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Research report

Effects of electrolytic lesions of the medial prefrontal cortex or its subfields on 4-arm baited, 8-arm radial maze, two-way active avoidance and conditioned fear tasks in the rat

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Abstract

The present study tested the effects of electrolytic lesions in two mPFC subregions, the dorsal anterior cingulate area (dACA) and prelimbic cortex, as well as the effects of a larger medial prefrontal cortex (mPFC) lesion which included both subregions, on 4-arm baited, 4-arm unbaited, 8-arm radial maze task and its reversal (Experiments 1 and 4), two-way active avoidance (Experiments 2 and 5) and conditioned emotional response (Experiments 3 and 6). Rats with large or small lesions of the mPFC learned the location of the 4 baited arms in the training and reversal stages of the radial maze task similarly to sham rats, indicating that these lesions did not affect animals' capacity to process and remember spatial information. dACA and mPFC lesions produced a transient deficit in the acquisition of the radial maze task, suggestive of an involvement of these regions in mnemonic processes. However, in view of the normal performance of these groups by the end of training and during reversal, this deficit is better interpreted as stemming from a difficulty to learn the memory-based strategy used to solve the task. Only mPFC lesion led to better avoidance performance at the beginning of training and tended to increase response during the presentation of a stimulus previously paired with shock, compared to sham rats. Both effects can be taken as an indication of reduced emotionality following mPFC lesion. The results are discussed in relation to known behavioral functions of the mPFC and the suggested functional specialization within this region. © 1997 Elsevier Science B.V.

Keywords: Frontal cortex; Medial prefrontal cortex; Dorsal anterior cingulate cortex; Prelimbic cortex; Learning; 4-Arm baited, 4-arm unbaited, 8-arm radial maze; Reversal; Conditioned emotional response; Two-way active avoidance; Emotional processes; Rat

1. Introduction

The rat medial prefrontal cortex (mPFC) has connections with diverse brain regions involved in perceptual, motor, cognitive, and autonomic-limbic functions [33,53,54,63,72]. This diversity is paralleled by multiplicity of function ascribed to the mPFC. Thus, the mPFC has been implicated in various cognitive functions, such as rule learning and the ability to use and shift between behavioral strategies [2,10,36,75], in working memory [17,36,68] (although less agreement exists with regard to this function; e.g. [60,76]), in spatial learning (e.g. [37–39,64]), and in emotional processes, particularly in the aversive domain [23,29–32,49,50,55].

The mPFC is a heterogeneous structure containing several cytoarchitectonically distinct subregions [71], which include along its dorsoventral axis the agranular medial cortex (AGm) (or Fr2 of [79]) (which has been suggested to include areas homologous to the primate premotor cortex, supplementary motor area, and frontal eye field [45,46,51,52,58,70]), dorsal anterior cingulate area (dACA), prelimbic cortex (PL), and infralimbic cortex (the latter is not considered by some writers to be part of the PFC, see [79]) [6,12,26,28,44,61,62,70]. These subregions differ in their pattern of connectivity [27,32,51–54,63,67,70,74], thus implying functional distinction. However, the establishment of structure–function relationships for these subregions still lags behind the anatomical refinement.

Most of the evidence used to ascribe different functions to different mPFC subregions has several shortcomings: (1) Some structure–function relationships are based on

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comparisons of partial and complete mPFC lesions, which may confound selective involvement of the different subregions and a 'mass action'. For example, Dunnett [17] reported that lesions of the pregenual shoulder area (AGm and dACA) result in a delay-specific deficit in the delayed non-match to sample task, implicating these regions in working memory, while larger lesions, including in addition the more ventral regions of the mPFC, result in a non-delay-dependent deficit, suggesting that the more ventral regions of the mPFC are involved in the acquisition of the more general aspects of the task (see also [19,34,64,68,78]). (2) Other conclusions are based on comparisons between different lesions in different experiments. For example, Brito et al.'s [3] conclusion that the PL cortex, rather than the shoulder area, is the critical site for spatial delayed alternation is not derived from a single study comparing the two lesions. (3) Finally, even studies using small mPFC lesions usually damage more than one mPFC subdivision (e.g. [8,9,19,35,76], but see [47]).

The present experiments sought to compare the behavioral functions of two mPFC subregions, dACA and PL. Anatomically, these regions differ in several sets of connections. The PL cortex has more extensive connections with autonomic and limbic brain regions compared with the dACA [32,33,43,51,53,54,63,66,67,72], while the dACA has more extensive connections with second-order association areas, posterior parietal cortex, and premotor areas [26,41,42,63,70,74]. This pattern of connectivity suggests that the PL might be more involved in the autonomic and limbic functions ascribed to the mPFC, while the dACA might be more involved in the cognitive functions ascribed to the mPFC.

Behavioral results thus far give only partial support to this suggestion. Thus, manipulations of the ventral mPFC (including PL) but not the dorsal mPFC (including dACA) result in alterations of autonomic responses, particularly those evoked by stress (e.g. [29–32,41,51,54]), although both regions appear to play a role in unconditioned and conditioned fear or anxiety [23,47,49,50]. There is a controversy with regard to the involvement of the shoulder area and PL cortex in the acquisition of spatial-delayed-alternation, a task involving both rule learning and working memory, and in which both regions have been implicated (shoulder area: [8,18,56]; PL: [3,4,24,68]). Some studies which assessed working memory in operant chambers suggested that the shoulder area might be more involved in the working memory component of the task, while the PL cortex might be more involved in the more general aspects of the task [17,58,73], but others concluded that the shoulder area is involved in the acquisition of skill- and rule-based behavior [77]. In addition, lesions of the ventral and dorsal mPFC (including PL and dACA, respectively) were found to induce similar effects on behavioral flexibility [9].

The present study tested the effects of electrolytic lesions in two mPFC subregions, the dACA and PL, on 4-arm baited, 4-arm unbaited, 8-arm radial maze task and its reversal (Experiment 1), two-way active avoidance (Experiment 2) and conditioned emotional response (CER; Experiment 3), as well as the effects of a larger mPFC lesion, which included both subregions, on these tasks (Experiments 4, 5, and 6, respectively). We have chosen the avoidance and CER tasks to test the involvement of the mPFC and its subregions in emotional processes [7,15,25], and the radial maze task to test their involvement in working and reference memory. We added a reversal stage to the radial maze task in order to obtain a better assessment of reference memory, since a rat with an impaired reference memory should be less affected by reversal.

2. Materials and methods

2.1. Subjects

Male Wistar rats (Tel-Aviv University Medical School, Israel) approximately 4 months old, weighing 300–420 g, were housed in pairs under reversed cycle lighting (lights on 1900–0700). Animals were maintained on ad lib food and water except for a week prior to and during the radial maze and CER tasks (see below).

2.2. Surgery

Rats were anesthetized with an i.p. injection of Equithesin (3.0 ml/kg). They were placed in a stereotaxic frame and an incision was made into the scalp to expose the skull. The vertical coordinates of bregma and lambda were measured in order to align them in the same (level head) plane. A small square of bone was removed starting approximately 1.5 mm anterior to bregma and extending rostrally about 3 mm. Bilateral electrolytic lesions were made by passing a 0.5-mA, 5-s current via a 0.3-mm electrode, insulated except for the tip. A constant current DC source was used. Each dACA animal was exposed to two anterior and two posterior lesions bilaterally. The coordinates were: anterior: 3.7 mm anterior to bregma, 0.8 mm lateral to the midline, and 1.5 mm ventral to dura; posterior: 2.7 mm anterior to bregma, 0.8 mm lateral to the midline, and 1.8 mm ventral to dura [59]. Each PL animal was exposed to two anterior and two posterior lesions bilaterally. The coordinates were: anterior: 3.7 mm anterior to bregma, 0.8 mm lateral to the midline, and 2.7 mm ventral to dura; posterior: 2.7 mm anterior to bregma, 0.8 mm lateral to the midline, and 3.1 mm ventral to dura [59]. Each mPFC animal was exposed to four anterior and four posterior lesions bilaterally. The coordinates were: anterior: 3.7 mm anterior to bregma, 0.8 mm lateral to the midline, and 1.5 and 2.7 mm ventral to dura; posterior: 2.7 mm anterior to bregma, 0.8 mm lateral to the midline, and 1.8 and 3.1 mm ventral to dura [59]. Control (sham-operated) animals underwent the same surgical procedure but without the insertion of the electrodes. Sterispon was used

to cover the hole in the bone, the scalp incisions were sutured by Michel clips, and Sulphonamide powder was sprinkled on the wound.

2.3. Apparatus

2.3.1. Radial arm maze task

The radial eight-arm maze was constructed of unpainted wood. The octagonal center platform was 31 cm in diameter, and each arm, which radiated from a side of the octagon, was 76 cm long and 12 cm wide. A 1.5-cm high wooden rim extended the length of each arm. The maze was elevated 50 cm off the floor. Holes, 3 cm in diameter and 0.6 cm deep, drilled 1 cm from the end of each arm, served as food wells. A round opaque box, 26 cm high and 28 cm in diameter, was used to cover the animal at the start of each trial. The maze was situated in a well-lit room that contained several prominent extra-maze cues, including a table, chairs, shelves, pictures, and an experimenter. These cues always remained in the same position with respect to the maze.

2.3.2. Two-way active avoidance

The apparatus consisted of four Campden Instruments shuttle boxes, each set in a ventilated sound-attenuated Campden Instruments chest (Model 412). The barrier between the two compartments of the box consisted of an aluminum wall with a central inverted U-shaped gate (12 cm high, 10 cm wide). The conditioned stimulus was a 10-s light flashing in bursts of 1.3 Hz. Shock was supplied to the grid floor by a Campden Instruments scrambled shock generator (Model 521C) set at 1 mA intensity. Equipment programming and data recording were computer controlled.

2.3.3. Conditioned emotional response

The apparatus consisted of four Campden Instruments rodent test chambers (Model 410), each set in a ventilated sound-attenuated Campden Instruments chest (Model 412). A drinking bottle could be inserted into the chamber through a 0.5-cm diameter hole which was at the center of the left wall of the chamber, 2.5 cm above the grid floor. When the bottle was not present, the hole was covered with a metal lid. Licks were detected by a Campden Instruments drinkometer circuit (Model 453). The conditioned stimulus (CS) was a 10-s, 2.8-kHz, 80 dB, tone produced by a Sonalert module (Model SC 628). Shock was supplied by a Campden Instruments shock generator (Model 521/S) set at 0.5 mA, 1 s duration. Equipment programming and data recording were computer controlled.

2.4. Procedure

2.4.1. Radial arm maze task

Prior to the beginning of the experiment, animals were handled for about 2 min daily for 7 days. Food restriction schedule was initiated simultaneously with handling. Animals were fed approximately one and a half hours per day until their body weights were reduced to 85%. This weight level was maintained throughout the experiment. Animals were weighed twice during the first week of food deprivation and every week during the behavioral testing. Water was freely available.

2.4.1.1. Adaptation to reward. On the three days preceding pretraining, rats were adapted to 45 mg food pellets (Noyes, England) in their home cages.

2.4.1.2. Pretraining. On each of 3 days, rats were trained to run on an elevated arm, using a T-maze in a different room. Each rat was individually placed at the stem of the maze and given 10 min to run and collect the food reinforcement from the wells at the end of both arms. The experimenter ensured that each rat consumed the food pellets.

2.4.1.3. Radial arm maze training. During training, four arms were baited and four arms were unbaited. For each rat, the 4 baited arms were randomly chosen with the restriction that no more than two arms were adjacent. The location of the baited arms with respect to extramaze cues did not vary. Each rat was given 1 trial a day for 6 days a week. On each trial, the food wells of the four baited arms were baited with four food pellets each. The rat was placed in the center of the maze and covered with an opaque round box that was lifted after a few seconds. This was done in order to ensure a random initial orientation of the rat and to enable the experimenter to return to his seat. The rat remained on the maze until all 4 rewards had been consumed or until 10 min had elapsed, whichever came first. The order of arm choices was recorded. An entry was recorded only if the rat reached the last quarter of the arm. Training continued for 24 days in Experiment 1 and 36 days in Experiment 4.

2.4.1.4. Reversal. On the following day, reversal training began and continued for 27 days in Experiment 1 and 15 days in Experiment 4. The procedure was identical to that of initial training, but for each rat the previously baited arms were now unbaited and the previously unbaited arms were now baited.

The number of errors made by each rat during training and reversal was classified into reference-memory errors (RME), working-memory errors (WME), and working-reference-memory errors (W-RME) (see [57]). RME consists of the first entry into an unbaited arm; WME consists of entry into arms that had been previously baited, but which had already been visited on that trial; and W-RME consists of reentry into already visited unbaited arms. The data of acquisition and reversal were analyzed using one way ANOVAs with a main factor of lesion and a repeated measurements factor of 3-day blocks, for each error type

and for the total number of errors. The effects of reversal were analyzed using one-way ANOVAs comparing the number of errors in the last 3 trials prior to reversal with the first 3 trials after the reversal for each type of error.

2.4.2. Two-way active avoidance

2.4.2.1. Activity. Each animal was placed in the shuttle box with the house light on for 60 min. The number of crossings between the two compartments of the shuttle box was recorded in blocks of 5 min. The data were analyzed using one-way ANOVA with a main factor of lesion and a repeated measurements factor of 5-min blocks.

2.4.2.2. Avoidance. On the next day, each animal was placed in the shuttle box with the house light on and received 100 avoidance trials, presented on a variable interval 60-s schedule ranging from 10 to 110 s. Each avoidance trial began with a 10-s flashing light followed by a 5-s shock, the flashing light remaining on with the shock. If the animal crossed the barrier to the opposite compartment during the 10-s flashing light, the stimulus was terminated and no shock was delivered (avoidance response). A crossing response during shock terminated the flashing light and the shock (escape response). If the animal failed to cross during the entire light-shock trial, the light and the shock terminated after 15 s. The number of avoidance responses was recorded. The data were analyzed using one-way ANOVA with a main factor of lesion and a repeated measurements factor of 10-trial blocks.

2.4.3. Conditioned emotional response

Prior to the beginning of the experiment, animals were handled for about 2 min daily for 6 days. A 23-h water restriction schedule was initiated simultaneously with handling. Animals were allowed to drink for 1 h between 2 and 4 p.m.

On each of the following 5 days, each subject was placed into the experimental chamber and allowed to drink for 15 min. On day 6, with the water bottle removed, each animal was put into the experimental chamber, and given five tone-shock pairings 5 min apart, with the shock immediately following tone termination. The first pairing was given 5 min after the start of the session. After the last pairing, animals were left in the experimental chamber for an additional 5 min.

On day 7, animals were retrained to drink in the experimental chamber. On day 8, each rat was placed in the chamber and allowed to drink from the bottle. When the rat completed 75 licks, the tone was presented, and lasted 5 min. The number of licks during tone presentation was recorded in 30-s blocks. The data were analyzed using one-way ANOVA with a main factor of lesion and a repeated measurements factor of 30-s blocks.

2.5. Histology

After the completion of behavioral testing, rats were anesthetized with an overdose of nembutal and perfused intracardially with physiological saline, followed by 10% formalin. Their brains were removed from the skulls and stored in 20% formalin–10% sucrose solution before being sectioned in the coronal plane at 80 microns thickness. Every second section was mounted and stained with thionin blue for histological examination. Verification of placements used the atlas of Paxinos and Watson [59].

2.6. Experimental design

2.6.1. Experiment 1: The effects of dACA and PL lesions on 4-arm baited, 4-arm unbaited, 8-arm radial maze

Thirty-six rats (12 dACA, 12 PL, and 12 sham) were used. Three rats (2 sham and 1 dACA) were excluded from the experiment during training because they did not enter any arm for 10 min for at least 5 days, and three rats (2 sham and 1 PL) fell ill during the experiment. Data of two rats (1 dACA and 1 PL) were discarded from statistical analysis after histological confirmation of the lesioned sites. The final analysis included 28 rats, 10 dACA, 10 PL, and 8 sham.

2.6.2. Experiment 2: The effects of dACA and PL lesions on two-way active avoidance

Thirty-one rats (11 dACA, 10 PL, and 10 sham) which participated in Experiment 1 (including the three that were excluded because of insufficient behavioral performance) were used. Testing began 14 days after the termination of the radial maze procedure.

2.6.3. Experiment 3: The effects of dACA and PL lesions on conditioned emotional response

Twenty-four rats (8 dACA, 8 PL, and 8 sham) were used. Data of 2 dACA animals were lost due to apparatus failure. The final analysis included 22 rats, 6 dACA, 8 PL, and 8 sham.

2.6.4. Experiment 4: The effects of mPFC lesion on 4-arm baited, 4-arm unbaited, 8-arm radial maze

Twenty-four rats (12 mPFC and 12 sham), from a larger batch of 48 rats operated (24 mPFC and 24 sham) were used. The other half (12 mPFC and 12 sham) participated in a delayed-non-match-to-sample task in a Skinner box (reported elsewhere). Three rats (1 mPFC and 2 sham) were excluded from the experiment during training because they did not enter any arm for 10 min for at least 5 days, and two sham rats fell ill during the experiment. Data of two mPFC rats were discarded from statistical analysis after histological confirmation of the lesioned sites. The final analysis included 17 rats, 9 mPFC and 8 sham-operated.

2.6.5. Experiment 5: The effects of mPFC lesion on two-way avoidance

Thirty-two rats, 16 (8 shams and 8 mPFC) which participated in Experiment 4, and 16 (8 shams and 8 mPFC) which participated in a delayed-non-match-to-sample task in a Skinner box (reported elsewhere) were used. Testing began 14 days after the termination of the radial maze or Skinner box procedures.

2.6.6. Experiment 6: The effects of mPFC lesion on conditioned emotional response

Twenty-eight rats (14 mPFC and 14 sham) were used. Data of three animals (2 mPFC and 1 sham) were lost due to apparatus failure. Data of one mPFC rat were discarded from statistical analysis after histological confirmation of the lesioned sites. The final analysis included 24 rats, 11 mPFC and 13 sham-operated.

3. Results

3.1. Experiment 1

3.1.1. Anatomical

Representative reconstruction of the dACA and PL lesions is presented in Plate 1, columns A and B, respectively. The dACA lesions obtained were triangular in cross-section and elongated in the anteroposterior axis. In most animals the lesion extended A–P 4.2–2.2 mm anterior to bregma. Restricted damage to the most dorsal

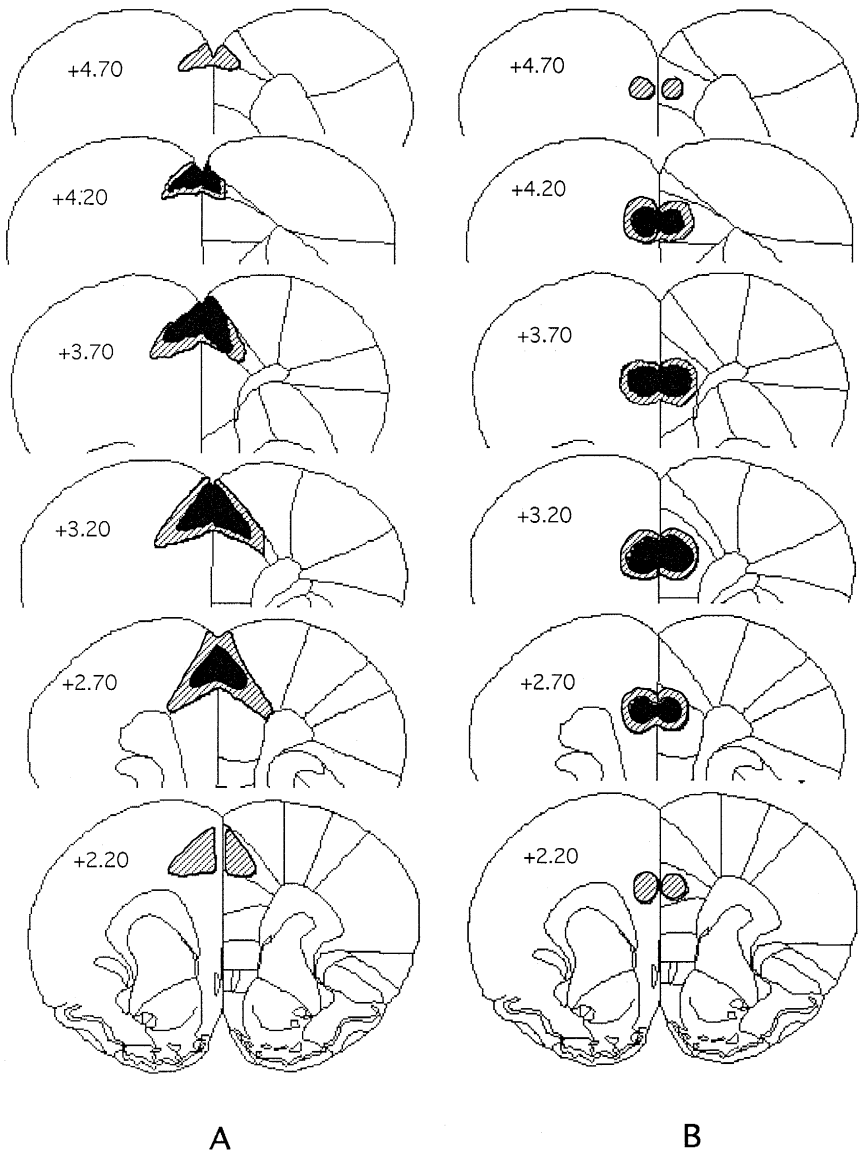

Plate 1. Reconstructions of dACA (column A, Experiments 1, 2 and 3) and PL (column B, Experiments 1, 2 and 3) lesions in successive brain sections taken from the atlas of Paxinos and Watson [59], representing the minimal (black) and the maximal (hatched) extent of the damage in common for all rats in the group.

aspect of Cg3 (PL) and the most medial aspect of Fr2 (AGm) was detected in most of the rats. The PL lesions obtained were circular in cross-section and elongated in the anteroposterior axis. The lesions extended A–P 4.2–2.2 mm anterior to bregma, in most rats. Restricted damage to the ventral aspect of dACA was detected in some of the animals. One dACA and one PL animal were excluded because of very short anteroposterior extent of the lesion.

3.1.2. Behavioral

3.1.2.1. Initial training. The number of RME, WME, W-RME, and total errors of the dACA, PL and sham groups, in 3-day blocks, is depicted on the left side of Fig. 1A–D, respectively. As can be seen, performance of the three groups in terms of RME, W-RME, and total errors, improved similarly with training. Analysis of each of the three types of error yielded only significant effects of blocks and of the linear trend of this factor (all P values < 0.0001). It can also be seen in Fig. 1B that performance in terms of WME improved similarly with training in the sham and PL rats, whereas the dACA rats exhibited a different pattern of errors, which was due to an increase in the number of WMEs in the first three blocks. Analysis of the number of WME yielded significant effects of blocks ($F(7,175) = 10.48$, $P < 0.0001$) and of the linear trend of this factor ($F(1,25) = 41.20$, $P < 0.0001$), as well as a significant quadratic trend of the Blocks x Lesion interaction ($F(2,25) = 3.71$, $P < 0.05$).

3.1.2.2. Reversal. The number of RME, WME, W-RME, and total errors of the dACA, PL and sham groups in 3-day blocks is depicted on the right side of Fig. 1A–D, respectively. As can be seen, following reversal, all three groups showed a marked increase in the number of RME, W-RME and total errors, and a less pronounced increase in

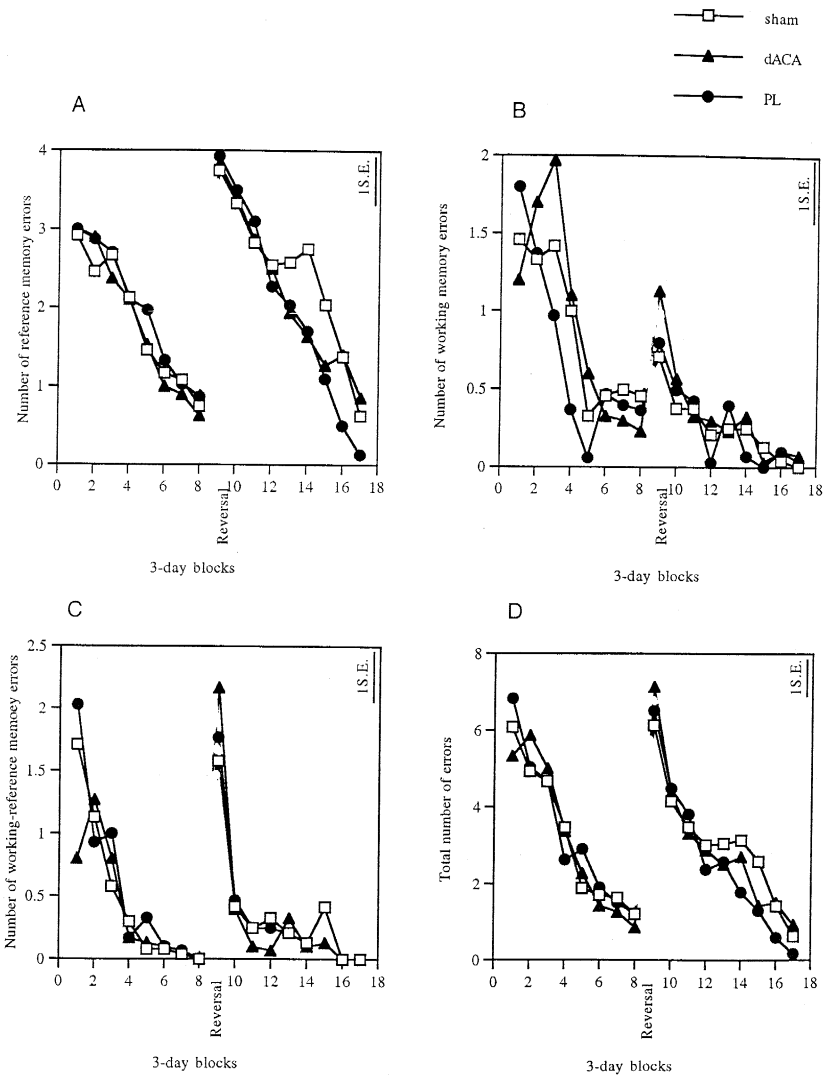

Fig. 1. The number of (A) reference memory errors, (B) working memory errors, (C) working-reference memory errors, and (D) total number of errors, in 3-day blocks, of dACA, PL and sham-operated rats in the radial arm maze, during initial training (left side) and following reversal (right side).

WME. This was supported by one-way ANOVAs comparing the number of errors in the last 3 trials prior to reversal with the first 3 trials after the reversal for each type of error, which yielded a significant effect of reversal for RME, WME, W-RME, and total errors ($F(1,25) = 619.26$, $P < 0.0001$, $F(1,25) = 6.79$, $P < 0.05$, $F(1,25) = 53.19$, $P < 0.0001$, and $F(1,25) = 121.44$, $P < 0.0001$, respectively). Lesion × Reversal interactions were not significant indicating that the effect of reversal was similar for the three groups.

In addition, it can be seen in Fig. 1A–D, right side, that all the groups reduced the number of RME, WME, W-RME and total errors as reversal progressed, and reached a similar level of performance by the end of training. Analyses of the number of WME, W-RME and total errors yielded only significant effects of blocks (all P values < 0.0001), as well as linear and quadratic trends of this factor (all $P < 0.05$). Analysis of the number of RME yielded, in addition to significant effect of blocks ($F(8,200) = 70.81$, $P < 0.0001$), and of the linear and cubic trends of this factor ($F(1,25) = 425.82$, $P < 0.0001$, $F(1,25) = 4.53$, $P < 0.05$, respectively), a significant effect of lesion ($F(2,25) = 4.50$, $P < 0.05$), and a significant Blocks × Lesion interaction ($F(16,200) = 2.18$, $P < 0.01$) and the linear trend of this interaction ($F(2,25) = 5.75$, $P < 0.01$). As can be seen in Fig. 1A, right side, these outcomes reflected higher number of RME in the sham group on the 5th and 6th blocks of reversal training.

3.2. Experiment 2

3.2.1. Anatomical

Same as Experiment 1.

3.2.2. Behavioral

Fig. 2A presents the mean number of crossings in 5-min blocks of the dACA, PL and sham rats. As can be seen, the three groups reduced their activity during the session, as reflected in significant effects of blocks ($F(11,308) = 53.23$, $P < 0.0001$) and of the linear, quadratic and cubic trends of this factor (all $P < 0.001$).

Fig. 2B presents the mean number of avoidance responses in 10-trial blocks of the dACA, PL and sham rats. As can be seen, the three groups improved similarly with training and reached similar levels of avoidance by the end of training (although dACA rats tended to show less avoidance responses than PL and sham rats at this stage). Analysis of the data yielded only significant effects of blocks ($F(9,252) = 25.76$, $P < 0.0001$) and the linear and quadratic trends of this factor ($F(1,28) = 116.98$, $P < 0.0001$, $F(1,28) = 16.51$, $P < 0.001$, respectively).

3.3. Experiment 3

3.3.1. Anatomical

The lesions were identical to those obtained in Experiment 1.

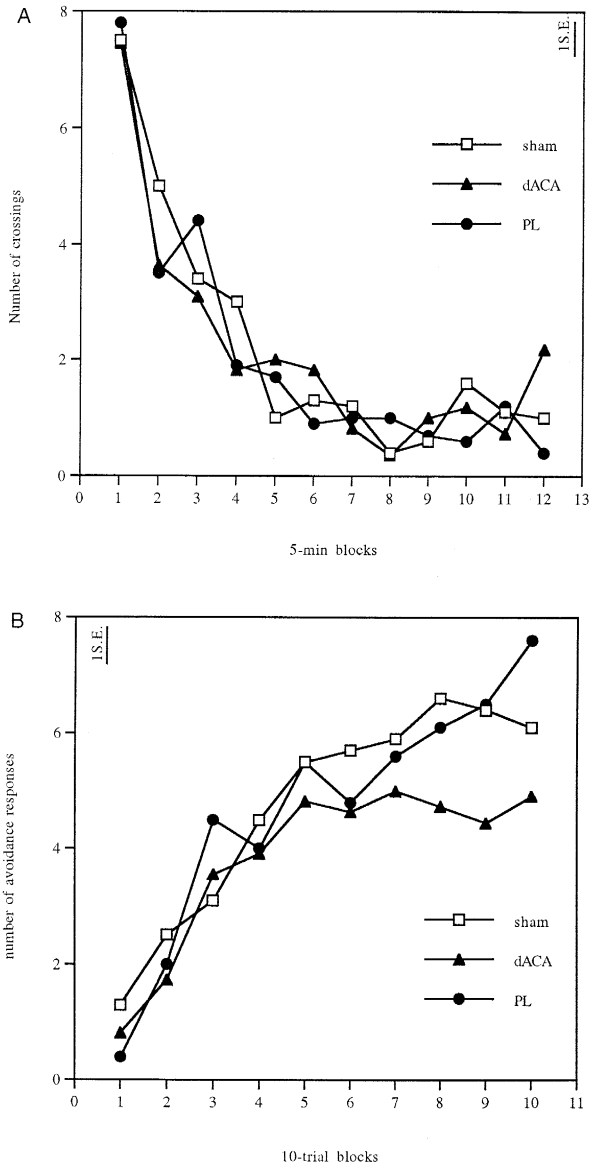

Fig. 2. A: mean number of crossings in 5-min blocks of the dACA, PL and sham-operated rats. B: mean number of avoidance responses in 10-trial blocks of the dACA, PL and sham-operated rats.

3.3.2. Behavioral

Fig. 3A presents the number of licks made during tone presentation, in 10 blocks of 30 s, of the dACA, PL, and sham rats. Although the two lesioned groups tended to drink more on most blocks, analysis of the data yielded only significant effects of blocks and the quadratic trend of this factor ($F(9,171) = 4.15$, $P < 0.0001$ and $F(1,19) = 18.40$, $P < 0.001$, respectively).

3.4. Experiment 4

3.4.1. Anatomical

Representative reconstruction of the mPFC lesions is presented in Plate 2. The lesions obtained were rectangular in cross-section and elongated in the anteroposterior axis.

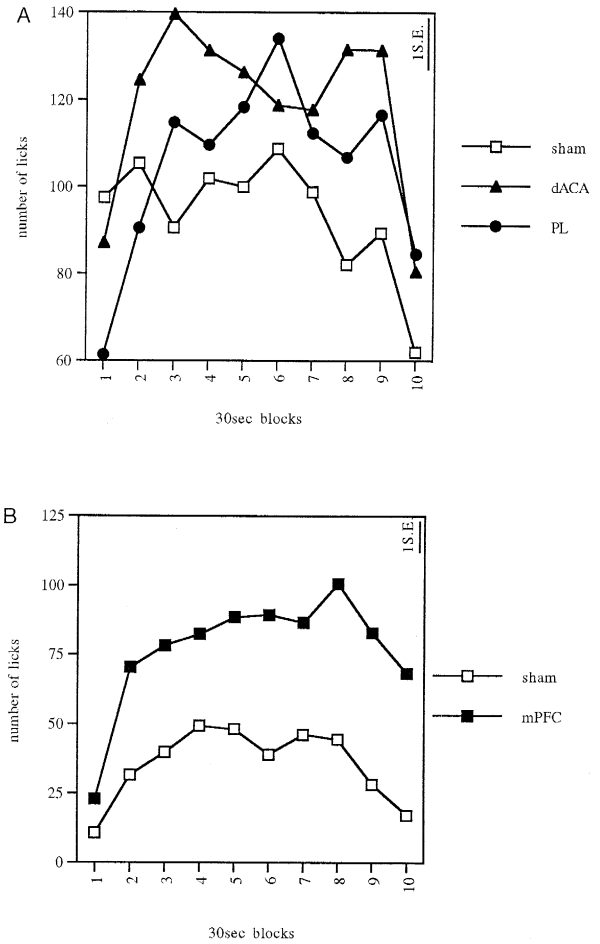

Fig. 3. Mean number of licks made during tone presentation, in 30-s blocks, of the dACA, PL, and sham-operated rats (A, Experiment 3) and of the mPFC and sham-operated rats (B, Experiment 6).

The lesions extended A–P 4.2–2.2 mm anterior to bregma in most rats. Restricted damage to the most medial aspect of Fr2 (AGm) was detected in most of the animals. One animal was excluded because of very short anteroposterior extent of the lesion. One animal was excluded because the damage to dACA was minimal.

3.4.2. Behavioral

3.4.2.1. Initial training. The number of RME, WME, W-RME, and total errors of the mPFC and sham groups, in 3-day blocks, is depicted on the left side of Fig. 4A–D, respectively. As can be seen, the performance of both groups on RME and W-RME improved similarly with training (although the mPFC rats tended to improve at a somewhat slower rate on RME). Analyses of the number of RME and W-RME yielded only significant effects of blocks and of the linear and quadratic trends of this factor (all $P < 0.0001$). In additon, the analysis of W-RME yielded a significant Lesion × Blocks interaction ($F(1,15) = 2.01$, $P < 0.05$), which reflects poorer performance of sham rats on the third block of training.

Analysis of the number of WME and total errors yielded significant effects of blocks and of the linear and quadratic trends of this factor (all $P < 0.01$). In addition, it can be seen in Fig. 4B and D that mPFC rats made more WMEs and total errors compared to sham rats on most blocks. This was supported for WME by the main effect of lesion which approached significance ($F(1,15) = 3.51$, $P = 0.08$), and for total errors, by a significant Lesion x Blocks interaction ($F(11,165) = 2.36$, $P < 0.01$).

3.4.2.2. Reversal. The number of RME, WME, W-RME, and total errors of the mPFC and sham groups in 3-day blocks is depicted on the right side of Fig. 4A–D, respec-

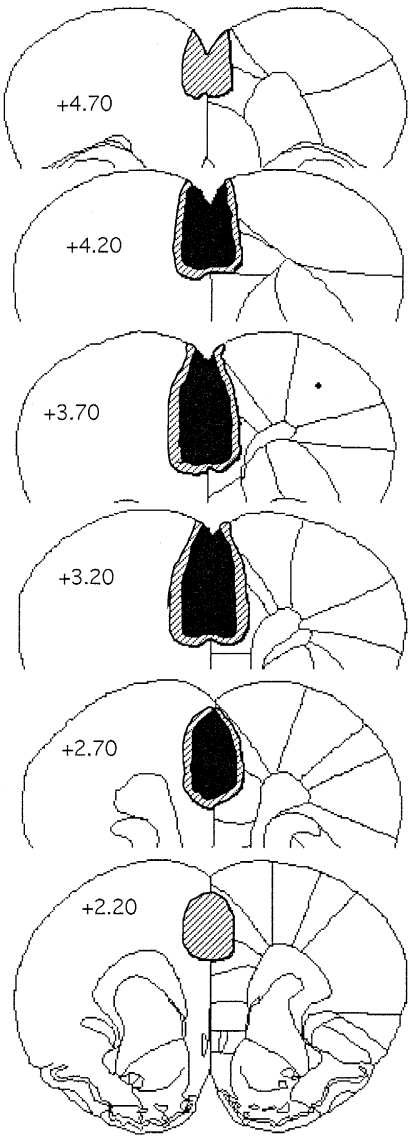

Plate 2. Reconstructions of mPFC (Experiments 4, 5 and 6) lesions in successive brain sections taken from the atlas of Paxinos and Watson [59], representing the minimal (black) and the maximal (hatched) extent of the damage in common for all rats in the group.

tively. As can be seen, both groups showed a substantial increase in the number of RME, W-RME, and total errors, but not in WME following reversal. This was supported by one-way ANOVAs comparing the last 3 trials prior to reversal with the first 3 trials after the reversal for each type of error, which yielded significant effects of reversal for RME, W-RME and total errors ($F(1,15) = 131.22$, $P < 0.0001$, $F(1,15) = 47.98$, $P < 0.0001$, and $F(1,15) = 105.14$, $P < 0.0001$, respectively). However, Lesion × Reversal interactions were not significant, indicating that the effect of reversal was similar for both groups.

It can be seen in Fig. 4A–D, right side, that as reversal progressed, both groups reduced the number of RME, WME, W-RME and total errors and reached a similar level of performance by the end of reversal training. This was supported by significant block effects (all $P < 0.05$), as well as the linear trend (all $P < 0.01$), obtained in the analyses of each error type, and no Lesion × Blocks interactions.

3.5. Experiment 5

3.5.1. Anatomical

Same as Experiment 4.

3.5.2. Behavioral

Fig. 5A presents the mean number of crossings in 5-min blocks of the sham and mPFC rats. As can be seen, activity was reduced in both groups during the session, and reached a similar level at the end. However, mPFC rats were less active than sham rats at the begining of the session. This was supported by significant effects of lesion and blocks ($F(1,30) = 5.76$, $P < 0.05$, $F(11,330) = 18.77$, $P < 0.0001$, respectively), and of the linear and cubic trends of blocks ($F(1,30) = 73.69$, $P < 0.0001$, $F(1,30) = 7.80$, $P < 0.01$, respectively), as well as by significant Blocks x Lesion interaction and the linear trend of this interaction ($F(11,330) = 18.77$, $P < 0.0001$, $F(1,30) = 4.81$, $P < 0.05$, respectively).

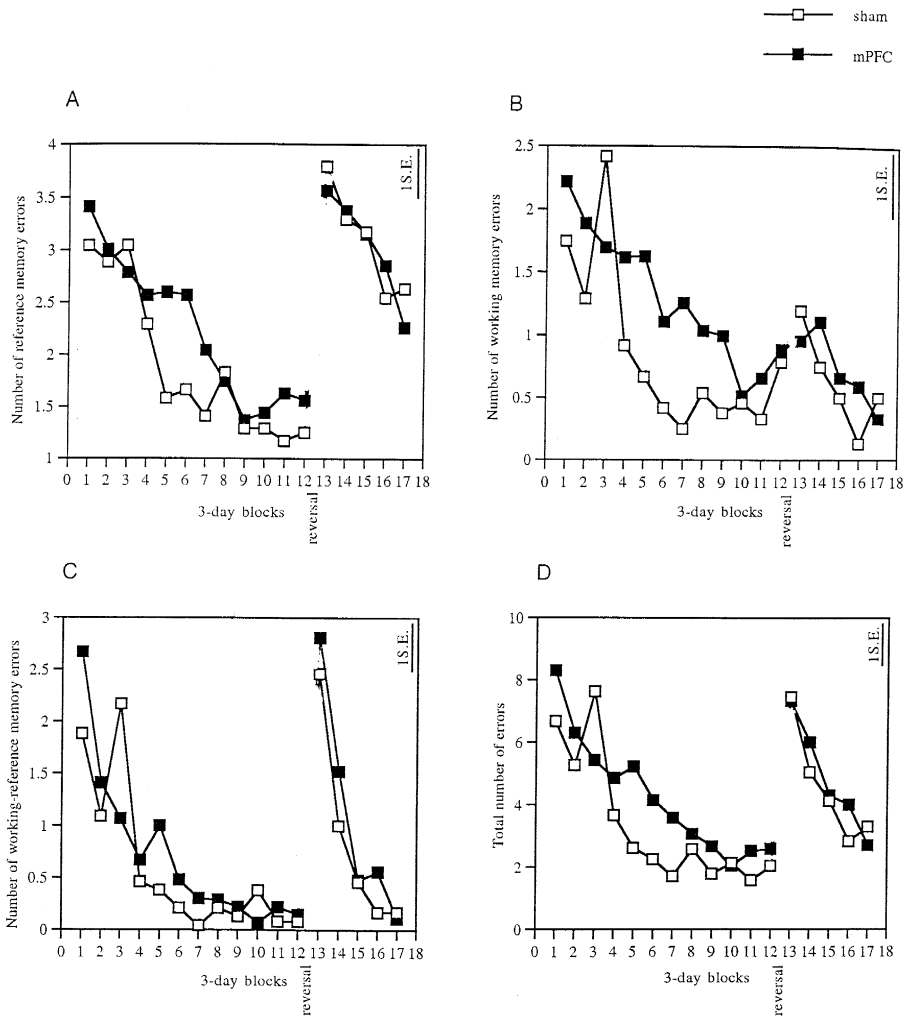

Fig. 4. The number of (A) reference memory errors, (B) working memory errors, (C) working-reference memory errors, and (D) total number of errors, in 3-day blocks of mPFC and sham-operated rats in the radial arm maze, during initial training (left side) and following reversal (right side).

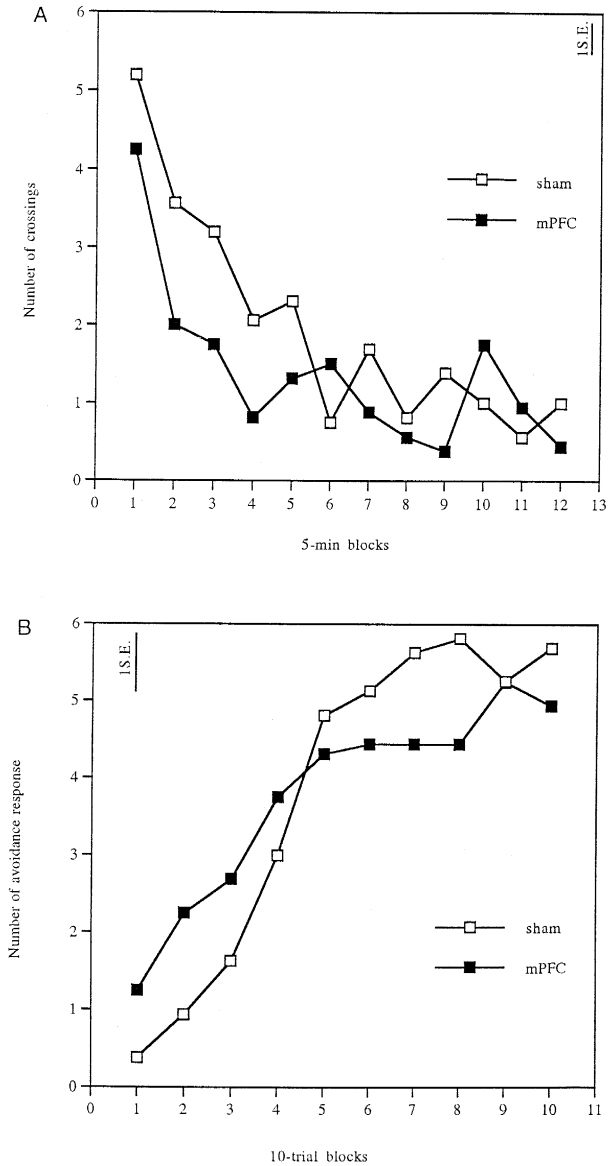

Fig. 5. A: mean number of crossings in 5-min blocks of the mPFC and sham-operated rats. B: mean number of avoidance responses in 10-trial blocks of the mPFC and sham-operated rats.

Fig. 5B presents the mean number of avoidance responses in 10 trial blocks of sham and mPFC rats. As can be seen, both groups improved with training, but mPFC animals made more avoidance responses than sham controls at the beginning of training, whereas this was reversed by the end of training. These outcomes were supported by significant effects of blocks ($F(9,270) = 29.63$, $P < 0.0001$) and of the linear trend of this factor ($F(1,30) = 99.70$, $P < 0.0001$), as well as by significant Blocks × Lesion interaction and the linear trend of this interaction ($F(9,270) = 2.50$, $P < 0.01$, $F(1,30) = 5.93$, $P < 0.05$, respectively).

3.6. Experiment 6

3.6.1. Anatomical

The lesions were identical to those obtained in Experiment 4. One animal was excluded because the damage to dACA was minimal.

3.6.2. Behavioral

Fig. 3B presents the number of licks during tone presentation in 30-s blocks of the mPFC and sham rats. Analysis of the data yielded significant effects of blocks and the quadratic trend of this factor ($F(9,180) = 4.38$, $P < 0.0001$ and $F(1,20) = 11.19$, $P < 0.01$, respectively), and a lesion effect which approached significance ($F(1,20) = 3.88$, $P < 0.1$). As can be seen in Fig. 3B, mPFC rats drank more throughout the presentation of the tone.

4. Discussion

Rats with large or small lesions of the mPFC learned the location of the 4 baited arms in the radial maze task similarly to sham rats, as reflected in similar acquisition curves of the RME and W-RME. dACA and mPFC lesions slowed down the acquisition of the 4-arm baited, 8-arm radial maze task, as a result of a slower rate of reduction in WME. However, impaired performance of the dACA and mPFC rats on the working memory component of the task was evident at the initial stages of training, whereas by the end of training the lesioned groups reached control level of performance. In addition, all the groups performed similarly following the reversal of the baited arms' location. The results obtained here in the acquisition phase contrast with those of Kolb et al. [37,38], who found that mPFC lesioned rats exhibited impaired reference- but not working-memory in a 4-arm baited, 8-arm radial maze task. It should be noted, however, that while in their earlier study [37], mPFC rats were found to exhibit more entries to unbaited arms throughout training, in the later study [38], this pattern was evident only on the first training day. Inspection of the representative lesions in these studies suggests that although in both studies AGm, dACA, and PL cortices were damaged, the lesions in the earlier study extended more caudally than those in the later study, which in turn were similar on the anteroposterior axis to the lesions in the present study. Thus, the different error pattern observed in the former compared with the two latter studies may stem, at least partly, from the difference in the caudal extent of the lesions. Interestingly, Silva et al. [64] tested mPFC rats in an 8-arm radial maze with all 8 arms baited, and found that lesions that extended more posteriorly resulted in impaired performance, whereas more restricted lesions, which were more similar to those used in the study by Kolb et al. [38], did not.

The similar RME and W-RME curves of the sham and lesioned groups in both acquisition and reversal obtained in the present study suggest that lesions of dACA and/or PL do not affect animals' capacity to process and remember spatial information. Although reduction of entries to unbaited arms (reduction in RME and W-RME) can be achieved by adopting an appropriate response strategy (i.e., a strategy that ensures one entry to each arm, baited or unbaited) rather than a memory-based strategy, the behavior of the rats following reversal indicated that their memory for the location of the baited arms was intact. Indeed, memory for the location of the baited arms was a strong determinant of the rats' behavior, as reflected in the consistency with which they visited all the previously baited arms (RME) on the initial trials following reversal, as well as in their tendency to re-enter these arms on the same trial (W-RME).

Likewise, the results do not support an existence of working memory deficit, because by the end of training mPFC and dACA rats reached sham's level of performance on the working memory component of the task, and the groups did not differ on this component following reversal. This, taken together with their normal performance on the reference memory component of the task, suggests that lesioned animals solved the task using a memory-based strategy. The slower rate of improvement of WME could stem from a difficulty of the dACA and mPFC rats to learn the memory-based strategy used to solve the task.

Other studies examining the behavior of rats with mPFC lesion in tasks requiring the processing of spatial information also concluded that mPFC lesions did not impair rats' ability to use spatial information (e.g. [1,9,24,42,60]), but rather impaired their ability to learn the general rule for solving the task. Thus, mPFC rats were able to learn the location of the goal box in a Maier's 3-table Y-maze when it was fixed, but not when its location was changed on every trial [60]. Similarly, de Bruin et al. [9] found that partial or complete mPFC lesions had no effects on the processing of spatial information in the water maze, but did retard the rats' ability to change from one behavioral strategy to another.

With regard to mPFC involvement in working memory, existing results are controversial. Most of the studies testing the effects of mPFC lesions on working memory used delay-type tasks. Although results from these tasks are consistent in demonstrating impaired performance of mPFC rats, they are inconsistent regarding the dependence of the deficit on the delay [17,36,56,68,76,77], which is required for a strict interpretation of a selective working memory impairment that can be separated from confounding lesion-induced alterations in learning capacity, motivational state, etc. [16,69]. Several studies that tested mPFC rats' performance as a function of delay, found impaired performance which was not delay-dependent [8,56,76], suggesting that mPFC rats may have a deficit in non-mnemonic aspects of these tasks, such as rule learning. In other types of tasks testing working memory, such as the radial arm maze and the Maier's three-table reasoning task, no deficits in working memory have been reported [36,60].

In a recent study [34] we found that introduction of delays after training on a Non-Match-To-Sample rule resulted in impaired performance of mPFC rats at all delays. However, the impairment could not be attributed to impaired working memory since (1) although mPFC rats showed decreased accuracy of performance after the introduction of each delay, this disappeared with training, and (2) although mPFC rats performed less accurately than sham rats by the end of training, this difference was not delay-dependent and was evident even on the no-delay trials. Rather, our results indicated that mPFC rats had difficulties in changing their strategy according to the changed task demands.

In light of the numerous reports of impaired reversal learning following mPFC lesions, the absence of such impairment in the reversal stage of the radial maze task may seem surprising. However, an important distinction should be made between reversal of a specific solution (e.g. reversing the location of the baited arms) and reversal of a general rule (e.g. changing the strategy needed to solve a task). The latter is impaired in frontal patients, as seen in their pattern of errors in the Wisconsin Card Sorting Test (e.g. [40,48]). Different lines of evidence suggest that the same may also be the case for mPFC rats. Thus, there are several studies reporting that mPFC lesions impair acquisition of a reversal learning set but do not impair reversal learning (e.g. [1,36,55]). Similarly, de Bruin et al. [9] found that mPFC rats were impaired when the strategy required for successful solution was changed but not when the location of the platform was changed (i.e., reversal of a specific solution). Recently we have found [34] that mPFC lesions identical to those used here, retarded a reversal from a Non-Match-To-Sample rule to a Match-To-Sample rule.

mPFC lesions tended to increase responding (licking) during the presentation of a stimulus previously paired with shock compared to sham rats. Such reduced conditioned emotional response can be taken as an indication of reduced emotionality, as is reliably produced in this test by anxiolytic compounds [7]. Although the mPFC is considered to play a major role in emotional behavior (e.g. [23,29–31,49,50,55]), the nature of mPFC involvement has remained a matter of debate. Thus, increased [29–31,50], decreased [22,47], and unchanged [11,29–31] emotionality following mPFC lesions have been reported. These inconsistencies are most probably due to the diverse procedures used to measure emotionality as well as to differences in lesion location and method. With regard to CER, Morgan and LeDoux, using freezing as an index for conditioned fear, reported that dorsal mPFC lesions resulted in increased fear conditioning as well as in increased resistance to extinction of conditioned fear [50], while ventral mPFC

lesions resulted in increased resistance to extinction of conditioned fear only [49]. In contrast, mPFC lesions were found to reduce freezing [22], and autonomic responses (sympathetic outflow and heart rate variability [23]) to a conditioned emotional stimulus. Likewise, using lever-pressing for food, Broersen et al. [5] found decreased CER after manipulation of the dopaminergic transmission of the mPFC.

Reduced emotionality following mPFC lesion may also account for the finding that this lesion led to better avoidance performance at the beginning of training, since facilitation of two-way avoidance is typically obtained with anxiolytic treatment, and is attributed in this context to the reduction in the impact of signals associated with shock [25]. It is also well documented that rats with low level of emotionality perform better in avoidance than highly emotional rats, and this difference in avoidance is not due to differences in learning ability but primarily to emotional factors [13–15,20,21]. Moreover, emotional factors appear to play a critical role at the initial stages of avoidance learning [15,21]. The fact that mPFC lesion-induced facilitation was observed only at the beginning of training is, therefore, consistent with reduced emotionality: since the effects of emotionality dissipate as avoidance training proceeds, the difference between mPFC and sham rats would be expected to disappear. Importantly, better avoidance performance of the mPFC rats cannot be attributed to increased activity, since mPFC lesion reduced spontaneous activity compared with the sham condition. Several studies testing avoidance in mPFC rats found poorer avoidance performance (see [39]), although intact avoidance and even improvement in some animals was reported following excitotoxic lesions of mPFC [65].

Neither dACA nor PL lesion affected CER or avoidance, although there was a tendency for the lesions to reduce CER similarly to the lesion of mPFC. Lack of effect of PL lesion on two-way avoidance was also found by Brito and Brito [4]; there are no reports regarding the effects of dACA lesions on learning. It is possible that both subregions contribute to emotional behaviors assessed in our experiments, so that only damage to both affects these functions. It should be borne in mind that our lesions were very small. Other studies using larger lesions of the ventral or dorsal aspects of the mPFC, did find effects on emotionality. Thus, Morgan and LeDoux [50] concluded that both dorsal and ventral subregions of the mPFC are involved in the fear system, but each modulates differential aspects of fear responsivity, and Frysztak and Neafsey [22] found that lesions of the dorsal and ventral subregions of the mPFC altered sympathetic activation, but in opposite directions. Apparently, the involvement of the two regions in emotional behavior depends on the specific emotional responses assessed.

To summarize, the present results do not provide evidence for mPFC involvement in spatial memory and behavior. They do provide limited support for the involvement of this region in complex learning and emotional processes. In addition, the results provide some support for the existence of functional specialization within the mPFC, since dACA but not PL lesion mimicked the effects of mPFC lesion in the radial arm maze.

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References

[1] J.T. Becker, D.S. Olton, Object discrimination by rats: The role of frontal and hippocampal systems in retention and reversal, Physiol. Behav. 24 (1980) 33–38.
[2] J.T. Becker, D.S. Olton, C.A. Anderson, E.R.P. Breitinger, Cognitive mapping in rats: The role of the hippocampal and frontal systems in retention and reversal, Behav. Brain Res. 3 (1981) 1–22.
[3] G.N.O. Brito, G.J. Thomas, B.J. Davis, S.I. Gingold, Prelimbic cortex, mediodorsal thalamus, septum, and delayed alternation in rats, Exp. Brin Res. 46 (1982) 52–58.
[4] G.N.O. Brito, L.S.O. Brito, Septohippocampal system and the prelimbic sector of frontal cortex: A neuropsychological battery analysis in the rat, Behav. Brain Res. 36 (1990) 127–146.
[5] L.M. Broersen, R.P.W. Heinsbroek, J.P.C. DeBruin, B. Olivier, Effects of local application of dopaminergic drugs into the medial prefrontal cortex of rats on latent inhibition, Biol. Psychiatry 40 (1996) 1083–1090.
[6] F. Cicirata, P. Angaut, M. Cioni, M.F. Serapide, A. Papale, Functional organization of thalamic projections to the motor cortex: An anatomic and electrophysical study in the rat, Neuroscience 19 (1986) 81–99.
[7] G.R. Dawson, M.D. Tricklebank, Use of the elevated plus maze in the search for novel anxiolytic agents, Trends Pharmacol. Sci. 16 (1995) 33–36.
[8] J.M. de Brabander, J.P.C. de Bruin, C.G. van Eden, Comparison of the effects of neonatal and adult medial prefrontal cortex lesions on food hoarding and spatial delayed alternation, Behav. Brain Res. 42 (1991) 67–75.
[9] J.P.C. de Bruin, F. Sanchezsanted, R.P.W. Heinsbroek, A. Donker, P. Postmes, A behavioural analysis of rats with damage to the medial prefrontal cortex using the Morris water maze: Evidence for behavioural flexibility, but not for impaired spatial navigation, Brain Res. 652 (1994) 323–333.
[10] L.D. Devenport, R.L. Hale, J.A. Stidham, Sampling behavior in the radial maze and operant chamber: Role of the hippocampus and prefrontal area, Behav. Neurosci. 102 (1988) 489–498.
[11] I. Divac, J. Mogenson, R.J. Blanchard, D.C. Blanchard, Mesial cortical lesions and fear behavior in the wild rat, Physiological Psychology 12 (1984) 271–274.
[12] J.P. Donoghue, C. Parham, Afferent connections of the lateral agranular field of the rat motor cortex, J. Comp. Neurol. 217 (1983) 390–404.
[13] P. Driscoll and K., Battig, Behavioral, emotional and neurochemical profiles of rats selected for extreme differences in active, two-way avoidance performance, in: I. Lieblich (Ed.), Genetics of the Brain, Elsevier, Amsterdam, 1982, pp. 95–123.

[14] P. Driscoll, J. Dedek, M. D'Angio, Y. Claustre, B. Scatton, A genetically-based model for divergent stress responses: Behavioral, neurochemical and hormonal aspects, Adv. Anim. Breeding Genet. 5 (1990) 97–107.
[15] P. Driscoll, P. Ferre, A. Fernandez-Teruel, M. Levi de Stein, C. Wolfman, J. Medina, A. Tobena, R.M. Escorihuela, Effects of prenatal diazepam on two-way avoidance behavior, swimming navigation and brain levels of benzodiazepine-like molecules in male Roman high- and low-avoidance rats, Psychopharmacology 122 (1995) 51–57.
[16] S.B. Dunnett, J.L. Evenden, S.D. Iversen, Delay-dependent short-term memory deficits in aged rats, Psychopharmacology 96 (1988) 174–180.
[17] S.B. Dunnett, Role of prefrontal cortex and striatal output systems in short-term deficits associated with aging, basal forebrain lesions, and cholinergic-rich grafts, Can. J. Psychol. 44 (1990) 210–232.
[18] H. Eichenbaum, R.A. Clegg, A. Feeley, Reexamination of functional subdivisions of the rodent prefrontal cortex, Exp. Neurol. 79 (1983) 434–451.
[19] B.D. Fantie, B. Kolb, An examination of prefrontal lesion size and the effects of cortical grafts on performance of the Morris water task by rats, Psychobiology 18 (1990) 74–80.
[20] A. Fernandez-Teruel, R.M. Escorihuela, P. Driscoll, A. Tobena, K. battig, Infantile (handling) stimulation and behavior in young Roman high- and low-avoidance rats, Physiol. Behav. 50 (1991) 563–565.
[21] A. Fernandez-Teruel, R.M. Escorihuela, J.F. Nunez, A. Zapata, F. Boix, W. Salazar, A. Tobena, The early acquisition of two-way (shuttle-box) avoidance as an anxiety-mediated behavior: Psychopharmacological validation, Brain Res. Bull. 26 (1991) 173–176.
[22] R.J. Frysztak, E.J. Neafsey, The effect of medial frontal cortex lesions on respiration, 'freezing', and ultrasonic vocalizations during conditioned emotional responses in rats, Cerebral Cortex 1 (1991) 418–425.
[23] R.J. Frysztak, E.J. Neafsey, The effect of medial frontal cortex lesions on cardiovascular conditioned emotional responses in the rat, Brain Res. 643 (1994) 181–193.
[24] S. Granon, E. Save, M.-C. Buhot, B. Poucet, Effortful information processing in a spontaneous spatial situation by rats with medial prefrontal lesions, Behav. Brain Res. 78 (1996) 147–154.
[25] S. Green and H. Hodges, Animal models of anxiety, in: P. Willner (Ed.), Behavioural Models in Psychopharmacology: Theoretical, Industrial and Clinical Perspectives, Cambridge University Press, Cambridge, UK, 1991, pp. 21–49.
[26] H.J. Groenewegen, Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the MD-prefrontal topography, Neuroscience 24 (1988) 379–431.
[27] H.J. Groenewegen, H.W. Berendse, J.G. Wolters, A.H.M. Lohman, The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: Evidence for a parallel organization, Prog. Brain Res. 85 (1990) 95–118.
[28] M. Herkenham, The afferent and efferent connections of the ventromedial thalamic nucleus in the rat, J. Comp. Neurol. 183 (1979) 487–518.
[29] R.R. Holson, Mesial prefrontal cortical lesions and timidity in rats. I. Reactivity to aversive stimuli, Physiol. Behav. 37 (1986) 221–230.
[30] R.R. Holson, C. Walker, Mesial prefrontal cortical lesions and timidity in rats. II. Reactivity to novel stimuli, Physiol. Behav. 37 (1986) 231–238.
[31] R.R. Holson, Mesial prefrontal cortical lesions and timidity in rats. III Behavior in a semi-natural environment, Physiol. Behav. 37 (1986) 239–247.
[32] K.M. Hurley-Gius, E.J. Neafsey, The medial frontal cortex and gastric motility: Microstimulation results and their possible significance for the overall pattern of organization of rat frontal and parietal cortex, Brain Res. 365 (1986) 241–248.
[33] M.J. Jay, M.P. Witter, Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin, J. Comp. Neurol. 313 (1991) 574–586.
[34] D. Joel, I. Weiner, J. Feldon, Electrolytic lesions of the medial prefrontal cortex in rats disrupt performance on an analog of the wisconsin card sorting test but do not disrupt latent inhibition: implications for animal models of schizophrenia, Behav. Brain Res. 85 (1997) 187–201.
[35] R.P. Kesner, G. Farnsworth, B.V. Mimattia, Double disssociation of egocentric and allocentric space following medial prefrontal and parietal cortex lesions in the rat, Behav. Neurosci. 103 (1989) 956–961.
[36] B. Kolb, A.J. Nonneman, R.K. Singh, Double dissociation of spatial impairment and perseveration following selective prefrontal lesions in rats, J. Comp. Physiol. Psychol. 87 (1974) 772–780.
[37] B. Kolb, K. Pittman, R.J. Sutherland, I.Q. Whishaw, Dissociation of the contributions of the prefrontal cortex and dorsomedial thalamic necleus to spatially guided behavior in the rat, Behav. Brain Res. 6 (1982) 365–378.
[38] B. Kolb, R.J. Sutherland, I.Q. Whishaw, A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats, Behav. Neurosci. 97 (1983) 13–27.
[39] B. Kolb, Functions of the frontal cortex of the rat: A comparative review, Brain Res. Rev. 8 (1984) 65–98.
[40] B. Kolb and I.Q. Whishaw, Fundamentals of Human Neuropsychology, W.H. Freeman and Company, New York, 1990.
[41] B. Kolb, Prefrontal cortex, in: B. Kolb and R.C. Tees (Eds.), The Cerebral Cortex of the Rat, MIT Press, Cambridge, 1990, pp. 438–458.
[42] B. Kolb, Animal models for human PFC-related disorders, Prog. Brain Res. 85 (1990) 501–519.
[43] D. Kooy, L.Y. Koda, J.F. McGinty, C.R. Gerfen, F.E. Bloom, The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in rat, J. Comp. Neurol. 224 (1984) 1–24.
[44] J.E. Krettek, J.L. Price, The cortical projetions of the mediodorsal nucleus and adjacent thalamic nuclei in the rat, J. Comp. Neurol. 171 (1977) 157–192.
[45] G.R. Leichnetz, S.G.P. Hardy, M.K. Carruth, Frontal projections to the region of the oculomotor complex in the rat: A retrograde and anterograde HRP study, J. Comp. Neurol. 263 (1987) 387–399.
[46] C.M. Leonard, The prefrontal cortex of the rat I. Cortical projection of the mediodorsal nucleus. II. Efferent connection, Brain Res. 12 (1969) 321–343.
[47] H. Maaswinkel, W.H. Gispen, B.M. Spruijt, Effects of an electrolytic lesion of the prelimbic area on anxiety-related and cognitive tasks in the rat, Behav. Brain Res. 79 (1996) 51–59.
[48] B. Milner, Effects of different brain lesions on card sorting, the role of the frontal lobes, Arch. Neurol. 9 (1963) 90–100.
[49] M.A. Morgan, L.M. Romanski, J.E. Ledoux, Extinction of emotional learning — contribution of medial prefrontal cortex, Neurosci. Lett. 163 (1993) 109–113.
[50] M.A. Morgan, J.E. Ledoux, Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats, Behav. Neurosci. 109 (1995) 681–688.
[51] E.J. Neafsey, E.L. Bold, G. Haas, K.M. Hurley-Gius, G. Quirek, C.F. Sievert, R.R. Terreberry, The organization of the rat motor cortex: A microstimulation mapping study, Brain Res. Rev. 11 (1986) 77–96.
[52] E.J. Neafsey, K.M. Hurley-Gius, D. Arvanitis, The topographical organization of neurons in the rat medial frontal, insular and olfactory cortex projecting to the solitary nucleus, olfactory bulb, periaqueductal gray and superior colliculus, Brain Res. 377 (1986) 261–270.
[53] E.J. Neafsey, The complete ratunculus: output organization of layer 5 of the cerebral cortex, in: B. Kolb and R.C. Tees (Eds.), Cerebral Cortex of the Rat, MIT Press, Cambridge, 1990, pp. 197–212.
[54] E.J. Neafsey, Prefrontal cortical control of the autonomic nervous

system: Anatomical and physiological observations, Prog. Brain. Res. 85 (1990) 147–166.
[55] A.J. Nonneman, J. Voigt, B.E. Kolb, Comparisons of behavioral effects of hippocampal and prefrontal cortex lesions in the rat, J. Comp. Physiol. Psychol. 87 (1974) 249–260.
[56] A.J. Nonneman, J.W. Corwin, Differential effects of prefrontal cortex ablation in neonatal, juvenile, and young adult rats, J. Comp. Physiol. Psychol. 95 (1981) 588–602.
[57] H. Okaichi, Y. Oshima, Choice behavior of hippocampectomized rats in the radial arm maze, Psychobiology 18 (1990) 416–421.
[58] R.E. Passingham, C. Myers, N. Rawlins, V. Lightfoot, S. Fearn, Premotor cortex in the rat, Behav. Neurosci. 102 (1988) 101–109.
[59] G. Paxinos and C., Watson, The Rat Brain in Stereotaxic Coordinates, 2nd edn., Academic Press, Sidney, 1986.
[60] B. Poucet, T. Herrmann, Septum and medial frontal cortex contribution to spatial problem-solving, Behav. Brain Res. 37 (1990) 269–280.
[61] R.L. Reep, J.V. Corwin, A. Hashimoto, R.T. Watson, Afferent connections of medial precentral cortex in the rat, Neurosci. Lett. 44 (1984) 247–280.
[62] R.L. Reep, G.S. Goodwin, J.V. Corwin, Topographic organization in the corticocortical connections of medial agranular cortex in rats, J. Comp. Neurol. 294 (1990) 262–280.
[63] S.R. Sesack, A.Y. Deutch, R.H. Roth, B.S. Bunney, Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: An anterograde tracing study with PHL, J. Comp. Physiol. 94 (1989) 808–818.
[64] M.G. Silva, M.A. Boyle, S. Finger, B. Numan, A.A. Bouzrara, C.R. Almli, Behavioral effects of large and small lesions of the medial frontal cortex, Exp. Brain Res. 65 (1986) 176–181.
[65] J.D. Sokolowski, L.D. McCullough, J.D. Salamone, Effects of dopamine depletions in the medial prefrontal cortex on active avoidance and escape in the rat, Brain Res. 651 (1994) 293–299.
[66] L.W. Swanson, G.J. Mogenson, Neural mechanisms for the functional coupling of autonomic, endocrine and somatomotor responses in adaptive behavior, Brain Res. Rev. 3 (1981) 1–34.
[67] R.R. Terreberri, E.J. Neafsey, Rat medial frontal cortex: A visceral motor region with a direct projection to the solitary nucleus, Brain Res. 278 (1983) 245–249.
[68] G.J. Thomas, G.N.O. Brito, Recovery of delayed alternation in rats after lesions in medial frontal cortex and septum, J. Comp. Physiol. 94 (1980) 808–818.
[69] G.J. Thomas, D.N. Gash, Differential effects of hippocampal ablations on dispositional and representational memory in the rat, Behav. Neurosci. 102 (1988) 635–642.
[70] H.B.M. Uylings, C.G. van Eden, Qualitative and quantitative comparison of the prefrontal cortex in rats and in primates, including humans, Prog. Brain Res. 85 (1990) 31–62.
[71] C.G. Van Eden, H.B.M. Uylings, Cytoarchitectonic development of the prefrontal cortex in the rat, J. Comp. Neurol. 241 (1985) 253–267.
[72] C.G. Van Groen, J.M. Wyss, Extrinsic projections from area CA1 of the rat hippocampus: Olfactory, cortical, subcortical, and bilateral hippocampal formation projections, J. Comp. Neurol. 302 (1990) 515–528.
[73] F. Van Haaren, J.P.C. Debruin, R.P.W. Heinsbroek, N.E. Vendepoll, Delayed spatial response alternation: Effects of delay-interval duration and lesions of the medial prefrontal cortex on response accuracy of male and female Wistar rats, Behav. Brain Res. 18 (1985) 481–488.
[74] B.A. Vogt, M.W. Miller, Cortical connections between rat cingulate cortex and visual, motor, and postsubicular cortices, J. Comp. Neurol. 216 (1983) 192–210.
[75] F. Winocur, M. Moscovitch, Hippocampal and prefrontal cortex contribution to learning and memory: Analysis of lesion and aging effects on maze learning in rats, Behav. Neurosci. 104 (1990) 544–551.
[76] G. Winocur, Functional dissociation of the hippocampus and prefrontal cortex in learning and memory, Psychobiology 19 (1991) 11–20.
[77] G. Winocur, A comparison of normal old rats and young adult rats with lesions to the hippocampus or prefrontal cortex on a test of matching-to-sample, Neuropsychologia 30 (1992) 769–781.
[78] C. Wolf, D. Waksman, S. Finger, C.R. Almli, Large and small medial frontal cortex lesions and spatial performance of the rat, Brain Res. Bull. 18 (1987) 1–5.
[79] K. Zilles, Anatomy of the neocortex: cytoarchitecture and myeloarchitecture, in: B. Kolb and R.C. Tees (Eds.), The Cerebral Cortex of the Rat, MIT Press, Cambridge, 1990, pp. 77–112.