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Research report

NMDA lesions in the medial prefrontal cortex impair the ability to inhibit responses during reversal of a simple spatial discrimination

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Abstract

Although lesion studies suggest that the rat medial prefrontal cortex (mPFc) is involved in the process necessary for reversal of a particular set of contingencies, the nature of lesion-induced deficits is unclear. The involvement of rat mPFc in reversal of a simple spatial discrimination was examined in the present study. Our hypothesis was that lesion-induced deficits may reflect a failure to inhibit a learned instrumental response. Lister Hooded rats were trained on a spatial discrimination task (SD), which required a correct barpress matching the cue location, then they were trained on reversal of SD (SDR), which required a correct barpress opposite to the cue location. Rats with mPFc lesions showed a slower learning rate compared to the controls. However, behavior of the lesioned rats during early and later reversal differed. During the initial SDR, the lesioned rats showed a greater number of barpresses during the intertrial interval and a slightly higher percent correct responses than that of the controls. Our data suggest that damage to mPFc may produce a lack of response inhibition, leading to an increase in nondiscriminated bapresses, thereby yielding a 'facilitation' during early reversal. mPFc lesion did not affect either open field activity or prepulse inhibition (PPI), a frequently used measure of sensorimotor gating. Disruption of reversal learning following damage to mPFc is partly due to a failure to inhibit instrumental responses, rather than to disruption of other processes involved in sensorimotor gating or general activity.

Keywords: Response inhibition; Spatial learning; Openfield activity; Prepulse inhibition

1. Introduction

A wide variety of tasks have been used in an effort to characterize the functions of rat medial prefrontal cortex (mPFc). Factors have varied across these tasks, including the degree of dependence on non-spatial versus spatial information, mnemonic requirements, selective attention demands, the complexity of stimulus discrimination and response requirements, and the inclusion of responses incompatible with previously learned responses. On the basis of deficits following lesions, mPFc has been hypothesized to be involved in behavioral flexibility [1–3], inhibition of responses [4–6], performance involving difficult discriminations [7–9] or significant working memory loads [8,10], shifting of attentional sets [11], shifting between rules [12], and shifting strategies [13,14].

Rat mPFc has been implicated in reversal learning. Reversal learning, unlike the acquisition of a new task, requires the animal to withhold a learned response appropriate to a previously learned set of contingencies. mPFc lesions produce reversal-specific impairment, without affecting initial acquisition, across different reversal tasks [15–18]. During reversal of a spatial [16] or of an olfactory [19] discrimination task, rats with mPFc lesions made perseverative errors, defined as the repetition of consecutive similar incorrect responses ("inflexibility"). Impaired performance was observed when animals were required to shift from a delayed non-matching-to-sample to a delayed matching-to-sample rule in an operant chamber [12], from a stimulus-matching to a stimulus-non-matching rule [18], from a place to a cue recognition rule in water maze [1], and from one perceptual dimension to another in attentional shift procedures [11]. Although these deficits have been interpreted as either failures of rule shifting [20] or extradimensional shifting in attentional sets [11], in all of

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these tasks contingencies are reversed, and to perform well on reversal formerly learned responses have to be inhibited. This raises the possibility that the involvement of the mPFc could be specific to reversal of a particular set of contingencies and that lesion-induced deficits may reflect a failure to inhibit a learned instrumental response. In this regard, lack of inhibition produced by mPFc lesions [4,5] may be specific to an instrumental response rather than general response inhibition. A lack of general response inhibition would be indicated by an increase in behaviors unrelated to the instrumental response and by an increase in spontaneous activity in a non-training context. A lack of inhibition of specific response would be indicated by an increase in behaviors related to the instrumental response in a training context (e.g. increased number of barpresses responses during acquisition, irrespective of contingency in effect).

The present study examined the involvement of mPFc in the reversal of a simple visuospatial discrimination task. Initially, rats were trained on a spatial discrimination task, which required a correct barpress that matched the cue location, then they were trained on reversal of spatial discrimination, which required a correct barpress opposite to the cue location. Our manipulation paralleled those used in previous reports, which allowed the animals to select between equally rewarded and available responses [8,9]. Our task involved simple stimuli and no delay component and so minimized the likelihood of performance deficits due to either stimulus complexity or working memory requirement. A reversal required an animal to stop making an approach response in the presence of a stimulus and to perform responses that are incompatible with those acquired during initial acquisition. Thus, our task enabled us to monitor the capacity of animals to inhibit formerly learned responses. Our hypothesis was that mPFc lesion effects would be specific to reversal learning, and that lack of inhibition related to the instrumental response would be indicated by an increase in non discriminated barpresses and a decrease in response latencies. In addition, we examined mPFc lesion effects on additional behavioral procedures, prepulse inhibition (PPI) and openfield behavior, that may be sensitive to mPFc damage [21]. PPI, a reduction in a startle response to a strong tone when this stimulus is preceded by a weaker tone or a prepulse, was measured to explicitly examine intactness of basic sensory processing. Openfield behavior was used to test for an increase in spontaneous activity in a non-training context. Our hypothesis was that mPFc lesion would be specific to the instrumental response and would affect neither PPI nor openfield behavior.

2. Materials and methods

2.1. Subjects

Forty-five male adult Lister Hooded rats, weighing 300–450 g, were used in this study. The animals were bred at the research facility in Schwerzenbach, Switzerland, and were singly housed under a reversed light–dark cycle (19:00–07:00 h). Rats were handled 5 min per day for at least 3 days prior to the start of the experiments. All experimental procedures were carried out in accordance with Swiss federal regulations for animal experimentation. Rats were food-deprived (85% body weight) with free access to water during instrumental training. Prior to surgery, all animals were shaped to barpress both left and right bars an equal number of times.

2.2. Surgical procedure

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and mounted on a Kopf stereotaxic device, with the incisor bar positioned at −3.0 mm. Rats received either NMDA lesions ($n = 15$) or sham lesion ($n = 13$). A dose of 10 mg NMDA (Sigma, St. Louis, MO, USA) was dissolved in 1 ml of phosphate-buffered saline (0.1 M, pH = 7.4). NMDA solution was freshly prepared on the day of surgery and was infused in eight sites of the mPFc (AP: +4.4, +3.2, +2.2 mm; ML: ±0.7 mm; DV: −1.2, −3.6, −1.6, −2.6 mm; 0.1, 0.075, and 0.125 μl, rostral to caudal). The sham rats were subjected to the same surgical procedure, but received an equivalent volume of phosphate buffer. A recovery period of two weeks was allowed.

2.3. Behavioral procedures

Behavioral procedures included a spatial discrimination task (SD), spatial discrimination reversal (SDR), prepulse inhibition (PPI), and openfield behavior (OF). Rats were maintained on food-deprivation (85% of their body weight) with water ad libitum during the entire SD and SDR training period. Unoperated rats ($n = 15$) were included in tests of prepulse inhibition and openfield behavior in addition to lesioned and sham groups.

2.4. Spatial discrimination task and reversal

2.4.1. Apparatus

Four standard operant chambers (Coulbourn Instruments; 25 cm × 30 cm × 30 cm), equipped with a house light (1.12 W), two retractable bars (2.5 cm above the chamber floor), two stimulus windows (red and green cues at 4 cm above each bar. Sixteen centimeters apart, with a luminance level of 9 mCd at 20 mA; intensities of red and green were 48 and 54 when readings were taken with ambient light intensity of 2 lx), and a pellet dispenser located between the two bars, were used. Each test chamber was housed inside a sound attenuation cubicle (inside dimensions: 45 cm × 70 cm with a height of 47.5 cm) equipped with a fan for ventilation. A computer controlled input–output flow to/from each operant chamber via an interface board. The computer controlled stimulus onset and offset, trial types (location and color of stimulus), and

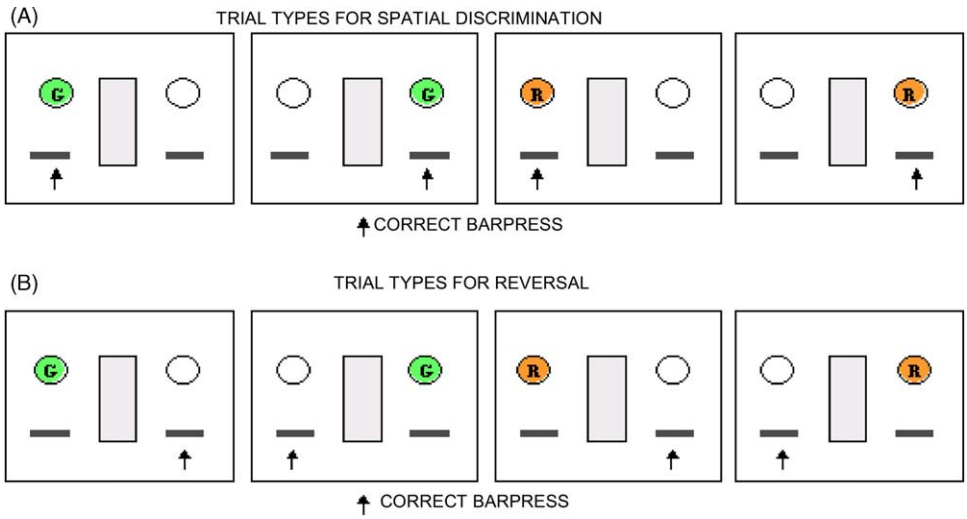

Fig. 1. Spatial discrimination task (top) and reversal task (bottom). The circles represent stimulus windows, and a shaded window indicates a stimulus (G, green; R, red). Arrows indicate the correct barpress for a particular trial type. The gray rectangle represents the hopper.

delivery of reward. Behavioral output information (right and left barpresses and response latencies) was recorded by a computer during the trial and the intertrial interval (ITI).

2.4.2. Training

In SD, a stimulus (red or green) was randomly presented above either the right or the left bar (Fig. 1A). A correct response was defined as a barpress that matched the location of the stimulus regardless of its color. Correct responses turned off the stimulus, turned on the hopper light, and delivered a pellet-reward (45 mg, Noyes) with a delay of 1 s. An incorrect response was defined as a barpress opposite to the stimulus location. Incorrect responses terminated the trial. Response time limit was 6 s. A barpress prior to the stimulus onset postponed the start of the trial by 2.5 s. All trials were preceded by an ITI of 8 s. Rats were trained until they reached a behavioral criterion ($\geq$85% correct responses, three consecutive sessions) but with a minimum of seven sessions and a maximum of ten sessions. A session was completed when the animals received a total of 100 rewards (each trial type was equally probable) or after 1 h. One session was given per day.

The reversal (SDR) training began when the animal reached the behavioral criterion on SD. In SDR, a correct response was defined as a barpress opposite to the stimulus location, and an incorrect response was defined as a barpress that matched the location of the stimulus regardless of its color (Fig. 1B). All other conditions were identical to the conditions in SD. SDR lasted 25 days. Acquisition was defined as a behavioral criterion of $\geq$80% correct responses for four consecutive sessions. As the percent correct responses during SDR was on average lower than during SD, the behavioral criterion was different from the one used during SD.

2.4.3. Data analysis

Behavioral data included percent correct responses (%CR), response latency (correct and incorrect), and number of barpresses during the ITI. Missed trials (trials with no response) and trials with a response latency shorter than 100 ms were omitted from the data analysis. One sham rat that did not reach the behavioral criterion on SD and one unoperated rat that stopped responding during SD (<50 responses across five consecutive sessions; $n = 1$) were eliminated from the data analysis. Statistical analyses were conducted by ANOVAs with the different treatments (lesion and sham) as a between-subjects factor and daily sessions as a repeated measure. Post hoc analyses included one-way ANOVAs on the different daily sessions (treatment as a factor) and on the different treatments (daily sessions as a repeated measure) and post hoc pair-wise comparisons were conducted with Fisher's PLSD test. One-way ANOVA (treatment as a factor) was used to compare the number of days to reach criterion. For the rats that did not reach the behavioral criterion on SDR, a value of 25 days was assigned.

2.5. Openfield behavior (OF)

Upon the completion of SDR, rats were kept in their home cages with free access to food and water for 4–5 days prior to testing in the open field. On the day of testing, the animals were placed in the room containing the openfields (an initial 15 min habituation to the room), followed by a 30 min habituation to the open field arena (four square open field arenas: 76.5 cm × 76.5 cm × 49 cm, made of dark gray plastic; 20 lx in the middle of the four arenas). Each open field arena was divided into two zones (the complete arena and a 20 cm × 20 cm center). Following habituation, the behavior of each animal was recorded for 30 min by a video camera mounted on the ceiling, and images were

relayed to a monitor and a video tracking system (Motion Analysis and Behavior Recognition System, EthoVision, Noldus, Wageningen, The Netherlands).

2.5.1. Data analysis

The tracking system measured the total distance traveled, the number of entries into the center, and the time spent in the center per 5 min-block. 3×6 ANOVAs with the different treatments as a between-subjects factor and the 5 min-blocks as a repeated measure were performed. Post hoc analyses included one-way ANOVAs on the different 5 min-blocks (treatment as a factor) and pair-wise comparisons conducted with post hoc Fisher's PLSD test.

2.6. Prepulse inhibition (PPI)

The animals were tested in squads of four with startle chambers counterbalanced across the different experimental groups. The testing was conducted in four ventilated startle chambers (SR-LAB, San Diego Instruments, San Diego, CA), which contained a transparent plexiglas tube (diameter 8.2 cm, length 20 cm) mounted on a plexiglas frame. Acoustic pulses and prepulses were delivered via a speaker, which was mounted 24 cm above the tube. Movement inside the tube was detected by a piezoelectric accelerometer below the frame. The amplitude of the whole body startle to an acoustic pulse was defined as the average of 100 one-microsecond accelerometer readings collected from pulse onset. Delivery of the acoustic stimuli and recording of startle responses was controlled by a computer. Once the animals were placed inside the tube, the startle session started with a 5-min acclimatization period, with a background noise level of 68 dB (A), which was maintained throughout the session. Following the acclimatization period, four startle pulses (30 ms, 120 dB (A)) were presented. The four initial startle pulses served to achieve a relatively stable level of startle reactivity for the remainder of the test session because the most pronounced habituation of the startle response occurs during the first four pulse presentations [22,23]. The prepulses were broad band noise bursts of either 72, 76, 80, or 84 dB (A) and were 20 ms in duration. The interval between the prepulse and pulse was 80 ms. Each session consisted of six blocks of 11 trials. Each block included four different trial types: two pulse-alone trials, four prepulses at different intensities followed by pulse, four prepulses alone at four intensities, and one no stimulus trial. The different trial types were presented pseudorandomly with a variable intertrial interval of 10–20 s. One session lasted about 23 min. Rats were tested on PPI four to five days after OF testing.

2.6.1. Data analysis

For each of the four 'prepulse-pulse' trial types, the percentage PPI (%PPI) was calculated: %PPI $= 100 \times (1 -$ (startle amplitude on prepulse trial/startle amplitude on pulse-alone trial)). The overall mean %PPI was calculated at the four prepulse intensities. The mean startle amplitude was calculated as the average response to the 12 'pulse-alone' trials across the entire six blocks. The PPI values were calculated for the total duration of the test session as well as for the first and second halves of the test session. Statistical analysis was conducted by ANOVA.

3. Results

3.1. Histology

Lesions were located along the medial wall in the medial prefrontal cortex between 4.2 and 2.2 mm anterior to bregma (Fig. 2A). Some variation in the size and extent of the bilateral lesions (range: $4.6 \pm 0.4\,\text{mm}^2$) was obtained but all lesions encompassed at least two of three subregions of the mPFc: the infralimbic, the prelimbic, and the ventral part of the cingulate cortex (Cg1) according to Preuss [24]. In general, the lesions were situated more in the dorsal than in the ventral part of the mPFc. Only rats with bilateral lesions greater than $4.0\,\text{mm}^2$ were included in our data analysis. Possible degeneration of other brain regions was not examined in the present study.

3.2. NMDA lesion effects on spatial discrimination (SD)

The percent correct response (%CR) of lesioned and sham rats increased across the days of training (Fig. 3A). A 2×7 ANOVA (treatment $\times$ day) on the %CR yielded no significant treatment effect [$F(1, 29) = 0.536$, $P > 0.05$], a significant effect for day [$F(6, 174) = 190.499$, $P < 0.0001$] but no interaction [$F(6, 174) = 0.458$, $P > 0.05$].

One-way ANOVA on the number of days to reach the behavioral criterion revealed no significant effect of treatment [$F(1, 29) = 2.047$, $P > 0.05$].

Latencies for both correct and incorrect responses decreased across days of training (Fig. 4A and B). A 2×7 ANOVA (treatment $\times$ day) on correct response latency revealed no significant treatment effect [$F(1, 29) = 2.332$, $P > 0.05$], but a significant main effect for day [$F(6, 174) = 25.652$, $P < 0.0001$]. No significant interaction [$F(6, 174) = 1.532$, $P > 0.05$] was found. A 2×7 ANOVA (treatment $\times$ day) yielded no significant lesion effect on incorrect response latency (Fig. 4B; [$F(2, 28) = 0.024$, $P > 0.05$]), but a significant main effect for day [$F(6, 174) = 21.490$, $P < 0.0001$] and no significant interaction [$F(6, 174) = 0.512$, $P > 0.05$].

The number of barpresses during the ITI of different treatment groups was measured (Fig. 4C). A 2×7 ANOVA (treatment $\times$ day) yielded a significant main effect for day [$F(6, 174) = 91.191$, $P < 0.0001$] but no significant treatment effect [$F(1, 29) = 0.326$, $P > 0.05$] nor interaction [$F(6, 174) = 1.181$, $P > 0.05$].

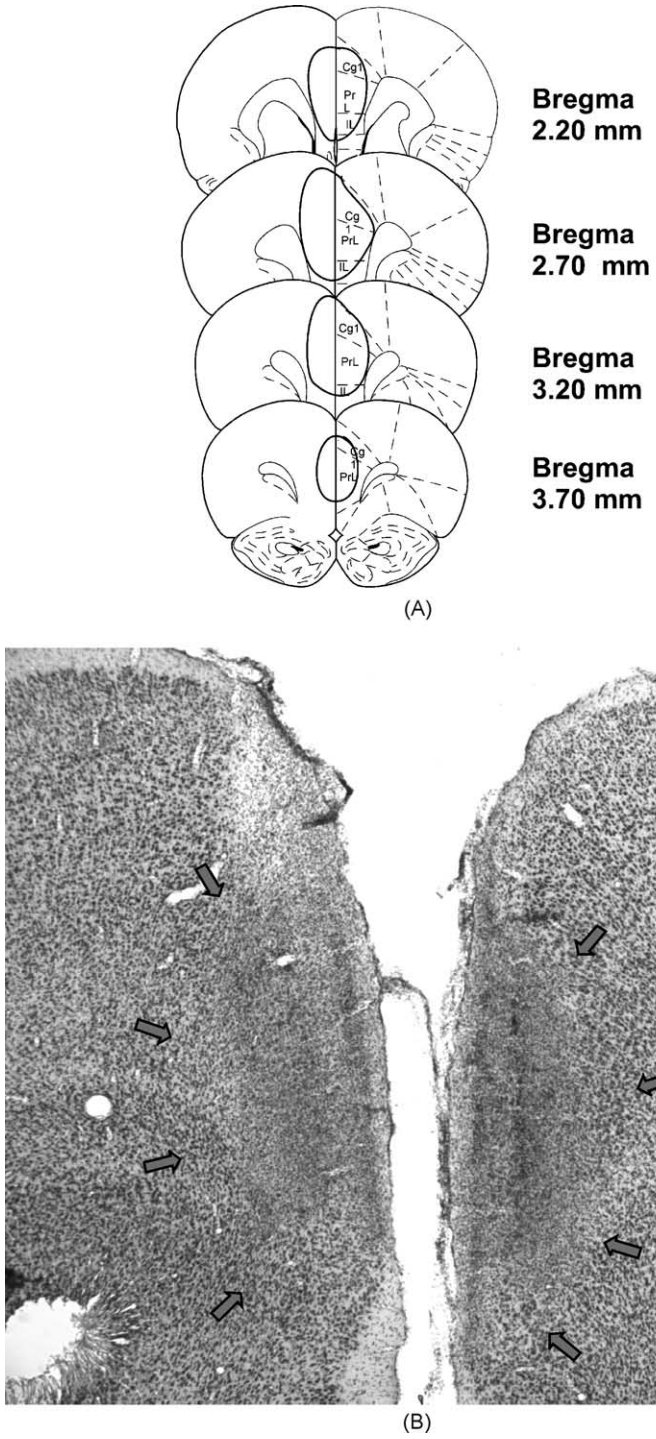

Fig. 2. (A) Schematic drawings of medial prefrontal cortex lesions. Numbers on each histological section represent the distance anterior to Bregma. IL, infralimbic cortex; PrL, prelimbic cortex; Cg1, ventral part of the cingulate cortex. Adapted from Paxinos and Watson (1997). (B) Photomicrographic image (2.5× magnification) of a coronal brain section stained with Nissl at 3.2 mm anterior to Bregma. Arrows indicate a shrinkage along the medial wall of the frontal cortex. Note that lesions were located along the medial wall in the medial prefrontal cortex encompassing the infralimbic cortex, the prelimbic cortex and the ventral part of the cingulate cortex.

3.3. NMDA lesion effects on reversal of spatial discrimination

During SDR, the %CR of all the groups increased across the days of training (Fig. 5A). A 2 × 25 ANOVA (treatment × day) on the %CR showed no treatment effect [$F(1, 29) = 0.050$, $P > 0.05$], a significant main effect of day [$F(24, 696) = 302.235$, $P < 0.0001$], and a significant interaction [$F(24, 696) = 3.497$, $P < 0.0001$]. This interaction was due to a significant main effect of

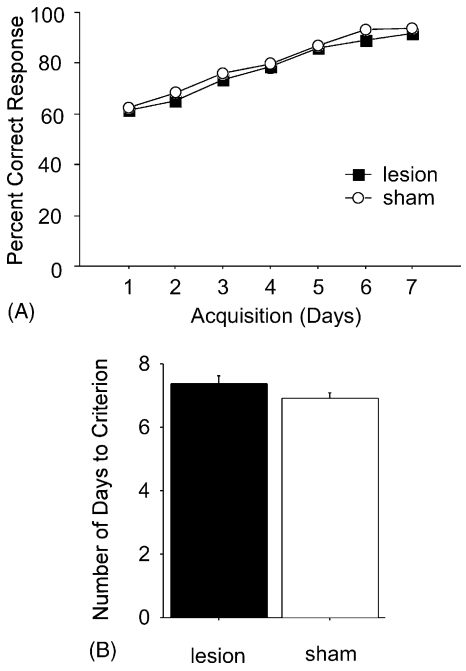

Fig. 3. Effects of NMDA lesions in the medial prefrontal cortex on acquisition of spatial discrimination. The numbers on the *Y*-axis represent the mean percentage of correct responses (A) or the number of days to reach the behavioral criterion (B) for lesioned (lesion; $n = 12$) and sham (sham; $n = 13$) animals. The numbers on the *X*-axis represent the daily session. In this and other figures, parentheses represent the standard error of the mean (S.E.M).

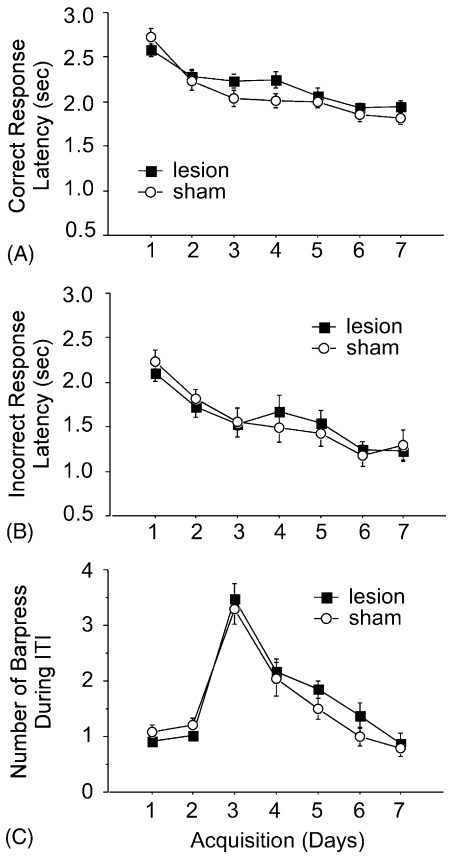

Fig. 4. Effects of NMDA lesions in the medial prefrontal cortex on response latencies and barpress during spatial discrimination. The numbers on the *Y*-axis represent the mean latency of correct (A) or incorrect responses (B), or the mean number of barpresses during the intertrial interval (C) for lesioned (lesion; $n = 12$) and sham (sham; $n = 13$) animals. The numbers on the *X*-axis represent the daily session. Asterisks denote significant differences (ANOVA; $^{*}P < 0.05$). Parentheses represent the standard error of the mean (S.E.M).

treatment on days 3, 4, 5, 6, and 22 (one-way ANOVA; [$F(1, 29) = 12.960$, 10.320, 6.721, and 4.222 and 4.726 respectively, $P < 0.05$]). Thus, during the early phase of SDR, when rats' performance was below 50% (Fig. 5A), the %CR of mPFc lesioned animals was higher than the %CR of sham controls, which was not the case during the late phase of SDR (>50% CR). We found that errors (incorrect barpresses) of lesioned rats on days 3, 4, 5, and 6 were randomly distributed between the two levers ($P > 0.05$).

Lesioned rats took longer than sham to reach a behavioral criterion (≥80% correct responses, four consecutive days; Fig. 5B). One-way ANOVA on the number of days to reach behavioral criterion yielded a significant main effect of treatment [$F(1, 29) = 4.711$, $P < 0.05$]. Reversal training was stopped on day 25. For rats that did not reach the criterion, a maximum of 25 days to reach the criterion was used. Seven out of 12 (58%) lesioned rats did not reach the criterion on SDR, in contrast to two out of 13 (15%) sham rats.

In contrast to sham rats, the lesioned rats did not decrease their correct response latencies across the days of training (Fig. 6A). A 2 × 25 ANOVA (treatment × day) on correct response latencies showed no significant effect of treatment [$F(1, 25) = 0.825$, $P > 0.05$], but a significant main effect of day [$F(24, 696) = 4.193$, $P < 0.0001$] and a significant interaction [$F(24, 696) = 3.965$, $P < 0.0001$]. A significant treatment effect on correct response latencies (one-way ANOVA) was found on days 2, 4, 15, 16, and 18–25 [$F(1, 25) = 7.248$ to 10.612, $P < 0.05$]. Post hoc analysis showed that on day 2 and 4, lesioned rats had shorter latency than sham rats ($P < 0.05$), whereas on days 15, 16, and 18–25, lesioned rats had longer latencies than sham rats ($P < 0.05$).

The incorrect response latencies of both lesioned and sham rats decreased across the days of training (Fig. 6B). In the early phase of SDR, the incorrect response latencies of lesioned rats were shorter than latencies of the shams, and in the late phase, the incorrect response latencies of lesioned rats was longer than latencies of sham controls. A 2 × 25 ANOVA (treatment × day) on the incorrect response latencies revealed no significant main effect of treatment [$F(1, 29) = 1.868$, $P > 0.05$], a significant main effect of day [$F(24, 696) = 62.213$, $P < 0.0001$] and a significant interaction [$F(24, 696) = 5.774$, $P < 0.0001$]. This interaction was due to a significant main effect of treatment on days 2, 3, 4, 6, 12, 16, 19, 20, 22, and 24 (one-way ANOVA; [$F(1, 29) = 4.40$ to 13.584, $P < 0.05$]). Post hoc analysis showed that incorrect response latencies were significantly

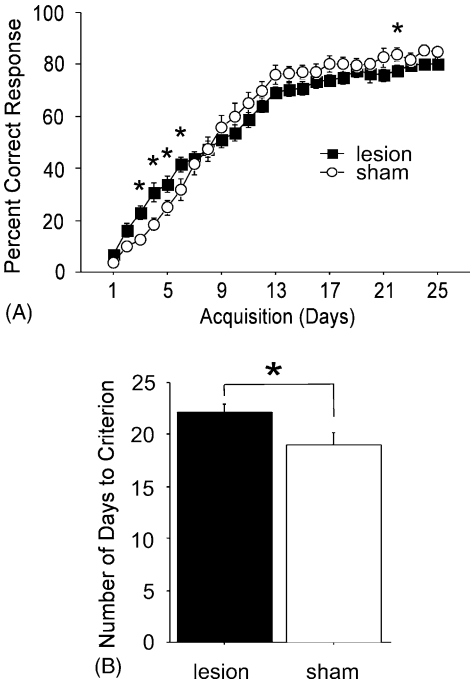

Fig. 5. Effects of NMDA lesions in the medial prefrontal cortex on reversal of spatial discrimination. The numbers on the *Y*-axis represent the mean percentage of correct responses (A) or the number of days to reach the behavioral criterion (B) for lesioned (lesion; $n = 12$) and sham (sham; $n = 13$) animals. The numbers on the *X*-axis represent the daily session. Asterisks denote significant differences (post hoc comparison; $^{*}P < 0.05$). Parentheses represent the standard error of the mean (S.E.M).

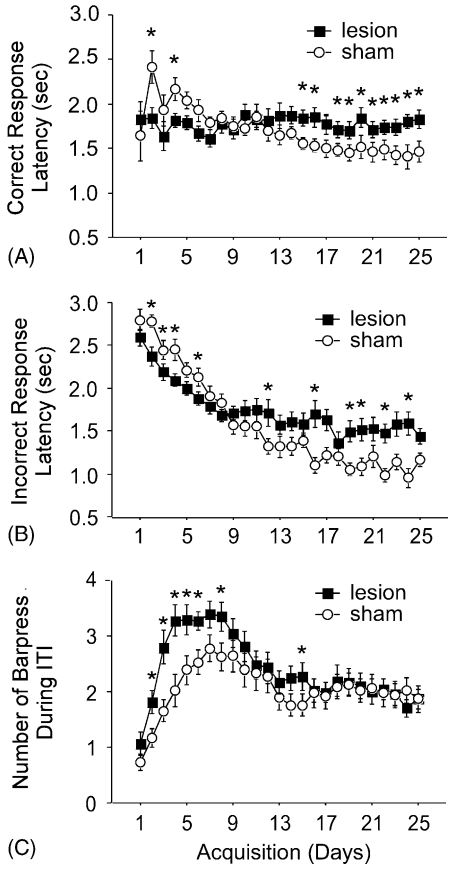

Fig. 6. Effects of NMDA lesions in the medial prefrontal cortex on response latencies and barpress during reversal of spatial discrimination. The numbers on the *Y*-axis represent the mean latency of correct (A) or incorrect responses (B), or the mean number of barpresses during the intertrial interval (C) for lesioned (lesion; $n = 12$) and sham (sham; $n = 13$) animals. The numbers on the *X*-axis represent the daily session. Asterisks denote significant differences (ANOVA; $^{*}P < 0.05$). Parentheses represent the standard error of the mean (S.E.M).

shorter in lesioned rats on days 2, 3, 4, and 6 compared to sham rats ($P < 0.05$), but longer on days 12, 16, and 19–24 compared to sham rats ($P < 0.05$).

No overall treatment effect was found but a significant interaction was obtained for ITI barpresses during SDR (Fig. 6C). A 2 × 25 ANOVA (treatment × day) revealed no main effect of treatment [$F(1, 29) = 2.785$, $P > 0.05$] but a significant effect for day [$F(24, 696) = 20.660$ $P < 0.0001$, $P < 0.0001$] and a significant interaction [$F(24, 696) = 3.023$, $P < 0.0001$]. This interaction was due to differences between treatments on days 2–6, 8, and 15 [$F(1, 29) = 5.028$ to 14.446, $P < 0.05$]. Post hoc analysis showed a significantly greater number of ITI barpresses in mPFc lesioned rats compared to the sham rats on days 2–6, 8, and 15 ($P < 0.05$). Thus, in parallel with the increase of %CR, the early phase of reversal was characterized by lesioned animals making more non-discriminated ITI barpresses than sham rats. In the late phase, the two groups continue to improve (i.e. the number of errors were comparable).

3.4. NMDA lesion effects on openfield activity and prepulse inhibition

3.4.1. Open field activity

With the exception of the first 5 min-block, lesioned rats did not differ from sham and unoperated controls in the open field arena according to the total distance moved (Fig. 7), center-crossings and time spent in the center. A 3 × 6 ANOVA (treatment × 5 min-bin) on the total distance moved revealed no main effect of treatment [$F(2, 37) = 0.011$, $P > 0.05$] but a significant main effect of 5-min blocks [$F(5, 185) = 92.792$, $P < 0.0001$], and a significant interaction [$F(10, 185) = 2.907$, $P < 0.01$]. This interaction was due to a treatment effect in the first 5 min-bin [$F(2, 37) = 3.517$, $P < 0.05$] during which lesioned rats had a higher total distance moved compared to sham rats ($P < 0.05$). The 3 × 6 ANOVAs (treatment × 5 min-bin) yielded no significant treatment effect on center-crossings and on the time spent in the center [$F(2, 37) = 1.556$ and 1.610, respectively, $P > 0.05$], a significant effect of 5 min-bin [$F(5, 185) = 2.951$ and 10.125, respectively, $P < 0.05$] and no interaction [$F(10, 185) = 1.766$ and 1.770, $P > 0.05$].

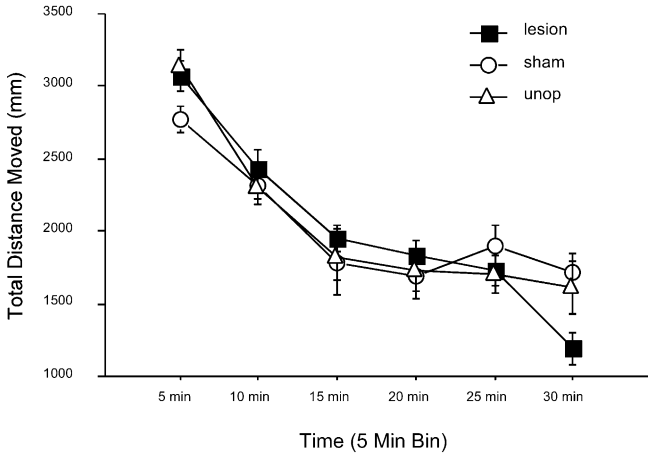

Fig. 7. Total distance moved in an open field arena. The numbers on the *Y*-axis represent the mean distance moved for lesioned (les; $n = 12$), sham (sham; $n = 13$) and unoperated (unop; $n = 15$) rats. The numbers on the *X*-axis represent bins of 5 min. Asterisks denote significant differences (ANOVA; $^{*}P < 0.05$). Parentheses represent the standard error of the mean (S.E.M).

3.4.2. Prepulse inhibition (PPI)

mPFc lesions did not affect %PPI (Fig. 8) and startle response. A 3 × 4 ANOVA (treatment × prepulse intensity) yielded a significant effect of prepulse intensity [$F(3, 111) = 173.636$, $P < 0.0001$], but no significant effect of treatment [$F(2, 37) = 0.571$, $P > 0.05$] nor an interaction [$F(6, 111) = 1.171$, $P > 0.05$]. The startle amplitude of lesioned rats did not differ from that of sham and unoperated rats. A 3 × 16 ANOVA (treatment × pulse) revealed no significant main effect of treatment [$F(2, 37) = 2.786$, $P > 0.05$], but a significant effect of pulse [$F(15, 555) = 6.982$, $P < 0.0001$] and a significant interaction [$F(30, 555) = 1.489$, $P < 0.05$]. This interaction was due to treatment differences (one-way ANOVA) on pulses 8, 10 and 13 [$F(2, 37) = 5247$, 5.403 and 4.846, respectively, $P < 0.05$): unoperated animals differed from both sham and lesioned rats on pulse 10 and 13, and from lesioned rats only on day 8 ($P < 0.05$).

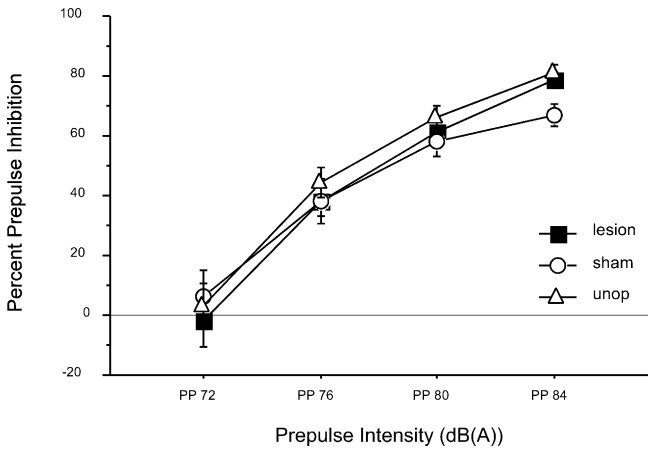

Fig. 8. Percent prepulse inhibition (%PPI). The numbers on the *Y*-axis represent the mean %PPI for lesioned (les; $n = 12$), sham (sham; $n = 13$) and unoperated (unop; $n = 15$) rats. The numbers on the *X*-axis represent the different intensities of the prepulse. Parentheses represent the standard error of the mean (S.E.M).

4. Discussions

Damage to the mPFc did not impair acquisition of a simple visuospatial discrimination task (SD). Lesioned and sham rats showed comparable performance across days on all behavioral measures. During the early part of reversal, until around day seven, lesioned rats showed higher percent correct responses, more ITI barpresses, shorter correct response latencies, and shorter incorrect response latencies. Lesioned rats had correct response latencies that monotonically decreased across days. During the later part of reversal, lesioned rats took longer to reach behavioral criterion than the controls, though learning curves were very similar, and the lesioned rats showed longer incorrect response latencies than the controls. mPFc lesions did not affect spontaneous activity in the open field arena and failed to disrupt prepulse inhibition.

4.1. Effects of NMDA lesions on spatial discrimination (SD)

mPFc lesion did not impair acquisition of SD. Performance of lesioned and sham rats was comparable on all behavioral measures, including percent correct responses (an indicator of learning rate), number of intertrial barpresses, correct response latencies, and incorrect response latencies. Lack of effect of mPFc lesions on acquisition has been reported previously for tasks involving visuospatial discrimination [18] and spatial orientation [3,15,20,25].

One feature that may engage mPFc function during acquisition is task difficulty [8,9,25], and mPFc involvement may vary depending on degree of difficulty. For example, mPFc lesion effects were observed when either the stimulus components of a stimulus-response association were difficult to learn, or when the response components were difficult to select [9]. Similarly, spatial orientation deficits in the Water maze following mPFc damage [26,27] may be particularly likely when features such as pool size and the nature of distal orientation cues increase difficulty in locating a hidden platform and render task performance susceptible to mPFc lesions. Our SD task was designed to be low on the dimension of task difficulty by involving simple stimuli and requiring simple responses. Thus, a lack of lesion effects in our study may have been due to the simplicity of our SD task.

Another feature that may engage rat mPFc function is the inclusion of a delay component that may recruit working memory processes and is thought to be critical to assess prefrontal function [10]. mPFc lesion disrupted acquisition of both a delayed matching [8] and a delayed non-matching-to-sample task [28]. Moreover, mPFc lesion

affected the acquisition of a non-matching-to-sample task only when a delay component was introduced [12]. Our SD task had similar features (e.g. equally rewarded and available responses) and was comparable to tasks used by Granon et al. [8] and Winocur and Eskes [9]. Our task did not include a delay component, and perhaps precluded mPFc dependent deficits based on working memory requirements. However, increasing evidence suggests that inclusion of a delay component does not always appear to be sufficient to produce an mPFc lesion effect. Impairment due to mPFc damage has been reported to be independent of delay components [29]. Moreover, with sufficient training, mPFc lesioned rats improved their performance [30] to the level of controls [20]. Taken together, our data support the notion that rat mPFc is not involved in the acquisition of simple spatial learning or spatial rules [12,18,20].

4.2. Effects of NMDA lesions on reversal of spatial discrimination

During reversal, lesioned rats took longer to reach a behavioral criterion than the controls. Our data are consistent with previous reports that mPFc lesions disrupted reversal learning without affecting acquisition [17–19]. Our data are inconsistent with other findings that mPFc lesions failed to affect simple reversal learning [11,12,27]. This discrepancy may be explained by the different types of reversals used in these studies and the present study. For example, Harrison and Mair [28] used serial reversal which required rats to reverse their position habit (side preference). Our findings are in agreement with previous reports that mPFc lesions impaired performance on tasks which required rats to switch from a non-matching-to-sample to a matching-to-sample rule [20] or from a stimulus-matching to a stimulus-non-matching rule [18]. Our SDR task, like our SD task, involved simple stimuli and responses and entailed no delay components. SDR performance, unlike SD performance, was affected by mPFc lesions. Response-inhibition demand during SDR was greater than during SD because the reversal required an animal to stop making an approach response in the presence of a stimulus and to make responses that were incompatible with those acquired during initial acquisition. Our data provide evidence for the involvement of rat mPFc in a situation where a response incompatible with prior training has to be performed.

An interesting observation was that during early reversal, when the animals' performance was below chance level (50%), mPFc lesioned rats made slightly more correct responses than the controls. One interpretation is that prefrontal lesions facilitated initial acquisition of the reversal [31,32]. In our case, however, facilitation was probably not due to the enhanced efficacy of a learning process. During the early phase of reversal, relative to sham rats, lesioned rats made more barpresses during the ITI and made shorter-latency responses. These measures indicate an increase in responses that were not discriminated. Transient facilitation may have been due to a reversal-specific increase in instrumental responses that were not under stimulus control. An increase in nondiscriminated responses would also explain the slower rate of reversal in lesioned rats in the present study. Similar behavioral changes have been reported previously: rats with prefrontal damage increased their responses rates [6], made a greater number of anticipatory responses prior to the stimulus onset, had shorter response latencies than controls on a three choice serial reaction time task [33], and rats had more difficulty inhibiting incorrect responses [19]. These results and the present findings support the hypothesis that rat mPFc is involved in inhibition of responses during reversal-type tests [4]. While a contribution of a response inhibition deficit to our results could not be eliminated, it is noteworthy that this contribution to the learning rate functions was limited by having a 5 s 'punishment' for an error response during a trial and by delaying (2.5 s) the onset of the discriminative stimulus if any barpress response was made within 1 s prior to stimulus onset. Simple response perseveration did not seem to play an important role in our results. Our lesioned rats made a greater number of correct responses than our sham rats did, and both lesioned and sham rats showed a similar seemingly random shifting between the two levers during early reversal. In fact, in an additional experiment with Wistar rats we observed a similar pattern (unpublished observation). Our observations are not consistent with the operations of response perseveration [3].

mPFc lesion effects on learning rate during later reversal were small. Lesioned and sham rats were comparable on most performance measures, including ITI barpresses and correct response latencies. However, incorrect response latencies of the lesioned rats tended to be longer than in the controls. These results suggest that although lesioned rats took longer to reach a behavioral criterion than the controls, their performance became comparable to that of the controls with sufficient training. Thus, in our view, the performance deficit following mPFc lesions may be specific to the early stage of reversal and may reflect the inability to inhibit nondiscriminated instrumental responses. This raises the possibility that if the animals were required to shift multiple times within a session, then demands for response inhibition would be higher. Thus, increasing response inhibition demand by requiring mPFc lesioned animals to shift multiple times would be expected to produce a marked impairment. Perhaps, such impairment following mPFc lesions can also be reflected in shifting to new strategies [3,13], shifting from an already learned rule to a new rule [20], or shifting attentional sets to new perceptual dimensions [11].

An issue that must be considered in order to interpret the effects of mPFc lesion is the size and location of lesions. It has been proposed that mPFc lesion effects in rats may require large, not small, lesions ([17,34]; see below for further discussion) and that ventral and dorsal lesions produce differential effects on performance [14,18]. Our rats, however,

had extensive lesions comparable in size to those reported to produce behavioral deficits in other tasks, including DNMS, DMS, three panel runway, water maze, and visual discrimination tasks [8,18,20,35]. In fact, we observed that small lesions did not affect performance measures during SDR (unpublished observation). In the present study, we avoided the confound of differential lesion size by excluding subjects with smaller lesions from our data analysis (see our methods section for details). Moreover, selective NMDA lesions in the infralimibic region failed to affect any performance measures during SDR, though disruption of both prelimbic and infralimbic regions via microinfusions of GABA agonist, muscimol, produced a severe impairment on SDR [36]. Thus, large lesions that encompass both prelimbic and infralimbic regions of mPFc may be required to induce a behavioral deficit during reversal.

4.3. Effects of NMDA lesions on prepulse inhibition and openfield activity

Percentage of prepulse inhibition (PPI) and startle response amplitude were comparable in mPFc lesioned and control rats. Our results are consistent with previous findings, showing a lack of mPFc lesion effect on the startle reflex measured by PPI [37,38], but they are inconsistent with other reports showing a disruption of prepulse inhibition after mPFc lesions [21,39,40]. This discrepancy may be due, in part, to the size of the lesions. For example, large lesions of the mPFc, which presumably include the infralimbic area [21] were shown to disrupt prepulse inhibition whereas smaller lesions did not [37]. On the other hand, Swerdlow et al. [38] made large lesions comparable in size to those produced in Yee's report [21], but found no mPFc lesion effect on PPI. Another interpretation is that mPFc lesion effects may depend on the type of lesions (ibotenic acid versus NMDA). For example, ibotenic [38] and NMDA lesions [37] did not affect PPI whereas 6-hydroxydopamine lesions did [41]. Our lesions included most of the prelimbic and cingulate (Cg1), and part of the infralimbic subregions of mPFc. The lack of lesion effect on PPI in the present study may have been partly due to lesion type and lesion size.

Open field activity of lesioned rats was also comparable to that of control rats, except during the first 5 min period, during which lesioned rats were more active than shams. A lack of prefrontal lesion effect on activity in a novel environment was also reported following excitotoxic lesion [32,41], high frequency lesion [43], and aspiration lesion of the mPFc [44]. Excitotoxic lesions in mPFc have also been reported to increase locomotor activity in an open field [21,37,45,46]. The size or type of the lesions may again explain part of the discrepancy. For example, large lesions were shown to increase activity [21,45,46] whereas small dorsomedial cortex lesions made with suction techniques [43] or small excitotoxic lesion limited to the prelimbic area [42,46] did not. Whether or not lesions encompass the infralimbic area may again be important. Our data are consistent with Burns et al.'s report [46] of no change in open field activity following quinolinic acid lesion in Lister hooded rats. Given that our procedural features (room illumination, type of lesions, etc.) were similar to those used by Lacroix et al. [37], the discrepancy between our results and Lacroix et al.'s [38] may have been due partly to strain difference: we used Lister hooded rats whereas Lacroix et al. [37] used Wistar rats.

In summary, mPFc lesion affected reversal of spatial discrimination without disrupting initial acquisition of spatial discrimination. The tendency of lesioned rats to barpress irrespective of the contingency during the early phase of the reversal suggests that mPFc lesions diminished the ability to inhibit instrumental responses. Barpressing without validation of which contingency was in effect may account for performance of mPFc lesioned rats. The lack of lesion effects on PPI and openfield activity suggests that slower reversal learning and enhanced ITI barpressing observed in mPFc lesioned rats was not due to disruption of sensorimotor gating or an overall increase in behavioral activity. Lesion effects on reversal were not large. This may have been partly to due the simplicity of our task and the use of a single reversal across days. Varying task complexity or introducing delay components so as to recruit other processes ascribed to the mPFc may yield larger effects. Finally, alternating tasks repeatedly within a session would increase demands for flexible shifting between sets of contingencies, and may reveal the involvement of mPFc in such shifting function. Whether rat mPFc mediates processes that involve shifting to new strategies [2,11], shifting from an already learned rule to a new rule [19], or shifting attentional sets to new perceptual dimentions [18] demands further testing.

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