

RESEARCH ARTICLE | *Control of Movement*

Impairment but not abolishment of express saccades after unilateral or bilateral inactivation of the frontal eye fields

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Submitted 22 March 2019; accepted in final form 2 April 2020

Dash S, Peel TR, Lomber SG, Corneil BD. Impairment but not abolishment of express saccades after unilateral or bilateral inactivation of the frontal eye fields. *J Neurophysiol* 123: 1907–1919, 2020. First published April 8, 2020; doi:10.1152/jn.00191.2019.—Express saccades are a manifestation of a visual grasp reflex triggered when visual information arrives in the intermediate layers of the superior colliculus (SCi), which in turn orchestrates the lower level brainstem saccade generator to evoke a saccade with a very short latency (~100 ms or less). A prominent theory regarding express saccades generation is that they are facilitated by preparatory signals, presumably from cortical areas, which prime the SCi before the arrival of visual information. Here, we test this theory by reversibly inactivating a key cortical input to the SCi, the frontal eye fields (FEF), while monkeys perform an oculomotor task that promotes express saccades. Across three tasks with a different combination of potential target locations and unilateral or bilateral FEF inactivation, we found a spared ability for monkeys to generate express saccades, despite decreases in express saccade frequency during FEF inactivation. This result is consistent with the FEF having a facilitatory but not critical role in express saccade generation, likely because other cortical areas compensate for the loss of preparatory input to the SCi. However, we also found decreases in the accuracy and peak velocity of express saccades generated during FEF inactivation, which argues for an influence of the FEF on the saccadic burst generator even during express saccades. Overall, our results shed further light on the role of the FEF in the shortest-latency visually-guided eye movements.

NEW & NOTEWORTHY Express saccades are the shortest-latency saccade. The frontal eye fields (FEF) are thought to promote express saccades by presetting the superior colliculus. Here, by reversibly inactivating the FEF either unilaterally or bilaterally via cortical cooling, we support this by showing that the FEF plays a facilitative but not critical role in express saccade generation. We also found that FEF inactivation lowered express saccade peak velocity, emphasizing a contribution of the FEF to express saccade kinematics.

express saccades; frontal eye fields; motor preparation; oculomotor system

INTRODUCTION

Express saccades are the shortest-latency visually guided saccades, with saccadic reaction times (SRTs) approaching the sensorimotor conduction delays between the retina and extra-

ocular muscles (Fischer and Boch 1983; Fischer and Ramspurger 1984). Express saccades are a low-level visual grasp reflex that, somewhat paradoxically, is potentiated by high-level preparation about the location, valence, or timing of an upcoming visual target (Paré and Munoz 1996; Schiller et al. 2004). The interaction between a low-level reflex and high-level preparation is seen in the activity profiles within the intermediate superior colliculus (SCi), a structure whose integrity is essential for express saccade generation (Schiller et al. 1987). Before regular (or nonexpress) latency saccades, a subset of visuomotor neurons within the SCi emit a “visual” burst of action potentials shortly after visual target presentation, as well as a second “motor” burst of action potentials shortly before the onset of a target-directed saccade (Dorris et al. 1997; Edelman and Keller 1996; Sparks et al. 2000). Prior to express saccades, visuomotor neurons emit only a single burst of action potentials; effectively, the visual and motor events become a singular event linked to both target and saccade onset (Dorris et al. 1997; Edelman and Keller 1996; Sparks et al. 2000). Within the SCi, a neural correlate of high-level preparation that potentiates express saccades is the level of low-frequency activity on SCi visuomotor neurons attained just before the arrival of the visual burst of activity. Although the exact details determining saccade triggering within the SCi and downstream brainstem burst generation remain to be determined, greater levels of low-frequency activity within the SCi are thought to bring the system closer to saccade threshold, increasing the probability of express saccade generation when the visually related burst of activity arrives in the SCi (Dash et al. 2018; Dorris et al. 1997; Krauzlis 2003; Rezvani and Corneil 2008).

There are many cortical and subcortical areas, including the lateral intraparietal area (Chen et al. 2013), dorsolateral prefrontal cortex (Johnston et al. 2014), primary visual cortex (Kim and Lee 2017), basal ganglia (Wurtz and Hikosaka 1986), and cerebellum (Ashmore and Sommer 2013; Ohmae et al. 2017) that exhibit preparatory-related activity and project to the SC, and hence could provide the high-level signals needed to potentiate express saccade generation. The frontal eye fields (FEF) in particular are an important source of top-down signals conveyed to the SCi (Sommer and Wurtz 2000, 2001; Wurtz et al. 2001), and many FEF neurons also display increased levels

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of low-frequency preparatory activity that correlates with increased express saccade probability (Dias and Bruce 1994; Everling and Munoz 2000). Somewhat surprisingly in light of these findings, the influence of temporary or permanent lesions of the FEF on express saccade occurrence is quite varied. On one hand, monkeys with permanent unilateral lesions of the FEF are still capable of generating contralesional express saccades after a short period of recovery (Schiller et al. 1987), and both ipsilesionally and contralesionally directed express saccades are also spared, and sometimes potentiated, by unilateral damage to the FEF in human patients (Guitton et al. 1985; Rivaud et al. 1994). In contrast, we have recently shown in monkeys that low-frequency preparatory activity and visual- and saccade-related activity in the ipsilesional SCi decrease with reversible cryogenic inactivation of a large-volume of the unilateral FEF (Dash et al. 2018; Peel et al. 2017). The study by Dash and colleagues (2018) also showed that the profile of SCi activity before express saccades generation is largely unchanged during FEF inactivation providing one compares saccades with the same SRTs, suggesting that low-frequency preparatory activity in the SCi reaches sufficient levels presumably due to inputs from other non-FEF sources (Dash et al. 2018). Thus, at least when long-term compensation is not involved, the FEF appears to play a facilitatory but not critical role for express saccade generation.

The overall goal of the current study is to further the understanding of the FEF's role in express saccade generation, here by examining express saccade proportion, accuracy, and peak velocity in monkey before, during, and after either unilateral or bilateral cryogenic inactivation of the FEF. To our knowledge, there has been no study of the effect of transient bilateral FEF inactivation on express saccade generation. Our overall goal is motivated by recent findings that challenge the prevailing view that the FEF only contributes to generation of contralateral saccades. For example, some FEF neurons represent ipsilateral portions of space (Crapse and Sommer 2009), and ipsilateral RTs may also increase after large-volume cryogenic inactivation or ablation of the unilateral FEF (Kunimatsu et al. 2015; Peel et al. 2014). Furthermore, as SCi activity was not recorded in the current study, we were not limited to the two-target configuration used in the study by Dash and colleagues (2018) and here introduced multiple potential target locations on one or both sides of midline during FEF inactivation. Doing so allowed us to examine how unilateral or bilateral FEF inactivation interacts with different potential target locations to influence express saccade generation. Finally, we also examined express saccade accuracy and peak velocity during FEF inactivation to gain further understanding into the FEF's potential influence on the movement parameters of express saccade. We found that although express saccade frequency decreased after either unilateral or bilateral FEF inactivation, the ability for monkeys to generate express saccades was never completely abolished. When generated, express saccades directed contralesional to the side of FEF inactivation tended to be slower and more inaccurate. Overall, our study reaffirms the potentiating but not critical role for the FEF in express saccades and highlights the FEF's continuing influence on saccadic kinematics regardless of reaction time.

METHODS

Subjects and surgical procedures. Two male rhesus monkeys (*Macaca mulatta*; DZ and OZ, weighing 9.8 and 8.6 kg, respectively) were prepared for head immobilization and cryogenic inactivation of FEF. All training, surgical, and experimental procedures were in accordance with the Canadian Council on Animal Care policy on the use of laboratory animals and approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care. Surgical procedures describing drug regimes, postsurgical care and implantation of head post and cryoloops, cryoloop dimensions, and estimated volume of inactivation for the monkeys used in this study can be found elsewhere. Briefly, each monkey was implanted bilaterally with two stainless steel cryoloops in the inferior and superior aspects of the arcuate sulcus. In this study we have cooled only the inferior arm, as our previous study (Peel et al. 2014) showed that combined cooling of both loops compromised subject performance. This earlier paper also showed that cooling the inferior arm alone produces SRT deficits ~70% as large as cooling both loops together. We have estimated that cooling the inferior arm inactivates a 90 mm³ volume of tissue. Further details about the cryoloops and pictures of their placement (including pictures of cryoloop placement) can be found in our previous studies (Peel et al. 2014, 2017).

Experimental procedures. Monkeys were seated in a custom-made primate chair with their head immobilized, facing a rectilinear grid of >500 red light-emitting diodes (LEDs) covering $\pm 35^\circ$ of the horizontal and vertical visual field. Using a Minolta CS-100 chromameter, background light was measured to be < 0.01 cd/m² and LED (Fairchild Semiconductors, MV5437) luminance was measured at 110 cd/m². Eye movements were recorded using a single, chair-mounted eye tracker (EyeLink II, resolution = 0.05° , sampling rate 500 Hz). All experiments were conducted in a dark, sound-attenuated room. The behavioral tasks were controlled by customized real-time Lab-View programs running on a PXI controller (National Instruments) at a rate of 1 kHz.

An experimental data set consisted of precooling, pericooling, and postcooling sessions, with 80–150 correct trials within each session (i.e., between 240 and 450 trials total). After the precooling session, the cooling pumps were turned on, allowing the flow of chilled methanol through the lumen of the cryoloops. The pericooling session was initiated when cryoloop temperature reached and stayed stable at 3°C. Once sufficient data were collected for the pericooling session, cooling pumps were turned off, which allowed the cryoloop temperature to rapidly return toward body temperature. When cryoloop temperature exceeded 35°C, the postcooling session was initiated.

Behavioral task. Monkeys performed a visually guided gap-saccade task to look to peripheral targets placed on the horizontal meridian. The disengagement of active visual fixation during this task promotes express saccades, but fixation disengagement is not a prerequisite for express saccade generation (Munoz et al. 2000). Monkeys fixated the central fixation LED for 750–1,000 ms until it disappeared, which was followed by a period of 200 ms (the gap interval), during which the monkeys maintained central fixation. A peripheral visual target was then presented for 150 ms (the locations of potential peripheral targets are described below). To receive a fluid reward, monkeys had to initiate a saccade to the peripheral target within 500 ms and land within a circular spatial window with a diameter of 60% of target's visual eccentricity. The large spatial tolerance window was based on the expected decreases in saccade amplitude and accuracy following FEF inactivation (Dias and Segraves 1999; Peel et al. 2014; Sommer and Tehovnik 1997).

All behavioral analyses were carried out using customized MATLAB programs (MATLAB, The MathWorks Inc., MA). Eye position traces were filtered using a third-order low pass Butterworth filter and differentiated to estimate eye velocity. Eye velocity was used to determine the onset and offset times of saccades with a velocity criterion of 30°/s, and the maximum instantaneous velocity between

saccade onset and offset was deemed the peak velocity. We analyzed the first saccade following peripheral target presentation. The eye position at saccade offset was used to calculate the mean saccade amplitude (horizontal and vertical) and end-point scatter, which was calculated as the mean angular distance between the displacements of mean and individual saccade end points from the central fixation position. Visual inspection of the data offline confirmed the validity of automatic marking.

Experimental design and statistical analysis. We performed 3 different experiments, with each experiment consisting of a unique combination of target locations and cooling configurations. In *experiment 1* (8 or 9 sessions in monkeys DZ or OZ, respectively), unilateral inactivation of left FEF was paired with contralateral target presentation at 3 potential locations. In *experiment 2* (18 or 13 sessions in monkeys DZ or OZ, respectively), unilateral inactivation of the left FEF was paired with potential target locations on the left or right (6 potential locations, 3 per side). In *experiment 3* (6 or 5 sessions in DZ or OZ, respectively), bilateral FEF inactivation was paired with potential target locations on the left or right (same targets as *experiment 2*). In all experiments, potential targets were placed at 3 different eccentricities (small, middle, and large) along the horizontal meridian (Monkey DZ: 10, 14, and 20; Monkey OZ: 6, 12, and 20).

We report the comparison of data collected from pre- and post-cooling sessions (termed FEFwarm) to data collected from the pericooling sessions (FEFcool); similar results were obtained if data from pericooling sessions were compared with data from pre- and post-cooling sessions separately. Data collected from different sessions in the same experiment were pooled together. In this study, we compared the effects of FEF inactivation on SRT, the proportion of express saccades, peak velocity, saccade amplitude, and saccade end-point scatter (i.e., the vectorial difference between mean saccade end point and individual saccade end point). Because SRTs differed for different target amplitudes and SRT distributions were not normal, SRTs were compared separately for each target amplitude using a Wilcoxon rank sum test. Because other measures also differed for different target locations (see RESULTS), we performed separate two-way ANOVAs to compare how a given measure (e.g., saccade amplitude) changed with amplitude (small, medium, and large) and inactivation (FEFwarm and FEFcool) for the three experimental configurations. Post hoc analyses comparing the subgroups were performed using Tukey–Kramer test corrected for multiple comparisons. Analysis of covariance was used to compare the changes in peak velocity (across all saccade amplitudes) resulting from FEF inactivation.

Range of express saccades SRTs. Rather than relying on ranges of SRTs published previously for express saccades, we objectively determined the lower and upper bound of the SRTs of express saccades. For the lower bound, we examined saccade gain (ratio of eye amplitude to horizontal target position) as a function of SRT, reasoning that the gain will be less variable for stimulus-driven express saccades versus anticipatory saccades. Figure 1, *A* and *B*, shows the gain for monkey DZ and OZ, respectively. Across our data set (i.e., precooling SRTs from all 3 experimental configurations), we observed accurate saccades to visual targets with SRTs as low as 50 ms, even in blocks where targets could be located to the left or right. Saccades with SRTs < 50 ms were rarer and more inaccurate. Thus, we set the lower SRT range for express saccade at 50 ms and discarded saccades with lower SRTs as anticipatory.

To determine the upper bound for express saccade SRTs, we examined the distribution of precooling SRTs, separately analyzing data for each monkey, saccade direction, and unique potential target configuration (Fig. 1, *C* and *D*; SRTs from *experiments 2* and *3* were pooled because the potential target configurations are identical). First, we used Hartigan's Dip test (Hartigan and Hartigan 1985) to test whether the SRT distribution was unimodal or not; the only unimodal distribution was the SRT distribution for rightward saccades in the 6-target configuration for monkey DZ (middle subplot, Fig. 1*C*). Nonunimodal distributions were smoothed with a kernel smoothing

function (Matlab function `ksdensity`, bandwidth 5). For bimodal distributions, the minimum value between the first and second modes of the smoothed SRT distributions was determined as the upper bound for express saccades (Monkey DZ unilateral targets/rightward SRTs: 128 ms; Monkey DZ leftward SRTs from 6-target configuration: 129 ms; Monkey OZ unilateral targets/rightward SRTs: 93 ms). To be conservative, for the unimodal distribution in monkey DZ (i.e., middle subplot in Fig. 1*C*), we used the 128-ms value determined for this monkey when targets were only placed to the right (i.e., the value from the left subplot in Fig. 1*C*). SRT distributions in monkey OZ were often multimodal (e.g., right two subplots in Fig. 1*D*), and for these we took the minimum value between the second and third modes of the smoothed SRT distributions as the upper bound for express saccades (Monkey OZ bilateral targets/rightward SRTs: 110 ms; Monkey OZ leftward SRTs from 6-target configuration: 109 ms; Fig. 1*D*). Importantly, we confirmed that we obtained qualitatively similar results if we used a fixed upper bound for express saccades (e.g., 100 or 120 ms) that was the same for both monkeys.

RESULTS

Express saccades persist during bilateral FEF inactivation. We studied the effects of reversible unilateral or bilateral FEF inactivation on saccade reaction times (SRTs), express saccade frequency, and metrics and kinematics of both express saccade and regular-latency saccades during a gap-saccade task. Figure 2 shows results from an exemplary experimental session (Fig. 2, *A* and *B*) and pooled group results across all sessions (Fig. 2, *C* and *D*) for the configuration expected to yield the maximum effect when the FEF was inactivated bilaterally (*experiment 3*). In this figure, red traces and histograms represent data collected when the FEF was warm, and blue traces and histograms represent data collected when the FEF was inactivated.

Recall in *experiment 3* that there were six possible target locations (3 in each direction). Despite this uncertainty, both animals generated very short RT saccades in the express saccade range when the FEF was not inactivated. Close inspection of these trials reveals that even the shortest RT movements landed near the flashed visual target, as shown by the banding of eye movement traces in each direction, corresponding to the 3 target amplitudes. Monkey DZ also exhibited a prominent SRT asymmetry, with leftward saccades having shorter SRTs than rightward saccades. Furthermore, and as is particularly clear in the SRT histograms of data collected across all sessions in *experiment 3* (Fig. 2, *C* and *D*), monkey OZ exhibited a bimodal SRT distribution, whereas monkey DZ exhibited a unimodal SRT distribution.

Upon bilateral FEF inactivation (blue traces in Fig. 2, *A* and *B* and blue inverted histograms in Fig. 2, *C* and *D*), SRTs increased by between ~30 and 50 ms for saccades in either direction in both monkeys. A decrease in express saccade frequency paralleled this increase in SRT, with the proportion of express saccades decreasing from 61.2 to 11.1% and 95.8 to 45.9% for rightward and leftward saccades for monkey DZ, respectively, and from 44.5 to 28.7% and 56.6 to 40.2% for rightward and leftward saccades for monkey OZ, respectively. Furthermore, individual express saccades at even the shortest SRTs persisted when the FEF was inactivated (e.g., compare the shortest SRTs for monkey OZ when the FEF was inactivated or not). These results demonstrate the persistence of express saccade generation, even when a large volume of FEF was inactivated bilaterally.

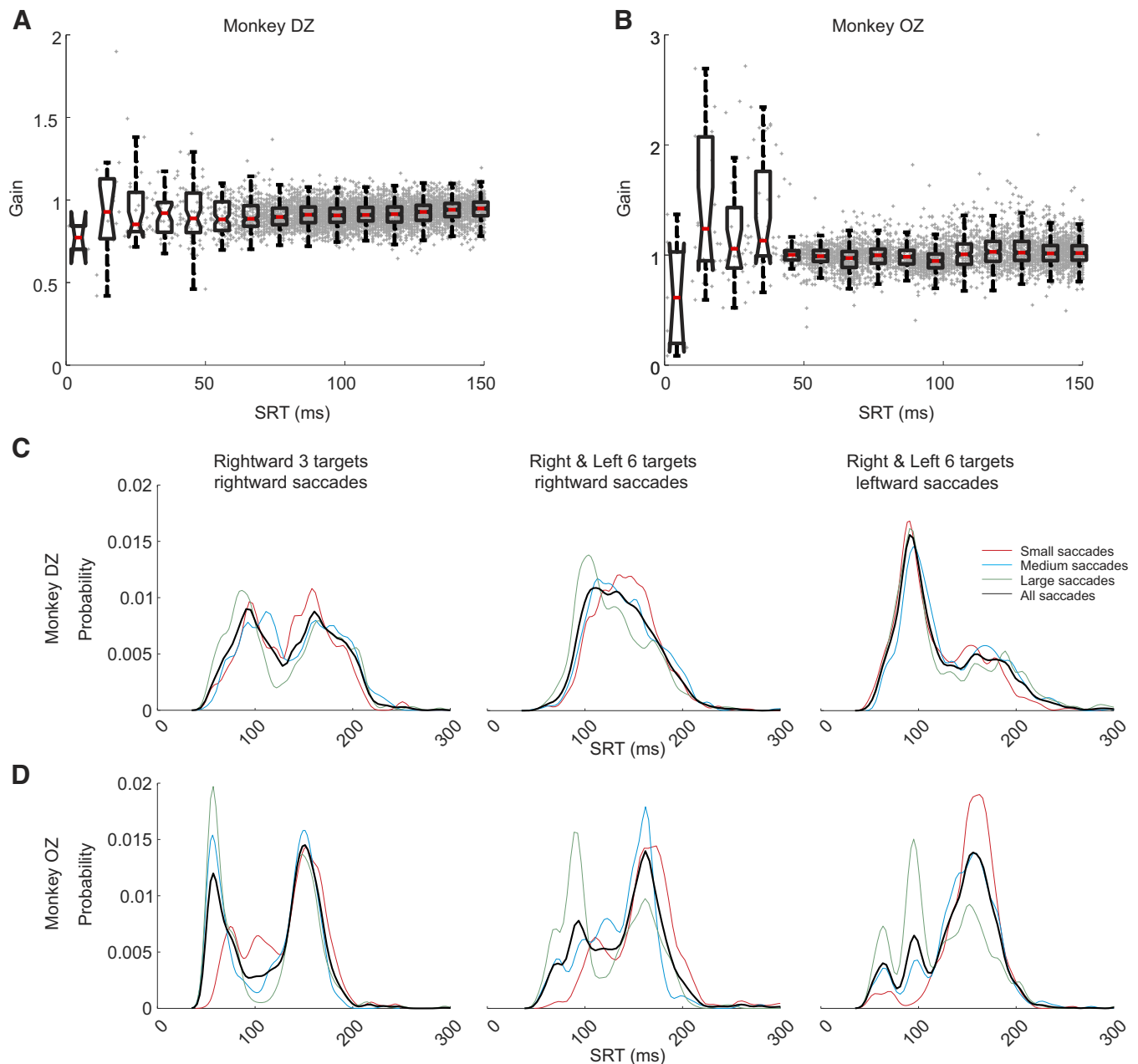


Fig. 1. Lower and upper bounds of express saccades: *A* and *B*: saccade gain (saccade amplitude/target amplitude) of all precooling saccades plotted as a function of saccadic reaction times (SRTs) between 0 and 150 for monkey DZ and OZ, respectively. Data are represented as individual trials (gray dots) and also as median, interquartile interval, and range in 15 bins of 10 ms each. *C* and *D*: the probability distribution of precooling SRTs of all the saccades (black), small saccades (red), medium saccades (blue), and large saccades (green) for various target configurations in monkey DZ and monkey OZ, respectively.

The effects of FEF inactivation on SRTs and express saccade frequency depend on target configuration. Next, we quantified SRT and the change in express saccade frequency with unilateral or bilateral FEF inactivation. Rightward and leftward SRT distributions are represented as probability density functions in Figs. 3 and 4, respectively, segregated by subject and experimental condition. Recall that unilateral FEF inactivation was combined with potential targets located contralaterally in *experiment 1* (i.e., there were no leftward saccades in *experiment 1*) or potential target locations on both sides in *experiment 2* and that bilateral FEF inactivation was combined with potential target locations on both sides in *experiment 3*. Each pair of red and blue probability density plots represent the SRT distribu-

tions in the FEFwarm and FEFcool conditions, respectively; the numbers above the plots represent the median SRT and the percentage of express saccades. The *P* value represents the results of a comparison of SRTs between the FEFwarm and FEFcool condition using a Wilcoxon rank sum test.

Figure 3, *A* and *B*, shows the probability density distribution of FEFwarm (red) and FEFcool (cool) conditions segregated based on target amplitudes (rows; small through to large amplitudes) and experimental configuration (columns; *experiment 1* through *experiment 3*) for rightward saccades in monkey DZ and monkey OZ, respectively. Without exception, unilateral (left FEF) or bilateral FEF inactivation increased rightward SRTs across all amplitudes and experimental con-

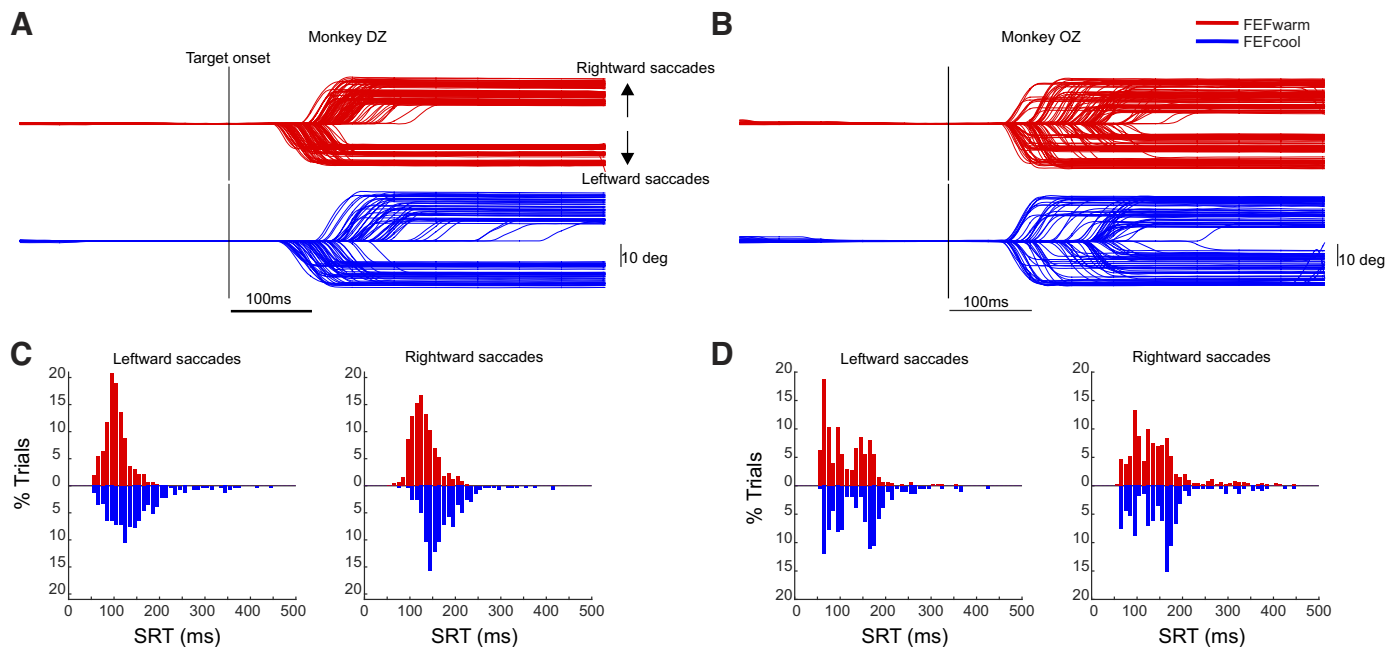


Fig. 2. Effect of frontal eye field (FEF) inactivation on saccadic reaction time (SRT): *A* and *B*: example session with bilateral FEF inactivation in monkey DZ and monkey OZ, respectively. Rightward and leftward saccades are represented by upward and downward deflections in eye position, respectively. Red traces represent FEFwarm trials and blue traces represents FEFcool trials. *C* and *D*: SRT histograms for FEFwarm (red) and FEFcool (blue) trials across all the sessions of bilateral FEF inactivation in monkey DZ and monkey OZ, respectively. All target amplitudes are pooled together in these histograms.

figurations. The probability density functions across monkeys, amplitudes, and experimental configuration also showed a reduction in the proportion of express saccades. This is best exemplified in monkey OZ, in *experiment 1* (Fig. 3*B*, first

column) with the blue distribution decreasing during the first mode. Distributions with clear bimodalities during the FEFcool condition (e.g., large saccades during *experiment 1* in monkey DZ and during all the experiments in monkey OZ) emphasize

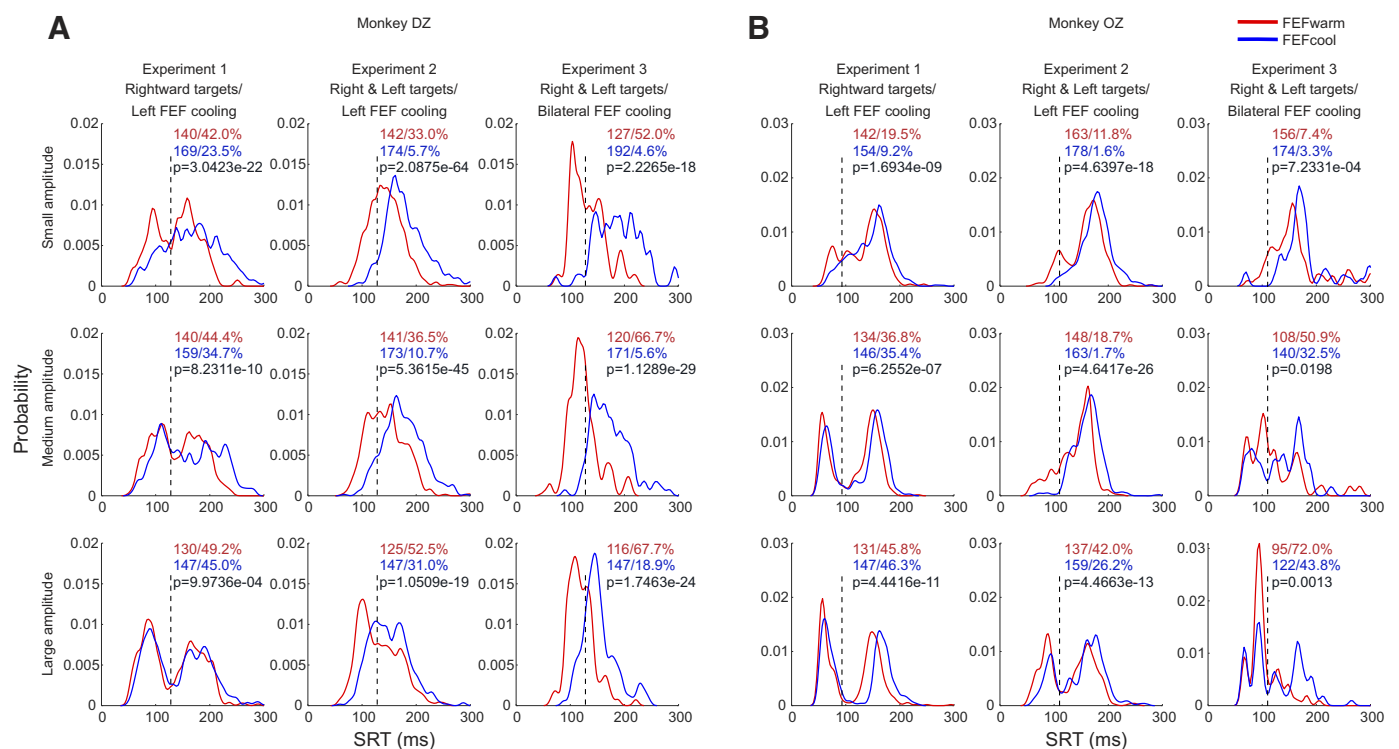


Fig. 3. Effect of frontal eye field (FEF) inactivation on rightward saccadic reaction times (SRTs): SRTs for rightward saccades for monkey DZ (*A*) and monkey OZ (*B*). Within each subplot, data are represented as SRT probability distributions for data collected in the FEFwarm (red) or FEFcool (blue) condition; vertical dashed line shows express saccade cutoff. Each subplot shows data for *experiments 1–3*, and different rows show data for different target amplitudes. The numbers associated with each subplot provide the associated median SRT and % express saccade for FEFwarm (red) and FEFcool (blue) conditions. *P* values show the result of a Wilcoxon rank sum test of SRT across FEF inactivation.

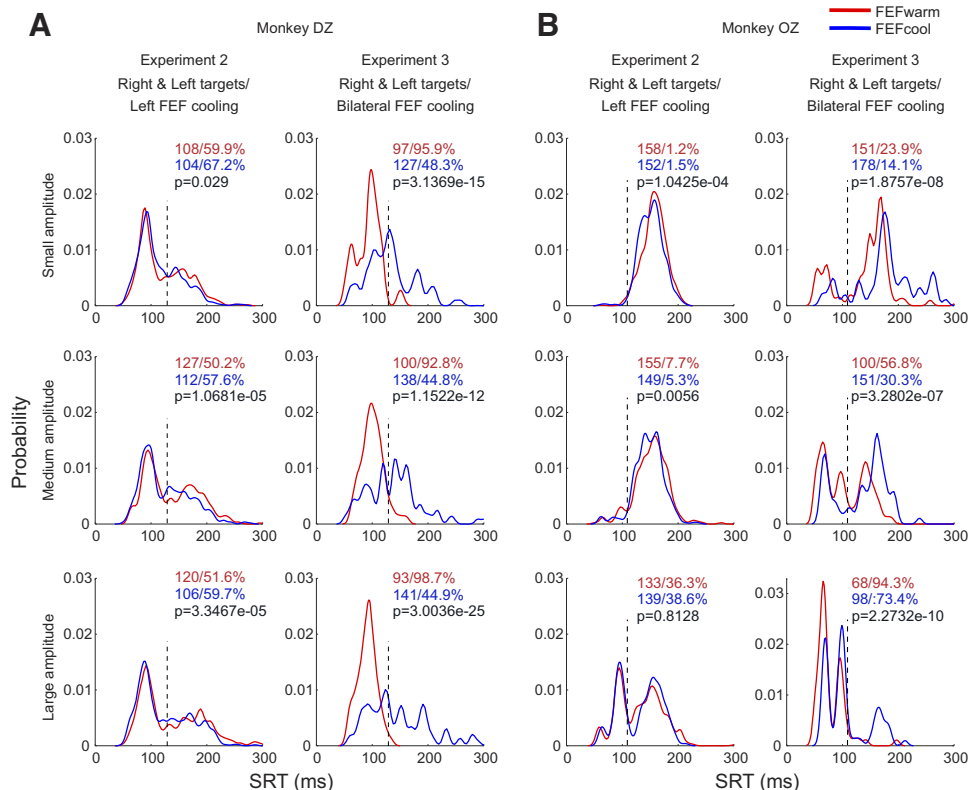


Fig. 4. Effect of frontal eye field (FEF) inactivation on leftward saccadic reaction time (SRT): SRTs for leftward saccades. Same format as for Fig. 3. Recall that there were no leftward saccades in *experiment 1*.

that the express saccade range is not shifted as a result of FEF inactivation. However, the part of the distribution representing regular SRT saccades showed a clear tendency of shifting toward higher SRT. In some conditions, widening of the SRT distribution was also observed during inactivation (e.g., right subplots for *experiment 3* in monkey DZ).

Apart from this main result of increased SRT following FEF inactivation, there are a number of other pertinent observations. First, when the FEF was not inactivated, we observed that SRTs decreased and express saccade frequency increased with increasing target eccentricities in both the monkeys. These observations differ from previous observations of a robust bowl-shaped SRT-eccentricity relationship with a minimum $\sim 5\text{--}6^\circ$ (Kalesnykas and Hallett 1994). Such differences may be attributable to the smaller number of potential targets in our study. Furthermore, our targets lay beyond the eccentricities associated with an express saccade “dead zone,” which is reported for eccentricities less than 2° (Weber et al. 1992). Second, the increase seen in median SRT during FEF inactivation was largest for small amplitude saccades in monkey DZ. For example, during *experiment 3* (bilateral FEF inactivation) the difference in median SRT was 52 versus 32 ms for small or large amplitude saccades in monkey DZ, respectively. Such a trend was not apparent in monkey OZ.

Next, we briefly report the effect of unilateral (left FEF) or bilateral FEF inactivation on the SRT of leftward saccades. Previous work has shown that pharmacological inactivation of the FEF may decrease ipsilesional SRTs (Dias and Segraves 1999; Sommer and Tehovnik 1997), whereas large volume cryogenic inactivation or radiosurgical ablation increases ipsilateral SRT (Kunimatsu et al. 2015; Peel et al. 2014). When the FEF was not inactivated, both monkeys generated a substantial proportion of leftward express saccades (red probability distributions in Fig. 4). Across both monkeys, unilateral (left FEF) inactivation tended to either decrease leftward SRTs or have no effect (Fig. 4, A and B, first column). Bilateral FEF inactivation increased leftward SRT in both monkeys (Fig. 4, A and B, second column).

FEF inactivation decreases but does not abolish express saccades. We next investigated the effects of unilateral and bilateral FEF inactivation on the proportion of express saccades. The changes in express saccade proportion were largely predictable given the changes in SRT noted above, with greater decreases or increases in express saccade proportion accompanying larger increases or decreases in SRTs upon FEF inactivation. Accordingly, we will only emphasize the most important points of an analysis of the changes in express saccade proportion conducted separately for each monkey for leftward and rightward saccades.

Without FEF inactivation, express saccade proportion depended on target configuration, being higher in *experiment 1* with only rightward potential targets (exceeding 50% in both monkeys; Fig. 3) than in *experiments 2* and *3* with potential target locations on both sides. In both monkeys, express saccades were generated most often to the most eccentric target locations. FEF inactivation almost always decreased express saccade proportion to a given contralateral target location. Bilateral FEF inactivation caused a greater decrease in express saccade proportion in monkey DZ than unilateral FEF inactivation, but this trend was not observed in monkey OZ. Bilateral FEF inactivation also decreased the prevalence of leftward express saccades in *experiment 3*, although unilateral inactivation of the left FEF increased the prevalence of leftward express saccades in *experiment 2* (Fig. 4). Regardless of these influences of FEF inactivation on express saccade proportion, it is important to stress that bilateral FEF inactivation de-

creased but never completely abolished the production of express saccades and that both monkeys could generate express saccades of essentially identical SRTs regardless of whether the FEF was inactivated or not.

FEF inactivation modestly increases saccade scatter, with weaker increases for express saccades. Pharmacological or cryogenic FEF inactivation is known to decrease saccade amplitude and increase end-point scatter of both visually and memory-guided saccades, with larger deficits accompanying more complex tasks (Dias and Segraves 1999; Peel et al. 2014; Sommer and Tehovnik 1997). Would such effects also be seen for express saccades? We first looked at the changes in horizontal saccade amplitude of express saccades as a result of FEF inactivation. Figure 5 plots the probability distribution of horizontal saccade amplitude for FEFwarm (red) and FEFcool (blue) conditions across all experiments and both the monkeys. The clear separation of distributions of express saccades corresponding to different amplitude further attests to the overall accuracy of saccades even in express saccade range. However, the effects of FEF inactivation on saccade accuracy were not consistent across both monkeys. Monkey DZ exhibited hypometria in *experiments 1* and *2* during contralateral FEF inactivation but hypermetria for rightward saccades during bilateral FEF inactivation (*experiment 3*). In contrast, saccade amplitude in monkey OZ did not systematically change in any experimental configuration.

Next, we analyzed the scatter of saccade end points, doing so for both express saccades only and all SRTs. This analysis confirmed the impression from Fig. 2 that eye position traces disperse only moderately during bilateral FEF inactivation in *experiment 3*, which is broadly consistent with the relative simplicity of the gap-saccade task. To analyze this quantitatively, we performed a two-way ANOVA to analyze changes in rightward saccade end-point scatter with amplitude (small, medium, and large) and inactivation (FEFwarm and FEFcool) as the factors. We did separate analyses for all saccades irrespective of SRT (squares in Fig. 6) and for just express saccades (diamonds in Fig. 6). We observed the expected significant main effect of saccade amplitude, with end-point scatter increasing for larger saccades across all experimental conditions, saccade directions, and monkeys (Fig. 6, *A* and *B*; $P < 0.005$). This trend persisted if we analyzed just express saccades or all saccades regardless of SRT.

Focusing now on the effect of FEF inactivation on end-point scatter, we observed a number of trends. First, in both monkeys and in all three experiments, the end-point scatter of contralateral saccades regardless of SRT increased during FEF inactivation (i.e., rightward saccades in *experiments 1* and *2*, leftward or rightward saccades in *experiment 3*; see P values in Fig. 6). This increase in contralateral end-point scatter tended to be larger in Monkey DZ (Fig. 6*A*) than Monkey OZ (Fig. 6*B*). Second, when we repeated this analysis exclusively for

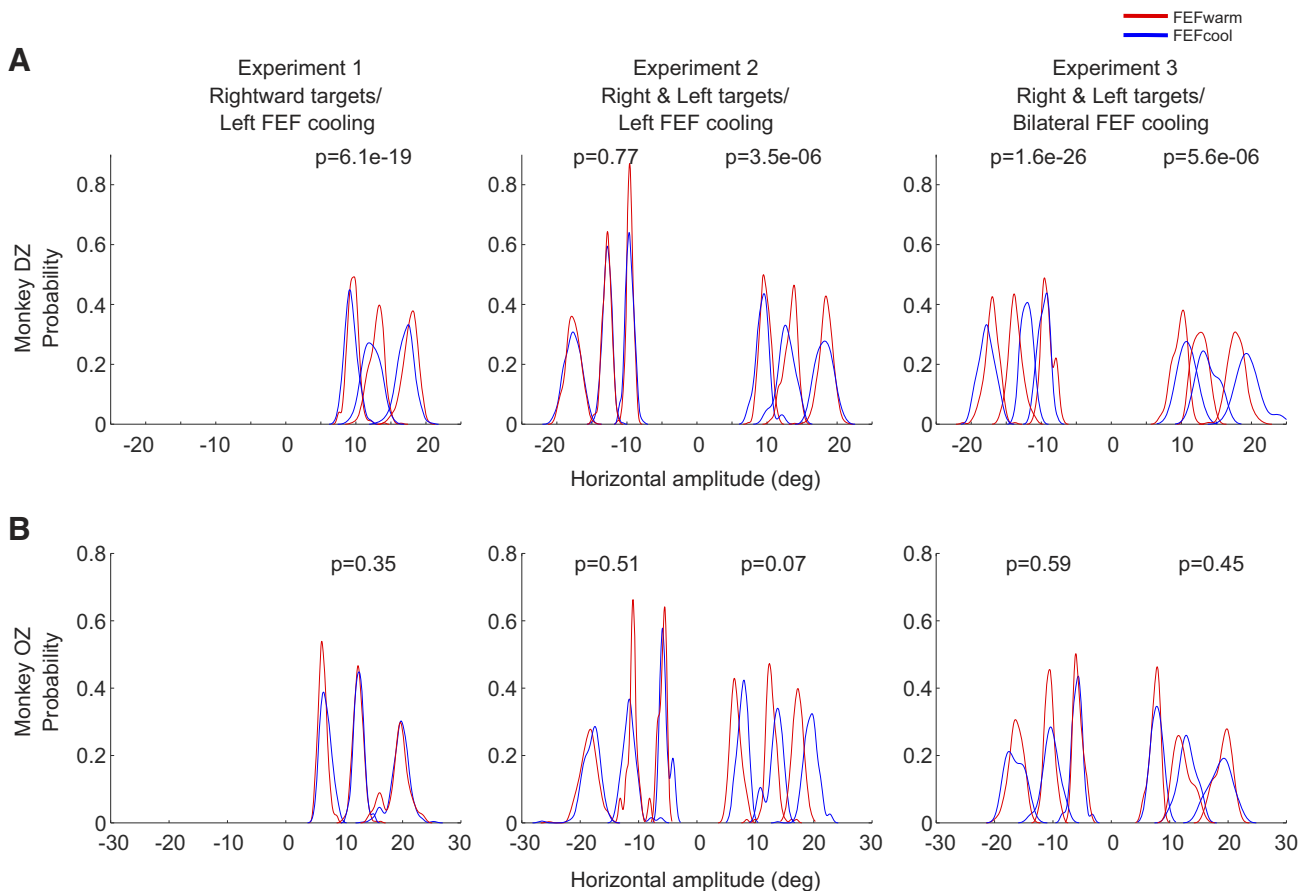


Fig. 5. Effect of frontal eye field (FEF) inactivation on saccade amplitude: probability distribution of horizontal saccade amplitudes in monkey DZ (*A*) and monkey OZ (*B*). The 3 columns represent *experiments 1–3*. FEFwarm data are represented in red and FEFcool data are represented in blue. P value indicates the result of main effect of FEF cooling for 2-way ANOVA.

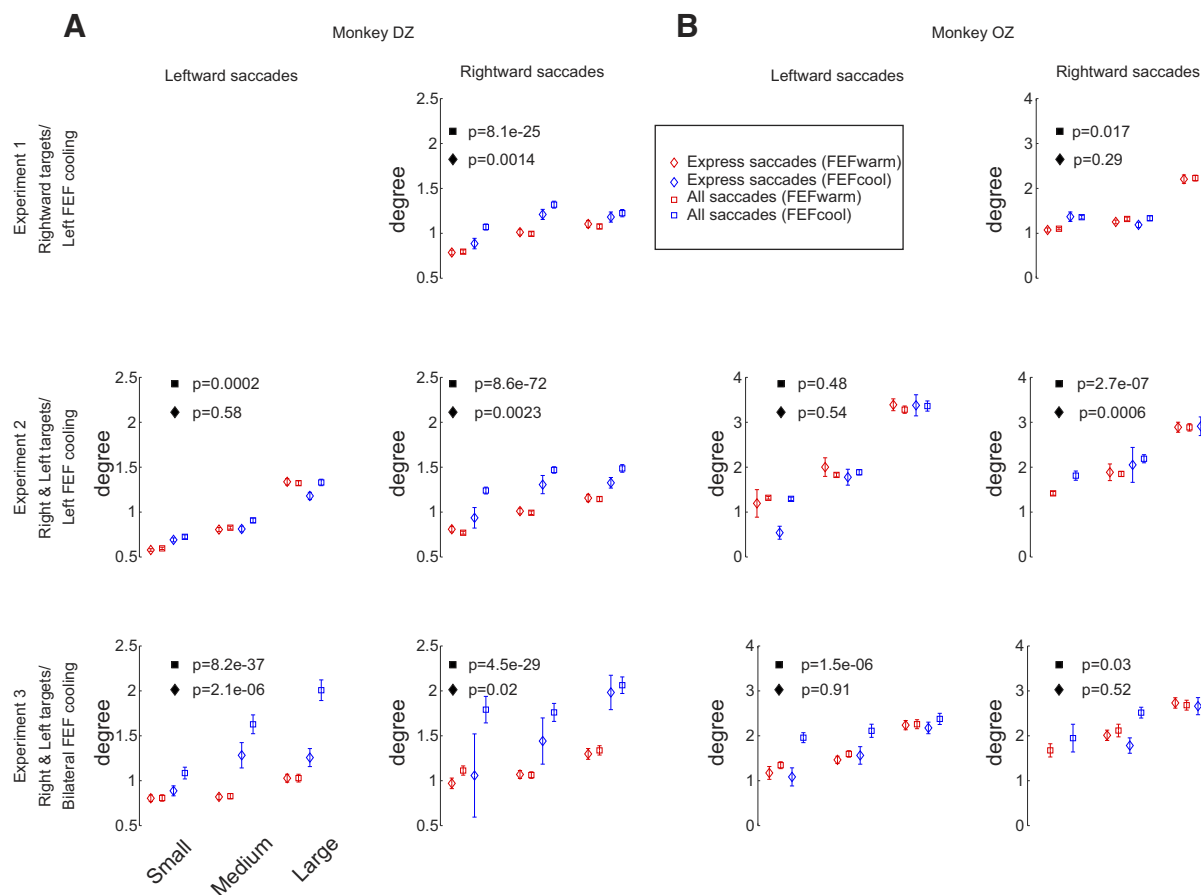


Fig. 6. Effect of frontal eye field (FEF) inactivation on saccade end-point scatter: saccade end-point scatter as mean and SE for FEFwarm (red) and FEFcool (blue) in monkey DZ (A) and monkey OZ (B). Data representing exclusively express saccades are shown with a diamond symbol, and square represents all the saccades irrespective of saccadic reaction time. Data are not shown in conditions when not enough express saccades were elicited (e.g., small saccades during *experiments* 2 and 3 for monkey OZ).

express saccades, we found that the scatter still increased significantly in monkey DZ, although the effect size was reduced (Fig. 6A). In contrast, Monkey OZ did not show any consistent increase in the end-point scatter of express saccades (Fig. 6B). Finally, the end-point scatter for ipsilateral saccades after unilateral FEF inactivation (i.e., leftward saccades in *experiment 2*) increased moderately in monkey DZ but did not change systematically in monkey OZ.

Overall, although FEF inactivation did influence the metrics of contralateral saccades, such influences were relatively modest in magnitude. Specific analysis of the metrics of express saccades showed even more modest effects, with the effects of FEF inactivation significantly increasing contralateral saccade end-point scatter in Monkey DZ but not Monkey OZ. The relatively modest influence of FEF inactivation on saccade metrics likely relates to the relative simplicity of the gap-saccade task, as past work in both animals have reported increased contralateral saccade scatter and hypometria in tasks involving a delayed component (Peel et al. 2017).

FEF inactivation decreases peak velocity of express saccades. Pharmacological or cryogenic FEF inactivation also decreases the peak velocity of contralaterally directed saccades generated in a delayed visually guided or memory-guided task (Dias and Segraves 1999; Peel et al. 2014; Sommer and Tehovnik 1997). Previous studies have not addressed whether FEF inactivation affects peak velocity of express saccades. To

address this, we plotted the velocity-amplitude main sequence relationship for express saccades across FEF inactivation (Fig. 7). Because the velocity-amplitude main sequence relationship is linear over the range of saccade amplitudes we studied, we analyzed whether peak velocity changes across saccade amplitude with FEF inactivation using an ANCOVA and whether the slope of a linear regression fit to these data changes during FEF inactivation.

The results of this comparison show that FEF inactivation decreases the peak velocity of express saccades directed contralateral to the side of inactivation (e.g., rightward saccades in *experiments 1* and *2*, leftward or rightward saccades in *experiment 3*). The influence of FEF inactivation on the main sequence relationship appears both as an overall decrease in peak express saccade velocity during inactivation (i.e., a clustering of the blue dots below the red dots in Fig. 7) and often as a decrease in the slope of a linear fit to this data (e.g., a clockwise rotation in the blue compared with red lines in Fig. 7), indicating that larger decreases in peak velocity accompanied larger saccades. These changes were observed in both monkeys, and similar trends were observed when all saccades were considered regardless of the SRT (results not shown in Fig. 7). Overall, all individual comparisons revealed a significant decrease in peak velocity of express saccades ($P < 0.05$; ANOVA) as well as when all saccades were considered regardless of SRT ($P < 0.05$; ANOVA).

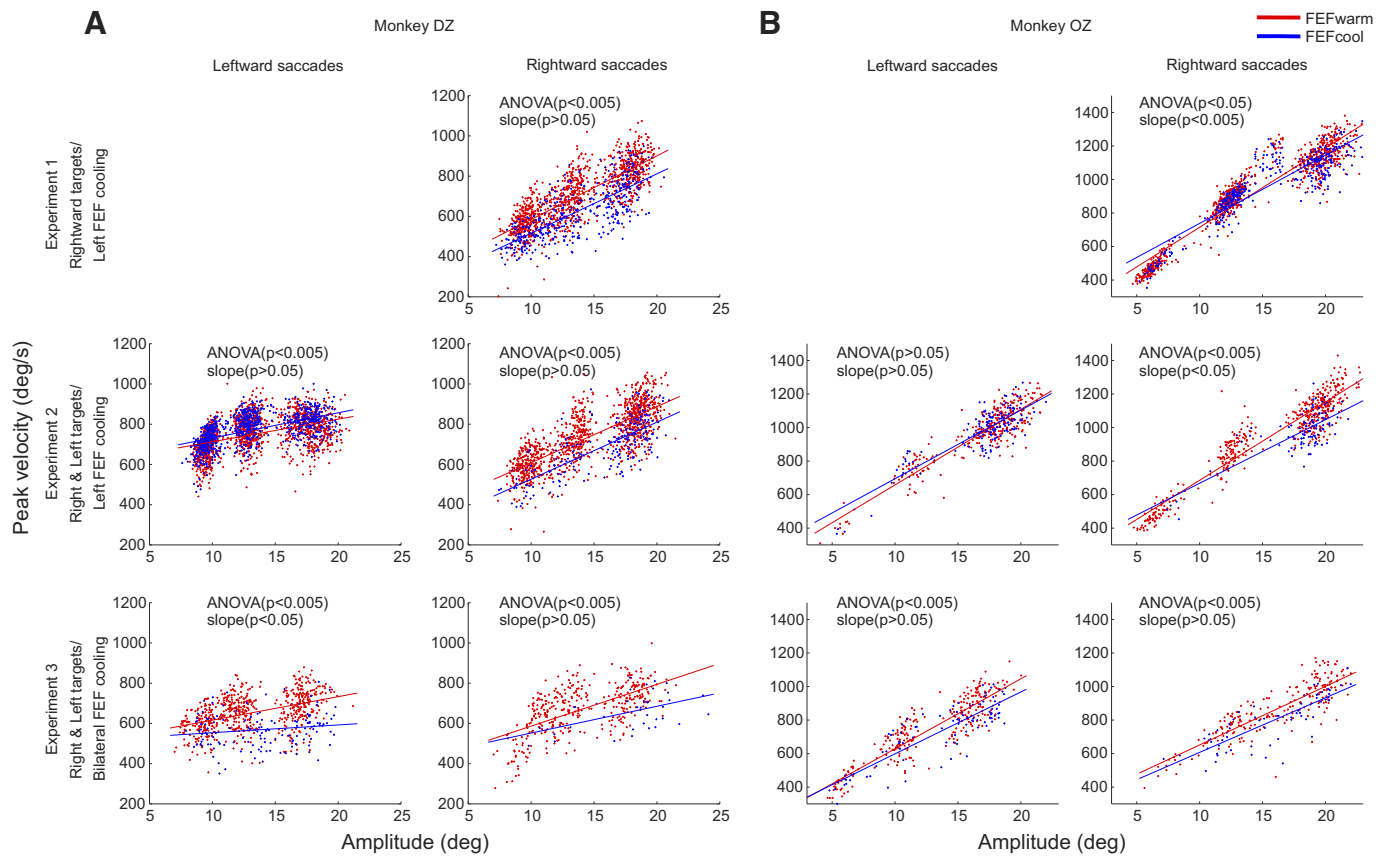


Fig. 7. Effect of frontal eye field (FEF) inactivation on express saccade peak velocity in Monkey DZ (A) and Monkey OZ (B): a comparison of velocity-amplitude main-sequence relationship for FEFwarm (red) and FEFcool (blue) conditions. The dots represent individual express saccade trials and the solid line represents the linear relationship between peak velocity and amplitude. The result of corresponding ANOVA comparison and slope change is given in each subplot.

Finally, we looked at the peak velocity of ipsilateral saccades during unilateral FEF inactivation (i.e., leftward saccades during left FEF inactivation in *experiment 2*). A previous study with unilateral FEF inactivation reported no changes in the peak velocity of ipsilateral saccades made during memory and delayed visually guided saccade tasks (Peel et al. 2014). Consistent with this, the main sequence relationship for ipsilaterally-directed saccades did not change if all saccades were considered regardless of SRT (results not shown). However, and somewhat unexpectedly, although the main sequence relationship for ipsilateral express saccades did not change in monkey OZ (Fig. 7B, left column in second row), the main sequence relationship increased in monkey DZ for ipsilaterally directed express saccades (Fig. 7A, left column in second row; note how blue dots and line cluster above red dots and line). However, this increase was relatively modest, with peak velocities increasing by only $\sim 2\%$ during FEF inactivation.

DISCUSSION

To our knowledge, this is the first study examining the influence of large but reversible inactivation of unilateral or bilateral FEF on express saccades. We emphasize a number of novel results. First, even after bilateral FEF inactivation, express saccades were not completely abolished despite decreasing in frequency. Second, the impact of FEF inactivation depended in part on the configuration of potential targets, being greater when potential targets were located on either side of

midline compared with being only presented on one side. Third, the peak velocity of contralateral express saccades decreased during FEF inactivation. Together, these findings contribute to the understanding of the role of the FEF in express saccades, reaffirming the important but not critical role in setting up the conditions necessary for express saccade generation and emphasizing an influence of the FEF on saccade velocity regardless of SRT.

Methodological considerations. In this study, we inactivated the FEF using reversible cryogenic inactivation. This technique has a number of advantages compared with other temporary inactivation techniques or permanent ablation (Lomber 1999), including a large volume of inactivation that impacts behavior and neural activity within 2–3 min of changes in the temperature of the cooling loop. Such rapid onset and cessation of action, as well as the repeatability of inactivation within and across days, facilitates simultaneous electrophysiological recordings in interconnected areas (Dash et al. 2018; Peel et al. 2017). Another advantage of cryogenic inactivation is that it permits assessment of the functional consequences of loss of a large volume of tissue without confounds arising from neuroplasticity. The oculomotor performance of monkeys is surprisingly unimpaired after permanent ablation of the FEF following a period of recovery (Schiller et al. 1980, 1987), and express saccades are either unchanged or potentiated in human patients with frontal lesions (Braun et al. 1992; Guitton et al. 1985; Rivaud et al. 1994). Our results, by detailing the immediate decreases in express sac-

cade proportion that accompany FEF inactivation, suggest that patients' persistent capacity to generate express saccades arises not from an inability to suppress reflexive glances consequent to frontal lobe damage, but instead due to long-term functional recovery of oculomotor behaviors.

We also emphasize that we are not inactivating the entire FEF. Indeed, we cooled only the cryoloop placed in the inferior arm of the arcuate sulcus. Cooling this arm alone produced SRT deficits ~70% as large as the deficits observed when cooling both the inferior and superior arms together (Peel et al. 2014); hence, it is likely that cooling both arms would have further compromised express saccade generation, particularly for larger amplitude saccades. Cryogenic techniques inactivate tissue within a 1.5-mm radius of the cryoloop (Lomber et al. 1999), leading to an estimated volume of ~90 mm³ of inactivated tissue in the anterior bank of the arcuate sulcus with a 7- to 8-mm length cryoloop (Peel et al. 2017). This volume of inactivation is still ~3–6 times larger than the volume inactivation by pharmacological means, which range between 14 and 33 mm³ (Dias and Segraves 1999; Peel et al. 2014; Sommer and Tehovnik 1997). Although it is difficult to ascertain directly, visual inspection of the tissue ablated in the studies by Schiller and colleagues (Schiller et al. 1987; Schiller and Chou 1998) and Kunimatsu and colleagues (Kunimatsu et al. 2015) show damage extending along the length of the arcuate sulcus and into the gyral crown, meaning that the total volumes of ablated tissue in these studies are perhaps ~25–50% larger than the volume that we inactivated in the current study (see Peel et al. 2014 for further considerations). Our estimated volume of inactivation also only considers the volume lying on the anterior bank of the arcuate sulcus. We did not insulate the posterior aspect of our cryoloops; hence, tissue lying on the posterior bank of the arcuate sulcus was inactivated as well. We have considered the potential impact of inactivation on the posterior bank more thoroughly in a previous publication (see Peel et al. 2014). Briefly, permanent (Rizzolatti et al. 1983) or reversible lesions (Schieber 2000) of tissue within the posterior bank of the arcuate do not produce the oculomotor deficits that we observed either in this or previous studies (Peel et al. 2014). Furthermore, electrical stimulation of the posterior bank of the arcuate sulcus mainly evokes ipsilaterally directed saccades (Neromyliotis and Moschovakis 2017) and saccade-related activity in the posterior bank is not biased contralaterally (Neromyliotis and Moschovakis 2018), in contrast to the FEF. We are therefore confident that the majority of the results reported here are attributable to the FEF within the anterior bank of the arcuate sulcus but again emphasize that the entire FEF was not inactivated.

Finally, each animal was implanted bilaterally. Although every attempt was made to ensure the symmetry of the implant relative to the spur of the arcuate, our results exhibit a fair degree of asymmetry both in the effects of inactivation and in the SRT distributions and asymmetries within each animal even before inactivation. For example, the SRT distributions in monkey OZ but not DZ exhibited a clear bimodality, and monkey DZ tended to have shorter leftward than rightward SRTs. The effects of either unilateral or bilateral inactivation also tended to be larger in monkey DZ than OZ. Studies in monkeys involve a limited number of animals, and these types of idiosyncrasies are unavoidable; hence, we place particular emphasis on results that were common in both animals.

Comparison with other inactivation or ablation studies. Unilateral lesion or inactivation of the FEF produce a triad of contralateral saccade deficits, increasing SRT and decreasing peak velocity and accuracy (Acker et al. 2016; Dias et al. 1995; Dias and Segraves 1999; Kunimatsu et al. 2015; Lynch 1992; Peel et al. 2014; Schiller et al. 1980, 1987; Schiller and Chou 1998, 2000; Sommer and Tehovnik 1997; van der Steen et al. 1986). Many of these studies examined saccade performance in the context of delayed or memory saccade paradigms in which express saccades are not reliably generated. One exception is the 1987 ablation study by Schiller and colleagues, which found that contralateral express saccade proportion initially decreased when assessed 3 days after the lesion but rapidly recovered back to preoperative levels by 7 days postlesion. Our findings show that the onset of FEF inactivation of contralesional express saccade proportion becomes apparent within minutes.

Recently, we reported that unilateral FEF inactivation decreases preparatory, low-frequency activity in the SCi during the gap interval (Dash et al. 2018). A component of this study found that express saccades persisted during FEF inactivation, similar to what we found here. Importantly, this past study employed only two potential target locations, one in the center of the SCi neuron's response field, and the other in the mirror opposite position. As we did not record SCi activity in the current study, we were free to use more configurations featuring more distributed target locations. The SRT distributions shown in Figs. 2 and 3 emphasize the influence of potential target configurations on the effects of FEF inactivation. Unilateral FEF inactivation caused a greater decrease in contralateral express saccade proportion and a corresponding larger increase in SRT in *experiment 2* (potential targets located on either side) compared with *experiment 1* (potential targets located only on one side). This finding complements observations we and others have made on how the impact of FEF inactivation is greater for more complex oculomotor tasks involving a delayed saccadic response (Dias and Segraves 1999; Peel et al. 2014, 2017; Sommer and Tehovnik 1997), extending this notion to immediate saccade tasks where the only difference is the number and distribution of potential targets.

The configuration of potential targets, and of task complexity in general, may also help resolve some confusion regarding the impact of unilateral FEF inactivation on ipsilateral SRTs. Previous studies with unilateral FEF pharmacological inactivation (Dias and Segraves 1999; Sommer and Tehovnik 1997) have reported either negligible effects on ipsilateral saccades or an increase in premature ipsilateral saccades (Dias and Segraves 1999), in contrast to reports of increased ipsilateral SRTs following large cryogenic inactivation (Peel et al. 2014, 2017) or permanent ablation (Kunimatsu et al. 2015). Previously, we speculated that increases in ipsilateral SRT may be associated with larger volumes of inactivation (Peel et al. 2014), which could impact the contribution of sparsely distributed saccade-related neurons tuned to ipsilateral directions (Crapse and Sommer 2009). In the current study, however, we observed that ipsilateral SRTs can decrease during large-volume cryogenic inactivation (Fig. 4), resembling reports of decreased ipsilateral SRTs after permanent FEF ablation (Schiller and Chou 2000). In the work by Peel and colleagues (2014), targets could be presented at 32 potential locations distributed throughout oculomotor space, whereas in the current study there were either 3 or 6 potential target locations distributed along the horizontal meridian. Thus, an additional factor in

determining the influence of unilateral FEF inactivation on ipsilateral SRTs in immediate saccade tasks may be the number and distribution of potential target locations. Whether this result is attributable to the FEF neurons reported by Crapse and Sommer (2009) with response fields in the ipsilateral space remains to be determined. Alternatively, because different monkeys were used in the study by Peel and colleagues (2014) than those in the current study, the influence of unilateral FEF inactivation on ipsilateral saccades may attest to idiosyncratic tendencies of particular animals.

The FEF potentiates but is not critical for express saccade generation and contributes to express saccade metrics and kinematics. The ablation work of Schiller and colleagues (1987) established that the integrity of the SC, but not FEF, is critical for express saccade generation. Consistent with this, we found using cryogenic inactivation that express saccades are spared and can even be generated at the exact same SRT during either unilateral or bilateral FEF inactivation. The FEF has direct projections to the SC (Künzle et al. 1976; Stanton et al. 1988) that can relay sensory, cognitive, and motor information to SCi (Everling and Munoz 2000; Sommer and Wurtz 2000) to influence SCi activity and oculomotor behavior (Dash et al. 2018; Inoue et al. 2015; Peel et al. 2017). The FEF also projects directly to oculomotor regions downstream of the SC (Huerta et al. 1986; Leichnetz et al. 1984; Segraves 1992; Stanton et al. 1988), and this pathway presumably underlies the ability for monkeys to recover oculomotor abilities in the weeks to months after SC ablation. In the intact animal, however, it appears that the FEF predominantly influences saccadic behavior through the SCi (Hanes and Wurtz 2001).

How do our results add to the understanding of the neural mechanisms preceding and during express saccades? Our main result that express saccade proportion decreases but is not abolished by FEF inactivation is largely consistent with the idea that the FEF is one source of preparatory activity that helps establish the preconditions that potentiate the generation of express saccades. In a related experiment (Dash et al. 2018), we recorded SCi activity related to express saccades either with or without unilateral FEF inactivation and found that FEF inactivation reduced preparatory, pretarget information in the SCi. Such decreases in SCi preparatory activity related to both an increase in overall SRT and a decreased proportion of express saccades during FEF inactivation. Our previous work (Dash et al. 2018) showed that SCi preparatory activity was essentially unchanged when we specifically examined those rare instances where an express saccade with the same SRT was generated during unilateral FEF inactivation. Our interpretation, which we speculate would generalize to bilateral FEF inactivation, was that inputs from other cortical and subcortical areas compensated for the loss of the FEF, as many such areas also display increasing preparatory activity before target onset in related tasks (Ashmore and Sommer 2013; Chen et al. 2013; Hikosaka et al. 1989; Johnston et al. 2014; Kim and Lee 2017; Ohmae et al. 2017; Wurtz and Hikosaka 1986).

A novel finding in the current study is that inactivation of the FEF influenced express saccade parameters, decreasing both accuracy and peak saccade velocity of express saccades during either unilateral or bilateral FEF inactivation. Express saccades can be viewed as a form of a visual-grasp reflex, and the SCi does appear to be critical in the phenomenon of express saccades. That being said, our findings that FEF inactivation decreases the accu-

racy and peak velocity of express saccades are novel to our knowledge and emphasize that the subcortical mechanisms for express saccade execution do not operate in isolation from cortical inputs. In particular, the influence of FEF inactivation on express saccade velocity was quite substantial (Fig. 7). Correlates with saccade velocity have been found in saccade-related activity in the SCi but not the FEF (Reppert et al. 2018; Segraves and Park 1993; Smalianchuk et al. 2018; Waitzman et al. 1991). As mentioned above, decreases in contralateral saccade velocity are common following FEF inactivation, and our results emphasize that such velocity decreases persist for express saccades. Whether this persistent influence on saccade velocity relates to a decrease in the vigor of saccade-related information in the SCi and/or to a loss of FEF input into the brainstem burst generator remains to be determined.

ACKNOWLEDGMENTS

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GRANTS

This work was supported by operating grants from the Canadian Institutes of Health Research to B.D.C. (MOPs: 93796, 123247, and 142317) and the Natural Sciences and Engineering Research Council (NSERC; RGPIN-311680). S.D. and T.R.P. were supported by funding from an NSERC CRE-ATE grant, and T.R.P. was supported by an Ontario Graduate Scholarship.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

SD and B.D.C. conceived and designed research; SD and S.G.L. performed experiments; SD and T.R.P. analyzed data; S.D., T.R.P., and B.D.C. interpreted results of experiments; SD prepared figures; SD drafted manuscript; SD and B.D.C. edited and revised manuscript; S.D., T.R.P., S.G.L., and B.D.C. approved final version of manuscript.

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