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Unilateral deactivation of macaque dorsolateral prefrontal cortex induces biases in stimulus selection

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Johnston K, Lomber SG, Everling S. Unilateral deactivation of macaque dorsolateral prefrontal cortex induces biases in stimulus selection. *J Neurophysiol* 115: 1468–1476, 2016. First published January 20, 2016; doi:10.1152/jn.00563.2015.—Following unilateral brain injury, patients are often unable to detect a stimulus presented in the contralesional field when another is presented simultaneously ipsilesionally. This phenomenon has been referred to as extinction and has been conceptualized as a deficit in selective attention. Although most commonly observed following damage to posterior parietal areas, extinction has been observed following lesions of prefrontal cortex (PFC) in both humans and nonhuman primates. To date, most studies in nonhuman primates have examined lesions of multiple PFC subregions, including the frontal eye fields (FEF). Theoretical accounts of attentional disturbances from human patients, however, also implicate other PFC areas, including the middle frontal gyrus. Here, we investigated the effects of deactivating PFC areas anterior to the FEF on stimulus selection using a free-choice task. Macaque monkeys were presented with two peripheral stimuli appearing either simultaneously, or at varying stimulus onset asynchronies, and their performance was evaluated during unilateral cryogenic deactivation of part of dorsolateral prefrontal cortex or the cortex lining the caudal principal sulcus, the likely homologue of the human middle frontal gyrus. A decreased proportion of saccades was made to stimuli presented in the hemifield contralateral to the deactivated PFC. We also observed increases in reaction times to contralateral stimuli and decreases for stimuli presented in the hemifield ipsilateral to the deactivated hemisphere. In both cases, these results were greatest when both PFC subregions were deactivated. These findings demonstrate that selection biases result from PFC deactivation and support a role of dorsolateral prefrontal subregions anterior to FEF in stimulus selection.

prefrontal cortex; macaque; saccades; stimulus selection; deactivation

AT ANY GIVEN INSTANT, OUR sensory systems are faced with many more stimuli than can be reliably processed at one time. Successful goal-directed behavior requires the ability to select relevant stimuli from among many competing alternatives and carry out appropriate actions. Numerous lines of evidence have shown that this ability is dependent on a network of brain areas, including the frontal and parietal cortex, as well as subcortical structures (Corbetta and Shulman 2002; Kastner and Ungerleider 2000). Studies with patients suffering from disturbances of attention, such as neglect following lesions, have been particularly informative in this regard (Corbetta and Shulman 2011; Mesulam 1981).

One commonly observed consequence of unilateral brain injury is an inability to detect a stimulus presented in the hemifield contralateral to the damaged hemisphere when another stimulus is presented simultaneously in the ipsilateral hemifield, while detection for single stimuli presented to either hemifield remains intact. This deficit in detection upon double simultaneous stimulation is commonly referred to as extinction (Oppenheim 1885), as the stimulus in the intact hemifield appears to extinguish detection of the stimulus in the damaged hemifield when both are presented simultaneously. Extinction has been observed across many sensory modalities and has commonly been characterized as a pathological bias in attention resulting from a disruption of the normal competitive mechanisms underlying stimulus selection (de Haan et al. 2012; de Haan and Karnath 2012; Driver et al. 1997).

Disruptions of attentional processing, including extinction, have been linked closely to the posterior parietal cortex (PPC). Extinction is commonly observed as a consequence of PPC lesions in human patients (Di Pelligrino et al. 1997; Heilman and Valenstein 1972; Rorden et al. 2008), can be induced by transcranial magnetic stimulation over PPC in normal subjects (Fierro et al. 2000; Meister et al. 2006) and is observed following permanent lesions (Lynch and McLaren 1989) and reversible deactivation of PPC (Schiller and Tehovnik 2003; Wardak et al. 2002) in monkeys. Although commonly associated with damage to the PPC, disruptions of attention have been shown to be anatomically much more diverse, and to result from unilateral damage to a variety of cortical and subcortical structures (Driver et al. 1997), including prefrontal cortex (PFC) in both human patients (Heilman and Valenstein 1972) and monkeys (Bianchi 1895; Deuel and Farrar 1993; Eidelberg and Schwartz 1971; Kennard 1939; Kennard and Ectors 1938; Welch and Stutteville 1958), as well as in the cat (Lomber and Payne 2004).

In human patients, extinction effects are generally assessed verbally and require that the patient report his or her perceptual experience. In animal models, this is obviously not possible; however, experimental paradigms in which two stimuli are presented either simultaneously, or at various stimulus onset asynchronies (SOAs), and require that the animal select one stimulus have been used to evaluate selection biases which resemble those seen in human patients (Di Pellegrino et al. 1997; Rorden et al. 2008). In this case, deficits can be characterized by the lead time required for a contralesional stimulus

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to be selected with equal probability to one in the unaffected ipsilesional hemifield, or by the probability of selecting the contralesional stimulus when stimuli are presented simultaneously. In the rhesus macaque, oculomotor selection biases have been observed following lesions of circumscribed frontal subregions, including the frontal (FEF) and supplementary eye fields (SEF) (Schiller and Chou 1998; Schiller and Tehovnik 2003). Many of the experimental lesions in earlier studies included not just the FEF, but also more anterior areas of the PFC. It is thus possible that damage to the PFC anterior to FEF also contributes to the pathological attentional imbalance underlying extinction effects.

Here, we investigated the role of two PFC areas anterior to the FEF in attentional selection by unilaterally deactivating subregions of the PFC. Cryoloops (Lomber et al. 1999) were implanted into the cortex lining the caudal principal sulcus (cPS), the likely homologue of the human middle frontal gyrus, and on the immediately adjacent superior dorsolateral surface of the PFC (DPC). These areas were unilaterally deactivated both separately and simultaneously, while macaque monkeys performed a saccadic free-choice task similar to that used in previous studies to investigate stimulus selection (Schiller and Chou 1998; Schiller and Tehovnik 2003; Wardak et al. 2002). In this task, animals were required to fixate and subsequently presented with stimuli in the left and right visual fields. Stimuli were presented either simultaneously, or with the left or right stimulus leading, at a range of SOAs. A saccade to either of the two stimuli was rewarded, and thus choices were based on the animal's spatial bias toward one or the other hemifield. PFC deactivation resulted in consistent shifts in animals' choices such that responses to stimuli presented contralateral to the deactivated hemisphere were reduced. These data are consistent with a role of DPC subregions anterior to the FEF, in competitive attentional processes related to stimulus selection.

MATERIALS AND METHODS

Surgeries. Data were collected from two male rhesus macaque monkeys (*Macaca mulatta*) weighing 10 and 12 kg. Each animal was prepared for PFC deactivation experiments using previously described procedures (Koval et al. 2011). Briefly, each animal underwent two aseptic surgical procedures. In the first, animals were implanted with a plastic head restraint to enable training on oculomotor tasks. After animals had been trained in a basic battery of tasks, a second surgery was carried out. In this surgery, bilateral stainless steel cryoloops (6 mm $\times$ 3 mm) were implanted into the posterior portion of the cPS (cPS loops). This area was targeted as the likely macaque homologue of the human middle frontal gyrus (Hutchison et al. 2012; Petrides and Pandya 1999). Bilateral loops were also implanted on the cortical surface immediately dorsal to the principal sulcus (DPC loops). Given that the extent of deactivated tissue has been shown to extend ~2 mm around cryloops (Lomber et al. 1999; Lomber and Payne 2000), we estimated that cooling of the cPS loop deactivated portions of areas 46, 9/46d and v, and 8, while cooling of the DPC loop deactivated portions of areas 46, 9, 9/46d, and 8 (Fig. 1*A*). Technical details of the cryoloop technique have been described in detail previously (Lomber et al. 1999). All procedures were carried out in accordance with the guidelines of the Canadian Council of Animal Care Policy on the Use of Laboratory Animals and a protocol approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care. Animals received analgesics and antibiotics postoperatively and were closely monitored by a university veterinarian. Daily records of the weight and health status of the monkeys were kept, and additional fruit was provided.

Task. Monkeys were trained to perform a saccade free-choice task (Fig. 1*B*). The task paradigm required the animals to saccade to one of two peripheral visual stimuli appearing in the left and right hemifields

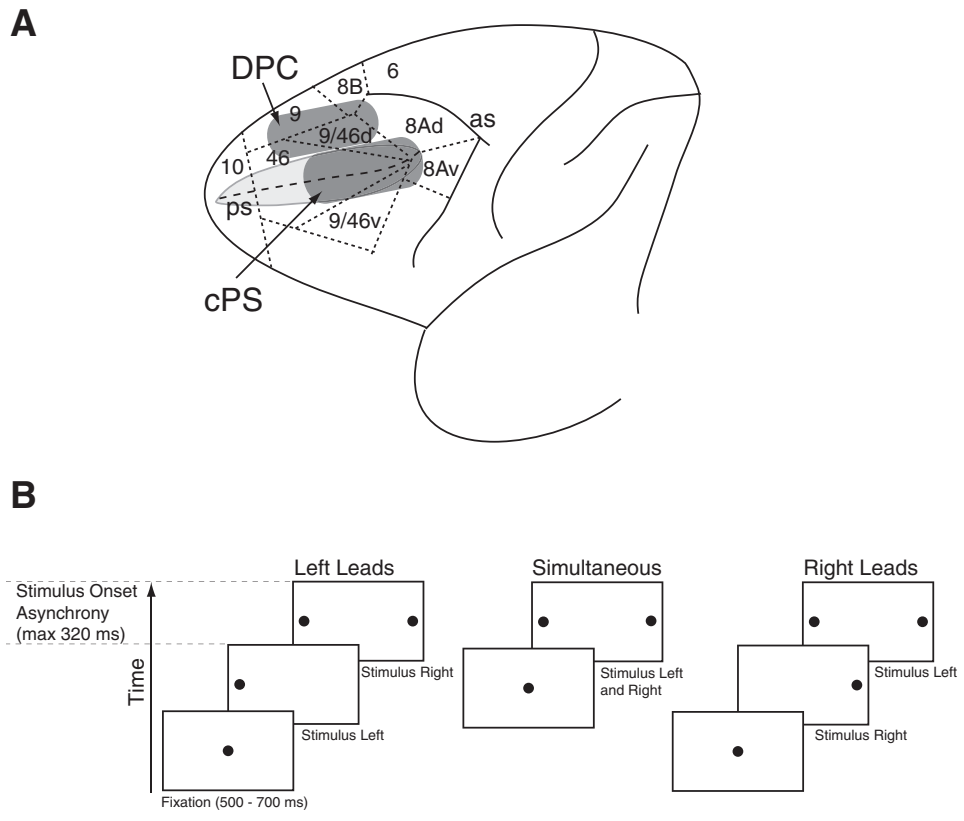

Fig. 1. *A*: lateral view of the frontal lobe of the left hemisphere of the macaque brain, showing extent of cortical area deactivated by cryoloops. Dark shaded regions depict estimated area of cortex deactivated by dorsolateral prefrontal cortex (DPC) and caudal principal sulcus (cPS) loops. as, Arcuate sulcus; ps, principal sulcus. Principal sulcus has been opened to show full extent of deactivation. *B*: free-choice task. Each trial began with presentation of a central fixation spot for a variable period. This was followed by presentation of visual stimuli in left and right hemifields, at stimulus onset asynchronies (SOA) ranging from 0 (simultaneous presentation) to 320 ms.

at a range of SOAs, including simultaneous presentation of both stimuli. Thus this task incorporated bilateral simultaneous stimulation as present in extinction tests used in human patients, but also allowed us to generate the full psychometric function describing each animal's choices.

Each trial began with the presentation of a small (0.2) white fixation stimulus at the center of the display monitor. The animals were required to fixate this stimulus within 2,000 ms, and to maintain fixation within a 0.5 × 0.5° window for a duration that varied randomly between 500 and 700 ms. Single small white visual stimuli (0.5°) were presented in each of the left and right visual fields at an eccentricity of 8° and at one of nine SOA values. At four SOA values, the stimulus in the left visual field was presented prior to that in the right, at four others the stimulus in the right visual field led that in the left, and a zero SOA condition was included in which stimuli were presented simultaneously in both hemifields. SOA conditions were presented in random order. Monkeys received a liquid reward for directing a saccade to either visual stimulus (within a 2 × 2° window) and were thus allowed to freely select either stimulus on a trial-by-trial basis. Eye movements were recorded at 500 Hz using a high-speed video eye tracker (EyeLink II, Kanata, ON, Canada). The experimental paradigm, behavior monitoring, and reward delivery were controlled using the CORTEX real-time operating system (National Institute of Mental Health, Bethesda, MD), running on two Pentium PCs.

Cryogenic deactivation. In each session we unilaterally deactivated either one or both PFC subregions into which cryloops were implanted. This resulted in three session types: *1*) combined unilateral deactivation of DPC and cPS; *2*) unilateral cPS deactivation; or *3*) unilateral DPC deactivation. The PFC of left and right hemispheres were unilaterally deactivated on separate days. Control and deactivation data were both collected within each session, during which the animals continuously performed the free-choice task. Single sessions were divided into three blocks: an initial "precooling" block in which control data were collected, a "cooling" block in which cortex was deactivated, and a "postcooling" block in which cortex was rewarmed and further control data were collected. Blocks were either 15 or 20 min in duration, and the duration of all blocks was the same within each session. The duration of each session was therefore either 45 or 60 min in total. This design allowed us to assess effects of PFC deactivation on the free-choice task independent of any day-to-day changes in the animals' behavior, and the postcooling block additionally allowed us to assess recovery from any cooling-induced bias of the animals' task performance.

During blocks of cooling trials, methanol at room temperature was pumped through Teflon tubing passing through a methanol ice bath which was reduced to subzero temperatures by the addition of dry ice. Chilled methanol was then pumped through a cryoloop and returned to the same reservoir from which it came. Cryoloop temperature was monitored by an attached microthermocouple. At the beginning of each cooling block, either one or two pumps (one for each cryoloop) were turned on, and the flow rate of the pumps was adjusted to gradually lower the temperature of the loops to 3°C. On average, this took 4 min. Data from this period of declining temperature were excluded from all analyses. Cryoloop temperature was maintained in the range of 1–5°C by adjusting the flow rate of the pump. This temperature range was chosen to inactivate as large an area of cortical tissue as possible, including the full depth of the cortex (layers I–VI), while avoiding potentially harmful subzero temperatures at the cortical surface (Lomber and Payne 2000). Using this technique, the extent of inactivated tissue is limited to a range of ~2 mm when cryoloop temperature is reduced to 1–3°C (Lomber et al. 1999). This procedure ensured that the cortical tissue adjacent to the cryoloop reached temperatures below 20°C, the threshold for neuronal deactivation (Adey 1974; Jasper et al. 1970; Lomber and Payne 2000). We were able to discount any long-term effects of repeated deactivations. Even after months or years of daily cooling deactivations, there is no alteration to either the structure or function of underlying cortex (Yang et al. 2006). We observed no indications of discomfort from the animals during any of the cooling sessions. At the end of each session, monkeys were given water until satiation and returned to their home cage.

Data analysis. For all analyses, data from the first 4 min of the cooling epoch, during which temperatures were decreasing, and the first 4 min of the postcooling epoch, during which cortical tissue was rewarming, were excluded. This ensured that all effects reported here were obtained at a steady state of cortical deactivation. To evaluate animal's performance in the free-choice task, and quantify any cooling-induced shifts in the probability of choosing stimuli in one direction, we computed the proportion of responses to stimuli presented in the hemifield contralateral to the deactivated hemisphere, and plotted these values as a function of SOA. These data were pooled for left and right hemisphere deactivations. We then fit these values with a logistic function, $y = 1/1 + e^{-k(x - x0)}$, where y is the proportion of contraversive responses at a given SOA value, $-k$ is the slope of the function, and $x0$ is the midpoint of the function. In this psychometric function, the midpoint represents the point of equal selection (PES), the SOA value at which the proportion of leftward responses was 0.5 (i.e., the probabilities of choosing the contralateral and ipsilateral stimulus were equal). To evaluate statistically any cooling-induced shifts in these psychometric functions, we carried out repeated z-tests comparing the proportion of contraversive responses between the pooled pre- and postcooling epochs, and the cooling epoch. These were performed at the three SOA values closest to the PES for the control data for each monkey, within each session type. As a further index of performance, we also carried out analyses of reaction times (RTs). The numbers of trials on which ipsi- and contraversive responses were made varied considerably as a function of SOA value, rendering an analysis of RT as a function of SOA impractical. For example, at value of 160 ms, animals often made ipsiversive saccades almost exclusively, leaving few or no contraversive saccades for analyses, while at an SOA value of 0 ms, approximately equal numbers of ipsi- and contraversive saccades were made. For this reason, and to obtain measures of deactivation-induced changes in RTs uncontaminated by the competitive influence of a second stimulus, we restricted analyses of RTs for ipsi- and contraversive saccades to the conditions with the longest SOA values. Since these values substantially exceeded the average RTs of the animals (roughly 180–250 ms), these were effectively single stimulus trials. The logic here is that, at an SOA value exceeding the animal's RT, a saccade will already have been made toward the first stimulus before the second appears on the display. Thus these first saccades at the longest SOA values could not be subject to a competitive influence from the stimulus appearing second.

These data were subjected to a 2 × 3 ANOVA with factors saccade direction (ipsiversive or contraversive to the deactivated hemisphere) and cooling epoch (precooling, cooling, or postcooling). Statistically significant interactions were investigated with Bonferroni-corrected post hoc t-tests. Saccade onset was defined as the time at which eye velocity exceeded 30°/s, and saccade end as the time at which velocity fell below 30°/s. Trials with broken or incorrect fixation were excluded from further analyses, as were trials with RTs < 80 ms (anticipations) or >1,000 ms (no response trials). All analyses were carried out using custom-designed software written in MATLAB (Mathworks, Natick, MA).

RESULTS

Data were collected in 58 sessions in which we carried out unilateral deactivations while monkeys performed the free-choice task (*Monkey B*, 31 sessions; *Monkey G*, 27 sessions). Within each session, *Monkey B* performed a mean of 700 trials, and *Monkey G* 618 trials. Within the three cooling epochs per session, an average number of 240, 233, and 237 trials were

performed by *Monkey B*, while *Monkey G* performed an average of 214, 216, and 187 trials. Because this design resulted in a relatively small number of trials at each of the nine SOA values within each epoch, within each session, data were pooled across all sessions of each session type for all analyses.

Effects of combined unilateral deactivation of DPC and cPS on free-choice performance. In the free-choice task, the performance of both animals was similar during control (pre- and postcooling) blocks, and these data were pooled for analysis. The proportion of contraversive saccades was distributed as a function of SOA, with values close to 1.0 at the greatest value on which the stimulus presented in the hemifield contralateral to the cooled hemisphere lead, declining at intermediate asynchronies, and reaching close to 0 at the greatest value on which the stimulus presented in the hemifield ipsilateral to the cooled hemisphere lead. We predicted that PFC deactivation would induce a selection bias between the stimuli such that those in the hemifield contralateral to the deactivated hemisphere would be selected less frequently, and that this would be reflected in shifts in the psychometric function relating the proportion of contraversive saccade choices and SOA value. In all cases, we predicted that the proportion of saccades to the hemifield contralateral to the cooled hemisphere would be reduced. This would lead to leftward shifts of the function and corresponding changes in PES values (see Fig. 2). Figure 3, *A* and *D*, depicts the performance of *Monkey B* and *Monkey G* during sessions in which unilateral deactivations of both DPC and cPS were carried out (*Monkey B*, Fig. 3*A*; *Monkey G*, Fig. 3*D*). For both animals, combined deactivation of DPC and cPS lead to significant leftward shifts of the psychometric function (z-test, $P < 0.016$). These changes were similarly reflected in changes in PES values for both animals (*Monkey B*, 28 ms shift; *Monkey G*, 149 ms shift). Altogether, combined unilateral deactivations of both PFC subregions resulted in selection biases such that the proportion of saccades to stimuli presented in the hemifield contralateral to the cooled hemisphere was significantly reduced. In *Monkey G*, we additionally observed a decrease in the slope of the psychometric function during deactivation, which we attributed to the fact that, for this animal, responses to contraversive stimuli never reached a proportion of 1.0, even at the longest SOA tested.

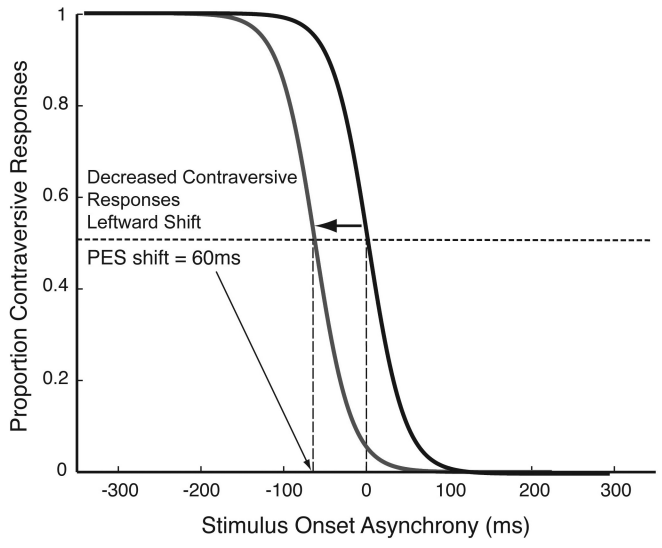

Fig. 2. Predicted shift in psychometric function relating proportion of contraversive responses to SOA values during prefrontal cortex (PFC) deactivation. At negative SOA values, stimulus in hemifield contralateral to deactivated hemisphere is presented first. At zero SOA, stimuli are simultaneous. At positive SOA values, stimulus in ipsilateral hemifield is presented first. Point of equal selection (PES) value is determined as SOA value at which psychometric function crosses 0.5 proportion of contraversive responses. During PFC deactivation, proportion of contraversive responses was predicted to decrease, leading to leftward shift of psychometric function and corresponding change in PES value relative to control (solid black line).

Effects of unilateral cPS deactivation on free-choice performance. The performance of each monkey on the free-choice task during sessions in which the cPS was deactivated is shown in Fig. 3, *B* and *E*. For *Monkey B*, we observed a slight leftward shift of the psychometric function during cPS deactivation (Fig. 3*B*), which was accounted for by significant decreases in the proportion of contraversive saccades during cooling at 0 and 40 ms SOA values (z-test, $P < 0.016$). For *Monkey G*, we observed a significant leftward shift of the function following unilateral cPS deactivation (Fig. 3*E*). These changes were also accompanied by shifts in PES values of 11 ms in *Monkey B* and 86 ms in *Monkey G*. Altogether, we observed significant selection biases following unilateral deactivation of cPS in *Monkey G*, while this effect was more limited in *Monkey B*.

Effects of unilateral DPC deactivation on free-choice performance. The performance of each monkey on the free-choice task during sessions in which the left or right DPC was unilaterally deactivated is shown in Fig. 3, *C* and *F*. For *Monkey B*, unilateral deactivation of DPC (Fig. 3*C*) resulted in negligible shifts in the psychometric function (z-test, $P > 0.05$). For *Monkey G*, we observed a significant leftward shift following unilateral DPC deactivation (z-test, $P < 0.016$, Fig. 3*F*), and a corresponding shift in PES values (24 ms). Thus unilateral DPC deactivation resulted in selection bias in one of two animals.

Effects of combined unilateral deactivation of DPC and cPS on RT in the free-choice task. To investigate cooling-induced changes in RT, we carried out for each animal, and for each area deactivated, a 2 × 3 repeated-measures ANOVA with factors saccade direction (ipsi- or contraversive) and epoch (precooling, cooling, or postcooling). Based on previous studies investigating unilateral PFC deactivation on RTs in saccade tasks (Johnston et al. 2014), we predicted that any effects would be evident as increases in RTs for saccades contraversive to the deactivated hemisphere, and decreases for those ipsiversive to the deactivated hemisphere. Such effects would be apparent as a direction × epoch interaction in these analyses. Figure 4, *A* and *D*, presents RTs for *Monkeys B* and *G* for sessions in which both DPC and cPS were unilaterally deactivated. For both animals, we observed significant direction × epoch interactions for unilateral deactivations [*Monkey B*, $F(1,2) = 10.853$, $P < 0.01$; *Monkey G*, $F(1,2) = 63.741$, $P < 0.001$]. In *Monkey B* (Fig. 4*A*), for ipsiversive saccades, post hoc t-tests revealed a trend toward a reduction in RTs between precooling and cooling epochs [$t(181) = 1.885$, $P = 0.061$] and a significant increase in RTs between cooling and postcooling epochs [$t(186) = -5.123$, $P < 0.01$]. For contraversive saccades, RTs increased significantly between precooling and cooling epochs [$t(183) = -7.947$, $P < 0.001$], and recovered following cooling, showing a significant decrease between cooling and postcooling epochs [$t(183) = 3.429$, $P < 0.01$].

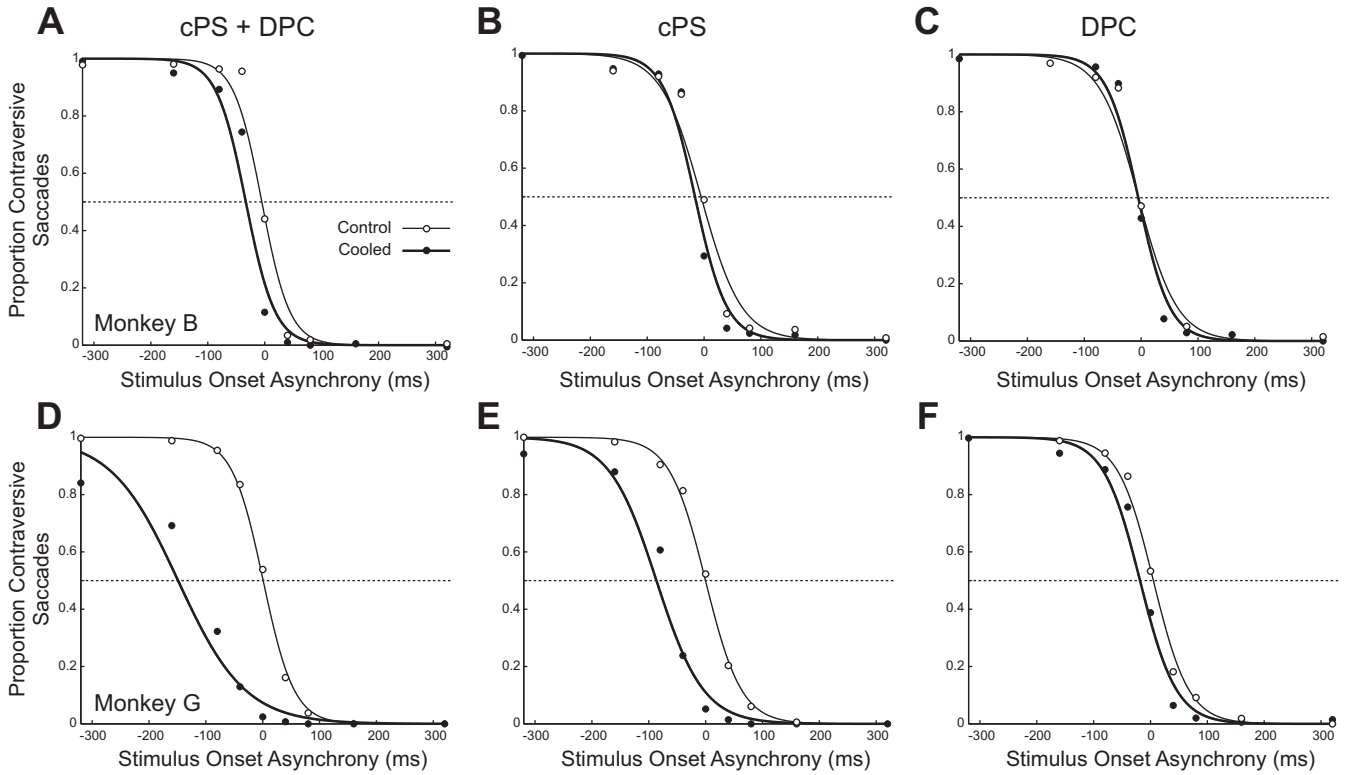

Fig. 3. Proportion of saccades to stimulus in hemifield contralateral to deactivated hemisphere as a function of SOA. In all panels, thin lines depict control data; thick lines, cooling data. *A* and *D*: deactivation of DPC + cPS for *Monkey B* (*A*) and *Monkey G* (*D*). *B* and *E*: deactivation of cPS for *Monkey B* (*B*) and *Monkey G* (*E*). *C* and *F*: deactivation of DPC for *Monkey B* (*C*) and *Monkey G* (*F*).

We also observed significant increases in RTs between pre- and postcooling epochs for both ipsiversive [$t(181) = -2.842$, $P = 0.005$] and contraversive [$t(183) = -4.310$, $P = 0.000$] saccades.

For *Monkey G*, we observed similar effects (Fig. 4*D*). RTs for saccades ispilateral to the deactivated hemisphere were decreased during cooling compared with the precooling epoch [$t(205) = 9.072$, $P < 0.001$] and recovered following cooling, showing a significant increase between cooling and postcooling epochs [$t(205) = -11.054$, $P < 0.001$]. RTs of saccades contralateral to the deactivated hemisphere were increased during cooling relative to the precooling epoch [$t(169) = -11.78$, $P < 0.001$] and significantly decreased between cooling and postcooling epochs [$t(169) = 9.553$, $P < 0.001$]. An RT increase between pre- and postcooling epochs was additionally observed for contraversive saccades [$t(169) = -3.886$, $P = 0.000$].

Overall, combined unilateral deactivations of both DPC and cPS lead to consistent increases in RTs for saccades contralateral to the deactivated hemisphere in both *Monkeys B* and *G*, and additionally to decreased RTs of saccades ipsilateral to the deactivated hemisphere in *Monkey G*.

Although we observed increases in RT between pre- and postcooling epochs in both animals, we believe this was attributable to incomplete recovery from deactivation, or within-session fatigue effects.

Effects of unilateral deactivation of cPS on RT in the free choice task. RTs for *Monkeys B* and *G* on sessions in which the cPS was deactivated are depicted in Fig. 4, *B* and *E*. *Monkey B* (Fig. 4*B*) exhibited a significant direction × epoch interaction [$F(1,2) = 12.91$, $P > 0.001$]. Post hoc *t*-tests revealed that, for ipsiversive saccades, RTs decreased significantly between precooling and cooling epochs [$t(647) = 3.248$, $P < 0.01$] and increased significantly between cooling and postcooling epochs [$t(542) = -8.615$, $P < 0.001$]. For contraversive saccades, RTs increased significantly between precooling and cooling epochs [$t(621) = -3.871$, $P < 0.001$], but failed to recover following cooling, showing instead a significant increase between cooling and postcooling epochs [$t(516) = -3.403$, $P < 0.01$]. Significant increases in RTs between pre- and postcooling epochs were additionally observed for both ipsiversive [$t(543) = -5.889$, $P = 0.000$] and contraversive [$t(517) = -8.195$, $P = 0.000$] saccades.

Monkey G also exhibited a significant direction × epoch interaction for sessions in which the cPS was unilaterally deactivated [$F(1,2) = 149.06$, $P < 0.001$; Fig. 4*E*]. For ipsiversive saccades, RTs were significantly lower during the cooling than precooling [$t(205) = 9.07$, $P < 0.001$] or postcooling [$t(205) = -11.05$, $P < 0.001$] epochs. RTs for contraversive saccades were elevated in the cooling, compared with precooling epoch [$t(169) = -11.78$, $P < 0.001$], and recovered following deactivation, showing a significant decrease between the cooling and postcooling epochs [$t(169) = 9.55$, $P < 0.001$]. We also observed a significant increase in RTs between pre- and postcooling epochs for contraversive saccades [$t(233) = -3.886$, $P = 0.000$].

Overall, for cPS deactivation we observed for both *Monkey B* and *Monkey G* decreased RTs for saccades directed ipsilateral to the cooled hemisphere which recovered following cooling. For contraversive saccades, we observed increases in RTs during cooling, which recovered only in *Monkey G*. As with combined DPC and cPS sessions, we observed RT in-

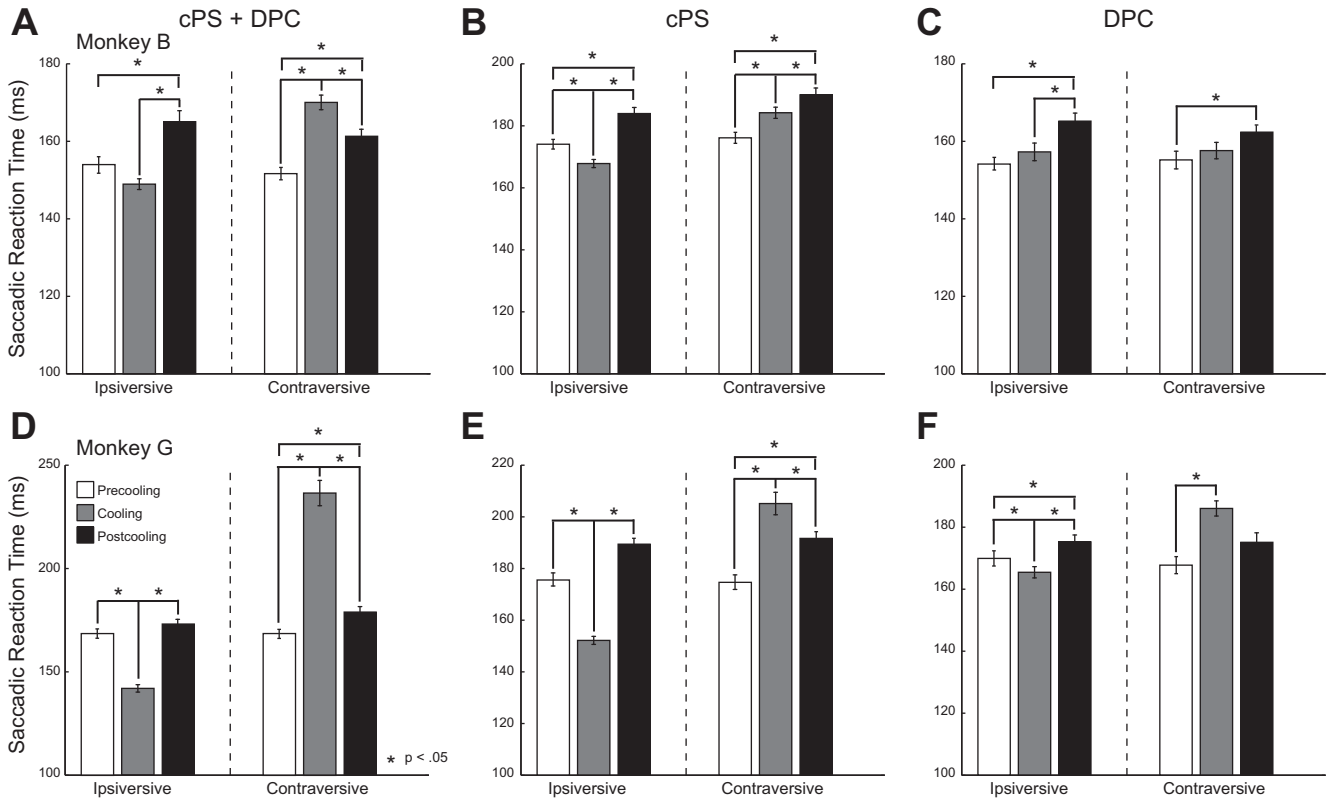

Fig. 4. Reaction times for saccades to stimuli in hemifield ipsilateral and contralateral to deactivated hemisphere for precooling, cooling, and postcooling epochs during unilateral PFC deactivation. *A–C*: reaction times for *Monkey B* during unilateral deactivation of cPS + DPC, cPS, and DPC, respectively. *D–F*: data for *Monkey G*. Values are means ± SE. *Significance at 0.05 level.

creases between pre- and postcooling epochs consistent with incomplete recovery or fatigue effects.

Effects of unilateral deactivation of DPC on RT in the free choice task. Figure 4, *C* and *F*, depict RTs for sessions in which the DPC was unilaterally deactivated. For *Monkey B* (Fig. 4*C*), we observed only a significant main effect of epoch [$F(1,2) = 10.408$, $P < 0.001$], resulting from an increase in RTs between cooling, and postcooling epochs for ipsiversive saccades [$t(110) = -2.274$, $P < 0.05$], and between pre- and postcooling epochs for contraversive saccades [$t(115) = -2.42$, $P < 0.05$]. An increase in RTs between pre- and postcooling epochs was also observed for both ipsiversive [$t(110) = -4.020$, $P = 0.000$] and contraversive [$t(115) = -2.421$, $P = 0.017$] saccades.

For *Monkey G*, a significant direction × epoch interaction was observed for DPC deactivation [Fig. 4*F*, $F(1,2) = 5.432$, $P < 0.01$]. Post hoc *t*-tests revealed a significant decrease in RT between the precooling and cooling epochs for ipsiversive saccades [$t(215) = 2.662$, $P < 0.01$], which recovered between cooling and postcooling epochs [$t(176) = -3.16$, $P < 0.001$]. For contraversive saccades, RTs increased between precooling and cooling epochs [$t(215) = 2.662$, $P < 0.01$], but recovery following cooling was incomplete, as evidenced by a nonsignificant decline in RTs between cooling and postcooling epochs [$t(163) = 1.652$, $P = 0.101$]. Finally, we observed an increase in RTs between pre- and postcooling epochs for ipsiversive saccades only [$t(176) = -3.886$, $P = 0.000$].

Altogether, we observed the predicted RT differences only for ipsiversive saccades and only in *Monkey G*. As with combined DPC and cPS, and cPS deactivation alone, we observed increases between pre- and postcooling epochs in both animals, which were consistent with within-session fatigue effects.

DISCUSSION

Numerous lines of evidence have linked PFC function to attentional processes. In both human patients (Heilman and Valenstein 1972; Knight et al. 1995), and nonhuman primates (Deuel and Farrar 1993; Rossi et al. 2007), PFC lesions have been shown to lead to impairments on a variety of tasks requiring the deployment of attention. A substantial and increasing body of neurophysiological evidence from single-neuron recordings in primates has also revealed neuronal responses consistent with this view (Everling et al. 2002; Kaping et al. 2011; Lebedev et al. 2004). Studies specifically investigating the role of primate PFC in selection processes analogous to extinction, an inability to detect a stimulus presented in the hemifield contralateral to the damaged hemisphere when another stimulus is presented simultaneously in the ipsilateral hemifield, have used almost exclusively the lesion approach and have removed large areas of tissue, including the FEF, as well as more anterior regions, including portions of areas 8a and 46 (Eidelberg and Schwartz 1971; Schiller and Chou 1998). Those using pharmacological approaches have been restricted to the FEFs (Moore and Noudoost 2011; Schiller and Tehovnik 2003). Here, we used unilateral reversible cryogenic deactivation to investigate the contribution of two prefrontal subregions anterior to the FEF in a free-choice task modeled on those used to investigate the extinction phenomenon in human patients (Di Pellegrino et al. 1997).

This task has also been used to investigate the roles of FEF (Schiller and Chou 1998; Schiller and Tehovnik 2003) and PPC (Lynch and McLaren 1989; Wardak et al. 2002) in stimulus selection in nonhuman primates.

PFC deactivation resulted in predictable shifts in both animals' selection of stimuli in the free-choice task, such that selection was biased away from the stimulus in the hemifield contralateral to the deactivated hemisphere. We quantified these effects using PES values, which may be taken to reflect the duration by which the contraversive stimulus was required to lead the ipsiversive stimulus to reach an equal probability of selection. This value varied substantially, depending upon which prefrontal subregion was deactivated. Effects were generally small and nonsignificant during left or right DPC deactivation, were larger for cPS deactivation, and greatest when both the DPC and cPS were unilaterally deactivated. We generally observed larger effects in *Monkey G* than *Monkey B*. Across all three conditions, the change in PES values during cooling ranged from 14 to 150 ms and 11 to 30 ms in each of the two animals, respectively. Thus, for combined DPC and cPS deactivations in *Monkey B*, the stimulus contralateral to the deactivated hemisphere was required to lead the stimulus ipsilateral to the deactivated hemisphere by 30 ms to reach equal selection, while for *Monkey G*, the lead-time required for the stimulus contralateral to the deactivated hemisphere was 150 ms. These values straddle those observed for lesions of the SEF or FEF in previous work. Schiller and Chou (1998) reported values of 31 ms for SEF lesions, and 117 ms for lesions of FEF. For reversible FEF deactivations using muscimol, this value was substantially smaller, being ~50 ms. Thus, for *Monkey B*, the maximal shifts we observed were roughly the same as those resulting from SEF lesions and roughly comparable with those observed during reversible pharmacological deactivation of FEF. In *Monkey G*, these values were substantially greater than those observed in previous studies of frontal cortex, but were comparable to those observed following pharmacological deactivation of cortex within the intraparietal sulcus, in which contralesional stimuli were either not selected, or selected at PES values of 200 ms or greater (Wardak et al. 2002). We attribute the variability between animals to variations in the degree of contact area of the cryoloops with the cortical surface and in the principal sulcus. Since loops were placed based on the position of sulcal landmarks, it is unlikely that variability in the cytoarchitectural areas deactivated by the loops accounts for the differences we observed here. Although we made every effort to ensure that the amount of cortical tissue contacted by the loops was consistent across animals and hemispheres, this is difficult to control surgically and can result in substantial variations in the volume of cortical tissue deactivated. Horel et al. (1984) reported temperature variations of 10°C over distances as little as 0.5 mm, which would be sufficient to change substantially the extent of cortex maintained below 20°C, the threshold for deactivation. Indeed, in human patients, the extent of attentional impairment has been associated with the volume of lesions in the PFC (Peers et al. 2005). Although an ideal approach would be to measure tissue temperature, or carry out neural recordings in the vicinity of the cryoloops within each animal to verify the degree of deactivation, this is technically difficult to accomplish, and the use of microelectrode arrays or implantation of arrays of cryodes within the cortex tends to produce undesirable tissue damage. Such studies have been carried out in both rhesus macaques and cats, to verify thermoclines and extent of neural deactivation surrounding cryoloops (Horel 1984; Lomber et al. 1996; Payne and Lomber 1999). These technical issues are discussed in detail by Lomber et al. (1999).

It is important to note that, although the magnitude of effects varied, the pattern of deficits across cortical areas was consistent between both animals. In particular, we note the substantial differences in the proportion of trials on which stimuli presented in the hemifield contralateral to the deactivated hemisphere were selected during simultaneous presentation of both stimuli between the control and cooling conditions. In *Monkey G*, the proportion of saccades to contralateral stimuli in the simultaneous condition declined by 0.51, 0.48, and 0.14 for cPS + DPC, cPS, and DPC cooling, respectively. In *Monkey B*, these values were 0.32, 0.19, and 0.04. While it is critical to acknowledge the limitations of our experimental paradigm, it is interesting that these effects resemble those observed in patients with extinction, in which the failure to report the contralesional stimulus is maximal when stimuli are presented simultaneously (Baylis et al. 2002; Di Pellegrino et al. 1997).

In addition to biasing selection away from contralateral stimuli, we observed changes in RTs for stimuli presented at the longest SOA values. These trials were effectively single stimulus trials, as they were presented at onset asynchronies greatly exceeding the animals' saccade latencies. As with the observed shifts in PES values, we found that these changes were smallest or nonsignificant for DPC activation, greater for cPS deactivation, and greatest when both areas were unilaterally deactivated. These changes in RT were observed as either increases for stimuli contralateral to the cooled hemisphere, decreases for stimuli ipsilateral to the cooled hemisphere, or both. These RT changes mirror previous observations from previous studies of unilateral cPS deactivation in our laboratory (Johnston et al. 2014), in which we observed increases in RTs for saccades made to stimuli in the hemifield contralateral to the deactivated hemisphere, as well as decreases in RTs for saccades made to stimuli in the hemifield ipsilateral to the deactivated hemisphere. The conditions in which the greatest PES shifts were observed corresponded with those in which the greatest changes in RTs were also observed. For example, we found the greatest shifts in PES values for combined DPC and cPS deactivation in *Monkey G* and also observed both increases in RT for contralesional stimuli and decreases for ipsilesional stimuli in this condition. Similarly, we found a nonsignificant shift in PES value for this animal during right DPC deactivation, and no effects on RT, while a significant shift in selection co-occurred with increases and decreases in stimuli in the hemifields ipsi- and contralateral to the deactivated hemisphere for left DPC deactivation. Altogether, we observed that deactivation-induced changes in the probability of selecting one of the two stimuli were associated with predictable shifts in RTs for single stimuli. This finding is consistent with observations from other studies investigating the effects of frontal cortex lesions in this task. Schiller and Chou (1998) observed similar increases in RTs of saccades to contralesional stimuli and decreases for ipsilesional stimuli following FEF lesions, in addition to stimulus selection biases. Interestingly, in that study, both selection biases and RT changes were less pro-

nounced following lesions of the SEF, similar to the differences in magnitude of both RT and selection biases between the DPC and cPS loops we observed here. Although our laboratory has previously reported decreases in saccade peak velocity and increases in saccade duration during unilateral dorsolateral PFC deactivation (Koval et al. 2014), these changes are generally small, and we do not believe they contributed to the selection biases we observed here, since they reflect changes in motor output occurring after the initial selection process.

It is important to note that there are some procedural differences between the oculomotor free-choice paradigm as used in the rhesus macaque and extinction tests in human patients. Patients are typically asked to report either verbally or via button-press the presence or identity of a stimulus (Di Pellegrino et al. 1997; Driver et al. 1997). In the oculomotor version of the task employed here, the visual stimulus and motor response used to select it are directly linked, thus leaving open that deficits are the result of deactivation-induced motor biases rather than impairments in attentional selection per se. We have obtained findings bearing on this issue in a previous study in which we investigated the effects of unilateral cPS deactivation on performance of the antisaccade task (Johnston et al. 2014). In this task, the animals are required to generate a saccade in the direction opposite the visual stimulus, thus dissociating processes related to stimulus processing and saccade generation (see for review Munoz and Everling 2004). We observed increases in RTs during presentation of the visual stimulus to the intact hemisphere, suggesting an impairment of motor processes, and on trials in which the visual stimulus was presented to the deactivated hemisphere, suggesting an impairment of processes related to stimulus processing. These changes were mirrored by alterations in the stimulus and saccade-related activity of single neurons recorded simultaneously in the superior colliculus an oculomotor structure critical for saccade generation. Another previous study observed alterations in free-choice task performance, as well as the magnitude and selectivity of visual responses of single neurons in V4 following manipulations of D1R in the FEF (Moore and Noudoost 2011). It would appear, therefore, that both motor and sensory, or attentional, processes are affected by deactivation of the PFC. Future work could directly investigate the contributions of deactivation-induced sensory and attentional impairments to changes in performance of the free-choice task by systematically varying the contrast, and hence relative strengths of saccade targets, a technique that has been employed in previous human work (Pavlovskaya et al. 2007).

Several theoretical accounts of the extinction phenomenon in human patients have been proposed. In general, these propose that deficits in selection of contralesional stimuli during conditions in which stimuli compete for selection result from either an imbalance between the cerebral hemispheres (Kinsbourne 1987), or an imbalance in weighting of stimuli (Desimone and Duncan 1995), which results when one stimulus is presented in the lesioned while the other is presented in the intact hemifield (de Haan et al. 2012). These accounts are consistent with both the reduced selection of contralesional stimuli and the increases in RTs of saccades to contralesional stimuli we found here. In addition, they predict a competitive advantage for ipsilesional stimuli. We observed partial support for this in the form of decreased RTs for saccades made to ipsilesional stimuli under some conditions. Ultimately, a full understanding of such mechanisms awaits further studies combining deactivation and electrophysiology.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: K.J. and S.E. conception and design of research; K.J. performed experiments; K.J. analyzed data; K.J. and S.G.L. interpreted results of experiments; K.J. prepared figures; K.J. drafted manuscript; S.G.L. and S.E. edited and revised manuscript; S.E. approved final version of manuscript.

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