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Abstract: Contrary to human and primate, working memory in the rodent is usually considered as a simple short term memory buffer and mainly investigated using delayed response paradigms. The aim of the present study was to investigate further the role of the rat prelimbic-infralimbic (PL/IL) cortex in different spatial delayed tasks in order to dissociate its involvement in the temporary storage from other information processes, such as behavioral flexibility and attention. In Experiment 1 rats were trained in a standard elimination win-shift task in a radial-arm maze after which a 1-min delay was inserted at mid trial. PL/IL lesions induced only a transient disruption of performance following introduction of the delay. In Experiment 2, rats were trained in a win-shift task with a 5-min delay that was subsequently extended to 30 min. PL/IL lesions did not significantly affect behavior. Nevertheless, transient disruptions of performance were noted repeatedly in lesioned rats when sets of interfering events were presented. The present findings indicate that PL/IL is not directly involved in the short term maintenance of specific information but is implicated when changes, such as sudden introduction of a delay or exposure to unexpected events, alter the initial situation.

It appears that working memory in rodent should be considered, as in human and primate, to encompass both storage and monitoring functions. The present results add to previous ones strongly suggest that PL/IL is not involved in the temporary on-line storage but rather in the control of information required to prospectively organize the ongoing action.

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The role of the rat prelimbic/infralimbic cortex in working memory: not involved in the short-term maintenance but in monitoring and processing functions

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Abstract

Contrary to human and primate, working memory in the rodent is usually considered as a simple short term memory buffer and mainly investigated using delayed response paradigms. The aim of the present study was to further investigate the role of the rat prelimbic-infralimbic (PL/IL) cortex in different spatial delayed tasks in order to dissociate its involvement in temporary storage from other information processes, such as behavioral flexibility and attention. In *Experiment 1* rats were trained in a standard elimination win-shift task in a radial-arm maze after which a 1-min delay was inserted mid trial. PL/IL lesions induced only a transient disruption of performance following introduction of the delay. In *Experiment 2*, rats were trained directly in a win-shift task with a 5-min delay that was subsequently extended to 30 min. PL/IL lesions did not significantly affect behavior. Nevertheless, transient disruptions of performance (correlated with lesion extent) were noted repeatedly in lesioned rats when sets of interfering events were presented. The present findings indicate that PL/IL is not directly involved in the short term maintenance of specific information but is implicated when changes, such as sudden introduction of a delay or exposure to unexpected interfering events, alter the initial situation. It appears that working memory in rodents should be considered, as in humans and primates, to encompass both storage and monitoring functions. The present results along with previous ones strongly suggest that PL/IL is not involved in the temporary on-line storage but rather in the control of information required to prospectively organize the ongoing action.

Key Words: Prefrontal cortex- spatial memory- lesions- behavioral flexibility- attention- delayed response

Introduction

Originally defined in humans, the concept of working memory combines, within a single model, a system for temporary storage and a mechanism for online manipulation of information that occurs during a wide variety of cognitive activities (Baddeley, 1996). Accordingly, working memory relies on several cognitive operations involving attentional resources, maintenance of information, concurrent processing of cues, selection processes, updating, cognitive flexibility, and planification of action.

Since the pioneering work of Jacobsen (1931), the prefrontal cortex (PFC) has been known to be involved in the organization of delayed responses and consequently in working memory function. The central role of the frontal lobes has been confirmed by studies of brain-damaged patients as well as functional neuroimaging of healthy volunteers. More recently, evidence from humans and primates indicated that working memory is subserved by multiple distributed systems. The same studies revealed a complex topographical regionalization of the PFC that may be related either to the sensory modality of information processed (domain hypothesis; eg Goldman-Rakic, 1996 ; Levy and Goldman-Rakic, 1999) or to the nature of the cognitive activities being carried out (Petrides, 1996; Owen, 1997).

In lower vertebrates (rodents and birds), working memory was originally defined in a similar way (Honig, 1978; Olton, 1979) but was rapidly restricted to refer to a memory buffer that maintains information on-line in order to perform the task correctly. Accordingly, in these species, working memory is generally investigated with delayed response tasks that are thought to tax working memory. One subregion of the rodent PFC, the prelimbic/infralimbic (PL/IL) area that lies on the ventral bank of the medial PFC and has remarkably dense reciprocal connections with the hippocampal formation (Jay and Witter, 1991; Condé et al., 1995; Delatour and

Witter, 2002), has been specifically implicated in working memory. There is growing evidence that PL/IL is a key structure involved in cognitive processes and its particular role in working memory is supported by experiments showing that lesions of this area induce deficits in the short-term maintenance of information (Brito and Brito, 1990; Granon et al., 1994; Aggleton et al., 1995; Seamans et al., 1995; Delatour and Gisquet-Verrier, 1996, 1999, 2000; Ragozzino et al. 1998, 2002; Taylor et al., 2003; Di Pietro et al., 2004). This seems to be particularly true for spatial information, leading some authors to claim that working memory capacities subserved by PL/IL are mainly restricted to the short term maintenance of spatial allocentric information (eg Ragozzino et al., 2002; Kesner and Ragozzino, 2003).

Over the last few years however, it appears that at least some of the impairments reported after PL/IL lesions may reflect problems more global than the capacity for short-term retention of information. PL/IL lesioned rats show deficits that may reflect a disruption of attentional processes (Williams et al., 1999 ; Granon et al., 2000 ; Chudasama and Muir, 2001; Dias and Honey, 2002; Dalley et al., 2004) or an impairment in behavioral flexibility (de Bruin et al., 1994; Granon and Poucet, 1995; Ragozzino et al., 1999a,b; Birrell and Brown, 2000; Delatour and Gisquet-Verrier, 2000; Dias and Aggleton, 2000). More importantly, it has been shown that delay-dependent deficits following PL/IL lesions may be caused by processes other than those required for memory buffering. For instance, we showed that performance of rats with PL/IL lesions trained in a delayed conditional discrimination was only disrupted when the delay was progressively increased, not when all delays were inter-mixed from the start of acquisition, questioning whether this latter procedure is really more taxing in terms of behavioural flexibility (Delatour and Gisquet-Verrier, 1999; Gisquet-Verrier et al., 2000). These data suggest that disruption resulting from

PL/IL lesions were more likely due to an inability to respond to changes to the initial training protocol (delay increases) rather than to difficulty retaining specific information over delays (Gisquet-Verrier et al., 2000). As PL/IL is implicated in the processing of spatial information, it is important to generalize the results from this delayed conditional discrimination to spatial delayed tasks.

The aim of the present study was to determine the exact function of PL/IL in the organization of spatial delayed memories, including short term maintenance and other information monitoring/processing. Rats with targeted neurotoxic lesions of the PL/IL area were trained in different spatial tasks using delays that were either progressively increased or static from the beginning of training. The effect of interfering events, designed to tax attentional resources during short-term maintenance of information, was also evaluated.

In Experiment 1, rats were trained in a standard non-delayed win-shift task; once the basic task was acquired, a one-min delay was interposed mid-trial. In Experiment 2, animals were first trained in a delayed win-shift task with a 5-min delay that was then increased to 30 min. Finally, we returned the delay to 5 min and introduced interfering events during this period.

Experimental procedures

Subjects

Rats obtained from the Iffa-Credo breeding center (St Germain sur l'Arbresle, France), served as subjects. They were 50-57 days old and weighed 250 g on arrival in the laboratory. Rats were housed in pairs in wire-mesh cages and maintained on a 12:12 hr day/night cycle with free access to food and water. Cages were located in a temperature-controlled room (21°C) in which the relative humidity was maintained at about 35%. All experiments were performed in accordance with standards of the guide for the care and use of laboratory animals (CNRS ILAR) and with respect to French and European Community rules.

Surgery

One week after their arrival, rats were randomly assigned to one of two surgical groups: prelimbic-infralimbic lesion and sham-operated groups. Rats were injected with atropine (i.p. injection, 0.3 ml), and anaesthetised 15 min later with pentobarbital (50 mg/kg i.p.) before being mounted in a stereotaxic frame (Narashige Instruments, model SR6, Tokyo, Japan). The skin on the skull was incised and underlying tissue removed. Holes were drilled in the skull in order to inject into the PL/IL area at the following coordinates adapted from the Paxinos and Watson atlas (1986): AP= +3.6 mm; ML= +/- 0.7 mm and DV= -3.4 mm. Ibotenic acid (Sigma Chemical Company, L'Isle d'Abeau Chesnes, France) dissolved in phosphate buffered saline (10mg/1ml, final pH 7.4) was injected bilaterally through a cannula (180 µm diameter) using a microinjector. Ibotenic acid (0.5 µl) was delivered over a 4 min period and the cannula was left in place for a further 10 min. Sham-operated rats were subjected to

the same surgical procedure with the exception of placing a cannula in the brain. Tissue penetration and saline injection in the brain of sham rats was omitted to avoid mechanical lesions in the PL/IL area. Histology from our previous studies indicated that lowering the cannula in the cortex did not induce any visible brain damage in areas dorsal to the PL/IL area (ie dorsal anterior cingulate and medial precentral cortices) (see also Figure 1A). Surgical procedures used in the present experiment were designed 1) to achieve neurotoxin-induced tissue loss selective to PL/IL in the lesioned group, 2) to preclude any possible brain damage in the sham-operated group. At the end of the surgery, the incision was sutured and sulfamides were applied locally.

After 1 week of postoperative recovery, rats were placed on a food-deprivation schedule to gradually reduce them to 85% of their free-feeding weight. They were maintained at this level throughout the training.

Histology

Following behavioral testing, rats were injected with an overdose of pentobarbital (120 mg/kg) and perfused transcardially with saline, followed by a 10% buffered formalin solution. Brains were then removed and post-fixed overnight in 10% buffered formalin and cryoprotected by immersion in a 30% sucrose-formalin solution for 48h. Brains were then cut on a freezing microtome into 40 μ m serial sections. Every second section was mounted on a gelatin-coated slide and stained with cresyl violet. Sections were subsequently observed under a microscope to examine the extent of the lesions, then digitized and redrawn using Canvas software (Deneba, Miami, USA). Percent of PL and IL damage were determined. To be included in the lesioned group it was necessary that cortical lesions showed more than 50% bilateral damage to the PL/IL area with minor, if any, encroachment on adjacent prefrontal areas.

Material

Radial maze

The apparatus was a wooden eight-arm maze painted in grey placed on a rotating device and elevated 70 cm above the floor surface. Each arm (60 x 12 cm) radiated from a central octagonal platform (30 cm diameter), had 2 cm high plastic walls and was equipped with a food cup at each end. The radial maze was placed in a room that had several distinctive visual cues (two doors, shelves, posters on walls, a computer and a chair for the experimenter). Chocolate cereal (Chocopops, Kellogg's, Rosny-Sous-Bois, France), was used as food reinforcer.

In Experiment 2, the radial maze was equipped with tunnels in transparent PVC with a slot placed close to the central platform on each arm. Black PVC doors could be inserted in the slot of each tunnel to prevent access to certain arms.

During the delays, rats were placed in the plastic holding cage that was used for transport to the experimental room.

Interference situations

For the last phase of *Experiment 2*, rats were placed in two different environments during the delay period in order to induce interference. The first environment was a beige PVC box (23 x 23 x 36cm) placed in a room situated between the colony and the experimental rooms. The second environment was a Y-maze constructed of beige PVC, consisting of three arms (13 x 60 x 38 cm) and a 25 cm equilateral triangular choice area.

Experiment 1: Radial maze – spatial win-shift task

Behavioral procedure

Twenty-four rats (15 with PL/IL lesions and 9 with Sham-operations) were trained using a standard elimination procedure in a radial maze for eight consecutive sessions. After this initial training, a 1 min delay was interposed mid-trial in order to increase the working memory load of the task.

Pretraining

Rats were given one 15 min free-exploration period a day for two consecutive days, with chocolate cereal randomly scattered across the different sections of the maze (central platform, arms, food cups). On the first day rats were placed in pairs on the maze and then individually on the second day.

Non-delayed win-shift task

Training began on the day following pretraining. Rats were given a single trial per day for eight consecutive days. At the beginning of each trial the rat was placed on the central platform and all food cups baited (2-3 pieces of cereal per cup). The rat was removed from the maze after the eight arms had been visited. The maze was cleaned and rotated, but remained in a constant position with regard to external cues between each rat.

Introduction of a 1 min. delay

During the following three days, each rat was given a single trial per day with a one min delay period after it had visited four different arms of its own choice. The rat was taken from the maze and placed in the holding cage, located under the maze, for the delay period. The remaining food was removed, the apparatus was rotated (with

preservation of the maze's general spatial orientation), and only the four non-previously visited arms were re-baited for the 2nd phase of the trial. At the end of the delay period the rat was replaced on the central platform of the maze until it retrieved all food reinforcements.

Data analysis

Entry into a previously visited arm was scored as an error.

During the 1 min delayed task, two different types of errors were recorded during the training and test phases (Seamans et al., 1995). *Within-phase errors* made either during the training or test phase corresponded to any re-entry into an arm that had been previously entered. Within-phase errors reflected to the ability to maintain specific information about spatial location of a previously visited arm within each particular phase. *Across-phase errors* were only made during the test phase and were defined as revisiting an arm that had been entered during the training phase. Across-phase errors reflected the ability to remember specific information about the location of the first 4 arms visited over the 1 min delay period.

To analyse circling strategies (visiting adjacent arms systematically in a clockwise or counterclockwise direction) we computed a degree of divergence based on previous studies (Ammassari-Teule & Caprioli, 1985; Dubreuil et al., 2003) : within a trial, all the transitions between two consecutive arm choices were quantified by counting one unit of divergence when the rat went successively in two adjacent arms, by counting two units of divergence when the rat went successively into arms separated by one arm, by counting three units of divergence when the rat went successively into arms separated by two arms, and so on. The units of divergence corresponding to the entire trial were summed and divided by the number of visited arms minus 1. According to this calculation, degree of divergence of 1 was obtained for rats that

systematically visited adjacent arms. On the other side, the higher the index, the higher the propensity for visiting arms that diverge.

All parameters were analyzed using a three-way ANOVA (with Lesion as a between-subjects factor and Session and Error-type as the within-subjects factors), using VAR3 software (Rouanet, Bernard, and Leroux, 1990) and Statistica 6 (StatSoft, Inc., Tulsa, USA).

Results

Histology

The nature and extent of brain damage resulting from injections of ibotenic acid was determined on the basis of the neuronal loss observed in the cresyl violet-stained sections. In most cases, the lesion was characterized by a central cavity (in the vicinity of injection sites; see Figure 1A) surrounded by necrotic tissues composed of glial cells and picnotic shaped neurons. No tissue alterations were noted in cortical regions dorsal to the PL/IL area: the cannula tract was hardly visible and both cortical layering and neuronal morphology were preserved (Figure 1A). Also no signs of inflammation (GFAP immunostaining; data not shown) were observed outside the targeted PL/IL region. In most of the lesioned rats, brain damage largely involves the IL cortex and, to less extent, the PL cortex, as already depicted in our previous studies (Delatour and Gisquet-Verrier, 1996, 1999, 2000). Eight rats were discarded from subsequent behavioral analysis for different reasons but mainly related to limited damage to the PL cortex (overall reduced lesion size, n=2; unilateral lesions, n=2; lesions restricted to rostral or caudal prelimbic cortex, n=2; lesions that did not damage the dorsal prelimbic cortex, n=2). In the remaining seven lesioned rats, ibotenic acid-induced lesions were found to be bilateral and to have damaged most

of the ventromedial PFC (see Figure 1B; global mean damage to the PL/IL area: 76% $\pm$ 4.78; minimum: 61% ; maximum: 95%).

Non-delayed win-shift task

Due to excessive anxiety two sham-operated rats were eliminated. Analyses were therefore carried out on 7 sham-operated rats and 7 PL/IL-lesioned rats. During the acquisition of the non-delayed win-shift task, lesioned rats did not differ from the sham-operated rats. The number of errors rapidly decreased across sessions ($F(7,168)= 9.27$; $p<.001$; see Figure 2) with no Lesion effect ($F(1,24)= 1.88$; ns) and no significant interaction between Lesion and Session ($F(7,68)= 1.48$; ns). Rats, in the two groups, made more across- than within-phase errors ($F(1,24)= 35.04$; $p<.001$) and there was no Session x Lesion nor Session x Lesion x Error-type interactions ($F_s<1$).

Analysis of degrees of divergence did not show any differences between sham-operated and PL/IL-lesioned rats ($F<1$). Degrees of divergence were close to 2 (mean value obtained over the eight training sessions - Sham: 2.02 ± 0.1 ; PL/IL: 1.93 ± 0.19) indicating that both groups did not use simple clockwise or anti-clockwise strategies to visit arms during the non-delayed win-shift task.

Introduction of a 1 min. delay

Contrary to sham-operated rats, lesioned rats were disrupted by the introduction of a 1 min delay (See Figure 2). Analysis of variance performed on the number of errors made by rats during the last non-delayed session and the first delayed session showed a significant Lesion effect ($F(1,24)= 5.49$; $p<.05$) and a Lesion x Session interaction ($F(1,24)= 8.32$; $p<.01$), indicating that the introduction of a 1 min delay mid-trial differentially affected sham-operated and PL/IL lesioned rats. More

precisely, introduction of the delay did not modify performance of sham-operated rats ($F < 1$) but caused a clear impairment in PL/IL-lesioned rats ($F(1,24) = 13.62$; $p < .005$). The Session x Error-type and Session x Error-type x Lesion interactions were not significant ($F_s < 1$) underlining that lesioned rats were similarly impaired on all measures of behavioral accuracy (across- and within-phase errors).

Delayed win-shift task

As indicated in Figure 2 (right part), the disruptive effect of the delay was very transient as sham-operated and PL/IL-lesioned rats showed a similar level of performance on the 2nd and 3rd days of testing with a 1-min delay ($F_s < 1$). An analysis of variance performed on the number of errors made during the three delayed sessions indicated no main effect of Session and Lesion ($F_s < 1$), but a significant interaction between Session and Lesion factors ($F(2,48) = 5.63$; $p < .01$). Such interaction was due to the progressive decrease of errors in the lesioned group ($F(2,48) = 3.85$; $p < .05$) that was not observed in the sham-operated group ($F(2,48) = 1.98$; ns). Lesioned rats made significantly more errors than sham-operated rats during the first delayed task training session, ($F(1,24) = 13.89$; $p < .001$), an effect that was not obtained thereafter. During the three delayed sessions, the number of across-phase errors was significantly larger than the number of within-phase errors ($F(1,24) = 34.73$; $p < .001$), with no Lesion x Error-type interaction ($F < 1$).

Degrees of divergence were not affected by the 1-min delay introduction as rats showed similar divergence in the non-delayed and delayed conditions ($F(1,12) = 1.22$; ns), with no interaction between Lesion and Task factors ($F(1,12) = 1.87$; ns). Replicating previous observations, degrees of divergence were above 1 (mean value

obtained over the three training sessions - Sham: 2 ± 0.07 ; PL/IL: 2.14 ± 0.12) and did not differ between groups ($F < 1$).

Summary

From the present results, it appears that PL/IL lesions did not affect performance in the standard elimination task in a radial maze and did not largely modify the foraging strategy used by control rats. Introduction of a one minute delay mid-trial, however, transiently affected the performance of PL/IL-lesioned rats without preventing the acquisition of the delayed task. These results suggest that the detrimental effects of PL/IL lesions are more likely associated with the introduction of the delay than to the length of the delay period.

Experiment 2: spatial delayed win-shift

There are several possible reasons that PL/IL-lesioned rats showed only a transient disruption of performance when the delay was imposed.

First, in Experiment 1, rats selected the first half of the arms themselves, allowing them to solve the task by using any strategy (e.g. stereotyped sequences of arms visits). To preclude such a possibility, in experiment 2 rats were tested with a spatial delayed win-shift procedure where the choice of the first set of 4 arms was arbitrarily determined by the experimenter (adapted from Seamans et al., 1995). Second, on the basis of our previous results (Gisquet-Verrier et al., 2000), we suspected that the transient disruption of PL/IL-lesioned rats obtained in Experiment 1 was caused by the sudden alteration in the initial experimental conditions (i.e. introduction of a delay) rather than by the delay itself. To test this, in experiment 2 rats were trained in the

delayed version of the task from the start. Third, as the transience of the impairment might result from the brevity of the delay period (1 min), rats in the present experiment were trained with longer delays (5 and 30 min). Finally, the possibility that disruption resulted from changes in the experimental situation was investigated by introducing interfering events during the delay period.

Thus, in Experiment 2 rats were initially trained in a 5-min delay version of the win-shift task. Subsequently two types of changes were introduced successively. First, the delay was increased from 5 to 30 minutes. Thereafter, the delay was reduced back to 5 minutes, and interfering events were introduced during the delay period.

Behavioral procedure

Twenty two male Sprague-Dawley rats (50-57 days old) were assigned to one of two groups: sham-operated rats (n=9) and PL/IL-lesioned rats (n=13).

Pretraining

During the two days of pretraining, rats were allowed to explore the radial arm maze with chocolate cereal available in the food cups at the end of each arm for 10 min. On the first day, rats were placed on the maze in groups of four and then individually on the 2nd day.

Training

Following pretraining, rats were trained in the spatial delayed win-shift task and were given one trial per day. Each trial consisted of a training phase and a test phase, separated by a delay. At the beginning of each trial, four arms chosen randomly by the experimenter were blocked and chocolate cereal was placed in the food cup of the other four arms. During the *training phase*, when rat had retrieved the food from the four open arms it was returned to its home cage for the delay. During this period,

the radial maze was rotated to prevent the use of olfactory cues, but the spatial orientation of the maze was kept constant as previously described. In the *test phase*, all arms were now opened but food was only available in arms positioned in previously non-visited spatial locations. The rat was re-placed on the central platform and the trial ended after all baited arms had been visited.

Rats were first trained with a five minute delay until they achieved a criterion of performance of retrieving the food reinforcers in five or fewer choices (i.e. maximum of one error) during the test phase for two consecutive days. Rats who did not reach this criterion in 22 training sessions were given a score of 22 and were eliminated from subsequent training phases. Rats were then trained in a 30 min-delayed version of the same task for 10 consecutive daily trials. Following that, the delay period was reduced to 5 min during which rats were exposed to interfering events. The interference phase comprised three daily trials. For the first interference phase, rats were placed in a novel PVC box during the delay. For the second interference phase, rats were placed in the Y-maze and allowed to explore this new environment.

Results

Histology

PL/IL cortical lesions induced in rats in Experiment 2 were very similar in terms of location and extent to those described in the first experiment. Seven rats were discarded because their lesions were considered too restricted (mostly invading only small part of the PL region). For the six remaining rats, lesions extensively damaged the PL and the IL cortices (global mean damage to PL/IL area: $63\% \pm 8.96$; minimum: 58%; maximum: 87 %) but largely spared the dorsomedial prefrontal cortex. The orbitofrontal cortex was marginally damaged in three rats but the encroachment was

very small and not considered sufficient to justify exclusion. Behavioral analyses were thus performed on 9 sham-operated rats and 6 PL/IL-lesioned rats.

Acquisition of the 5-min delayed task

Rats took a minimum of four consecutive training sessions before reaching criterion. As illustrated in Figure 3, PL/IL lesions did not affect the acquisition of the 5-min delayed win-shift task. An analysis of variance with one between group variable (Lesion) and two within group measure (Session and Error-type) was performed on the number of errors made by the two groups of rats during these first four training sessions (see Figure 3A, left part). The analysis revealed a significant effect of Error-type, with a significantly larger number of across- than within-phase errors ($F(1,13)=16.64$, $p<.0013$) and a main effect of Session ($F(3,39)=4.50$, $p<.01$) but no effect of Lesion and no interaction between any of these different factors.

The numbers of within- and across-phase errors made during the training and test phases before reaching criterion are illustrated in Figure 3B. Analyses of variance of these data indicated a main effect of the Error-type factor ($F(1,13)=49.57$, $p<.001$), with no effect of Lesion ($F<1$) and no interaction between these two factors ($F(2,26)=1.20$; ns). The number of within-phase errors which was very low during the training phase, was somehow higher during the test phase ($F(1,13)=4.48$; $p=.054$).

Analysis of variance performed on the number of trials to criterion (see Figure 3A, right part) showed no difference between sham-operated and PL/IL-lesioned rats ($F<1$). One rat in each group was unable to achieve the training criterion within 22 trials and both were eliminated for the rest of the experiment, leaving a total of 8 sham-operated rats and 5 PL/IL-lesioned rats.

Extending the delay from 5 to 30-min

Results of the acquisition of the 30-min delayed task are illustrated in Figure 4. Extending the delay between the training and test phases from 5 to 30 min resulted in a similar increase in the number of across-phase errors for both groups of rats. As the number of within-phase errors observed during the training phase was constantly very low (mean number of errors always under 1, in every trials), data analysis was conducted only on within- and across-phase errors made during the test phase.

A three-way analysis of variance on the number of errors performed during the last 5-min and the first 30 min sessions indicated a significant effect of Error-type and Session ($F(1, 11) = 6.74, p < .025$), with no Lesion effect ($F < 1$) and no Lesion by Session interaction ($F(1, 11) = 1.80, ns$). Complementary analyses indicated that the increase in delay affected only across-phase errors ($F(1, 11) = 5.40, p < .05$) and not within-phase errors ($F(1, 11) = 2.02, ns$).

Performing the 30-min delayed task

During the ten 30-min delayed sessions, the number of errors progressively decreased (see Figure 4), with no difference between the two experimental groups. Analysis of variance showed a significant effect of Error-type with across-phase errors significantly more numerous than within-phase errors ($F(1, 11) = 79.44, p < .001$) and a main effect of Session ($F(9, 99) = 2.02, p < .045$) but no effect of Lesion and no interaction between these different factors ($F_s < 1$).

There were no correlation between performance (total number of errors during the ten 30-min delayed sessions) and the extent of the lesions (PL, IL and PL-IL : $r_s < .11, ns$).

Interference phases

Interference I: As illustrated in Figure 4 (right part), exposure to a novel box during the 5 min delay period caused the lesioned rats to make more errors than sham-operated rats. Analysis of variance performed between the last 30-min delay session and the first session of *Interference I* revealed a significant effect of Error-type ($F(1,11)= 7.00, p<.025$), Session ($F(1,11)= 4.91, p<.05$) and of Session by Lesion interaction ($F(1,11)= 14.90, p<.005$). Additional analyses indicated that the disruptive effect due to the interfering events was observed in the PL/IL-lesioned rats ($F(1,4)= 8, p<.048$) but not in the sham-operated rats ($F(1, 7)= 2.33, ns$) and affected across-phase ($F(1,4)= 7.76, p<.05$) but not within-phase errors ($F<1$).

A three-way analysis of variance performed on the number of errors made during the three trials performed during the first interference revealed a significant main effect of Error-types ($F(1,11)= 33.41, p<.001$), did not indicate any main effect of Lesion or Session ($F(1,11)=2.09, ns$ and $F(2,22)=1.43, ns$, respectively) but revealed a significant interaction between these two factors ($F(2,22)= 5.50, p<.025$), resulting from a higher number of errors in PL/IL-lesioned rats than sham-operated rats, during the first trial of interference I ($F(1,11)= 7.73, p<.025$). This increase which only concerned across-phase errors ($F(1,11)= 8.15, p<.015$), was not seen during the second and the third trials ($F<1$ and $F(1,11)= 2.34, ns$, respectively).

Interference II: Modifying the interference conditions re-introduced a clear disruptive effect in the lesioned rats (see Figure 4). Comparison of the last *interference I* and the first *Interference II* trial revealed a significant Lesion by Session interaction ($F(1,11)= 15.11, p<.0025$) due to an increase of errors in the PL/IL group ($F(1,4)= 10, p<.05$) that was not observed in sham-operated rats ($F(1,7)= 2.33, ns$). Complementary analyses further indicated that the disruptive effect observed in

PL/IL-lesioned rats affected the across-phase errors ($F(1,4)= 13.5$, $p<.025$) but not the within-phase errors ($F<1$).

A three-way analysis of variance performed on the three trials of *interference II* indicated an effect of Error-type ($F(1,11)= 72.74$, $p<.001$), an effect of Lesion that nearly reached the statistical level of significance ($F(1,11)= 4.57$, $p<.056$), no effect of Session ($F<1$) and a significant Session by Lesion interaction ($F(2,22)=3.62$, $p<.05$). Similar to *Interference I*, lesioned rats made significantly more errors than sham-operated rats ($F(1,11)= 8.03$, $p<.025$) during the first trial of interference II but not thereafter ($Fs<1$ during the 2nd and 3rd trials of *Interference II*).

Correlations were obtained between performance (total number of errors during the interference sessions) and the lesions extent for PL, and PL-IL, but not for IL lesions ($r= 0.73$, $p<.05$; $r=0.70$, $p<.05$ and $r= 0.56$, ns, respectively).

Summary

Results obtained in Experiment 2 indicated that rats with PL/IL lesions were able to learn a spatial delayed win-shift task similar to control rats, even when stereotyped patterns of response were precluded by the use of a forced-choice procedure. Rats with PL/IL lesions were able to remember the spatial location of previously visited arms within a particular phase and even across the training and test phases when separated by a 5-min delay. Extending the delay period from 5 to 30 min resulted in a transient disruption of performance in both groups of rats. Rats with PL/IL lesions as well as sham-operated rats then demonstrated a progressive improvement in performance with additional training, showing similar abilities to acquire the 30-min delay version of the spatial task. When the delay was subsequently reduced to 5 min and interfering events were introduced during the delay (*interference I*), the performance of lesioned rats, but not of control rats, was transiently disrupted. Such

an effect could result from the combination of the reduction of the delay (from 30 min to 5 min) and the pretest exposure to the interfering events. Nevertheless, as similar transient disruptive effects were observed again in PL/IL-lesioned rats when placed in a new environment while still performing the 5-min delayed task (*interference II*), it appears that exposure to unexpected events *per se* is sufficient to cause behavioral impairments in lesioned rats. Subsequent analyses revealed that disruption due to interference was correlated with the extent of both PL and PL-IL damage.

In all, the present experiment revealed that rats with lesions of the PL/IL area were able to acquire a spatial delayed-response task and to maintain their performance even with an extended time delay. Contrary to control rats, however, PL/IL-lesioned rats were susceptible to interfering events introduced during the delay period.

Discussion

The present series of experiments examining the role of PL/IL in spatial delayed tasks revealed that lesions of this area disrupted behavioral performance only under specific circumstances.

First, from the present results it appears that rats with lesions to the PL/IL cortex are able to perform a spatial delayed working memory task. In *Experiment 1*, rats with PL/IL lesions were able to use trial unique information in a standard radial arm maze task similar to control rats, corroborating our previous results (DelaTour and Gisquet-Verrier, 1996). They were also able to perform the task when a one-minute delay was introduced at mid-trial. In both cases arm choice was free, so we cannot be sure that lesioned rats did not use subtle alternative strategies to compensate for a potential deficit. There were two main differences between Experiment 1 and 2. In Experiment 2, the first set of arms was fixed by the experimenter in order to prevent the use of any alternative strategy; from the beginning of training, we imposed a five-min delay. As rats with PL/IL lesions still presented normal acquisition in these conditions, we conclude that PL/IL is not required for holding on a specific information over a 5 min time period. This capacity may extend beyond 5 minutes, as lesioned rats still performed as control rats when required to remember the location of the first set of visited arm over a 30-min period.

Second, the present results indicated that PL/IL lesions induced clear, but short-lasting disruptions in performance in each experiment: in *Experiment 1*, when lesioned rats were shifted from a non-delayed to a 1-min delayed win-shift task and, in *Experiment 2*, when PL/IL-lesioned rats were exposed to a novel box or maze

environment during the delay period. It is possible that the latter disruption of performance was due to retroactive interference, interfering stimuli preventing remembering of the first set of baited arms. In all cases, PL/IL lesioned rats exhibited performance deficits only during the first session following a change in the initial experimental situation. Thereafter, lesioned rats rapidly recovered to achieve normal performance levels. In Experiment 1, the introduction of the delay induced a performance deficit that affected both within- and across-phase errors, indicating that such a change transiently disrupted the overall ability to perform the task. In Experiment 2, interfering stimuli only affected across-phase errors, indicating that rats were mainly impaired in their capacity to remember the location of the first set of visited arms over a 5-min period.

The role of PL/IL in temporary on-line storage

The present set of data provides evidence that PL/IL is not directly involved in the retention of specific information during a delay period. The data clearly indicate that PL/IL is not required for the maintenance of spatial information during time intervals up to 30 min. This finding strengthens our previous results indicating that PL/IL was not involved in holding relevant information in a go/no-go conditional discrimination task (Gisquet-Verrier et al., 2000) and extends this assertion for allocentric spatial information.

The role of PL/IL in maintaining information during a short interval arises from the putative homology between this region and the primate dorsolateral PFC (but see Delatour, 1999) than on conclusive data. Several studies, for example, do not report any effect of PL/IL lesions on short-term spatial memory. Large medial prefrontal lesions, including PL/IL, have repeatedly been shown to spare performance on a delayed non-matching-to-position task (DNMTP), nor did damage restricted to PL/IL

affect recognition of object position (Aggleton et al., 1995; Porter and Mair, 1997; Porter et al., 2000). In addition, when reported, deficits induced by mPFC lesions in spatial tasks are always mild or transient and not delay-dependent, arguing against its possible involvement in temporary on-line storage (Aggleton et al., 1995; Kesner et al., 1996; Young et al., 1996; Chudasama and Muir, 1997; Porter and Mair, 1997; Ragozzino et al., 1998; Porter et al., 2000; Taylor et al., 2003). Interestingly, Seamans and collaborators (1995) did report significant impairment of spatial delayed responses in rats following transient inactivations of the PL/IL area. Such a disruptive effect may come from the use of lidocaine that may provide unexpected side effects such as drug diffusion (Edeline et al., 2002) or inactivation of axons traversing the targeted area (Sandkühler and Gebbhart, 1991). In addition, it should be noted that the reported behavioral deficits were obtained using a single-trial testing paradigm, a procedure that may have exaggerated the transient deficit, resulting from mPFC lesions, often limited to the very first trials (eg de Bruin et al., 1994; see discussion below).

The literature focusing on non-spatial delayed tasks do not provide either a strong support a role for PL/IL in the maintenance of specific information, as when reported, disruption of performance were non delay-dependent. PL/IL damage does not affect short-term retention of gustatory information, egocentric stimuli, or performance in a temporal go/no-go task (Kesner et al., 1996; DeCoteau et al., 1997; Gisquet-Verrier et al., 2000). Although PL/IL lesions disrupt visual recognition (Ragozzino et al., 2003) the deficits were not increased by lengthening the time delay, suggesting that the lesion did not affect the capacity to hold the information “on-line”. Importantly, this is supported by most of the electrophysiological studies indicating that PL and IL cortices have a very low proportion of units showing altered firing during delay

periods (Jung et al., 1998; Pratt and Mizumori, 2001 but see Batuev et al., 1990). Collectively, these studies along with the present results provide strong evidence suggesting that PL/IL area does not play a direct role in short-term maintenance of information, whether spatial or not.

The role of PL/IL in information monitoring

The present results further confirm that PL/IL lesions induced transient performance deficits associated with changes in the original experimental context (de Bruin et al., 1994; Delatour and Gisquet-Verrier, 1996). Furthermore, we showed direct correlations between PL/IL damage and disruption of performance during the interference phases (Experiment 2). In our previous studies, changes concerned delay shifting (Delatour and Gisquet-Verrier, 1999; Gisquet-Verrier et al., 2000) and the present results indicate that changes may also concern introduction of distracting events during the delay, suggesting a role of PL/IL in the monitoring of interferences (see also Bubser and Schmidt, 1990; Kozlov et al., 2001) and/or planning of behavioral adjustments. This is in agreement with numerous other studies that showed disruptive effects of PL/IL permanent or transient lesions when rats must shift between behavioral strategies or rules. These changes included switching between place and visual, place and response, or place and odor discriminations (de Bruin et al., 1994; Ragozzino et al., 1999a and b, 2003), as well as between matching and non matching rules (Joel et al., 1997b; Dias and Aggleton, 2000). PL/IL has also been involved in attentional set-shifting tasks where shift in attention to different perceptual dimensions of complex stimuli is successively required (Birrell and Brown, 2000) and in extinction processes which involve changes in response contingencies (Quirk et al., 2000; Rhodes & Killcross, 2004; Mickley et al., 2005)

In the present experiment, not every alteration of the experimental paradigm systematically resulted in a performance deficit. For instance, in *Experiment 2*, extending the initial 5 min delay to 30 min induced a comparable behavioral disruption in both groups of rats that was progressively overcome with additional training. Such an observation, possibly due to a ceiling effect, has already been noted in rats trained in a delayed go/no-go alternation task when shifted from a 10 to 40 s delay (Delatour and Gisquet-Verrier, 1996). More generally, from our own experiments and from others, it must be pointed out that PL/IL is not unambiguously involved in all forms of behavioral flexibility. For example, PL/IL lesions generally do not affect reversal learning, but impair extradimensional shifting behavior (Joel et al., 1997a; Ragozzino et al., 1999a, 2003; Birrell and Brown, 2000) (eg Birrell and Brown, 2000; Delatour and Gisquet-Verrier, 2003). Nevertheless contradictory results have been reported (Bussey et al., 1997; Delatour and Gisquet-Verrier, 2003; Salazar et al., 2004). Accordingly, PL/IL may be viewed as a brain structure providing adequate adjustment of responses when the original experimental context is modified under certain conditions, yet to be determined.

Conclusions

In summary, the present results showed that PL/IL is not directly implicated in the capacity for temporary on-line storage of spatial information, a result already demonstrated for non-spatial information (Gisquet-Verrier et al., 2000). They further indicate that PL/IL lesions induced transient disruption of performance in some particular circumstances: (1) when rats had to unexpectedly delay their responses and (2) when unpredicted events were presented during the delay period. These findings, in addition to previous studies as described above, allow us to conclude that PL/IL is probably not involved in the on-line maintenance of information and thus not

implicated in working memory, at least under its current conception as defined by the literature on rodent.

The present data indicated that PL/IL is transiently brought into action when significant changes occur within the experimental situation. Our own results further indicate that PL/IL damage may disrupt rats' abilities to respond to even subtle changes. Hence introducing a delay, extending it, or presenting interfering events, may constitute conditions requiring the integrity of PL/IL. Accordingly, PL/IL could be seen as playing a role in situations requiring rats to overcome distraction and to adapt their behavioral response to new contingencies. Such attentional and flexible control of action is required for the prospective planning of delayed responses in an attempt to bridge temporal gaps between physical cues and behavioral outcome.

The present study indicates that PL/IL does not play a direct role in the short-term maintenance of information, but is involved in monitoring / processing functions related to the regulation of working memories. This supports the contention that, in lower mammals as in higher species working memory encompasses both storage and monitoring functions.

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Legends

Figure 1-Histological evaluation of lesion extent

A- Representative lesion of the PL/IL area (left part). Non-damaged tissue from a sham-operated rat is shown in the right part of the figure. Note: even the PL/IL lesioned rats show preserved morphology of dorsomedial PFC regions suggesting that the neurotoxic lesion was confined to ventromedial areas.

B- Reconstruction of PL/IL cortical lesions (adapted from Paxinos and Watson, 1986), at different rostro-caudal levels (+4.7 to +1.7 mm from bregma), from rats trained in Experiment 1. Lesions were centred around the ventromedial prefrontal cortex. Dark gray area: region lesioned for 75-100% of rats; hatched area: region lesioned for 50-75% of rats; light gray area: region lesioned for 25-50% of rats. Nomenclature is adapted from Krettek and Price (1977): PrCm = medial precentral cortex; ACd = dorsal anterior cingulate cortex; ACv = ventral anterior cingulate cortex; PL = prelimbic cortex; MO/VO = medial orbital and ventral orbital cortices; VLO = ventrolateral orbital cortex; LO = lateral orbital cortex; IL = infralimbic cortex; dp = dorsal peduncular cortex; tt = taenia tecta; Acb = accumbens nucleus; Cpu = caudate putamen.

Figure 2- Experiment 1

Mean number of (across- and within-phase) errors (+/- SEM) made by sham-operated (Sham) and PL/IL-lesioned rats (PL/IL) in the standard elimination radial maze (non-delayed win-shift) and in the delayed tasks (1-min delay).

No differences were observed between sham-operated and PL/IL-lesioned rats in the non-delayed task. Introducing a one-minute delay period mid-trial caused a transient

disruption of performance in PL/IL-lesioned rats but not in sham-operated rats. Note that lesioned rats showed an increased number of both across- and within-phase errors.

Figure 3- Experiment 2

A- Left side: Mean number of across- and within-phase errors (+SEM) made by sham-operated (SHAM) and PL/IL-lesioned (PL/IL) rats during the first four training session of the 5 min delayed win-shift task (Experiment 2). Right side: Mean number of trials (+/- SEM) required to reach a training criterion in the 5-min spatial delayed win-shift task. No difference was observed between SHAM and PL/IL rats.

B- Mean number errors by trial (+ SEM) made by PL/IL-lesioned and Sham-operated rats until they reached the training criterion in the 5-min spatial delayed win-shift task. All rats made more across- than within-phase errors, with no difference between sham-operated (SHAM) and PL/IL-lesioned (PL/IL) rats.

Figure 4- Experiment 2

Mean number of (across- and within-phase) errors (+/- SEM) observed during the test phase of the spatial delayed win-shift task in sham-operated (SHAM) and in rats with lesions to PL/IL (PL/IL). Extending the delay from 5 to 30 min led to an increase in the number of across-phase errors in both groups of rats, with no effect of the lesion. Exposing rats to new environments, such as a box (Interference 1) and a Y-maze (Interference 2) resulted in transient disruptions of performance in PL/IL-lesioned rats.

figure 1: : Gisquet-Verrier & Delatour

4.7

4.2

3.7

3.2

2.7

2.2

1.7

figure 2: : Gisquet-Verrier & Delatour

EXPERIMENT 1

A

B

Figure 3: Gisquet-Verrier & Delatour

EXPERIMENT 2

A

B

Figure 4: Gisquet-Verrier & Delatour

EXPERIMENT 2