task, when possible (27 of 61 sites, or 44%; monkey N, 23 of 44; monkey F, 4 of 17) we also collected data while the monkeys performed a simple visually guided saccade task without the stop signal. This test was done in the same experimental session without moving the electrode. As observed previously, overall response time adapted to the presence of the stop signal; pooled across all sessions, saccade latency in visually guided saccade trials without stimulation was much shorter than that in the no stop signal trials (mean difference 94 ms, $t = 51.16$, d.f. $= 38{,}867$, $P < 0.0001$; monkey N, mean difference 139 ms, $t = 64.44$, d.f. $= 19{,}092$, $P < 0.0001$; monkey F, mean difference 88 ms, $t = 34.14$, d.f. $= 19{,}773$, $P < 0.0001$). Direct comparison of the saccade latency difference in the two tasks measured in the same session showed identical results independent of the effect of the stimulation on the frequency of canceled saccades. First, microstimulation more often had a significant ($P < 0.05$) effect during the countermanding protocol (88% of sessions for contraversive saccades, 77% for ipsiversive saccades) than during simple visually guided saccades (23% of sessions for contraversive, 8% ipsiversive). Second, whereas during the countermanding task microstimulation almost always increased saccade latency (96% contraversive, 88% ipsiversive), it almost always decreased it during simple visually guided saccades (77% contraversive, 62% ipsiversive).

An example of this effect can be seen in the RT distributions of a typical experiment (**Fig. 5**). During the countermanding task, microstimulation increased the latencies of contraversive (33 ms; permutation test $P = 0.008$) and ipsiversive (32 ms; $P < 0.001$) saccades. However, during the visually guided saccade task, in which the stop signal never occurred, microstimulation with the same parameters at the same site decreased latencies of both contraversive (–21 ms; $P < 0.001$) and ipsiversive (–12 ms; $P = 0.09$) saccades. Thus, whereas subthreshold microstimulation of the SEF during the stop signal task produced slower saccade RTs, stimulation during visually guided saccades produced faster RTs.

Relationship of microstimulation effect and neuron types

The SEF includes neurons with apparent movement-related activity, those with error-related signals, others with conflict-related activity and still others with reinforcement-related activity[3]. To determine whether effective sites coincided with particular functional types of neurons, the distribution of functional neuronal types recorded at the sites with the different stimulation effects immediately before stimulation was compared with the distribution of all neurons recorded in the two monkeys. Sites at which stimulation had no effect had marginally more movement-related activity than the overall population ($\chi^2 = 12.45$, d.f. $= 5$, $P = 0.05$; **Table 2**). However, sites with significant stimulation effects were not distinguished by any particular incidence of neuron types. Furthermore, we recorded the most neurons at sites with either a bilateral performance improvement effect (**Figs. 2a** and **3a**) or no significant effect (**Fig. 3d**). No significant difference in the frequency of neuron types was observed between sites with these two effects ($\chi^2 = 5.69$, d.f. $= 5$, $P = 0.46$) and there was no indication of anatomical clustering of sites with particular stimulation effects.

Table 1 Effect of SEF stimulation on saccade latency

	Saccade latency change due to microstimulation (ms)			
Trial type	C+/I+	C–/I+	C+/I–	Co/Io
Contraversive, no stop signal trials	21***	–19***	7	3
Ipsiversive, no stop signal trials	31***	13***	–50***	10***
Contraversive, noncanceled trials	–1	–46 **	–11	–2
Ipsiversive, noncanceled trials	0	–19	6	0

Mean saccade latency during stimulated trials minus mean saccade latency in nonstimulated trials is shown for the different types of trials (rows) and sites of microstimulation (columns). C+/I+, sites at which stimulation increased the probability of canceling both contraversive and ipsiversive saccades; C–/I+, sites at which stimulation decreased the probability of canceling contraversive saccades and increased that of ipsiversive saccades; C+/I–,sites at which stimulation increased the probability of canceling contraversive saccades and decreased that of ipsiversive saccades; Co/Io, sites at which stimulation had no significant effect on the probability of canceling either contraversive or ipsiversive saccades. $^{**}P < 0.01$; $^{***}P < 0.001$.

DISCUSSION

Consistent with numerous other studies of medial frontal cortex in other conditions[18–21], microstimulation in the SEF during the countermanding saccade task had mixed effects. This is most likely due to the fact that

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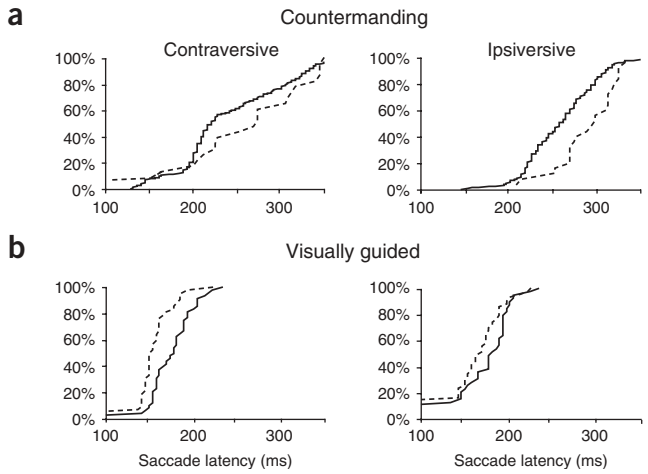

Figure 5 Context-dependent effect of SEF microstimulation on saccade latency. (**a**,**b**) Cumulative distributions of saccade latencies without (dashed) and with (solid) microstimulation at the same site in the SEF, during the countermanding task (**a**) and during a visually guided saccade task with no stop signal (**b**), for contraversive and ipsiversive saccades.

the SEF, like many other parts of the frontal cortex, is a mosaic of functionally different groups of neurons.

In a few locations, the stimulation effect depended on the direction of the affected saccades: in most of these, the result of stimulation was that contraversive saccades were facilitated whereas ipsiversive saccades were inhibited. This observation matches previous findings in the frontal eye field (FEF)[22]. A likely interpretation is that subthreshold microstimulation results in an increased tendency to generate a contraversive saccade toward the endpoint of the saccade that is evoked by a superthreshold current at the same site. By itself, the subthreshold current was too weak to evoke saccades, but during countermanding experiments, saccades toward a visual target at this location received additional activation and were initiated faster. On the other hand, during the generation of saccades toward visual targets in the ipsiversive location, the subthreshold stimulation activated neurons whose movement fields were opposite to those of the neurons activated by the visual target. In the FEF, movement neurons that generate similar saccades enhance each other, whereas neurons that generate saccades in mutually exclusive directions inhibit each other[23]. Thus, the electrically evoked activation in the motor map competes with the visually evoked one, and it takes longer for the ipsiversive saccade–generating neurons to reach their threshold[24]. This pattern of behavioral effects can therefore be explained by a straightforward manipulation of an oculomotor function without an executive control component.

In most cases, microstimulation of the SEF resulted in better control over the generation of saccades during the countermanding task. It is possible that microstimulation served as an enhanced stop signal through another modality; redundant stop signals do reduce stop signal RTs (SSRT)[25]. However, this does not explain how the stimulation could act as a supplementary stop signal, why the effect would vary across sites within the SEF or how opposite effects on saccade latency could occur when visual stop signals occur. Furthermore, in most of the stimulation experiments, microstimulation occurred infrequently in trials with no visual stop signal, weakening the association between the visual stop signal and microstimulation. It is also possible that the stimulation increased the monkeys' attention in the task. In fact, this is just what many authors suggest that executive control does (for example, ref. 26.). Of course, referring to attention does not explain how the effect on performance is accomplished. Our data indicate that microstimulation of the SEF exerted a context-dependent influence on saccade generation. If no stop signal occurred, and thus no executive control was necessary, stimulation of the SEF reduced saccade latency. However, if stop signals occurred in some trials calling for executive control, stimulation of the SEF delayed saccade generation. This is adaptive because saccades generated later have a greater chance of being canceled if a stop signal is presented than saccades generated earlier. Our results are consistent with the original and more current descriptions of the effects of stimulating the supplementary motor area (SMA) in humans: namely, the interruption of actions[18,27,28]. The inhibitory influence of SEF stimulation also fits with preliminary reports that the activity of FEF movement neurons is reduced during SEF stimulation (S. Sadeghpour, J. Schlag, M. Schlag-Rey, A. Mohempour & A. Dorfman. *Soc. Neurosci. Abstr.* 24.522, 1998) and that saccade latencies are prolonged (S.J. Heinen & A.N. Anbar. *Soc. Neurosci. Abstr.* 24.1147, 1998). Notably, though, this inhibitory effect of SEF microstimulation is context dependent, which is consistent with a previous report that the effect of microstimulation in the SEF depends on the behavioral state of the animal[19].

The observation that low microstimulation currents inhibit saccade generation during countermanding seems to be in contrast to the fact that higher currents at the same site evoke saccades, as reported previously[29–31]. However, electrical stimulation can evoke saccades from parts of the brain that only indirectly influence primary ocular motor structures. For example, saccades can be evoked by electrical stimulation of primary visual cortex (V1)[32–36] under certain conditions with very small currents[37]. This effect is mediated through the projection from V1 to the superior colliculus[35,36]. Thus, it is plausible that microstimulation of the SEF with higher currents also evokes saccades indirectly through the stronger activation of the FEF and superior colliculus. Microstimulation with lower currents influences fewer neurons[38] and may therefore more accurately reveal the function of local circuits.

Table 2 Comparison of distributions of neuron types at sites with different effects

Neuron population	V	VM	M	R	E	U	Sum	χ^2	*P*
All neurons	20% (85)	31% (130)	11% (44)	7% (29)	4% (15)	27% (115)	418		
Neurons from C+/I+ sites	15% (13)	34% (29)	15% (13)	4% (3)	5% (4)	27% (23)	85	4.07	0.67
Neurons from C–/I+ sites	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	100% (1)	1	2.62	0.86
Neurons from C+/I– sites	30% (3)	40% (4)	10% (1)	0% (0)	0% (0)	20% (2)	10	1.95	0.92
Neurons from Co/Io	10% (3)	50% (15)	24% (7)	3% (1)	3% (1)	10% (3)	30	12.45	0.05

A χ^2 test was performed to compare the distributions of neuron types at sites with different effects with the overall distribution of neuron types sampled in the SEF of the two monkeys. In some sessions, we recorded from neurons without microstimulation, so the number of neurons from microstimulation sites is different from the total number of recorded neurons. Neuron categories: visual (V), visuomovement (VM), movement-related (M), reinforcement (R), error (E) and unmodulated (U). Number in parenthesis indicates the number of neurons recorded from.

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In summary, our results demonstrate that microstimulation of the SEF with low currents can either enhance or inhibit saccade production according to the need for executive control. This observation provides additional evidence supporting the hypothesis that the SEF is part of the executive system providing top-down control signals to achieve adaptive behavior.

METHODS

Data were collected from two male macaque monkeys (*Macaca radiata*) using procedures described previously[3,39]. The SEF is defined as the area in the dorsomedial convexity in which saccades can be reliably evoked with electrical currents below 50 μA. To characterize the threshold and movement field of each site, microstimulation was delivered when monkeys performed visually guided saccades to one of four target locations.

Behavioral task. The primary data were collected when monkeys performed a saccade stop signal task (**Fig. 1**). Monkeys fixated a central stimulus, and then a peripheral target appeared 1,000–4,000 ms later at one of two locations on opposite sides of the hemifield (at the same eccentricity); one of these was at the endpoint of the saccade evoked by microstimulation of a given site. Simultaneously, the fixation light was removed. Monkeys had to shift their gaze to the target within 800 ms in order to earn a fluid reinforcement, which was delivered after the monkeys fixated the target for 300–800 ms. On one-third to one-half of trials, the fixation light reappeared after target presentation as an imperative stop signal after a variable stop signal delay (SSD, 25–475 ms). On these stop signal trials, monkeys earned a reward by canceling the saccade and maintaining fixation (canceled trial). Reward was given after 600–1,000 ms of maintained fixation of the central spot. Production of the saccade before or in spite of the stop signal was considered an error and resulted in no reward (noncanceled trials). The intertrial interval varied between 200 and 2,500 ms.

Performance in the countermanding task is probabilistic because of the variability in RTs across trials. The probability of not canceling the movement increases as the delay between the signal to initiate the movement and the signal to inhibit the movement (stop signal delay) increases. Movements that are generated with a short latency tend to be initiated before the stop signal can influence the system. On the other hand, movements generated with long latencies tend to be inhibited because there is enough time for the stop signal to influence the system. The time needed to cancel the movement, known as stop signal RT, can be estimated from a simple race model that determines the response time on no signal trials that corresponds to the probability of canceling a movement at each stop signal delay[15,40]. The mean stop signal RT calculated from the behavioral data collected while recording from the SEF neurons was 100 ms (N, 104 ms; F, 95 ms). The estimate of stop signal RT allows direct comparison of activation between canceled stop signal trials and the subset of no stop signal trials with latencies long enough that the movement would have been canceled if the stop signal had been presented.

On one-half of the stop signal trials, microstimulation was delivered synchronously with presentation of the stop signal. The current was adjusted to be no more than one-half of that needed to evoke a saccade. To measure its effect on saccade latency, stimulation was delivered on a randomly chosen fraction of trials with no stop signal, at the same range of times.

Microstimulation procedure. In a typical experiment, we advanced an electrode (1–4 MΩ) into the SEF. We recorded neuronal activity at different depths. Well-isolated neurons were recorded from, regardless of task relevance. Next, we switched from a recording circuit to a stimulation circuit and applied microstimulation through the same electrode, in order to test whether it was possible to evoke saccades at this site. If it was, we determined the threshold and the metric of the evoked saccade. For the countermanding protocol, one target was placed at the endpoint of the saccade (originating from the fixation point) that was evoked by superthreshold microstimulation (contraversive), the other 180° opposite (ipsiversive). If no saccades could be evoked with currents below 100 μA, the targets were placed at the endpoint of the evoked saccade that was last recorded at the penetration site. During the stimulation in the countermanding protocol, we used a current strength of one-half the threshold current or 10 μA, whichever was smaller. We stimulated with trains of biphasic pulses (0.2 ms pulse width), with a frequency of 333 Hz, starting at the onset of the stop signal and lasting for 200 ms. We stimulated on one-half of all stop signal trials. To test the effect of stimulation on saccadic latency within the countermanding protocol, in some experiments we stimulated during no stop signal trials using the same stimulation parameters. In the countermanding task, we excluded all saccades with latencies in the express saccade range from the analysis. To test the effect of stimulation on saccadic latency outside the countermanding protocol, we recorded two sets of saccades to four different target positions with and without stimulation before some experiments. One of the target locations was at the endpoint of the saccade evoked by the superthreshold stimulation; the others were opposite and perpendicular to it. The stimulation parameters were identical to the ones used during countermanding.

All training, surgery and experimental procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Vanderbilt University Animal Care Committee.

Statistical analysis. The performance of the monkey in the countermanding protocol is described by the inhibition function, which plots the probability of the monkey generating a saccade to the target (noncanceled trials) as a function of stop signal delay. The inhibition function has a sigmoid form. Following short stop signal delays, monkeys have a greater likelihood of successfully withholding saccades to the target. As the stop signal delay increased, the monkeys increasingly failed to withhold the saccade. We used generalized linear model analysis to measure the influence of the microstimulation on performance in the countermanding task[16]. Four factors could potentially influence the inhibition function: stop signal delay (SSD), saccade (and target) direction (DIR), presence of microstimulation (STIM), and interaction between saccade direction and presence of microstimulation (STIM × DIR). We estimated maximum-likelihood fits of four nested general logistic regression models and determined the significance of each factor through log-likelihood ratio statistics[16]. We only analyzed best fits with R^2 values > 0.69. We fitted the following logistic regression function independently for each target: $\log[P/(1-P)] = b0 + b1 \times SSD + b2 \times DIR + b3 \times STIM$. We determined the SSD values at which the function reached the 0.5 level if the STIM factor is set to either 1 or 0. We quantified the amplitude of any shift as the difference of the two values.

We used a permutation test[41] with 30,000 permutations to determine whether microstimulation had a significant effect on saccadic latency, because this test has a high power and is free of mathematical assumptions. All analyses were done in MATLAB (Mathworks).

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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