Neuronal Correlates of Post-Error Slowing in the Rat Dorsomedial Prefrontal Cortex

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INTRODUCTION

The rodent prefrontal cortex is comprised of the anterior cingulate, prelimbic, and infralimbic areas (Gabott et al. 2005). During simple reaction time tasks, inactivation of the dorsal part of the prelimbic cortex and the adjacent pregenual anterior cingulate cortex, which we collectively call the dorsomedial prefrontal cortex (dmPFC), has been shown to increase the frequency of premature responding (Narayanan et al. 2006; Risterucci et al. 2003). Single neurons within dmPFC fire prominently on error trials (Narayanan and Laubach 2006). Furthermore, the activity of delay-modulated neurons in dmPFC is predictive of premature responding (Narayanan and Laubach 2006). Rodent dmPFC is necessary for goal-directed behavior (Corbit and Balleine 2003; Killcross and Coutureau 2003; Ostlund and Balleine 2005) and is engaged in spatial working-memory tasks (Baeg et al. 2003; Batuev et al. 1990; Ragozzino et al. 1998). These studies suggest that error-sensitive neurons within dmPFC may maintain information about past outcomes (i.e., errors) to guide future actions.

In primates, neurons in medial prefrontal regions, including anterior cingulate, supplementary motor regions, and superior frontal gyrus, are prominently modulated after errors (Amiez et al. 2006; Niki and Watanabe 1976; Ridderinkhof et al. 2004; Rushworth et al. 2004, 2007; Schall et al. 2002; Walton et al. 2007). These studies implicate primate medial frontal areas in posterior processing. dmPFC in primates may not be directly homologous to rodent dmPFC (Preuss 1995; Uylings et al. 2003). Nevertheless, it has been argued that these areas mediate similar behavioral functions (Uylings et al. 2003). Across species, medial prefrontal cortex may monitor behavioral performance (van Veen et al. 2004) and integrate information about prior behavior to control future actions (Bush et al. 2002; Dalley et al. 2004; Ridderinkhof et al. 2004; Rushworth et al. 2004; Schall et al. 2002; Shima et al. 2007).

In the present study, we tested the hypothesis that rodent dmPFC is involved in posterror processing. Rats were trained to perform a simple reaction time task in which they held a lever down over a delay period of 1.0 s. Lever releases that occurred too early or too late were scored as errors and were unrewarded. After errors, animals showed a slowing of reaction times (RTs). That is, RTs were longer on trials that were preceded by an error than on trials that were preceded by a correct response. In one experiment, we inactivated dmPFC and found that rats showed attenuated posterror slowing of RTs. In a second experiment, we recorded neural activity in dmPFC and found that many dmPFC neurons increased their firing rates after errors and maintained such elevated firing into the delay period on the following trial. This pattern of neural activity was not observed in motor cortex. Together, our results suggest that dmPFC neurons are involved in posterror slowing and may mediate a form of retrospective working memory that improves task performance following errors.

METHODS

Twenty-two Long-Evans rats (aged 3–4 mo, male) were trained to perform a simple RT task using standard operant procedures and by motivation through water restriction (Narayanan et al. 2006). To perform this task correctly, animals had to press and hold a lever for a 1.0-s delay period and release the lever promptly (within 0.6 s) to receive a liquid reward (0.15 ml of water). The end of the delay period was signalled by a 100-ms, 72-dB, 8-kHz tone. Reaction time was defined as the latency between the end of the delay period and lever release. In some recording sessions, tones were omitted on 50% of trials (catch trials); no difference in behavior or neural activity has been found on such trials (Narayanan and Laubach 2006; Narayanan et al. 2006). If animals released the lever prior to the end of the 1.0-s delay or after the 0.6-s response window, then these trials were scored as errors (premature or late, respectively), and all behavioral devices (pump, lever, and houselight) were extinguished for 4–8 s (Fig. 1A). Seven animals were tested in sessions without posterror timeouts and in sessions with distractors [lever vibration at 50 Hz using a vibration shaker (Bruel and Kjaer, Norcross, GA) and a 72-dB, 8-kHz tone presented for 1 s immediately after pump inactivation on 50% of pseudorandomly chosen correct trials].

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Reversible inactivation of dmPFC was performed according to procedures described previously (Narayanan et al. 2005, 2006). Briefly, 33-gauge cannulae (Plastics One) were implanted into the dorsal prelimbic region (coordinates from bregma: AP +3.2, ML ±1.4, DV −3.6 @ 10° in the frontal plane; Fig. 1B) (Paxinos and Watson 1982) of seven fully trained animals via aseptic surgical procedures. One week after surgery, animals were lightly anesthetized with halothane via a nosecone for 7 min and tested in the simple RT task 45 min after recovery from anesthesia. On the first day of testing, animals were run without manipulation. Infusion was conducted by inserting injectors into the guide cannula and 0.5 ml saline (Phoenix Scientific, St. Joseph, MO) was infused into dmPFC at 0.1 mg/ml (inactivation sessions). On the second day of testing, muscimol, a GABA_A receptor agonist (Sigma-Aldrich, St. Louis, MO) (Lomber 1999; Martin and Ghez 1999; Narayanan et al. 2006), was infused into dmPFC at 0.1 mg/ml (inactivation sessions). On the third day of testing, animals were run without manipulation. Infusion was conducted by inserting injectors into the guide cannula and 0.5 ml of infusion fluid was delivered per site at a rate of 15 μl/h (0.25 μl/min) (Martin and Ghez 1999) via a syringe infusion pump (KDS Scientific, Holliston, MA). After injection was complete, the injector was left in place for 2 min to allow for diffusion. Rats were tested in the simple RT task 45 min after the start of the infusions.

In these animals, microelectrodes configured in 4 × 4 arrays of 50-μm stainless steel wires (250 μm between wires; impedance measured in vitro at 100–300 kΩ; Neurolinc) were implanted into rat motor cortex (7 animals; coordinates from bregma: AP −0.5, ML ±2.5–3.5, DV −1.5 @ −25° in the frontal plane; 1 animal had poor recordings and was excluded from neural analyses) according to methods described in detail previously (Laubach et al. 2000; Narayanan and Laubach 2006; Narayanan et al. 2005). In eight additional animals, microelectrode arrays were implanted into the dorsal prelimbic region of rat frontal cortex (8 animals; coordinates from bregma: AP +3.2, ML ±1.4, DV −3.6 @ 10° in the frontal plane; ~94% of electrodes were in precentral cortex) targeting coordinates of previous inactivation (Narayanan and Laubach 2006; Narayanan et al. 2006) (Fig. 1B).

Neuronal ensemble recordings were made using a multi-electrode recording system (Plexon, Dallas, TX). Putative single neuronal units were identified on-line using an oscilloscope and audio monitor. The Plexon off-line sorter was used to analyze the signals off-line and to remove artifacts. Principal component analysis and waveform shape were used for spike sorting. Single units were identified as having consistent waveform shape, separable clusters in PCA space, average amplitude estimated at least three times larger than background activity, a consistent refractory period of 2 ms in interspike interval histograms, and consistent firing rates around behavioral events (as measured by a runs test of firing rates across trials around behavioral events; neurons with [z] scores >4 were considering “nonstationary” and were excluded). Analysis of neuronal activity and quantitative analysis of basic firing properties were carried out using Stranger (Biographics, Winston-Salem, NC), NeuroExplorer (Nex Technologies, Littleton, MA) and with custom routines for MATLAB. Peri-event rasters and average histograms were constructed around lever release, lever press, and tone offset.

Once experiments were complete, rats were anesthetized and killed by injections of 100 mg/kg sodium pentobarbital and then were transcardially perfused with either 10% formalin or 4% paraformaldehyde. Brains were sectioned on a freezing microtome, mounted on gelatin-subbed slides, and stained for Nissl with thionin.

The Animal Care and Use Committee at the John B. Pierce Laboratory approved all procedures.
responses (lever released before the end of the delay period) and 17 ± 2% of these being late responses (lever released >0.6 s after the end of the delay period). Animals exhibited posterror slowing of RTs (defined as the time between stimulus onset at the end of the delay period and lever release); that is, RTs on correct trials that were preceded by errors were slower (0.283 ± 0.09 s) than RTs on correct trials that were preceded by correct responses [0.253 ± 0.008 s; paired \( T_{(1,24)} = 3.08, P < 0.005 \); repeated-measures ANOVA \( F(1,1622) = 28.85, P < 0.05 \); within-subjects analysis revealed that 9 of 15 animals exhibited posterror slowing; group analyses included 25 sessions from all 15 animals]. RTs were slowed following both premature errors [paired \( T_{(1,24)} = 2.24, P < 0.03 \)] as well as following late errors [paired \( T_{(1,20)} = 3.61, P < 0.002 \)]. There was no difference between RTs on trials preceded by premature errors (0.286 ± 0.01 s) and by late responses (0.292 ± 0.01 s; paired \( T_{(1,20)} = 0.82, P < 0.42 \); 4 sessions with <5 late trials were excluded]. Posterror slowing was also observed in sessions without posterror timeouts [i.e., where houselight and devices were not extinguished after errors; paired \( T_{(1,6)} = 3.84, P < 0.009 \)]. However, if a distracting stimulus (tone and lever vibration lasting 1 s after pump activation after 50% of correct trials] was presented after correct trials, posterror slowing was eliminated [paired \( T_{(1,6)} = 0.41, P < 0.70 \) on postdistractor trials]. This effect may have been due to increased variability of RTs following the distractor [postdistractor RT SEs = 0.02 s; postcorrect RT SEs = 0.014 s; paired \( T_{(1,6)} = 2.19, P < 0.07 \); no difference between postdistractor and postcorrect RTs: paired \( T_{(1,6)} = 1.05, P < 0.33 \)].

Posterror slowing developed consistently after 16 days of training [paired \( T_{(1,6)} = 3.43, P < 0.01 \); learning experiments done with 7 separate animals; Fig. 2A], several days after animals’ performance reached criterion of 60% correct responses (reached on day 11 at 62 ± 7%; Fig. 2B). Early in training, animals rarely exhibited posterror slowing [only on day 5; paired \( T_{(1,6)} = 3.28, P < 0.02 \)], suggesting that posterror slowing emerged as a feature of skilled performance and not of learning the basic procedure associated with the simple RT task.

To assess the role of dmPFC in posterror slowing, muscimol was used to inactivate dmPFC in seven additional rats. With dmPFC inactivated, animals did not exhibit posterror slowing. That is, posterror RTs (0.252 ± 0.018 s, 95% CI = 0.217–0.288 s across animals) were equivalent to postcorrect RTs [0.250 ± 0.037 s, CI = 0.178–0.321 s; paired \( T_{(1,5)} = 0.09, P < 0.94 \)]. Importantly, these animals exhibited posterror slowing of RTs in control sessions [posterror RTs: 0.288 ± 0.011 s, CI = 0.267–0.3101 s; postcorrect RTs: 0.245 ± 0.011 s, CI = 0.224–0.265 s; paired \( T_{(1,6)} = 2.95, P < 0.03 \)] and in recovery sessions run 24 h after the inactivation sessions [posterror RTs: 0.265 ± 0.015 s, CI = 0.235–0.294 s; postcorrect RTs: 0.241 ± 0.021 s, CI = 0.200–0.283 s; paired \( T_{(1,6)} = 2.49, P < 0.05 \)]. The loss of posterror slowing of RTs in dmPFC inactivation sessions was due to speeding of posterror RTs in dmPFC inactivation sessions [paired \( T_{(1,6)} = 2.61, P < 0.04 \); Fig. 2C]. Note that with dmPFC inactivated, animals’ correct responding significantly decreased.

**FIG. 2.** Posterror slowing of reaction times (RTs). A: RTs following correct trials (black line) decreased over learning but were consistently faster than RTs following error trials (gray line) only after the 16th day of training. Asterisk indicates significant posterror slowing (\( P < 0.05 \)). B: learning of the simple RT task. Animals increased their correct responses (black line) over the course of several days, reaching 60% on the 11th day of training. Premature errors (dark gray line) decreased over training [paired \( T_{(1,6)} = 10.2, P < 0.001 \) on day 1 vs. 11], and late errors (light gray line) decreased somewhat [paired \( T_{(1,6)} = 2.00, P < 0.09 \) on day 1 vs. 11]. C: posterror slowing in control sessions was attenuated in sessions with dmPFC inactivated, as RTs became equivalent following errors and correct trials. Asterisk indicates significant posterror slowing (\( P < 0.05 \)).
compared with control sessions [dmPFC inactivation sessions: 40 ± 4% of responses, CI = 33–48%; control sessions: 58 ± 3%, CI = 52–64%; paired T_{(1,6)} = 4.01, P < 0.007], primarily because of increased premature responding [dmPFC inactivation sessions: 41 ± 5% of responses, CI = 30–52%; control sessions: 25 ± 3%, CI = 22–30%; paired T_{(1,6)} = 2.80, P < 0.03].

FIG. 3. Errors influence persistent activity of dmPFC neurons. A: sequence of events following correct and error trials. Following correct trials (top line, black), a pump is activated at a latency of 100 ms after lever release and is kept on for 1 s. Following error trials (bottom line, gray) the house lights and all behavioral devices are extinguished for 4–8 s. House lights then come on 1.5 ± 0.2 s prior to posterror lever presses. B–E: examples of neurons that fired differently depending on trial outcome are shown. B and C: neurons fired more if trials ended in error (gray colors, left panel) than if trials were correct and rewarded (black, left panel). Increased posterror firing (gray colors, right panel) persisted into the delay period of the following trial, and for these neurons, was more than postcorrect firing (black, right panel). Shaded region after lever press on right panel was used to identify delay-related posterror differences in firing rate. No difference was observed between premature (dark gray) and late (light gray) errors; see text.
These results implicate dmPFC in posterror slowing of RTs. To test this idea, we recorded from 194 single rodent dmPFC neurons (15 sessions, 8 animals) during simple RT task performance. Of these, 30% (58 of 194) of dmPFC neurons had significant posterror differences in delay-related firing (0.25–1 s after lever press; Wilcoxon rank-sum \( P < 0.05 \), more than were influenced by the outcome (correct vs. error) of the second trial back (14 of 194, or 7%; \( \chi^2 = 33.02, P < 0.001 \)). That is, neural activity of these neurons during the delay period while animals were waiting to respond was significantly different depending on whether the previous trial was correct or resulted in an error.

Two-thirds of neurons (39 of 58, or 64%) with posterror differences in delay-related firing rate also had significant posterror differences in firing rate during the intertrial interval (ITI; 1–2 s after lever release; Wilcoxon rank-sum \( P < 0.05 \); mean ITI = 7.21 ± 0.45 s). Most of these neurons (26 of 39, 67%) had increased firing rates after errors. Of these, 16 neurons (of 58, or 28%; 8% of all dmPFC neurons) had significantly increased posterror activity that persisted into the delay period of the following trial. Examples of such neurons are shown in Fig. 3. Sometime after the lever release, the firing rates of such neurons diverged by ~25% (left panels) depending on trial outcome (correct or error). On the following trial, when rasters were sorted by the previous trial outcome (i.e., if the previous trial was correct or an error; right panels), differences in firing rate could persist into the delay period of the following trial when animals were holding down the lever. Few of these neurons fired differently on premature errors (dark gray) versus late errors (light gray; 3 of 39; 7%; no different from chance at \( P < 0.05 \); \( \chi^2 = 0.21, P < 0.64 \)).

Some dmPFC neurons showed increased delay-related firing after errors as well as after correct trials. To quantify this effect, we calculated a modulation index for posterror differences in delay-related firing rate according to the following formula: 

\[
\text{FR}_{\text{postCorrect}} - \text{FR}_{\text{postError}}) / (\text{FR}_{\text{postCorrect}} + \text{FR}_{\text{postError}})
\]

This index is close to 1 if delay-related firing is stronger after correct trials. Conversely, this index is close to -1 if delay-related firing is stronger after error trials. For the subpopulation of neurons that showed significant differences in delay-related firing rate as a function of preceding trial outcome, 45% of 58 neurons had stronger delay-related firing following correct trials and 55% of 58 neurons had stronger delay-related firing following error trials (Fig. 4A).

To compare these patterns of neural activity to that of the motor cortex, we recorded from 80 motor cortical neurons (6 animals, 8 sessions) during the simple RT task. Of these, 18 neurons (23%) had both delay- and ITI-related differences in firing rates following correct and error responses, a fraction that was similar to our data from dmPFC (\( \chi^2 = 0.20, P < 0.67 \)). In contrast to dmPFC, there were no neurons in motor cortex that showed significantly increased posterror activity that persisted into delay period of the following trial. Furthermore, most motor cortex neurons with delay-related posterror differences in firing rate had stronger delay-related firing following correct trials (23 of 29, or 79%; \( \chi^2 = 6.41, P < 0.01 \)) and less delay-related firing following error trials (6 of 29, or 20%; \( \chi^2 = 5.73, P < 0.02 \)) when compared with dmPFC (Fig. 4B).

**DISCUSSION**

We tested the hypothesis that rodent dmPFC is involved in posterror processing. We inactivated and recorded from rodent dmPFC during a simple RT task in which animals exhibited posterror slowing of RTs. We report two main findings: inactivation of dmPFC attenuated posterror slowing by speeding posterior RTs and many dmPFC neurons increased their firing after errors. These data establish that, in rats, dmPFC neuronal activity is sensitive to posterror slowing in simple RT tasks. As neuronal signals in dmPFC persisted from one trial to the next, the subpopulation of dmPFC neurons that are sensitive to posterror slowing may mediate a form of retrospective memory that is used to monitor prior task performance.

Neurons in motor cortex also had posterror differences in firing rate. However, these neurons tended to fire more after correct trials (Fig. 4B), and we could find no examples of neurons with persistent posterror differences in firing rate, suggesting that motor cortex patterns of posterror activity were distinct from that observed in rodent dmPFC.

Previous work from our lab has demonstrated that dmPFC inactivation dramatically increases premature responding and speeds RTs (Narayanan et al. 2006), leading to the hypothesis that dmPFC exerts an inhibitory influence over responding (Narayanan et al. 2005, 2006; Risterucci et al. 2003). The present data suggest that rats may benefit from dmPFC inhibitory control to slow RTs after errors. We note that RTs in dmPFC inactivation sessions are more variable; suggesting that without dmPFC-mediated inhibitory control, RTs may become faster and more erratic.

![Fig. 4. Posterror modulation indices. Posterror (gray) or postcorrect (black) delay-related modulation indices plotted for dmPFC (A) and for motor cortex (B). Although dmPFC neurons could have greater firing rate following errors and correct trials, dmPFC had significantly more neurons with posterror delay activity than motor cortex, while motor cortex had significantly more neurons with postcorrect delay activity than dmPFC.](http://jn.physiology.org/ by 10.220.33.6 on November 3, 2016)
In addition to inhibitory control, rodent dmPFC has been implicated in maintaining task-relevant information (Baeg et al. 2003; Batuev et al. 1990; Ragozzino et al. 1998). We find further evidence for mnemonic processing in our simple RT task based on the posterior activity of dmPFC neurons. This activity may function in a type of retrosp ective memory that could be used to improve task performance following an error.

Behavioral experiments were used to assess the effects of distractors on posterior slowing. This manipulation attenuated posterior slowing, suggesting that animals may be attending to recent trial outcomes. By contrast, removing the normal contextual cues associated with the ITI (such as the houselights) had no effect of posterior slowing. We note that dmPFC neural activity did not simply transiently increase and then decrease after errors. Instead, activity increased and persisted to the next trial following an error. Taken together, these data suggest that such neural processing might represent a form of retrospective working memory for trial outcome, i.e., a form of distractor-sensitive working memory (Baddeley 1987). In the present study, it is difficult to determine which aspect of past task performance animals are maintaining—i.e., whether it is trial outcome (correct vs. error) or reward history (rewarded vs. unrewarded). Future experiments that manipulate reward contingencies will be needed to dissociate these possibilities.

Our findings converge with reports that primate medial frontal regions are prominently involved in posterior processing (Emeric et al. 2007; Schall et al. 2002; van Veen et al. 2004; Walton et al. 2007) as well as in action selection (Bush et al. 2002; Rushworth et al. 2004, 2007; Shima et al. 2007). During simple RT performance, inactivation of dmPFC decreased delay-related activity in motor cortex (Narayanan and Laubach 2006), suggesting that rodent dmPFC may exert top-down control over neurons in rodent motor cortex that are responsible for executing movements (Donoghue et al. 1992; Laubach et al. 2000; Neafsey et al. 1986). Taken together, these data suggest that in both primates and rodents, medial prefrontal regions may be involved in integrating information about task performance to achieve supervisory control of sensorimotor processes (Dalley et al. 2004; Rushworth et al. 2004; Schall et al. 2002).

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