Research report

Spatial delayed alternation of rats in a T-maze: effects of neurotoxic lesions of the medial prefrontal cortex and of T-maze rotations

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Abstract

The medial prefrontal cortex (mPFC) is usually considered to be a brain area important for working memory processes. In rats this statement is evidenced by their diminished performance in delay-type tasks following mPFC damage, notably in spatial delayed alternation (SDA) in a T-maze. This study has addressed two questions. First, to examine whether the functional deficiency in SDA, observed in rats with (usually large) mPFC damage, can be ascribed to an anatomically defined subarea of mPFC, the dorsal anterior cingulate area (ACd). Small, bilateral, NMDA-induced lesions were made, restricted to the dorsal part of mPFC. The performance of such animals in a T-maze paradigm, using delays of 0 and 15 s, was compared with sham-operated animals. Although these small lesions resulted in an increased number of perseverative errors, this effect was not delay-dependent, and, moreover, by the end of the training group differences had disappeared. The second aim was to study whether or not spatial (extra-maze) cues are important for the performance of this task. This was achieved by subjecting the well-trained sham-operated animals to a series of systematic trial-to-trial variations in the position of the maze in the experimental room. These spatial manipulations severely impaired the performance of the SDA task, indicating that extra-maze information is required to solve this task. In animals with ACd lesions, subjected to the same manipulations, the deficiency was comparable to that of the sham-operated animals.

Keywords: Medial prefrontal cortex; Neurotoxic lesion; Spatial delayed alternation; T-maze; Working memory; Spatial memory; Rat

1. Introduction

The prefrontal cortex (PFC), defined as the cortical projection area of the mediodorsal nucleus of the thalamus [25], occupies in the rat two distinct regions, a medial and a lateral one [18]. Based on cytoarchitectonical criteria and connectivity both areas are subdivided in a number of subareas. Along its dorsoventral axis the medial PFC (mPFC) consists of frontal area 2 (Fr2), dorsal anterior cingulate area (ACd), prelimbic area (PL) and infralimbic area (IL) [16,33,34].

The mPFC is involved in different functions [13], which include behavioural flexibility needed for an adaptive interaction with the environment, response inhibition [13,24], attentional processes [20] and spatially guided behaviour ([14], but see [7,31]). Involvement in short-term memory functions is often considered a key function of mPFC. This statement is based on a number of studies, both in non-human primates and rodents, using delay-type tasks, which reflect such functions [10,13]. In rats, as well as in various other mammalian species (e.g. [27]), the classical example of such a task, used to show functional impairment in mPFC-damaged animals, is spatial delayed alternation (SDA) in a T-maze. In this task, rats have to alternate between the two goal arms of the maze in order to obtain a reinforcement. In each particular trial, the correct choice of the arm depends on the previous choice. The interposing of delays between trials produces a delay-dependent effect on task accuracy. We have previously carried out a number of studies which illustrate that rats with damage to the mPFC are deficient in the performance of this task, especially when the delay between the trials is
In the present study we have addressed two unresolved issues. The first one is concerned with the anatonical locus in the mPFC, responsible for the deficiency in SDA learning in a T-maze. The second one is concerned with the question to which degree SDA performance in a T-maze is governed by spatial orientation.

Data on T-maze performance of rats with mPFC damage are usually derived from studies which involved suction or electrolytic lesions. Such lesions vary from relatively small ones, restricted to the dorsal parts of mPFC (Fr2, ACd) [5, 9, 32, 37], to large ones, also including the ventral parts of mPFC (PL, IL) [17, 32, 37]. Large mPFC lesions have consistently been found to impair performance in T-maze delayed alternation tasks, notwithstanding differences in procedures. With small lesions the effects on these tasks are less equivocal. On the one hand there are data which indicate that dorsal mPFC is clearly involved in the mediation of SDA in a T-maze [5, 9], while others failed to find an impairment with lesions restricted to dorsal mPFC, and deficiencies in task performance only seen when the lesion also included ventral mPFC [32, 37]. This has led Brito and Brio to hypothesise that ventral mPFC, especially its PL subarea, is essential for SDA performance [2]. Support for this hypothesis came from a study in which ventral mPFC lesions were made which resulted in a clear impairment in the T-maze task. However, these lesions also caused damage to the adjacent ACd subarea [3]. Therefore, and also in view of our earlier data, which showed delay-dependent SDA performance impairment in animals with dorsal mPFC lesions which did not invade the PL subarea [5], we think that it is still unclear which anatonical locus in the mPFC is critical for this behaviour. In order to characterise this functional deficiency anatomically, we have first examined whether a small bilateral lesion restricted to its ACd subarea would result in a deficient SDA performance. We have opted for a neurotoxic NMDA (N-methyl-D-aspartate) lesion to inflict a small-sized lesion and to prevent damage to passing fibre systems. Moreover, to minimize damage to the more dorsal area of mPFC (Fr2) we have used iontophoretic administration, which allows the use of a very small pipette, preventing 'leakage' of the neurotoxic agent along the pipette.

The second point concerns the task requirements. The suggestion that mPFC is involved in short-term memory functions is based on tasks which indeed require the operation of short-term memory processes but which most often also encompass other components, notably spatial ones. The T-maze task serves as an example. The question arises to which degree impairment in spatial orientation is responsible for impaired T-maze performance. When solving the T-maze task rats can use cues outside the maze (allocentric orientation), or they can use an egocentric orientation, based on memory for responses that depend on accurate assessment of body orientation in space. A combination of both strategies is also possible. By manipulating the extra-maze cues and measuring the performance in the T-maze, it is possible to determine whether allocentric orientation is the governing strategy in this task. This question has previously been examined by Barnes and co-workers [1]. By rotating the T-maze in between trials it could be concluded that adult rats predominantly used an allocentric orientation, while aged rats were more prone to use an egocentric orientation. Since in the Barnes' study the T-maze was rotated 180° in a predictable way, we may assume that rats could adapt to such a change. In order to completely block the use of allocentric cues, we have taken this a step further by rotating the T-maze in a for the rat completely unpredictable manner. Therefore, if rats rely on extra-maze cues to perform the SDA T-maze task, their performance should drop to chance level. On the other hand, if the task can be performed with the use of an egocentric orientation, their performance should not, or hardly, be impaired.

This issue is also of interest because of the differential involvement of brain areas in these two types of spatial orientation. The hippocampal formation is considered to be the focal brain region for allocentric spatial orientation. It has been shown that damage to the hippocampal formation not only results in impaired spatial orientation, but also leads to an impaired SDA performance in a T-maze [29]. With regard to the involvement of the PFC in spatial information processing data are less equivocal. On the one hand, data are available assigning the PFC a role in the processing of egocentric spatial information in several mammalian species, including rats [12], non-human primates [22] and humans [26]. On the other hand, a number of studies with rats has yielded data pointing to a mediating role of the PFC in allocentric spatial learning (e.g. [14, 15]). However, we have recently reported that mPFC lesions did not affect the acquisition and retention of the allocentric spatial navigation task in the water maze [7], but did impair acquisition of an egocentric version of the water maze task [31]. Based on these latter findings, one may assume that, if damage of the mPFC affects SDA performance in the T-maze, the spatial component of this working memory task requires the processing of egocentric information.

2. Materials and methods

2.1. Animals

In this study 24 male Wistar rats were used, born in the Institute. After birth, litters were randomised and
culled to 6 male and 2 female pups per litter. After weaning the males were socially housed in large macroconon cages (54 x 33 x 20 cm) in groups of four, with food and water available ad libitum. The light schedule was reversed with lights on between 19:00 and 07:00. Temperature ranged between 20°C and 22°C, humidity between 50% and 60%.

2.2. Surgery

When the animals were 10 weeks old, they were randomly divided in two groups, one group to receive NMDA-induced lesions (NMDA: n= 16, mean weight 314.5 g, range 298–345 g) and one group to be sham-operated (sham: n=8, mean weight 315.8 g, range 292–348 g). Each cage contained both NMDA and sham animals. Under Hypnorm anesthesia (0.1 ml/100 g, i.m.) the NMDA animals were placed in a David Kopf stereotaxic apparatus. Following longitudinal incision of the skin the skull was exposed and two small holes were drilled on each side of the midsaggital suture line, at 2.2 and 3.4 mm anterior to bregma and 1.2 mm lateral to the midsaggital line. At each position the dura was incised to allow the lowering of a glass micropipette (tip diameter 80–100 μm). Once the tip of the micropipette was positioned at a distance of 1.6 mm ventral of the dura, iontophoretic application of 50 mM NMDA (N-methyl-D-aspartate, Sigma), dissolved in 0.1 M Tris-HCl (pH 8.2), began and lasted for 20 min for each site. An electrode was placed inside the micropipette and connected to a locally made iontophoretic apparatus. During this 20 min period NMDA was expelled by a negative pulsed current (30 V, 6 μA; 5 s on and 5 s off). Sham operations were restricted to anesthesia, placing the animal in the stereotaxic apparatus, and exposing the skull as described above.

2.3. Histology

At the end of behavioural training (see below) animals were anaesthetised with Nembutal (0.1 ml/100 g, i.p.) and perfused transcardially with 0.9% saline followed by phosphate-buffered 4% glutaraldehyde fixative. Brains were cut with a vibroslicer at 50 μm and sections were Nissl stained. Sections containing lesions were drawn at standard levels [21] using a camera lucida. Both the assessment of lesion site and size, and the decision which animals – on the basis of anatomical criteria – to exclude from further analysis were made without knowledge of behavioural performance.

2.4. Behavioural procedures

The T-maze and SDA procedures used in the present experiment have been previously described [5,6,30]. Briefly, 1 week after operation and 1 week before starting pretraining animals were food-deprived and handled. Rats were then adapted to the maze on 5 consecutive days, and training lasted 20 days, 5 days a week, in two phases of 10 days, one phase for each intertrial interval (ITI 0 s and ITI 15 s). Errors were scored as type I or ‘first’ error and type II or ‘perseverative’ error [5,30].

After completion of the ten ITI-15 sessions allocentric cue manipulation began with the following procedures. During the first and second day the animals were adapted to the manipulation procedures. With the holding cage placed in the centre of the room, the maze was placed on a cart which during the ITI-15 was moved forward or backward, but returned to its original position. On the third day the maze was moved during the ITI-15 (with the animal in the holding cage), placed in a different position in the room and rotated 0°, 90° or 180°, either clockwise or anti-clockwise, when compared with its previous position. The sequence of spatial changes was random but identical for all animals.

3. Results

3.1. Anatomical observations

Fig. 1 shows a representative NMDA-induced lesion. Based on anatomical criteria (damage restricted to ACd, a minimum anterior-posterior lesion of 2 mm, and comparable bilateral damage) five animals of the NMDA group were selected for behavioural analysis.

3.2. Behavioural results

Fig. 1 shows a representative NMDA-induced lesion. Based on anatomical criteria (damage restricted to ACd, a minimum anterior-posterior lesion of 2 mm, and comparable bilateral damage) five animals of the NMDA group were selected for behavioural analysis.
3.2. Behavioural observations

In addition to the direct variables, first and second order errors, we calculated the percentage of correct responses. Each variable was subjected to a MANOVA with one between-subjects factor, Group (sham and NMDA), and two within subjects factors, Delay (with two levels, 0 s and 15 s) and Session (with 10 levels, days) as repeated measurement factors.

After maze adaptation and shaping, spatial alternation training began with the ITI-0 stage. Performance, computed as percentage of correct responses, increased over days reaching in both groups scores above 80% on the 10th day of training. ITI was lengthened to 15 s on the 11th day and this change clearly affected the performance of both groups. Prolonged training allowed animals to improve, with both groups finishing the ITI-15 stage above 90% of correct responses. MANOVA showed a strong effect of Delay (F(1,11)=40.14, P<0.001) and Session (F(9,99)=19.01, P<0.001), but no interaction, either between these factors (F<1), or with the main factor Group. Similar effects were found for the MANOVA's of type I and type II errors.

Despite the absence of an interaction effect, the main effect of the factor Group was statistically significant for the percentage of correct responses (mean ± SEM, sham: 84.92 ± 1.17; NMDA: 79.4 ± 2.5; F(1,11)=5.1, P=0.045), and the number of type II (perseverative) errors (sham: 0.28 ± 0.05; NMDA: 0.55 ± 0.07; F(1,11)=11.39, P=0.006), but not for the type I errors (F(1,11)=2.62, P>0.1). MANOVAs, performed separately for each delay, showed that this effect was due to a lower level of percentage of correct responses (sham: 83.3 ± 1.32; NMDA: 76.0 ± 2.43; F(1,11)=8.22, P=0.015), and a higher number of type II errors (sham: 0.28 ± 0.08; NMDA: 0.66 ± 0.11; F(1,11)=9.17, P=0.011) in the animals of the NMDA group during the ITI-0 stage. Performance during the ITI-15 stage was very similar for both groups, and no significant differences were detected.

We also compared the performance of sham and NMDA animals during the first day of training. As can be seen in Fig. 2, animals of the sham group started performing at a higher level of (spontaneous) alternations than the NMDA group (t(11)=2.48, P=0.031). Again, this effect was due to a larger number of type II errors in the NMDA animals (t(11)=2.83, P=0.016).

The second phase of the experiment consisted of a systematic trial to trial variation in the position of the maze in the experimental room to assess whether or not rats use allocentric spatial information when solving this SDA task. In the sham animals the introduction of the changes in the experimental set-up (Fig. 3, session 2) initially slightly disrupted their performance. This effect was transient: an additional session with the same set up (session 3) restored accuracy to its final ITI-15 level (session 1 in Fig. 3). When the ‘real’ manipulation was introduced (session 4 in Fig. 3) accuracy dropped abruptly to around 60%. In contrast with the 2 previous ‘control’ days, prolonging this kind of manipulation by another day, decreased accuracy even more. The rats now performed at chance level. Fig. 3 illustrates that the effects of these manipulations were similar in sham and
NMDA animals. Statistical analysis demonstrated that the effect of Session (sessions 3, 4 and 5 of Fig. 3) was highly significant \((F(2,22)=110.94, P <0.001)\). This was also true for the number of type I and type II errors, \(F(2,22)=69.79, P < 0.001\), and \(F(2,22)=25.33, P < 0.001\), respectively. Neither the main factor Group, nor its interaction with Session reached significance.

4. Discussion

The classical and widely used task to demonstrate functional impairment in mPFC-damaged rats is SDA in a T-maze, a task which provides a measure of working memory performance and encompasses spatial components. It is still uncertain whether or not deficiencies in T-maze alternation can be attributed to a specific subarea within the mPFC. On the one hand data are available implicating the dorsal mPFC (subareas ACd and Fr2) in the functional impairment \([5,9]\), while on the other hand data have been presented pointing at the ventral mPFC (subarea PL and possibly also IL) as the neural focus responsible for the impairment \([3,32,37]\). In order to contribute to the solution of this discrepancy, this study has first examined whether or not the ventral subarea of the dorsal mPFC (ACd) is critical for SDA learning in a T-maze. To restrict the damage to this anatomically specified subarea of mPFC neurotoxic lesions were made using iontophoretic application of the neurotoxic agent NMDA. These lesions resulted in a slower acquisition only during the first day of training with ITI-0, and performance rapidly improved to a level similar to that of sham-operated animals. Although there was a strong delay effect, it did not differentially affect the groups. Thus, we can conclude that small lesions of the mPFC, restricted to its ACd subarea, did not affect memory processes.

In the NMDA animals acquisition was impaired because their initial alternation was around chance level in contrast to the sham animals, which showed higher values comparable with those reported before \([6,32]\). The low level of alternation in the experimental group was due to a significantly higher number of perseverative errors. Thus, the lesion resulted in a transient increase in perseverative responses processes, which quickly diminished with practice. There are a number of papers in the literature supporting this effect of mPFC damage on perseverative tendencies in different kinds of tasks including SDA in the T-maze \([13,24,28,36,37]\). Since such damage usually includes a large area of mPFC, it is difficult to assess whether a particular subarea of the mPFC is responsible for this deficiency. Our data indicate that damage to ACd results in perseveration, be it transient. Previous experiments have shown that the perseverative effect of mPFC damage is related with the size of the lesion \([7]\).

It might be argued that the small and transient differences between the animals with damage of ACd and the sham-operates were due to the limited sham operation procedure, which did not include the lowering of the micropipette into the dorsal part of ACd. We used this type of sham operation because in previous studies we failed to detect any behavioural effects in related tasks, either following the infusion of a vehicle solution in the mPFC (ACd, PL), or following mechanical damage (Fr2) inflicted by the implantation of a guide cannula \([4]\).

Our failure to find any deficiency in working memory processes can be due to a number of factors. (i) Perhaps the damage of just one subarea is insufficient to produce impaired working memory. In most studies showing impaired working memory, lesions were considerably larger, both in an anterior-posterior and in dorso-ventral direction (see introduction for references). Perhaps mass action, sensu Lashley, is critical, and impaired working memory might have been witnessed if subarea Fr2 had been included in the lesion. (ii) Another possibility \((vide supra)\) is that the effects of dorsal mPFC lesions on working memory are caused by damage of PL. Although a re-analysis of our previous data \([5,6]\) did not support this hypothesis, this possibility cannot be dismissed in view of a number of data \([2,3,32,37]\). An examination of the effects on SDA performance of neurotoxic lesions, inflicted in the same manner as in the present study, and restricted to subarea PL, is the logical follow-up experiment. (iii) Another point concerns the difference between suction lesions employed in most studies relating mPFC damage with behavioural performance and the neurotoxic (NMDA) lesions of the present study. With lesions of the latter type damage is restricted to cell bodies, while passing fibres are spared. Thus, it is possible that behavioural deficiencies in animals with suction lesions are caused by – at least partly – the interruption of passing fibers. This issue can only be resolved by comparing the behavioural deficiencies of animals with similarly sized lesions induced by either suction or a neurotoxin.

Rotation of the T-maze in a random trial-to-trial fashion produced a random trial-to-trial rotation of the external landmarks without affecting the intra-maze environment. When subjected to these procedures behavioural performance dropped to chance levels. Thus, it can be concluded that rats use allocentric information when memorising the SDA task in a T-maze and do not rely on their egocentric information processing. This drop to chance level occurred equally in sham and NMDA animals, thus precluding a conclusion whether or not the ACd lesion had affected spatial orientation. Whether they can learn this task when the T-maze is rotated remains a question for future research.

The finding that allocentric spatial information is important for maze learning in rats is supported by data from literature showing that rats are prone to use this
kind of information when managing maze tasks [1,11,19,31]. The study of Barnes et al. [1] is of particular interest in this context. By rotating the T-maze 180°, they witnessed, in their adult rats, a drop in performance in the SDA-task, although not to the degree reported here. This could be due to the more limited manipulation, only involving a 180° turn in position. Thus, allocentric information could still be used, that is, available spatial information was still present and only the view of the animal was changed; one of the characteristics of cognitive (spatial) maps is a flexible use of the information they represent [23].

Our results demonstrate that the memorizing of SDA in a T-maze encompasses a strong allocentric spatial component. A previous study [7] has indicated that restricted damage (affecting either dorsal or ventral mPFC) or large damage (affecting the whole of mPFC) did not impair performance in the classical reference memory task in the water maze. Therefore, it is unlikely that impaired SDA performance in a T-maze in rats with mPFC damage is caused by a malfunctioning allocentric cognitive map. Although experimental data indicate that damage of mPFC results in impaired egocentric spatial learning [12,31], this impairment is unlikely to account for deficient SDA in a T-maze, since the allocentric map – most likely – is not affected [7]. Therefore, impaired SDA performance in a T-maze of mPFC-damaged rats is not the result of an impairment in spatial orientation, but in other processes, most likely working memory processes.

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