The Effect of Catecholaminergic Depletion Within the Prelimbic and Infralimbic Medial Prefrontal Cortex on Recognition Memory for Recency, Location, and Objects

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There is good evidence that the medial prefrontal cortex (mPFC) is involved in different aspects of recognition memory. However, the mPFC is a heterogeneous structure, and the contribution of the prelimbic (PL) and infralimbic (IL) cortices to recognition memory has not been investigated. Similarly, the role of different neuromodulators within the mPFC in these processes is poorly understood. To this end, we tested animals with 6-hydroxydopamine (6-OHDA) lesions of the PL and IL mPFC on three tests of object recognition memory that required judgments about recency, object location, and object identity. In the recency task, lesions to both PL and IL severely impaired animals' ability to differentiate between old (earlier presented) and recently presented familiar objects. Relative to sham and PL animals, the IL lesion also disrupted performance on the object location task. However, both lesions left novel object recognition intact. These data confirm previous reports that the mPFC is not required for discriminations based on the relative familiarity of individual objects. However, these results demonstrate that catecholamines within the PL cortex are crucial for relative recency judgments and suggest a possible role for neural processing within the IL in the integration of information about object location.

Keywords: object recognition memory, prelimbic, infralimbic, dopamine

Recognition memory depends on the ability to discriminate a novel stimulus from one that has been encountered previously and is central to our ability to remember. Tests of object recognition, which exploit rodents' natural tendency to preferentially explore novel objects, have been widely used to investigate the neurobiological basis of recognition memory (e.g., Dere, Huston, & De Souza Silva, 2007; Winters, Saksida, & Bussey, 2008; Warburton & Brown, 2010). These studies have demonstrated that neural plasticity within the perirhinal cortex is necessary for discriminations based on the relative familiarity of objects (e.g., Aggleton, Keen, Warburton, & Bussey, 1997; Bussey, Muir, & Aggleton, 1999; Brown & Aggleton, 2001) as well as processing the relative recency of encountered stimuli (e.g., Barker, Bird, Alexander, & Warburton, 2007). However, there is also good evidence that the medial prefrontal cortex (mPFC) contributes to familiarity discriminations. Although the mPFC does not appear to be involved in standard tests of novel object recognition in rodents, perfor-

mance on recency tasks, as well as object-in-place memory, has been shown to be susceptible to damage to the mPFC (e.g., Hannesson, Howland, & Phillips, 2004; Hannesson, Vacca, Howland, & Phillips, 2004; Barker et al., 2007). Moreover, electrophysiological studies have revealed object-selective unit activity within PFC neurons (Rainer & Miller, 2000, 2002; Xiang & Brown, 2004).

The mPFC is richly innervated by dopaminergic fibers originating in the ventral tegmental area (e.g., Lindvall, Bjorklund, Moore, & Stenevi, 1974). The modulation of neural functioning within the mPFC by dopamine (DA) has been shown to play an important role in regulating working memory and other higher cognitive functions (e.g., Goldman-Rakic, 1998; Robbins, 2000) that may in turn support performance on recognition memory tasks. In line with this suggestion, blockade of D1 receptors within the mPFC impairs long-term recognition memory (Nagai et al., 2007), and there is a phasic increase in DA release in the mPFC during both the acquisition and retrieval phases of a delayed response task (Phillips, Ahn, & Floresco, 2004). However, the modulatory role of the mPFC dopaminergic innervation in different aspects of object recognition memory is not firmly established. Moreover, evidence from neuroanatomical studies has demonstrated that the rodent mPFC is a heterogeneous structure comprising the anterior cingulate as well as the prelimbic (PL) and infralimbic (IL) cortices that are hodologically distinct (Fisk & Wyss, 1999; Vertes, 2004). On the basis of this divergent connectivity, there are good grounds to expect that these regions may be differentially involved in recognition memory.

To assess the role of DA within different subregions of the mPFC on distinct components of recognition memory, we tested rats with pretraining 6-hydroxydopamine-induced lesions to DA

terminals within the PL and IL mPFC on three tests of spontaneous object recognition memory. First the rats were tested in a recency task that requires the animals to differentiate between two familiar objects that are presented at different time intervals. Subsequently, we assessed the animals' ability to detect changes in the spatial array of a familiar set of objects (location memory), and finally, we examined whether animals with 6-hydroxydopamine (6-OHDA) lesions to the PL and IL mPFC can discriminate a novel from a familiar object (novel object recognition memory).

Methods

Subjects

The subjects were 60 adult male Wistar rats (Charles River, United Kingdom) and were caged in pairs on a 12:12h light/dark cycle with food and water ad libitum. Rats were handled for approximately 10 min per day for 1 week and then, at mean weight 265 g (range 225–307 g), underwent surgery. Twenty rats were randomly allocated to each of the PL and IL groups and a total of 20 rats were allocated to the sham condition (10 rats were sham operated at the PL coordinates and 10 rats were sham operated at the IL coordinates). The animals were subsequently tested on a conditioned emotional response task (Nelson, Thur, Marsden, & Cassaday, 2010a).

All procedures were carried out in accordance with the United Kingdom Animals Scientific Procedures Act (1986), project license number PPL 40/3163.

Stereotaxic Infusion of 6-OHDA

In order to protect noradrenergic terminals, animals received subcutaneous administration of the noradrenaline (NA) reuptake inhibitor desipramine (20 mg/kg) 40 min prior to surgery. Anesthesia was induced by isoflurane (4%) in a N_2O/O_2 (1:2, vol/vol) mixture and maintained thereafter with isoflurane (1–2%). Stereotaxic surgery was conducted with the incisor bar set at -3.3 mm below the intraaural line. The bone above the mPFC was removed and the dura was cut to expose the cortex. Rats received bilateral infusions of 6-OHDA or vehicle into either PL or IL mPFC at the following stereotaxic coordinates: prelimbic, AP $+$ 3.8 mm; ML $\pm$ 0.6 mm; DV -3.8 mm; AP $+$ 3.2 mm; ML $\pm$ 0.6 mm; DV -3.6 mm; AP $+$ 2.5 mm; ML $\pm$ 0.6 mm; DV -3.4 mm; infralimbic, AP $+$ 3.0 mm; ML $\pm$ 0.7 mm; DV -5.4 mm (Paxinos & Watson, 2005). DV coordinates were taken from dura. Infusions were made via a 31-gauge stainless steel injector attached by polythene tubing to a 1 μl Hamilton syringe. 6-OHDA hydrobromide (24 mg/ml as salt dissolved in vehicle; Sigma, United Kingdom) or vehicle (0.9% saline/ascorbic acid 0.01% wt/vol) was infused manually over 2 min bilaterally in a volume of 0.2 μl per injection site. The injectors were left in situ for 5 min to allow absorption of the bolus and to minimize spread of the toxin. Rimadyl (0.03 ml s.c.) provided postoperative analgesia. Animals were allowed a minimum of 7 days recovery before the commencement of behavioral testing.

Quantification of 6-OHDA Lesion by HPLC-ECD

Following the completion of behavioral testing, the rats were humanely terminated by dislocation of the neck and decapitated.

The dissection and micropunch technique has been described previously (Nelson et al., 2010a). Sample tissue was taken from the following brain regions: PL, IL, orbitofrontal cortex (OFC), nucleus accumbens (NAc) core, NAc shell, caudate-putamen (CPu), and amygdala. Subsequently, neurotransmitter levels in the samples were determined by high-pressure liquid chromatography with electrochemical detection (HPLC-ECD). The tissue samples were homogenized in 0.1M PCA solution by sonication and centrifuged at 17400 g for 20 min at 4 °C. Neurotransmitter levels were detected using a glassy carbon flow cell (VT-03 Antec) with an ISAAC reference electrode. An external standard consisting of DA, NA, serotonin (5-HT), and metabolites in concentrations of 10^{-7}, 0.5×10^{-7}, and 10^{-8}M was injected at a volume of 4 μl for calibration. Samples were injected onto the column at 4 μl volumes, except for the PL, IL, OFC, and amygdala samples, which were injected at 8 μl because of the higher detection thresholds in these regions. Results were analyzed using Alexys software data system. Bradford assay was used to adjust for protein content using the pellet remaining after sample centrifugation.

Behavioral Apparatus

All testing was conducted in a rectangular arena that was made of opaque plastic and measured 38 cm $\times$ 40 cm. The walls were 54 cm high. An overhead camera was used to record animals' behavior for subsequent analysis.

The stimuli consisted of duplicate copies of objects made of glass, metal, or plastic that varied in shape, color, and size, and were too heavy to be displaced by the animal. Pairs of objects were placed in opposite corners of the arena. Objects used included bottles, flasks, and cans. The objects differed markedly and did not appear to share common features. The test box and objects were cleaned with an alcohol-based solution (20% wt/vol) before each trial to remove odor cues. The particular set of objects used was counterbalanced and, at test, the placement (left or right of arena) of the recent and old object (Experiment 1), displaced object (Experiment 2), or novel object (Experiment 3) was counterbalanced between animals. The test objects were always identical copies of the object or objects seen at sampling. Animals were always placed in the center of the arena at the start of the sample and test sessions. Time spent exploring each object was defined as directing the nose at the object at a distance of less than 1 cm and actively exploring it (i.e., sniffing and/or interacting with the object). Object exploration was not scored if the animal was in contact with but not facing the object or if it sat on the object or used it as a prop to look around or above the object (Ennaceur & Delacour, 1988; Dix & Aggleton, 1999). The animal was returned to the home cage with its respective cage mate in an adjoining holding area between sample and test phases.

Behavioral Testing

Pretraining. Prior to the start of testing, animals received one habituation session. The rats were placed individually into the arena for 10 min.

Experiment 1: Recency task. This task comprised two sample phases and one test. In each sample, the rats were allowed to explore two identical objects for a total of 5 min, but different objects were used in each sample. There was a delay of 1 hr

between the two sample phases. The identity of the objects in each sample phase was counterbalanced across experimental groups. The test was given 15 min after the second sample phase. In the test, an identical copy of one of the objects seen in *each* of the sample phases was placed in the arena for the animals to explore. If recency memory is intact, animals should preferentially explore the object seen least recently, that is, the object seen in Sample 1.

Experiment 2: Object location recognition memory. Four identical objects (glass bottles) were used. During the sample phase, the animals explored two identical objects for 5 min. The total time spent exploring the two identical objects was recorded. After a delay of 10 min, the animals were returned to the arena and allowed to explore two identical copies of the objects sampled earlier. One object was in the identical spatial location as in the sample phase, while the other was now placed in a novel location (adjacent corner to other object rather than opposite corner). Object location recognition memory is demonstrated when animals show a preference for the displaced object.

Experiment 3: Novel object recognition memory. During the sample phase, animals were allowed to explore two identical copies of the sample object for a period of 5 min. The total time spent exploring the two identical objects was recorded. After a delay of 10 min, each rat was returned to the arena, which now contained a novel object and an identical copy of the object previously seen during the sampling phase. Each rat was tested once for 3 min. Time spent exploring the familiar and novel object was recorded. Successful novel object recognition is indexed by greater exploration of the novel compared to the familiar object.

Interrater Reliability

An independent experimenter blind to the lesion group and object contingencies rescored 20% of all test phases from the original video footage. The rescored results significantly correlated with the original scores ($r = .82$, $p < .001$), indicating robust interrater reliability.

Design and Analysis

The discrimination ratio, the total time spent exploring the least recently seen object (Experiment 1), the displaced object (Experiment 2), or the novel object (Experiment 3), divided by the time exploring both objects sampled at test, was calculated. The behavioral data for each test were analyzed in separate analyses of variance with lesion as the between-subjects factor. The alpha level was set as 0.05. Where appropriate, differences between lesion groups were explored with two-tailed independent t tests. In order to establish whether animals' performance at test was above chance, one-sample t tests were performed (with the test value set at 0.5, indicating equivalent exploration of the two objects). Four animals in Experiment 1, five in Experiment 2 and six in Experiment 3 failed to explore the objects during one of the stages of the experiment and, consequently, these animals' scores are not included in the analyses.

Results

Neurochemical

Quantification of the selectivity of the lesions by HPLC revealed that six animals (three IL and three PL operated animals)

showed suboptimal levels of dopaminergic depletion ($<40\%$) and, consequently, these animals were excluded from subsequent behavioral and neurochemical analysis. One additional IL-lesioned animal was also excluded from the neurochemical analysis, as it failed to perform any of the behavioral tasks (see Design and Analysis). Thus, after these exclusions, there were 20 sham-operated animals, 16 IL-lesioned animals, and 17 PL-lesioned animals.

The levels of DA, NA, and 5-HT in the seven brain regions assayed are displayed in Table 1 as absolute levels and in Table 2 as the percentage depletion relative to sham levels.

The 6-OHDA infusions into the PL cortex selectively depleted DA in the target structure ($\sim71\%$) and produced only minimal changes in DA in the more ventral IL cortex. However, the IL lesions were less anatomically selective, as they resulted in robust depletions in the IL ($\sim74\%$) and also, to a lesser extent, depleted DA in the PL cortex ($\sim49\%$), suggesting some spread of the toxin dorsally. Desipramine pretreatment did not provide uniform neurochemical selectivity, as there was a significant reduction in NA within the IL following infusion of the neurotoxin into both regions. Nonetheless, there was no evidence of nonspecific neuronal damage, as 5-HT levels in the mPFC were unaffected by the 6-OHDA infusions. Moreover, there were no significant changes in catecholamine or 5-HT levels in any of the five other brain regions assayed.

Behavioral

6-OHDA mPFC lesions and recency judgments (Experiment 1).
Sample phases. There was no overall effect of lesion on total exploration during either sample phase (max $F_{(2,47)} = 1.17$, $p > .10$): Sample Phase 1 mean total exploration time (s) ($\pm$S.E.M.) sham $= 38.9$ (±2.8), IL $= 37.5$ (±3.5), PL $= 30.9$ (±3.5); Sample Phase 2 mean total exploration time (s) ($\pm$S.E.M.) sham $= 40.9$ (±4.2), IL $= 34.2$ (±3.9), PL $= 31.3$ (±3.3).
Test. Analysis of object exploration at test also showed no difference in total time spent interacting with the two objects at test ($F_{(2,47)} = 2.48$, $p = .09$), mean total exploration time (s) ($\pm$S.E.M.) sham $= 26.6$ (±2.2); IL $= 20.1$ (±2.9); PL $= 19.3$ (±2.6). However, as is clear from the discrimination ratios displayed in Figure 1, the groups differed markedly in the proportion of time spent exploring the two objects. Shams were clearly able to discriminate the recently seen object (in Sample Phase 2) from the least recently seen object (from Sample 1). However, recency memory was severely impaired in both lesion groups, as these animals failed to discriminate between the two objects at test. The description of the data was confirmed by ANOVA, which yielded an effect of lesion ($F_{(2,47)} = 21.99$, $p < .001$). This effect arose because both the IL ($t_{(32)} = 6.28$, $p < .001$) and PL ($t_{(32)} = 6.11$, $p < .001$) groups had lower discrimination ratios than the shams. Moreover, one-sample t tests confirmed that shams readily discriminated the objects and performance in these animals was above chance level (i.e., a discrimination ratio higher than 0.5). However, test performance in neither the IL nor the PL group differed statistically from chance (max $t_{(15)} = -1.28$, $p > .10$).

6-OHDA mPFC lesions and object location memory (Experiment 2).
Sample. There were no differences by lesion group in the time spent exploring the two objects in the sample phase ($F < 1$),

Table 1
Mean Absolute Levels (±S.E.M) of DA, NA, and 5-HT (Pmoles/μg Protein) of Sham (Pooled), PL-, and IL-Lesioned Animals in PL, IL, OFC, NAc Core and Shell, CPu, and Amyg

	Dopamine			Noradrenaline			Serotonin		
	Sham	PL lesion	IL lesion	Sham	PL lesion	IL lesion	Sham	PL lesion	IL lesion
PL sample	0.07 (±0.011)	0.021 (±0.004)	0.032 (±0.007)	0.179 (±0.018)	0.065 (±0.011)	0.091 (±0.032)	0.126 (±0.014)	0.103 (±0.015)	0.12 (±0.031)
IL sample	0.115 (±0.016)	0.102 (±0.024)	0.03 (0.008)	0.378 (±0.039)	0.369 (±0.181)	0.40 (±0.256)	0.349 (±0.05)	0.215 (±0.036)	0.414 (±0.117)
OFC sample	0.784 (±0.203)	0.617 (±0.169)	0.681 (±0.194)	n.d.	n.d.	n.d.	0.128 (±0.014)	0.118 (±0.012)	0.105 (±0.011)
NAc core sample	9.22 (±1.01)	7.75 (±0.96)	9.91 (±1.31)	n.d.	n.d.	n.d.	0.352 (±0.043)	0.284 (±0.038)	0.388 (±0.06)
NAc shell sample	4.07 (±0.53)	3.81 (±0.59)	5.42 (±0.65)	1.022 (±0.159)	1.205 (±0.16)	1.373 (±0.19)	0.452 (±0.048)	0.442 (±0.056)	0.579 (±0.083)
CPu sample	7.17 (±0.44)	6.58 (±0.82)	7.23 (±0.62)	n.d.	n.d.	n.d.	0.168 (±0.027)	0.129 (±0.018)	0.179 (±0.035)
Amyg sample	0.393 (±0.097)	0.366 (±0.055)	0.314 (±0.07)	0.226 (±0.018)	0.226 (±0.024)	0.21 (±0.019)	0.323 (±0.032)	0.324 (±0.045)	0.302 (±0.03)

Note. PL = prelimbic; IL = infralimbic; OFC = orbitofrontal cortex; NAc = nucleus accumbens; CPu = caudate-putamen; Amyg = amygdala; n.d. = not determined.

mean total exploration time (s) (±S.E.M.) sham = 39.9 (±2.5), IL = 36.6 (±2.8), PL = 36.2 (±3.9).

Test. Lesion did not affect total time spent exploring the objects at test ($F_{(2,46)}$ = 2.2, $p >$ .10), mean total exploration time (s) (±S.E.M.) sham = 28.1 (±3.6), IL = 21.6 (±2.5), PL = 20.1 (±2.1). However, as is clear from Figure 2, the groups explored the displaced and nondisplaced objects differentially. ANOVA revealed an effect of lesion ($F_{(2,46)}$ = 6.35, $p <$ .01), as both the sham ($t_{(32)}$ = 3.47, $p <$ .01) and PL ($t_{(28)}$ = 2.3, $p <$ .05) groups had higher discrimination ratios than the IL group but did not differ from each other ($t <$ 1). One-sample t tests confirmed that the sham ($t_{(18)}$ = 10.67, $p <$ .001) and PL ($t_{(14)}$ 6.69, $p <$ .001) readily detected the change in the spatial array of the objects at test. Although test performance in IL animals was impaired relative to the sham and PL groups, the IL group's performance was statistically greater than chance ($t_{(14)}$ = 2.6, $p <$ .05).

6-OHDA mPFC lesions and novel object recognition memory (Experiment 3).

Sample. Total exploration times did not differ by lesion group in the sample phase ($F <$ 1), mean total exploration time (s) (±S.E.M.) sham = 33.4 (±4.4), IL = 33.9 (±3.7), PL = 27.9 (±3.4).

Test. Similarly, there was no effect of lesion on total amount time spent exploring the objects at test ($F <$ 1), mean total exploration time (s) (±S.E.M.) sham = 33.0 (±3.2), IL = 35.9 (±4.0), PL = 33.9 (±2.2). As is clear from Figure 3, and in contrast to the findings in Experiments 1 and 2, there was no evidence of an effect of either lesion on animals' ability to discriminate a novel from a familiar object ($F_{(2,44)}$ = 1.33, $p >$ .10), and test performance in all groups was above chance (min $t_{(14)}$ = 8.67, $p <$ .001).

Discussion

The current set of experiments examined the involvement of the mPFC and its dopaminergic innervation in different aspects of recognition memory. Consistent with previous findings suggesting that the mPFC is not necessary for familiarity judgments per se (e.g., Ennaceur, Neave, & Aggleton, 1997), we found no evidence that DA denervation within either the PL or IL mPFC had any effect on rats' ability to discriminate a novel from a familiar object. However, both lesion groups were severely impaired on the recency task and were unable to discriminate objects on the basis of the recency of their occurrence. Moreover, 6-OHDA lesions to the IL, but not PL, mPFC impaired rats' ability to detect changes in the spatial array of objects.

Neuroanatomical and Neurochemical Specificity of the 6-OHDA Lesions

Infusion of 6-OHDA into the PL produced selective depletion within the target structure and spared catecholamine content in the more ventral IL cortex. Consistent with previous data (e.g., Naneix, Marchand, Di Scala, Pape, & Coutureau, 2009), 6-OHDA IL-lesions were less selective and did deplete catecholamines in both the IL and more dorsally in the PL. Despite desipramine pretreatment, there was some evidence of changes in NA levels within the PL following lesions to both subregions. Importantly, there was no evidence of nonspecific neuronal damage, as there

Table 2
Mean Percentage Difference (±S.E.M) in DA, NA, and 5-HT Levels of PL- and IL-Lesioned Animals Compared to PL and IL Vehicle-Infused Sham Animals in the Seven Brain Regions Assayed

	Dopamine		Noradrenaline		Serotonin	
	PL lesion	IL lesion	PL lesion	IL lesion	PL lesion	IL lesion
PL sample	−71.3%* (±6.5)	−49.8%* (±11.9)	−66.9%* (±6.7)	−52.4%* (±11.9)	−16.3% (±11.9)	−6.3% (±23.2)
IL sample	−10.5%† (±21.2)	−74.0%*† (±6.8)	−7.5% (±44.6)	+8.2 (±70.4)	−25.2% (±13.4)	+10.2% (±28.1)
OFC sample	−12.2% (±24.8)	−14.8% (±25.3)	n.d.	n.d.	−9.6% (±9.5)	−13.4% (±8.5)
NAc core sample	−13.1% (±10.6)	+1.4% (±8.8)	n.d.	n.d.	−29.2% (±10.2)	+16.9% (±23.8)
NAc shell sample	−3.8% (±15.1)	+30.9% (±15.5)	+30.1% (21.1)	+25.9% (±17.4)	−0.7% (±12.4)	+26.9% (±18.1)
CPu sample	−14.1% (±10.5)	+4.0% (±8.8)	n.d.	n.d.	−29.2% (±10.2)	−16.9% (±23.5)
Amyg sample	−7.1% (±13.9)	−20.2% (±17.8)	+0.1% (±10.6)	−8.8% (±8.4)	+0.5% (±13.9)	−6.4% (±9.7)

Note. PL = prelimbic; IL = infralimbic; OFC = orbitofrontal cortex; Nac = nucleus accumbens; CPu = caudate-putamen; Amyg = amygdala; n.d. = not determined.
† Significant difference from other lesion group, $p < .05$, *t*-test. * Significant difference from sham, $p < .05$, *t*-test.

were no significant changes in 5-HT levels in either prefrontal subregion. Neither lesion had any significant effects on catecholaminergic function in any of the five other brain regions from which tissue samples were taken.

PL mPFC and Recency Judgments

In the recency task, sham-operated animals preferentially explored the object seen least recently, whereas lesioned animals explored the two objects equally. There has been some debate in the literature as to what is actually measured in tasks where animals are required to discriminate two familiar objects that have been experienced at different points in time. It has been suggested that performance on these tasks taxes temporal order memory such that animals are able to discriminate the order in which the objects were encountered (e.g., Mitchell & Laiacona, 1998; Hannesson, Vacca, et al., 2004; Barker et al., 2007; Barker & Warburton, 2011), while others have argued that temporal order memory cannot be inferred from these tasks but rather they are solved on the basis of relative recency (see Ennaceur, 2010, for a full discussion of these issues). Performance on this task could be mediated by an actual representation of the order in which the objects were encountered, but it is equally possible that animals preferentially explore the least recently seen object because the memory trace for that object is weaker than the most recently sampled object, that is, they discriminate on the basis of relative recency rather than the order of occurrence of the objects. It is, however, impossible to differentiate between these two accounts of performance on this task (Ennaceur, 2010; Barker & Warburton, 2011). Impairments on the recency task manifest behaviorally as equivalent exploration of the two objects; thus, in principle, deficits on this task could arise because both objects were recognized as familiar or because both were recognized as novel (Ennaceur, 2010). It is similarly difficult to distinguish between these two accounts of impaired performance on this task. Whatever the merits of these arguments, the mPFC-lesioned animals were clearly unable to discriminate between objects that had been sampled at different time points.

It could be argued that recency judgments amount to a simple test of relative familiarity in that the older object has been forgotten or the memory trace for that object is weaker, so that animals treat the old object as novel relative to the one seen in the more
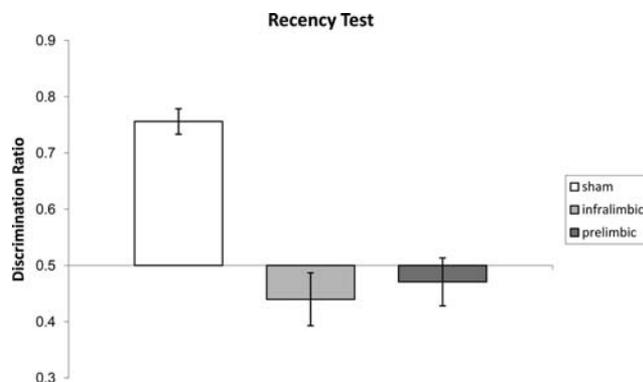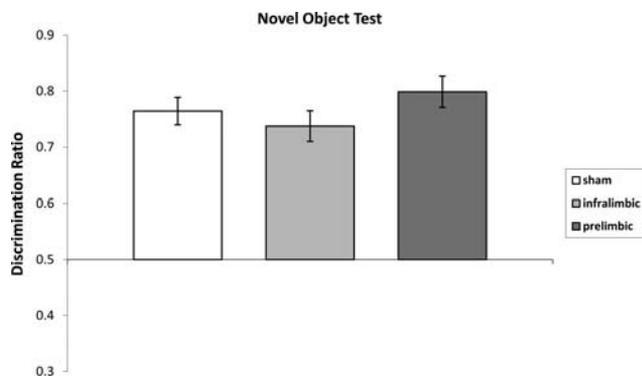

Figure 1. The effect 6-OHDA lesions to the prelimbic and infralimbic medial prefrontal cortex on recency judgments. Test performance for sham (white bars) infralimbic (light gray bars) and prelimbic (dark gray bars) are presented as discrimination ratios. Performance above 0.5 indicates a preference for the least recently seen object.

Figure 2. The effect 6-OHDA lesions to the prelimbic and infralimbic medial prefrontal cortex on location recognition memory. Test performance for sham (white bars) infralimbic (light gray bars) and prelimbic (dark gray bars) are presented as discrimination ratios. Performance above 0.5 indicates a preference for the displaced object.

Novel Object Test

Figure 3. The effect 6-OHDA lesions to the prelimbic and infralimbic medial prefrontal cortex on novel object recognition memory. Test performance for sham (white bars) infralimbic (light gray bars) and prelimbic (dark gray bars) are presented as discrimination ratios. Performance above 0.5 indicates a preference for the novel object.

recent sample phase. Thus, lesion effects on this task may not reflect impairments in the processing of recency information per se but, rather, task difficulty. Although we found no evidence that the mPFC animals were impaired on standard tests of relative familiarity, albeit after a short delay (Experiment 3), the deficit seen in Experiment 1 could nonetheless be due to task difficulty, that is, this task is more demanding due to the longer interval between sample and test, and, hence, performance is susceptible to 6-OHDA mPFC lesions. This, however, is an unlikely account of the pattern of results that we, and others, have obtained with this task. Normal animals are able to discriminate novel from familiar objects with delays of 24 hr and longer (e.g., Nelson, Thur, Marsden, & Cassaday, 2010b), and mPFC-lesioned animals have been shown to distinguish between a novel and familiar object with delays of up to 3 hr (Barker et al., 2007; Barker & Warburton, 2011). Moreover, if the impairment in the mPFC animals was due to task difficulty and the lesioned animals had simply forgotten the older object due to the longer interval between sample and test, the old object would have presumably appeared novel and, hence, would have been explored preferentially. In contrast, the lesioned animals explored both objects equally and, if anything, showed a mild preference for the more recently seen object, suggesting that the old object had not simply been forgotten.

Alternatively, the differential performance of sham and lesioned animals on the various tasks could be due to an order effect. As the lesioned animals were only severely impaired on the first task (recency), and their performance improved in the two subsequent tasks, it could be argued that these animals' performance does not reflect impaired processing of recency information but simply a test-order effect. One of the advantages of spontaneous object recognition tasks is that they do not require rule learning, extensive training, or reinforcement, but, in principle, factors other than the processing of mnemonic information could contribute to lesion-induced impairments on these tasks. For example, lesioned animals could be more neophobic or hypoactive and thus take longer to perform the task successfully. However, there was no evidence from the exploration times in any of the sample phases (when all objects were novel) of differences between the lesion groups, indicating that the lesioned animals were not more neophobic or

unable to detect novelty per se. Moreover, other groups that have tested the effects of excitotoxic mPFC lesions on recency judgments have similarly found deficits on this task even though the test order was different to ours (e.g., recency task run after unimpaired performance on the standard novel object task, e.g., Barker et al., 2007). Thus, it is unlikely that order effects provide a complete explanation of the pattern of results obtained here.

There is now good evidence that the mPFC is important for judgments about the recency of object presentation (e.g., Mitchell & Laiacona, 1998; Hannesson, Howland, et al., 2004; Hannesson, Vacca, et al., 2004; Barker et al., 2007), and other reports have shown mPFC lesions produce deficits on tasks that require the integration of temporal information (e.g., Kesner & Holbrook, 1987; Kesner, 1989; Seamans, Floresco, & Phillips, 1995). These findings are consistent with a prominent view that one of the functions of the mPFC is the temporal sequencing of behavior (e.g., Fuster, 2001; Dalley, Cardinal, & Robbins, 2004). The current experiments build on these previous reports in two respects.

First, the experiments provide evidence of the neuroanatomical locus of these effects within the different subregions in the mPFC. In principle the finding that both lesion groups were impaired on the task could be taken as evidence to suggest that performance on tasks that tax recency judgments is susceptible to DA loss in both the PL and IL subregions of the mPFC. However, the PL lesion was anatomically highly selective and spared catecholamine content in the more ventral IL, and, thus, the current results demonstrate that catecholamine depletion within the PL is sufficient to impair discriminations based on the relative recency of two familiar objects. This, in turn, would suggest that the deficit seen in the IL-lesioned group is due to catecholamine loss produced by this lesion in the PL rather than the IL or entire mPFC. As such, the current results provide the first evidence that neuroplasticity specifically within the PL is critical for judgments based on the relative recency of object presentation. This suggestion is in line with neuroanatomical data. There are direct and reciprocal connections between the mPFC and the perirhinal cortex, and there is good evidence that functional interactions between the mPFC and perirhinal cortex support performance on recognition tasks that require recency judgments (e.g., Hannesson, Howland, et al., 2004; Barker et al., 2007). Consistent with the current data, these connections are, however, particularly strong between the perirhinal cortex and the PL rather than the IL mPFC (e.g., Vertes, 2004; Hoover & Vertes, 2007).

Second, the current findings further extend our knowledge of the neurobiological basis of recognition memory by demonstrating, for the first time, the importance of catecholamines within the PL in these processes. It is most unlikely that catecholamines within the PL are actually involved in the active storage of information relevant to the performance on this task. Studies that have temporarily inactivated the mPFC indicate that the test phase (i.e., retrieval) is the critical stage at which effects are found (Hannesson, Howland, et al., 2004; Barker et al., 2007). Indeed, the complete lack of any deficit on the object-identity task in Experiment 3 fits well with this assertion. Similarly, the sparing of object-familiarity discriminations suggests that the deficits seen in the processing of recency information are most unlikely to be due to impaired sensory motor, perceptual, motivational, or attentional processes. Rather, it would seem that the PL and its DA innerva-

tion are needed to retrieve or use task relevant information to facilitate recency judgments. Future experiments employing focal infusions of selective antagonists at different stages of the procedure (sample vs. test) will be required to provide a more definitive answer as to the role of DA and NA in recency discriminations.

IL and Object-Location Recognition Memory

It has long been known that the mPFC, and, in particular, its DA innervation, is important for spatial working memory (e.g., Brozoski, Brown, Rosvold, & Goldman, 1979). Indeed, since this seminal work, overwhelming evidence has accumulated to implicate the mPFC in spatial memory processes (e.g., Kolb, Sutherland, & Whishaw, 1983; Aggleton, Neave, Nagle, & Sahgal, 1995; Ragozzino, Adams, & Kesner, 1998). Nonetheless, several studies have shown little or no deficit on spatial tasks in animals with mPFC lesions (e.g., Kesner, Farnsworth, & DiMattia, 1989; Granon, Save, Buhot, & Poucet, 1996). This discrepancy more than likely arises from differences in task demands such as the requirement for rule learning or primary reinforcement.

In studies that have used tests of spontaneous object recognition, mPFC lesions have been shown to have behaviorally dissociable effects. It has previously been demonstrated that excitotoxic mPFC lesions and inactivation with lidocaine leave object-location discriminations (detecting the change of spatial location of a familiar object) intact but disrupt object-in-place memory (recognizing the topographical relationship between sets of objects; Ennaceur et al., 1997; Hannesson, Vacca, et al., 2004; Barker et al., 2007). In the current experiments, we similarly found no evidence that 6-OHDA lesions to the PL impaired performance on the object-location task. However, the IL lesion did disrupt animals' ability to detect a change in the spatial location of a familiar object. Although performance in the IL group was statistically above chance (i.e., a discrimination ratio higher than 0.5), these animals were nonetheless impaired relative to sham- and PL-lesioned animals. One possible explanation of the discrepancy between our results and previous demonstrations of a lack of an effect of neural manipulations to the mPFC on object-location memory could be the specific targeting of catecholamines within the mPFC in the current study. Previously, both 6-OHDA lesions to the mPFC and infusions of D1 antagonists have been shown to disrupt spatial memory on tasks with a delay between training and testing (Bubser & Schmidt, 1990; Seamans, Floresco, & Phillips, 1998). These and other studies point to a particular role of DA in the regulation of spatial working memory. Moreover, to our knowledge, no previous study has explicitly sought to delineate the contribution of the IL to recognition memory. Indeed, previous studies that have shown intact object location memory have tended to target the PL or entire mPFC rather than the IL per se (e.g., Hannesson, Vacca, et al., 2004). Although the IL lesions in the current study did also deplete catecholamines in the PL, animals with selective PL lesions performed this task at comparable levels to shams. The IL mPFC receives rich input from the hippocampus (Hoover & Vertes, 2007), and neural activity within the hippocampus is essential for recognition discriminations based on the spatial location of items (e.g., Wan, Aggleton, & Brown, 1999; Hardt, Migues, Hastings, Wong, & Nader, 2009). Moreover, the IL projects to the shell of the NAc (Berendse, Galis de Graaf, & Groenewegen, 1992), and we have recently found a critical role for DA

within the shell for object-location memory (Nelson et al., 2010b). Thus, these effects may underlie the impairment in object-location memory observed in the current study following 6-OHDA lesions to the IL.

Conclusions

The current findings establish the importance of catecholamines within subregions of the mPFC in different components of object-recognition memory. These results suggest that catecholamines within the PL are important for discriminations based on the relative recency of encountered stimuli and indicate that catecholamine depletion within the IL can produce deficits in object-location memory. These findings are consistent with a role of both DA and NA in working memory and other related executive functions, and suggest that catecholamines within the mPFC modulate the flow of mnemonic information from downstream corticolimbic structures involved in the different aspects of recognition memory to allow purposeful and adaptive behavior.

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