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Contributions of the amygdala and medial prefrontal cortex to incentive cue responding

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Abstract

Reward-seeking behavior is controlled by neuronal circuits that include the basolateral nucleus of amygdala (BLA), medial prefrontal cortex (mPFC), nucleus accumbens (NAc) and ventral tegmental area. Using a discriminative stimulus (DS) task in which an intermittently presented cue (DS) directs the animals to make an operant response for sucrose, we previously demonstrated that dopamine receptor antagonism in the NAc reduced reinforced cue responding, whereas general inactivation of the NAc increased behavioral responding in the absence of the cue. Because they send major glutamatergic projections to the NAc, the BLA and mPFC may also contribute to reward-seeking behaviors modulated by the NAc. In this study we compare the effects of BLA and mPFC inactivation on the performance of a DS task. BLA inactivation by combined GABA_A and GABA_B agonists impaired cue responding with minimal effects on operant behavior in the absence of cues. Dorsal mPFC (dmPFC) inactivation also inhibited cue-evoked reward-seeking. In contrast, ventral mPFC (vmPFC) inactivation disinhibited responding to unrewarded cues with less influence on reinforced cue responding. These findings demonstrate that the BLA and dmPFC facilitate cue-evoked reward-seeking, whereas, in the same task the vmPFC exerts inhibitory control over unrewarded behaviors.

Keywords

basolateral nucleus; infralimbic cortex; nucleus accumbens; prelimbic cortex; cingulate cortex; reward-seeking behavior

Cue-evoked reward-seeking behaviors require recognition of learned cues that predict reward availability and selection of appropriate motor responses. Performance of reward-seeking behavior requires integrated functions of multiple brain areas including the basolateral nucleus of the amygdala (BLA), medial prefrontal cortex (mPFC), nucleus accumbens (NAc) and ventral tegmental area (VTA). The BLA and mPFC play crucial roles in reward-related

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behaviors and are major sources of glutamatergic afferents to the NAc (McGeorge and Faull, 1989; Brog et al., 1993; Zahm, 2000). The NAc, which receives excitatory inputs from the BLA and mPFC and projects via both direct and indirect paths to output nuclei of the basal ganglia, has been proposed to function as an interface between corticolimbic and motor areas (Mogenson et al., 1980). The VTA provides a dense dopaminergic projection to the NAc (Swanson, 1982), and this projection is critical for the performance of learned appetitive responses to reward predictive cues (Nicola et al., 2000; Yun et al., 2004b; Fields et al., 2007; Nicola, 2007).

Electrophysiological studies have reported that BLA and mPFC neurons as well as VTA neurons respond to reward-predictive cues during many reward-seeking behaviors (Ljungberg et al., 1992; Muramoto et al., 1993; Schultz et al., 1993; Schoenbaum et al., 1998, 1999; Jodo et al., 2000). Different subpopulations of NAc neurons are excited or inhibited by discriminative stimuli (DSs) and other cues that predict reward availability (Nicola et al., 2004b; Ghitza et al., 2003; Wan and Peoples, 2006; Day et al., 2006). VTA inactivation selectively abolishes the responses of NAc neurons evoked by reward-predictive cues (Yun et al., 2004b). These findings have led to a model of neuronal circuits involving the NAc, VTA and corticolimbic structures (BLA and mPFC). According to this model (Nicola, 2007), dopamine released in the NAc at the time of cue presentation facilitates the firing of a subpopulation of neurons that are activated by corticolimbic structures and promote specific reward-seeking behaviors. Since the VTA is the sole source of dopamine input to the NAc, the fact that dopamine antagonists injected into the NAc reduce responding to DSs supports this model (Yun et al., 2004a,b). Strikingly, however, responding to incentive cues in the same task is not reduced by general inactivation of NAc neurons with glutamate receptor antagonists or tetrodotoxin. Instead, general inactivation of the NAc results in *increased* behavioral responding that is not appropriate for reward seeking, such as responding to a stimulus that does not predict reward, or uncued responding on levers during the inter-trial interval (Yun et al., 2004a). This finding suggests that the firing of some NAc neurons normally inhibits behaviors that are inappropriate to the learned task. Thus, corticolimbic input to the NAc can either inhibit or dopamine-dependently excite reward-seeking behavior. An important question, then, is which specific inputs promote and which inhibit reward-seeking.

The BLA plays a crucial role in reward representation and stimulus-reward learning (Baxter and Murray, 2002). Previous lesion studies demonstrated that BLA-lesioned rats showed deficits in appetitive Pavlovian second-order conditioning and Pavlovian-instrumental transfer (Hatfield et al., 1996; Blundell et al., 2001). On the other hand, the mPFC has been implicated in a variety of cognitive and executive processes. Lesions and inactivation of the mPFC induce deficits in formation of action-outcome associations, movement initiation, inhibition of responses, behavioral flexibility and working memory tasks (Granon et al., 1994; Ragozzino et al., 1999; Passetti et al., 2002; Risterucci et al., 2003; Salazar et al., 2004; Ostlund and Balleine, 2005; Narayanan et al., 2006). Furthermore, the mPFC can be functionally and anatomically subdivided into dorsal and ventral divisions (dmPFC and vmPFC, respectively) (Passetti et al., 2002; Heidbreder and Groenewegen, 2003). The dmPFC, including the anterior cingulate and dorsal prelimbic cortex, is particularly involved in the temporal organization and shifting of behavioral sequences, while the vmPFC, including ventral prelimbic cortex, infralimbic and medial orbital cortex, appears to be specifically responsible for maintaining behavioral flexibility and motor preparation of conditioned responses (Passetti et al., 2002; Heidbreder and Groenewegen, 2003; Risterucci et al., 2003).

Although both the BLA and mPFC participate in reward-related behaviors, the mPFC is likely to contribute to reward-associated action selection, whereas the BLA appears to be implicated in processing information about emotional or motivational significance. Because the tasks used previously to draw these conclusions differ from the DS task we have employed in our

behavioral and electrophysiological studies, it is difficult to predict which, if any, nucleus (BLA or mPFC) provides the critical input to the NAc that dopamine-dependently facilitates behavioral and neuronal responses to learned reward predictive cues, and which provides the input that results in behavioral inhibition. Therefore, in the present study, we asked how the BLA and mPFC contribute to performance of a DS task similar to the one we used earlier to explore the contribution of NAc neurons and dopamine to cue-evoked reward-seeking. Parts of these data have been presented in abstract form (Ishikawa et al., 2007).

Experimental procedures

Subjects

Thirty-two male Long-Evans rats (~350 g on arrival) were individually housed in a colony room maintained on a 12-h light, 12-h dark cycle. All experiments occurred during the light portion of the cycle. After receipt, rats were allowed at least 1 week of *ad libitum* food and water, followed by 1 week of restricted food and water before training. Animals were fed 13 g of BioServ formula F-173 pellets (1 g each) and 30 ml of water per day for the duration of the experiments. Animals were weighed daily, and those showing weight loss >10% of free feeding weight were given additional food until their weight stabilized. All procedures were approved by the Ernest Gallo Clinic & Research Center Institutional Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines.

Apparatus

Experimental sessions were conducted in standard operant chambers (29.5 cm long, 25 cm wide and 25 cm high) enclosed within a sound- and light-isolating plastic outer chamber (MED Associates, St. Albans, VT, USA). Two retractable response levers (6 cm above the grid floor) were situated on one wall of the operant chamber, with a reward receptacle between them. The reward receptacle contained a small well into which a liquid 10% sucrose reward was pumped using a syringe pump. Two white houselights were on throughout experiments, and white noise (65 dB) was present at all times. An additional speaker was used to present two auditory stimuli (85 dB). One was an intermittent 15 kHz tone that was on for 120 ms and off for 270 ms, and the other was a constant 5 kHz tone.

Behavioral tasks

Twenty-four animals were trained on the DS task. In the DS task, two auditory stimuli (up to 10 s long) were presented as tone cues: a DS, which predicted reward delivery after a correct lever-press during DS presentation, and a non-rewarded stimulus (NS), during which lever-pressing did not trigger reward delivery. For half the animals, the DS was the intermittent tone and the NS was the constant tone; the opposite relation held for the other half. The left lever was designated the active lever for half the animals, and the right lever was designated active for the other half. Responses on the inactive lever had no programmed consequence at any time. A response on the active lever during DS presentation always terminated the DS and resulted in delivery of 50 μ l of 10% sucrose into the reward receptacle. Cues were presented on a variable interval schedule with an average interval (from the end of the previous cue to the onset of the next) of 29.4 sec; the DS or NS was randomly presented at the end of each interval.

Eight additional rats were trained on a simpler uncued fixed ratio 1 (FR1) task in which there was no explicit auditory stimulus. As in the DS task, the left lever was active for half the animals, and the right lever was active for the other half. A single press on the active lever caused the delivery of 50 μ l of 10% sucrose reward. After reward delivery, additional reward was immediately available contingent on an active lever press. In both tasks, test sessions lasted for 1 h.

Training procedures

Animals progressed through several stages of training before undergoing surgical implantation of cannulae for drug microinjections. In stage 1 of DS task training, food-restricted animals were introduced to the chamber. Entry into the reward receptacle or pressing either of the two levers triggered delivery of 50 μ l of a 10% sucrose solution. A 20 sec time-out was imposed after reward delivery, during which reward could not be earned. After animals learned to obtain all 100 available rewards in < 1 h, they were advanced to stage 2. This stage consisted of a two-lever FR1 task in which a response on either lever triggered reward delivery followed by a 3 s time-out. Animals remained at this stage until they learned to obtain 100 rewards in < 1 h. In stage 3, animals were advanced to a one-lever FR task in which pressing the active lever (left or right, depending on the animal) during cue presentation triggered reward delivery followed initially by a 10 s timeout. The cue was presented at the end of the timeout and remained on until an active lever press. The timeout was increased to 20 s and then 30 s when animals obtained more than 100 rewards during the session. Lever-pressing in the absence of the cue was not rewarded. Animals were advanced to stage 4 (the final DS task) when their latency to press the lever was less than 15 s after cue presentation. Training procedures for the uncued FR1 task began with stages 1 and 2 as described for the DS task. Animals were then advanced to the uncued FR1 task.

Animals were trained on the DS task until the NS response probability averaged ~20% and the DS response probability averaged > 90%. For animals on the uncued FR1 task, training continued until animals performed more than 600 active lever-presses per session in 3 consecutive sessions. When animals met these criteria, surgery for cannula implantation was performed.

Surgery

Animals were anesthetized with isoflurane (0.5-2.0%) and placed in a stereotaxic apparatus. Nineteen rats (11, DS task; 8, uncued FR1 task) were implanted bilaterally with two sets of 27 gauge microinjection guide cannulae (Plastics One, Roanoke, VA, USA), one set in the BLA and the other in the mPFC. BLA coordinates were AP -3.0, ML \pm 4.8, DV -7.0 mm relative to bregma and mPFC coordinates were AP +3.2, ML \pm 0.75, DV -1.5 mm. In 5 additional rats trained on the DS task, guide cannulae were implanted bilaterally in both the caudate putamen (CPu) (AP -2.3, ML \pm 5.0, DV -4.5 mm) and motor cortex (MC) (AP +3.2, ML \pm 2.0, DV -1.5 mm). A final group of 8 rats on the DS task was implanted with cannulae only in the mPFC (AP +3.2, ML \pm 0.75, DV -2.0 mm) to compare the effects of dmPFC and vmPFC inactivation. Guide cannulae were secured to the skull with bone screws and dental acrylic, and wire obturators were inserted into the guide cannulae; the ends of the obturators were flush with the ends of the guide cannulae. Rats were given at least 7 days of recovery before they were retrained on their respective tasks and habituated to the close handling necessary for the microinjection procedure.

Microinjections

After retraining, animals were injected bilaterally in the BLA, mPFC, CPu or MC prior to every other session with either drug or vehicle (saline). The drug solution consisted of a mixture of muscimol and baclofen (M/B) (GABA_A and GABA_B agonists) dissolved in saline. The doses of muscimol and baclofen were 12.5, 25 and 50 ng of each drug for BLA injection; 25, 50 and 100 ng for mPFC injection; 12.5 ng for CPu injection; and 25 and 50 ng for the MC, dmPFC and vmPFC. Doses indicate the amount delivered per hemisphere. Injection volume was always 0.5 μ l per side.

To inject animals, the obturators were removed and 30 gauge injector cannulae were bilaterally inserted into the guides. Injectors extended 1 (MC and dmPFC), 1.5 (BLA and CPu), 2 (mPFC)

and 3 mm (vmPFC) below the end of the guides for final injection targets of AP -3.0 , ML ± 4.8 , DV -8.5 mm (BLA), AP $+3.2$, ML ± 0.75 , DV -3.5 mm (mPFC), AP -2.3 , ML ± 5.0 , DV -6.0 mm (CPu), AP $+3.2$, ML ± 2.0 , DV -2.5 mm (MC), AP $+3.2$, ML ± 0.75 , DV -3.0 mm (dmPFC) and AP $+3.2$, ML ± 0.75 , DV -5.0 mm (vmPFC). After a 1 min pre-injection period the entire volume was injected over 2 min. After a 1 min post-injection wait, the injectors were removed, the obturators were replaced and the animal was immediately placed into the behavioral chamber and the session began.

Animals with cannulae in both the BLA and mPFC received injections in these structures on alternating days (although animals were run on the task every day, 5 days per week), except that 3 DS-trained rats received BLA injections prior to mPFC injection. The order of drug doses applied for each brain site was randomly chosen for each animal except that injection of 12.5 ng M/B into the BLA for 5 animals was the last treatment. In the 8 rats receiving dmPFC and vmPFC inactivation, injection into the vmPFC was performed after all doses of M/B and saline were applied to the dmPFC.

Because each injection site received several injections over the course of the experiment, it is possible that cumulative tissue damage could have affected behavioral performance. To determine whether this was the case, we examined the animals' performance on the last (uninjected) session, one day prior to the last injection received. Performance at that time was similar to the animals' initial criterion performance. Specifically, in the 16 DS-trained rats (8 for BLA or mPFC injection; 8 for dmPFC or vmPFC injection), the DS ratio was 93.7 ± 1.0 and the NS ratio was 19.3 ± 2.3 .

Data analysis

For the DS task, several behavioral measures were examined: the DS and NS response ratios (proportion of these cues during which the animal made a response on the active lever during presentation of the cue), the DS response latency, and the rate of uncued responding on the active levers (the rate of responding in the absence of DS and NS). For the uncued FR1 task, the number of active lever presses in the 1-h session was determined. The effects of the drug were analyzed using paired *t*-test or repeated measures ANOVA followed by Fisher's PLSD post hoc test, in which significance was defined as $P < 0.05$.

Histology

At the end of experiments, animals were deeply anesthetized with sodium pentobarbital and perfused with saline and 10% formalin. Sections (50 μ m) from each brain site were cut on a cryostat and stained with neutral red, and cannula positions were determined. Data from improper placements were excluded from the analysis. Final *N* values were 8 DS-trained rats that received BLA and mPFC inactivation, 5 DS-trained rats receiving CPu and MC inactivation, 8 rats trained on FR1 that received BLA and mPFC inactivation, and 8 DS-trained rats receiving dmPFC and vmPFC inactivation.

Results

The role of the BLA in reward-seeking behaviors

Inactivating the BLA with GABA_A and GABA_B agonists (M/B) impaired cue responding behavior (Fig. 1). M/B induced a dose-dependent decrease in DS response ratio ($F_{3,21} = 22.77$, $P < 0.0001$; Fig. 1A). Responding to the NS was also reduced by BLA inactivation ($F_{3,21} = 12.31$, $P < 0.0001$; Fig. 1C). Although the DS response latency tended to increase following BLA inactivation, there was no significant difference among groups ($F_{3,21} = 1.58$, $P = 0.23$; Fig. 1B). The rate of uncued responding on the active lever was reduced when M/B was injected at a dose of 50 ng ($F_{3,21} = 4.17$, $P < 0.05$; Fig. 1D). As the injected drugs may diffuse away

from the BLA and affect neighboring brain areas over time, we implanted a separate group of animals with cannulae directed towards the CPU and monitored their performance on the DS task. M/B (12.5 ng) infused into the neighboring CPU had no effect on any behavioral measures (DS response ratio, $t = 2.59$, $P = 0.06$; NS response ratio, $t = 1.26$, $P = 0.28$; DS response latency, $t = 0.24$, $P = 0.82$) (Fig. 2).

To determine whether the behavioral effects of BLA inactivation could be due to a gross motor deficit or a reduction in the animals' motivation for reward, we determined the effects of BLA inactivation on lever-pressing in the uncued FR1 task (Fig. 5A). Although 25 and 50 ng of M/B microinjected into the BLA significantly reduced active lever responses, the 12.5 ng dose did not affect responding ($F_{3,21} = 12.61$, $P < 0.0001$). In contrast, the lowest (12.5 ng) dose of M/B reduced the DS response ratio by ~50% (Fig. 1A). Therefore, although the reduction in responding in the DS task by higher doses of M/B injected into the BLA may be due in part to a motor or motivational deficit, these factors cannot entirely explain the impaired cue responding observed in the DS task. Instead, elements of the DS task structure that differ from the FR1 task (e.g., a cue is presented at relatively long inter-trial intervals) likely cause the DS task to be more dependent on the BLA than the FR1 task.

The role of the mPFC in reward-seeking behaviors

In contrast to BLA inactivation, microinjection of M/B into the mPFC (between the dmPFC and vmPFC injection sites described in the next section) induced significant increases in NS response ratio ($F_{3,21} = 9.59$, $P < 0.001$; Fig. 3C) and in uncued responses on the active lever ($F_{3,21} = 7.74$, $P < 0.01$; Fig. 3D). At higher doses (50 and 100 ng), M/B injected into the mPFC also reduced the DS response ratio ($F_{3,21} = 8.75$, $P < 0.001$; Fig. 3A). The DS response latency increased after all doses of M/B ($F_{3,21} = 24.77$, $P < 0.0001$; Fig. 3B). To exclude the possibility that the effectiveness of M/B injected into the mPFC depends upon diffusion to neighboring brain areas, we examined the effects of MC inactivation on performance in the DS task. Inactivation of the neighboring MC with M/B had no effect on the DS response ratio ($F_{2,8} = 2.76$, $P = 1.22$ or NS response, $F_{2,8} = 1.18$, $P = 0.36$), although it did increase DS response latency ($F_{2,8} = 8.01$, $P < 0.05$) (Fig. 4). This indicates that the increase in unrewarded responding was most likely due to an effect within the mPFC.

To determine whether an increase in motivation for sucrose reward could explain the increased unrewarded responding, we asked whether M/B infused into the mPFC caused an increase in lever-pressing during the uncued FR1 task. M/B (25 and 50 ng) infused into the mPFC had no effects on the number of active lever responses on this task ($F_{2,14} = 0.49$, $P = 0.63$; Fig. 5B). Because an increase in lever pressing may not have been observed due to a ceiling effect, we increased the animals' motivation for sucrose by providing them with only 3 g of food (instead of the usual 13 g) on the day before the test session. When they were more food restricted, animals responded with more lever presses than when they were moderately restricted ($t = -3.86$, $P < 0.01$; Fig. 5C). These results suggest that the activity of the mPFC plays an essential role in responding to learned reward-predictive cues as well as in the inhibitory control of behaviors inappropriate to the learned task.

Dissociable contributions of dmPFC and vmPFC to reward-seeking behaviors

Inactivation of the mPFC both reduced responding to the reward-predictive DS and increased the probability of unrewarded behaviors, such as responding to the NS and uncued responding on the active lever. We previously reported a reduction in DS responding after injection of dopamine receptor antagonists into the NAc, and an increase in unrewarded responding after general inactivation of the NAc (Yun et al., 2004a). Recent evidence suggests that the ventral mPFC, which projects more strongly to the NAc shell than to the core, exerts a suppressive effect on reward-seeking behavior, whereas the dorsal mPFC, which projects more strongly to

the NAc core, exerts an activating effect (Passetti et al., 2002; Risterucci et al., 2003; Peters et al., 2008a,b). Therefore, we compared the effects of dmPFC and vmPFC inactivation on performance of the DS task.

DS responding was impaired by dmPFC inactivation at doses of 25 and 50 ng ($F_{2,14} = 11.27$, $P < 0.01$; Fig. 6A-1), whereas only the 50 ng dose of M/B injected into the vmPFC reduced the DS response ratio ($F_{2,14} = 9.50$, $P < 0.01$; Fig. 6B-1), and to a smaller degree than dmPFC inactivation. Furthermore, M/B injection into the dmPFC induced an increase in DS response latency ($F_{2,14} = 5.34$, $P < 0.05$; Fig. 6A-2), whereas vmPFC inactivation had no effect on this measure ($F_{2,14} = 0.35$, $P = 0.71$; Fig. 6B-2). On the other hand, inactivation of the vmPFC, but not the dmPFC, induced a significant increase in NS response ratio (vmPFC, $F_{2,14} = 13.67$, $P < 0.001$; dmPFC, $F_{2,14} = 1.97$, $P = 0.18$) (Fig. 6A-3, 6B-3). Both dmPFC and vmPFC inactivation augmented uncued responding on the active lever (dmPFC, $F_{2,14} = 4.75$, $P < 0.05$; vmPFC, $F_{2,14} = 8.70$, $P < 0.01$) (Fig. 6A-4, 6B-4). Thus, dmPFC but not vmPFC inactivation significantly reduced responding to reward-predictive cues. In contrast, vmPFC but not dmPFC inactivation increased responding to the unrewarded cue. Inactivation of either site enhanced uncued responding on the active lever.

To further confirm this conclusion, we analyzed the effects of individual injections of 25 ng M/B into the mPFC as a function of the depth of the injection site as determined by histological reconstruction. The DS and NS response ratios were significantly correlated with the depth of injection sites (DS: $r = 0.54$, $P < 0.01$, $n = 24$; NS: $r = 0.56$, $P < 0.01$, $n = 21$) (Fig. 7), whereas uncued responding showed no significant correlation with the injection sites ($r = -0.007$, $P = 0.98$, $n = 22$). The DS response ratio was reduced at more dorsal injection sites (Fig. 7A), while the NS response ratio (normalized to saline) increased at more ventral sites (Fig. 7B). These findings support the conclusion that the mPFC has dissociable functions in reward-seeking behaviors, with the dmPFC promoting cue-evoked responding and the vmPFC inhibiting responding to discrete unrewarded sensory cues, and both sites inhibiting uncued responding.

Histological verification of injection sites

The tips of microinjection cannulae were verified to be in the BLA, mPFC (including dmPFC and vmPFC), CPU and MC of animals trained on the DS task (Fig. 8). The injection sites for mPFC inactivation were distributed in the middle part of the prelimbic cortex. The locations of the dmPFC inactivations were in the anterior cingulate cortex and boundary between the cingulate and prelimbic cortex, while the injections sites for the vmPFC were mainly in the infralimbic cortex. Microinjection sites of animals trained on the uncued FR1 task were within the same area of the BLA and mPFC (middle part of the prelimbic cortex) as in the DS task-trained animals.

Discussion

To obtain reward in the DS task, animals must perform an operant response during DS presentation. During the long (30 sec) intervals between cue presentations, and during NS presentation, reward is not available. Previously, we showed that dopamine acting in the NAc is required for animals to respond during the DS (Yun et al., 2004a,b). However, NAc neurons also inhibit behaviors that animals have learned to suppress in the context of a reward seeking task, as demonstrated by an increase in the probability of unrewarded actions after general inactivation of the NAc (Yun et al., 2004a). Here, we show that, similar to dopaminergic input from the VTA, both the BLA and dmPFC are required for responding to the DS. In contrast, inactivation of the vmPFC resulted in disinhibition of unrewarded behaviors, similar to the effects of general inactivation of the NAc. Since the BLA, dmPFC and vmPFC all project to the NAc, our results suggest that NAc neurons that promote reward-seeking in response to cues are driven by BLA and dmPFC neurons, whereas NAc neurons that inhibit responding to

unrewarded cues are driven by inputs from the vmPFC. Furthermore, our results suggest that inputs from both dmPFC and vmPFC to NAc neurons inhibit uncued responding in this task.

Inactivation of the BLA

Inactivation of the BLA reduced both the DS response ratio and uncued responding in the FR1 task. The latter effect suggests that BLA inactivation either reduces motivation for reward or produces a motor impairment. However, we did not observe significant reduction in FR1 task responding at the 12.5 ng dose of M/B, and decreases in DS response ratio at each dose (12.5 ng, 43%; 25 ng, 64%; 50 ng, 85% relative to saline treatment) were greater than the reduction in responding on the FR1 task caused by equivalent doses (12.5 ng, 9%; 25 ng, 26%; 50 ng, 40%). Therefore, DS responding is more sensitive to BLA inactivation than FR1 responding, and this is likely due to differences in task structure. In particular, in the DS task, animals must respond to an explicit cue presented at relatively long (~30 sec) intervals, whereas no explicit cues are presented in the FR1 task and reward is always available in exchange for a lever press. Thus, BLA inactivation, particularly at low doses of M/B, relatively specifically impairs some aspect of reward-seeking triggered by discrete reward predictive cues.

Since the amygdala consists of multiple subdivisions such as the BLA, lateral and central nucleus, and these nuclei are small and adjacent to each other, it is possible that M/B injected into the BLA diffused to adjacent nuclei and affected behavioral performance. In particular, the central nucleus of the amygdala is required for performance of Pavlovian approach behavior, which is outwardly similar to the DS task: when a cue is presented, the animal locomotes towards an operandum, responds on it, and consumes reward (Parkinson et al., 2000). However, in Pavlovian approach behavior, the cue is usually the operandum itself, whereas the auditory DS sets the occasion for approach to the lever. In the former case, the cue may activate a general motivational process subserved by the central nucleus, whereas in the latter, the cue may activate an association between the DS and the specific reward it predicts. The ability to act on such an association requires the BLA rather than the central nucleus (Balleine and Killcross, 2006). Furthermore, lesions of the BLA that spare the central nucleus impair performance of a DS task for cocaine reward (Yun and Fields, 2003), suggesting that the effects of our injections were specific to the BLA. Nevertheless, our results do not rule out a contribution of the central nucleus to performance of the DS task for sucrose reward.

Although uncued responding in the DS task was not significantly reduced by BLA inactivation with low (12.5 and 25 ng) doses of M/B, the data show a trend towards a reduction in responding (Fig. 1D) especially when compared with the effects of the same doses of M/B on the uncued FR1 task (Fig. 5A). Because uncued responding in both the DS and FR1 task is not controlled by explicitly presented cues, it is paradoxical that uncued responding in the DS task should be more sensitive to BLA inactivation than uncued responding in the FR1 task. A potential resolution lies in the similarity of the effects of BLA inactivation and injection of dopamine receptor antagonists into the NAc. Specifically, the DS and NS response ratio, as well as active lever uncued responses in the DS task, decreased after injection of dopamine receptor antagonists into the NAc, whereas FR1 task performance was unaffected (Yun et al., 2004a). Recently, we showed that infusion of dopamine antagonists into the NAc reduced not only cue responding, but all responding that is initiated when the animal is not engaged in task-related reward-seeking behavior, such as pressing the lever or entering the reward receptacle (Nicola and Fields, 2007). These findings explain why uncued responding in the DS task, in which reward is only intermittently available and animals therefore become disengaged from task performance, is reduced by NAc dopamine antagonists, whereas uncued responding in the FR1 task, in which reward is always available and animals therefore rarely become disengaged, is unaffected. If the BLA's glutamatergic projection to the NAc is required for NAc dopamine

to promote responding from the disengaged state, one would expect the behavioral effects of BLA inactivation to parallel those of NAc dopamine antagonist injection.

This hypothesis is supported by evidence of anatomical and functional interaction of BLA and VTA afferents in the NAc. For instance, although dopamine attenuates excitation of NAc neurons evoked by stimulation of amygdalar afferents (Yim and Mogenson, 1982,1983, 1986;Charara and Grace, 2003), dopamine increases the relative amplitude of strong, as compared to weak, glutamate-induced excitation in NAc neurons (Kiyatkin and Rebec, 1996;Nicola et al., 2000;Horvitz, 2002;Hjelmstad, 2004;Nicola et al., 2004a). Indeed, excitation of NAc neurons by DSs depends on the VTA; these excitations are also dependent on the BLA (Ambroggi et al., 2007). Furthermore, the glutamatergic terminals of BLA neurons projecting to the NAc synapse in close apposition to VTA-derived dopaminergic varicosities (Brog et al., 1993;Johnson et al., 1994;Phillips et al., 2003). These synapses are a possible site where the BLA glutamatergic projection can act presynaptically to increase dopamine efflux in the NAc (Floresco et al., 1998). These mutually facilitatory interactions between dopaminergic and glutamatergic afferents to the NAc may explain the similarity of the effects of NAc dopamine receptor antagonism and BLA inactivation on performance of the DS task.

Inactivation of the mPFC

In contrast to BLA inactivation, inactivation of the mPFC increased both the NS response ratio and responding in the absence of cues in the DS task, although, at higher doses, mPFC inactivation also decreased the DS response ratio and increased the DS response latency. Since mPFC inactivation had no effect on responding in the uncued FR1 task, the effects on the DS task are unlikely to be due to changes in motivation. The seemingly opposing effects of mPFC inactivation (decreasing DS responding while increasing responding to unrewarded cues) are likely due to the different roles of the dmPFC and vmPFC. Specifically, vmPFC inactivation increased the NS response ratio, whereas dmPFC inactivation reduced DS response ratio and increased DS response latency. A growing body of evidence delineates distinct functions of the dmPFC and vmPFC in several tasks (Passetti et al., 2002; Heidbreder and Groenewegen, 2003; Vidal-Gonzalez et al., 2006). For instance, Risterucci et al. (2003) show that lesions of the prelimbic and infralimbic cortex but not the anterior cingulate cortex produce increased premature responding and disrupted motor readiness in a reaction time task, in which subjects have to release a lever at the onset of a cue light. Furthermore, in a study using a five-choice task (Passetti et al., 2002), where animals have to choose one correct aperture out of 5 that is guided by light stimulus at the rear, an anterior cingulate lesion strongly reduced correct responding, increased correct response latency, and increased frequency of omissions compared to prelimbic-infralimbic cortex lesion. In contrast, perseverative responses were induced by prelimbic-infralimbic cortex but not anterior cingulate lesions. Finally, a recent study found that dmPFC, BLA or NAc core inactivation tended to reduce spontaneous recovery of cocaine-seeking behavior in abstinent animals, whereas vmPFC or NAc shell inactivation tended to increase this behavior (Peters et al., 2008a,b). Thus, previous findings are consistent with a role for the dmPFC in activation of reward-seeking behavior, particularly in response to cues, whereas the dmPFC exerts an inhibitory effect on behavior that accounts for the disinhibition of unrewarded responding when vmPFC is inactivated.

Since cue responding is impaired by both dopamine antagonists in NAc and general dmPFC inactivation, it is possible that the glutamatergic projection from the dmPFC is linked to dopaminergic neurotransmission within the NAc. Indeed, excitatory NAc neuronal responses to the DS, which require an intact projection from the VTA (Yun et al., 2004b), also depend on the mPFC (Ishikawa et al., 2008). These findings are supported by anatomical and electrophysiological evidence that mPFC projections interact with dopaminergic afferents within the NAc. Specifically, single NAc neurons receive convergent inputs from the mPFC

and dopaminergic terminals (Sesack and Pickel, 1992). This structural relationship could underlie dopamine's modulatory effects on the excitatory drive from mPFC neurons (Brady and O'Donnell, 2004). Such mPFC and VTA interaction within the NAc could promote the cue-evoked firing of NAc neurons, which in turn could facilitate the reward-seeking response to cues in the DS task.

Role of BLA and mPFC neuronal activity in reward-seeking behavior

Inactivation of the BLA and dmPFC resulted in effects similar to injection of dopamine receptor antagonists in the NAc: reduction in responding during DS presentation (Yun 2004a,b). In contrast, inactivation of the vmPFC had effects similar to NAc inactivation: an increase in responding to unrewarded cues (Yun 2004a). These functional differences could be due to the distinct connections of the dmPFC and vmPFC with the core and shell of the NAc. Anatomical studies show that the dmPFC mainly projects to the NAc core, while the vmPFC selectively innervates the NAc shell (Brog et al., 1993; Zahm, 2000). Indeed, responding from a state of task disengagement (which is likely to occur often during the DS task because reward availability is sparse) is more dependent on dopamine release in the NAc core than in the shell (Nicola and Fields, 2007). Thus, current evidence is consistent with the hypothesis that the vmPFC projection to the shell serves to inhibit reward-seeking behavior, whereas the BLA and dmPFC projections to the core serve to dopamine-dependently promote reward seeking. This conclusion is further supported by recent evidence that vmPFC and NAc shell inactivations disinhibit cocaine-seeking behavior, whereas BLA and NAc core inactivations reduce it (Peters et al., 2008a,b).

Also consistent with this hypothesis, BLA and dmPFC neurons respond phasically to reward-predictive cues in several behavioral tasks (Muramoto et al., 1993; Schoenbaum et al., 1998, 1999; Jodo et al., 2000). Phasic, cue-evoked excitation of NAc neurons appears to be required for animals to respond appropriately to reward-predictive cues (Nicola et al., 2004b; Yun et al., 2004b; Ambroggi et al., 2007; Ishikawa et al., 2008), and this firing could be due to the phasic responses of BLA and dmPFC neurons to cues. Therefore, we predict that phasic firing of neurons in the BLA and dmPFC also occurs in response to DSs, and that inactivation of the BLA and dmPFC reduces DS responding because these phasic responses are abolished. On the other hand, disinhibition of unrewarded responding might be due to attenuation of tonic firing (occurring in the absence of discrete cues or behavioral events) of vmPFC neurons in the context of the task, because these behavioral changes occur continuously and are not triggered by external stimuli. Future work should focus on determining how neurons in the BLA and mPFC influence the firing of NAc core and shell neurons to excite and inhibit reward-seeking behavior.

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Comprehensive list of abbreviations

BLA	basolateral nucleus of the amygdala
CPu	caudate putamen
dmPFC	dorsal medial prefrontal cortex
DS	discriminative stimulus

FR	fixed ratio
M/B	muscimol and baclofen
MC	motor cortex
mPFC	medial prefrontal cortex
NAc	nucleus accumbens
NS	non-rewarded stimulus
vmPFC	ventral medial prefrontal cortex
VTA	ventral tegmental area

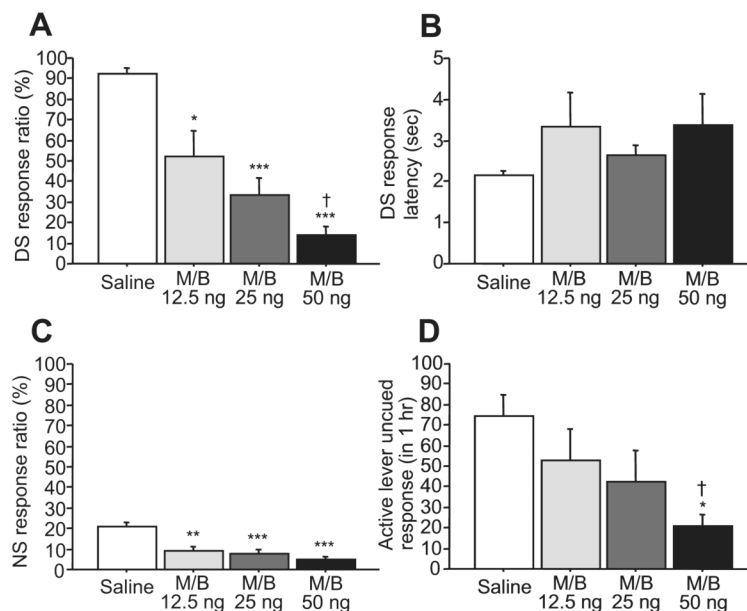
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BLA inactivation – DS task

**Fig. 1.**

The effects of BLA inactivation on DS behaviors. Bilateral microinjection of M/B into the BLA dose-dependently reduced the DS response ratio (A), although there was no significant difference in DS response latency (B). BLA inactivation decreased the NS response ratio (C). Uncued responses on the active lever were attenuated by 50 ng of M/B (D). * $P < 0.01$, ** $P < 0.001$ and *** $P < 0.0001$ compared with saline. † $P < 0.05$ compared with 12.5 ng of M/B.

CPu inactivation – DS task

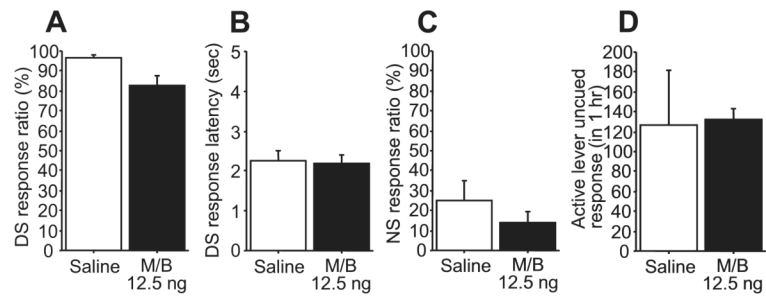


Fig. 2. Microinjection of M/B into the CPu. CPu inactivation had no effect on DS or NS response ratio (A, C), DS response latency (B) and active lever uncued response (D).

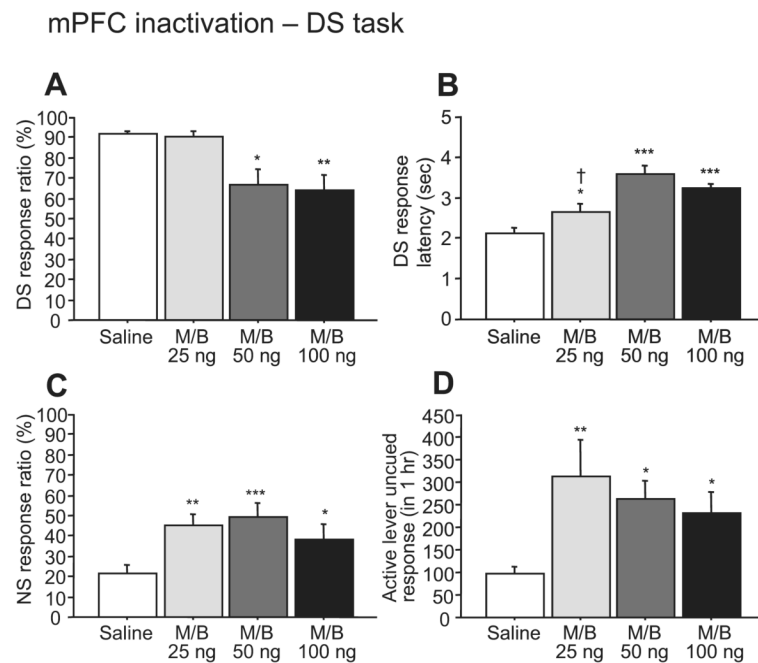


Fig. 3. The effects of mPFC inactivation on performance of the DS task. Injection of higher (50 and 100 ng) doses of M/B reduced the DS response ratio (A). Bilateral M/B injection into the mPFC increased DS response latency (B), NS response ratio (C) and uncued active lever responses (D). * $P < 0.01$, ** $P < 0.001$ and *** $P < 0.0001$ compared with saline. † $P < 0.05$ compared with 50 or 100 ng of M/B.

MC inactivation – DS task

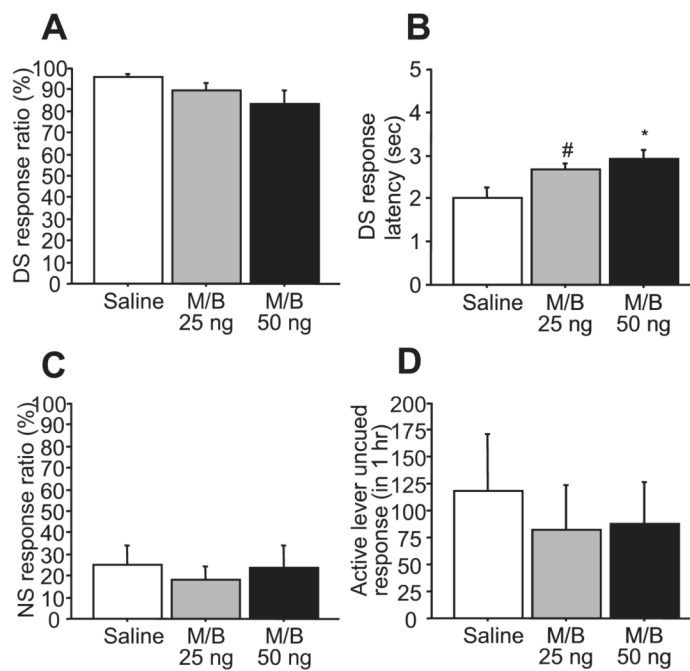


Fig. 4. Microinjection of M/B into the MC. MC inactivation had no effect on DS or NS response ratios (A, C) and active lever uncued responses (D), while there was a significant increase in DS response latency (B). # $P < 0.05$ and * $P < 0.01$ compared with saline.

BLA and mPFC inactivation – uncued FR1 task

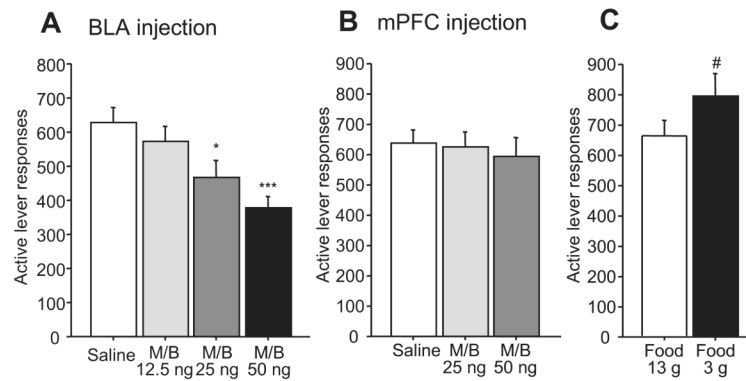


Fig. 5. Effects of BLA (A) and mPFC (B) inactivation on the performance of an uncued FR1 task. There was no significant difference in active lever response between saline and 12.5 ng M/B injection into the BLA, although M/B injection of 25 and 50 ng reduced active lever responses (A). M/B injection into the mPFC had no effect on active lever responses (B). Increased food restriction (3 g pellets and 15 ml water) for one day induced a significant increase in active lever responses, indicating that the absence of an increase in lever pressing due to M/B injection was not the result of a ceiling effect (C). * $P < 0.01$ and *** $P < 0.0001$ compared with saline. # $P < 0.01$ compared with animals given 13 g of food.

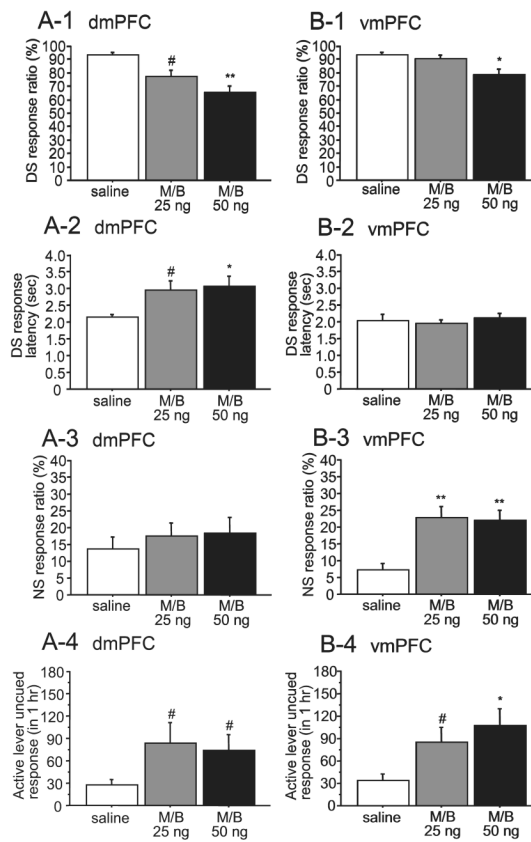


Fig. 6. Distinct effects of dmPFC and vmPFC inactivation on DS behaviors. DS responding was impaired by dmPFC inactivation using 25 and 50 ng M/B (A-1), whereas only the 50 ng M/B dose injected into the vmPFC reduced the DS response ratio (B-1). M/B injection into the dmPFC increased DS response latency (A-2), whereas vmPFC inactivation had no effect on the latency (B-2). On the other hand, inactivation of the vmPFC but not the dmPFC induced a significant increase in the NS response ratio (A-3, B-3). Both dmPFC and vmPFC inactivation augmented uncued responses on the active lever (A-4, B-4). # $P < 0.05$, * $P < 0.01$ and ** $P < 0.001$ compared with saline.

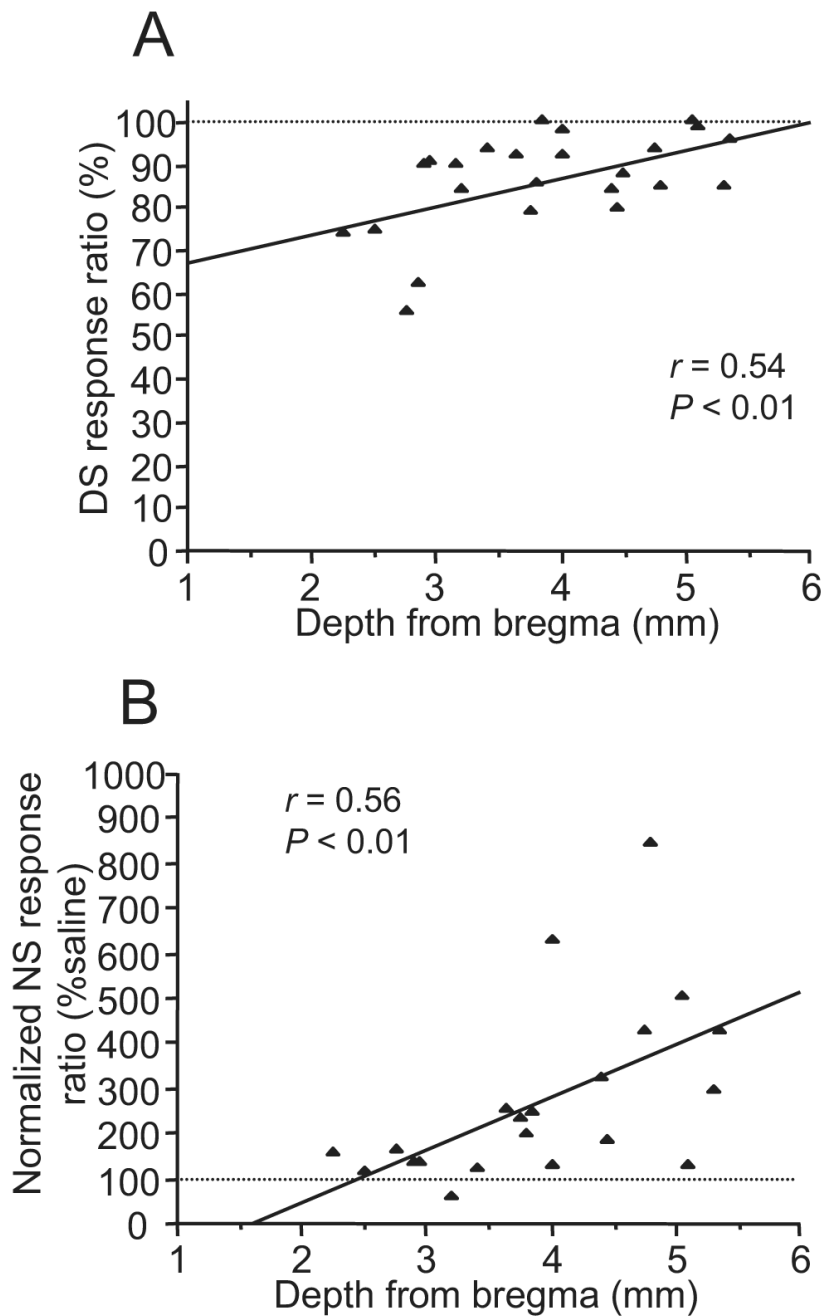


Fig. 7. Relationship between inactivation sites in the mPFC and performance of the DS task. There was a significant correlation between the depth of injection sites and the effect of inactivation on the DS ($n = 24$) (A) or normalized NS ratio ($n = 21$) (B). The number of injection sites is derived from 16 animals for mPFC and dmPFC/vmPFC injections.

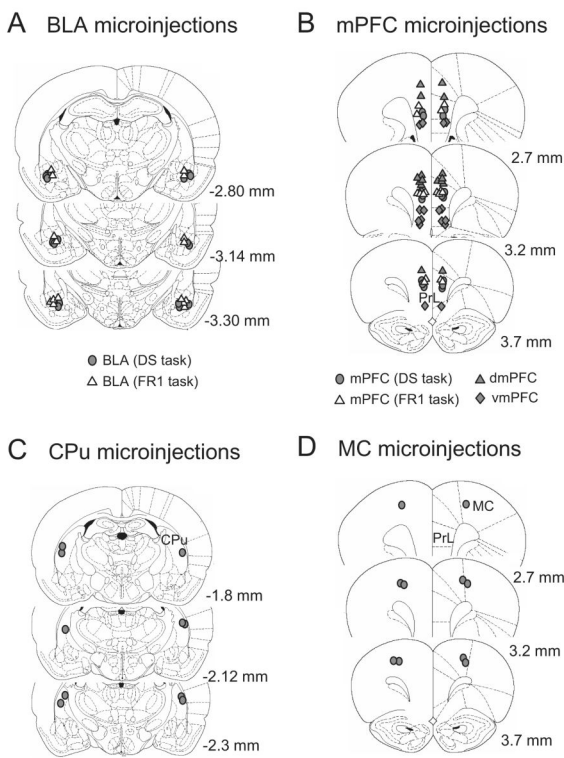


Fig. 8. Microinjection sites of the BLA (A), mPFC (B), CPu (C) and MC (D) in animals trained on the DS task (filled symbols) or uncued FR1 task (open triangles). The locations of the dmPFC inactivation were in the anterior cingulate cortex and boundary between the anterior cingulate and prelimbic cortex. mPFC injection sites were within the prelimbic cortex, while the locations for vmPFC inactivation were mainly in the infralimbic cortex. Numbers on each coronal section indicate distance from bregma according to Paxinos and Watson (1998).