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Research report

Performance of the odour span task is not impaired following inactivations of parietal cortex in rats

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ABSTRACT

Working memory (WM) is the ability to temporarily store information for use and manipulation. Working memory is thought to depend on a distributed set of higher cortical areas including the prefrontal and parietal cortex in primates while relatively little research has been conducted in rodents to elucidate the exact role of the parietal cortex (PC) in WM, particularly in relation to the construct of WM capacity. Previous work in our lab demonstrates that performance of the odour span task (OST), an olfactory incremental delayed nonmatching-to-sample task, relies on the medial prefrontal cortex (mPFC). However, the effects of inactivating the PC on the OST have not been studied. Therefore, the present experiment assessed the effects of inactivating the PC with the GABA receptor agonists muscimol/baclofen on performance of the OST. Infusions of muscimol/baclofen did not disrupt working memory performance, assessed by the mean number of odours each rat could remember before making an error on each day of testing. In contrast, performance of a positive control task, spontaneous cross-modal object recognition, was impaired by inactivating the PC. These results suggest that performance of the OST does not depend on the PC in rats. Our results are notable given past research demonstrating the importance of the parietal cortex for attentional processes and working memory in other tasks.

1. Introduction

Working memory (WM), the ability to temporarily store information for use and manipulation, is believed to depend in primates and rodents on a distributed set of higher cortical areas involved in executive function and attention such as the prefrontal and parietal cortices [1,2]. Considerable evidence has linked WM function with the parietal cortex in humans and non-human primates [3–10]. However, relatively little research has been conducted in rodents to elucidate the role of the parietal cortex (PC) in WM [11], and in particular, the construct of WM capacity – the number of items that can be simultaneously held in WM with enough accuracy for recall. Two previous studies found working memory impairments following manipulations of the PC. McDaniel, Compton and Smith [12] performed lesions of the posterior PC and found a WM impairment in a multiple T-maze; Espina-Marchant et al. [13] found WM impairments in an Olton 4 × 4 maze after temporary inactivation of the posterior and anteromedial parietal cortex with lidocaine. However, given that both of these studies used spatial WM tasks, the effects of parietal cortex manipulations on spatial navigation cannot be fully separated from the effects on WM capacity *per se*. Kolb, Buhrmann, McDonald and Sutherland [14] investigated the effects of posterior PC lesions in rats on WM and found no impairment in two nonmatching-to-sample tasks using objects with delays from 0 to 20 s. Importantly, these experiments required rats to maintain information over a delay period, but did not assess the effects of PC lesions on WM capacity, as there was no requirement to remember multiple items. Thus, it is possible that rats would be impaired in remembering a larger amount of information over a delay period after disruption of the PC.

The odour span task (OST), an incrementing olfactory delayed nonmatching-to-sample (DNMS) task that requires rats to maintain an increasingly large number of odours over a delay period [15], serves as a test of WM capacity in rodents [16,17]. Previous work from our lab demonstrates that the medial prefrontal cortex (mPFC) and striatum in rats are critical for WM capacity in this task [18,19], which complements the primate literature with respect to the role of both structures in WM [7,9,20,21].

In the present experiment, we assessed the effects of temporarily inactivating the PC on performance of rats in the OST to determine if the rat PC is involved in WM capacity [3,4]. In addition, we assessed the effects of these same inactivations on performance of cross-modal object recognition (CMOR), a task requiring rats to rely on multisensory integration to recognize objects, as well as tactile- and visual-only recognition tests [22–25]. The tactile and cross-modal sub-tests of the CMOR battery rely on the PC in rats [23] and as a result, acted as a

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positive control to determine the effectiveness of the PC inactivations. We anticipated that if the rat PC is necessary for normal WM capacity [3–9], performance of rats in the OST would be diminished. Performance in the CMOR and tactile tests should similarly be reduced, in line with previous findings showing a role of the rat PC in the task [23].

2. Materials and methods

2.1. Subjects

Thirty-one male Long Evans rats (Charles River Laboratories, Kingston, NY) weighing 300–500 g were used. Rats were individually housed in standard ventilated cages and kept on a 12 h light/dark cycle. For experiments requiring food restriction, rats were maintained at 85–90% of their free-feeding weight with water available *ad libitum*. For experiments not requiring food restriction, both food and water were available *ad libitum*. All experiments were approved by the University of Saskatchewan Animal Research Ethics Board and conformed to the guidelines of the Canadian Council on Animal Care.

2.2. Odour span task

The OST was performed in a similar fashion to previous experiments from our lab [18,26,27] but used a "lid-flipping" procedure adapted from MacQueen, Bullard, & Galizio [28]. Training was conducted on a table (cream-coloured corrugated plastic; 91.5 cm^2) with a 2.5 cm high border around the edges and elevated 95 cm above the floor with a metal frame and casters. Between trials, the rats were placed in a transparent Plexiglas enclosure (32 cm W × 50 cm H × 35 cm D) located in one corner of the table. During trials, the rats were let out of the enclosure and allowed to explore the table. Rewards were presented in plastic Dixie cups (59 mL) containing a small amount of sand with 2 reward pellets (45 mg rodent purified dustless precision pellets; Bio-Serv, Flemington, NJ) sitting on top of the sand. The portion cups were covered with scented plastic Dixie cup lids sitting loosely on top of them. The lids were scented with odours by storing them in Tupperware containers containing various spices (Allspice, Anise, Basil, Cacao, Caraway, Celery Salt, Cinnamon, Clove, Coffee, Cumin, Dill, Fennel, Garlic, Ginger, Lemon, Marjoram, Mustard, Nutmeg, Onion, Orange, Oregano, Paprika, Sage, and Thyme). The lids were repeatedly reused in training so that rats could not rely on marking individual 'fresh' lids with their own scents as a mediating strategy.

Training consisted of 3 phases: The first stage was initial shaping, in which rats were habituated to the table and trained to flip lids off the cups to obtain a reward. This was done by first presenting an open cup with a reward and, after each correct trial (the rat successfully obtaining the reward within 5 min), an unscented lid was placed on the cup covering incrementally more of the opening (1/2 covered, ¾ covered, fully covered). This stage continued with each rat receiving 3 trials per day until they could successfully obtain 3 rewards in one day from fully covered cups.

The second phase of training was delayed nonmatching-to-sample (DNMS) training. This stage was conducted by presenting a cup with a sample odour and allowing the rat to obtain the reward. After a delay of approximately 40 s (the time between stimulus presentations), a second cup containing a reward with a novel odour (S+) along with an unbaited cup with the original sample odour (S−) were placed on the table. Importantly, the locations of the novel and familiar odours were shuffled to remove any spatial cues from the task and to prevent rats from visually tracking where the novel odour was placed on the table. Rats were given 6 trials per day (with an intertrial interval (ITI) of 60 s) of DNMS until they performed with 80–100% accuracy for 3 out of 4 consecutive days with the 4th day being no less than 50% accuracy, or until 10 days of DNMS training had elapsed, at which point rats were moved to OST training regardless of whether they had reached criterion in DNMS. Rats that were slow learners in DNMS learned the OST after extensive training even if they had not mastered DNMS within 10 days.

The OST was performed in the same manner as DNMS; however, after each correct choice, an additional odour was added to the table in serial fashion until the rat made an error at which point that particular trial was ended and an ITI began before commencing the next trial. As with DNMS, the locations of the odours were thoroughly shuffled during the ~40 s delay to remove spatial cues from the task and prevent visually-guided mediating strategies. A schematic of this procedure is shown in Fig. 2A. Each rat was trained in this task for a maximum of 3 trials per day (with no repetition of odours across different trials) or a maximum of 30 min, whichever criterion was met first. Rats were trained for 6–7 weeks ~5 times/week before being tested with infusions. During training, the span performance, errors, and latency to make a choice were recorded by hand, and latency was tracked manually with a stopwatch.

2.3. Cross-modal object recognition

The cross-modal object recognition task was administered in the same basic fashion as previous literature [22,23]. Training was conducted in a Y-shaped maze (white corrugated plastic; 10 × 27 cm) with one entrance arm and two object arms. The entrance arm was blocked by a sliding plastic divider except during the task. Transparent Plexiglas dividers separated the objects from the rest of the maze for visual-only training. Objects consisted of a variety of plastic toys, Lego towers, and assorted ornamental items made of plastic, glass, or porcelain.

Training consisted of 4 phases: habituation, tactile object recognition, visual object recognition, and cross-modal object recognition. Habituation took place over 2 days and consisted of 2 10-min sessions that included a counterbalanced mix of the following conditions: Testing room lights on for 50% of each session and the Plexiglas dividers in for 50% of each session. Thus, rats were habituated to both the lit and unlit testing room and the presence/absence of the Plexiglas dividers. Training and testing for the tactile, visual, and cross-modal phases consisted of a 3-min sample phase, in which the rat was allowed to explore the maze with identical copies of an object in each object arm, followed by a 2-min test phase 1 h later. The tactile, visual, and cross-modal phases took place across 4 consecutive days, with the cross-modal test being conducted twice for each rat with two different object pairs in order to maximize the sample size in this test.

A schematic depicting the phases of behavioral training and testing is shown in Fig. 3A–C. During the tactile phase, the room lights were turned off, and the rats were only able to explore the objects using touch (the objects were thoroughly cleaned with ethanol to minimize any of their olfactory features). During the visual phase, the room lights were on, and Plexiglas dividers blocked the objects from direct contact, requiring the rats to explore using vision only. In the cross-modal phase, the sample phase was conducted with the lights off and dividers out so that the objects would be explored using touch only, and the test phase was conducted with lights on and dividers in so that rats would be tested on their memory using vision only. To recognize objects during the cross-modal test phase, rats must rely on multisensory integration, extrapolating from a tactile representation of the familiar object to recognizing it from its visual features alone [23,25]. In addition, different copies of the objects were used in the sample and test phases to minimize the influence of olfactory cues on performance.

During both the sample and test phases, the order of objects presented as 'familiar' and 'novel' was counterbalanced, as was the location of the novel object in either the left or right arm of the maze. Across the tactile and visual phases of the task, the rats receiving saline or drug was counterbalanced such that rats receiving saline during the tactile phase received drug during the visual phase and vice versa. The cross-modal phase of the task was run twice with two different sets of objects to allow all rats to perform the task under both the saline and drug conditions. As such, the order that rats received saline or drug was counterbalanced in addition to the other counterbalancing described above.

2.4. Surgery and intracranial drug infusions

2.4.1. Stereotaxic surgery

Surgeries were performed under isoflurane anesthesia (Janssen, Toronto, Ontario). After shaving the head and placing the rat in a stereotaxic frame (Kopf Instruments, Tajunga, CA), the scalp was cleaned and incised along the midline. Five to six small burr holes were drilled to accommodate jeweler's screws in order to secure the head caps. Larger holes were then drilled to allow the lowering of 4 11-mm 23-gauge guide cannulae into the PC. Each set of guide cannulae was fashioned into "double barrel" cannulae by soldering them side by side with a steel spacer in between (small section of 23-gauge steel tubing; ~1 mm). These double barrel cannulae were implanted −3.9 mm posterior and 2.2 and 3.4 mm lateral to bregma, with the mediolateral coordinate being measured from the inside cannulae of each set. Dorsoventral placement was based on lowering the cannulae tips to the brain surface and then advancing them ~0.2 mm deeper. Coordinates were derived from the Rat Brain Atlas [29]. The anatomical definition of the PC in rats varies somewhat in the literature. Our coordinates correspond to what some authors consider the posterior PC [30,31], whereas other authors consider the posterior PC as slightly more posterior to our coordinates [23]. In at least one case, the area we targeted is also defined as containing an "anteromedial" area [13]. Evidence from the tracing of cortical connectivity reveals little difference in the cortical inputs to the PC along its anteroposterior axis; however, differences in cortical connectivity along its mediolateral axis exist [32], of which our coordinates covered the full extent. Given previous evidence that 0.5 µL of infusate can spread 1–2 mm from the infusion site [33], and allowing for the aforementioned variation in the anatomical definition of the rat PC, we anticipated that our infusions would affect the full mediolateral extent of the PC, and likely between 50% and 100% of its anteroposterior axis.

Steel dummy stylets were placed in the guide cannulae to plug them between infusions. Importantly, due to the fact that the tips of the guides were very close to the brain surface, the dummy stylets were either of equal length to the guides or were left ~1 mm longer than the guides and beveled to a point in order to prevent repeated irritation of the dura mater in some rats. After fashioning a head cap from dental acrylic, rats were given a single subcutaneous injection of Anafen (0.5 mg/kg) for analgesia, the scalp was sutured, and the rats allowed to recover for a minimum of a week before recommencing behaviour testing.

2.4.2. Intracranial drug infusions

Rats were first habituated to the infusion procedure through handling in the room in which infusions were to take place. This included removal of their dummy stylets and mock infusions, which consisted of inserting shortened infusion needles that were not connected to the microinfusion pumps and running the pumps to simulate the noise and sensation of the infusions. Approximately 20–30 min before behavioral testing (prior to sample phase in CMOR), rats received intracranial drug infusions of either 0.9% saline or muscimol/baclofen (0.5 µg/µL, respectively; dissolved in saline and mixed together [18,34]). Infusions were performed using two microinfusion pumps (Harvard Apparatus, Holliston, MA) at all 4 sites simultaneously at a flow rate of 0.5 µL/min for 1 min. Infusion needles (27 gauge; 12 mm) were left in the guide cannulae for 1–2 min to allow the drug to diffuse, and rats were returned to their cages to await testing.

2.5. Perfusions and histology

Rats were overdosed with isoflurane and perfused transcardially with 200 mL of 0.1 M PBS followed by 200 mL of 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde for 24 h before being transferred to a cryoprotectant solution of 0.1% sodium azide/30% sucrose. After the brains had sunk in the cryoprotectant, they were sectioned coronally at 40 µm on a freezing sliding microtome (Leica Biosystems, Concord, Ontario). Sections containing cannula tracks were mounted to glass slides and cannulae placement was determined with the aid of a stereoscope and brain atlas for reference [29].

2.6. Data analysis

2.6.1. Odour span task

The mean span of each animal on each training day was calculated by taking the number of correct choices −1 within each trial and averaging the scores between all trials completed. To analyze the effects of infusions into the PC, rats' performance (mean span capacity) was compared in a dependent *t*-test between Saline and M/B days. Performance in the Saline condition was also compared to performance the day before infusions, and performance on 9 days of pre-infusion training was compared to ensure that performance was stable across days.

2.6.2. Cross-modal object recognition

Performance in CMOR was assessed separately for each phase of the task (tactile, visual, and cross-modal). A discrimination ratio (DR) was calculated for each animal using the following equation:

$$\frac{(Exploration\ Time^{Novel} - Exploration\ Time^{Sample})}{(Exploration\ Time^{Novel} + Exploration\ Time^{Sample})}$$

Importantly, rats were excluded from the analyses of each day of training if they 1) had 0 exploration of the sample objects, or 2) did not explore both objects in the test phase (i.e., a DR of 1 or −1), or had a DR that was ± 2 standard deviations or greater from the mean. The first minute of the test, as well as both combined minutes of the test, were used in the analysis.

3. Results

3.1. Location of infusion sites

The infusion sites are shown in Fig. 1. Twenty-one rats had injections that were deemed acceptably within the PC. Ten rats had injections that were anterior to the parietal cortex, located in the somatosensory cortex, or were too ventral and affected the dorsal hippocampus, and were thus removed from all subsequent analyses.

3.2. Odour span task

A one-way within-subjects ANOVA was conducted on 9 days of pre-infusion training (Fig. 2B) to verify that performance was stable on the days before infusions. No main effect of training day was found ($F_{(8,72)} = 0.40$, $p = .74$), indicating stable performance across the days leading up to infusions. A significant main effect of individual rat was found ($F_{(9,72)} = 11.28$, $p < .05$), revealing that performance significantly differed between rats. However, we do not consider this to be a concern, as the two infusion treatments were compared within subjects. Mean span in the saline condition also did not significantly differ from performance the day before infusions ($t_{(9)} = 0.29$, $p = .78$), indicating that performance was not affected by the infusion procedure. The mean spans (Fig. 2C) achieved by each rat in either the Saline or M/B conditions were compared in a dependent *t*-test. Mean span was not affected by M/B infusions ($t_{(9)} = 0.88$, $p = .40$, 2-tailed). In addition, there was no difference in the latency (Saline = 8.42 s, SEM = 1.98; M/B = 13.46 s, SEM = 5.62) to make a choice ($t_{(9)} = 0.99$, $p = .35$, 2-tailed). Due to the mean span being lower than previously-published studies [18,26,28] [Davies et al. 2017], a single-sample t-test was performed to confirm that the mean span of rats in the saline condition was significantly higher than a hypothetical mean span of 0 ($t_{(9)} = 3.14$, $p < .05$, 2-tailed).

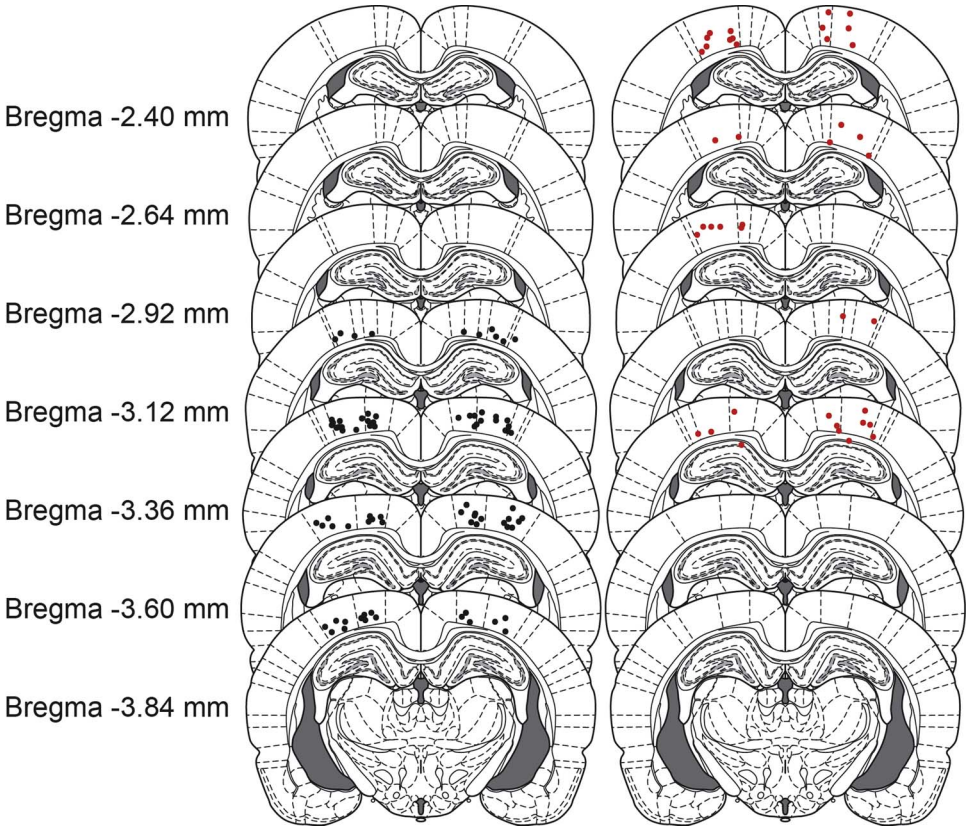

Fig. 1. Diagram showing the locations of correctly-placed (black, left) and incorrectly-placed (red, right) infusions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Source: Images were adapted from Paxinos & Watson (2006).

3.3. Cross-modal object recognition

Exploration times during the sample phases for tactile, visual, and cross-modal object recognition for Saline and M/B conditions are shown in Table 1. No significant differences in sample phase exploration between infusion conditions were found for the tactile ($t_{(18)} = 1.39$, $p = .18$, 2-tailed) or cross-modal tests ($t_{(33)} = 0.17$, $p = .87$) while rats infused with M/B explored the objects significantly more than rats infused with Saline ($t_{(16)} = 2.22$, $p = 0.04$). Total exploration times during test phases are shown in Table 2. No significant differences were noted between groups for minutes 1 and 2 of the tactile ($t_{(18)} = 1.69$, $p = .11$), visual ($t_{(16)} = 1.98$, $p = .07$), or cross-

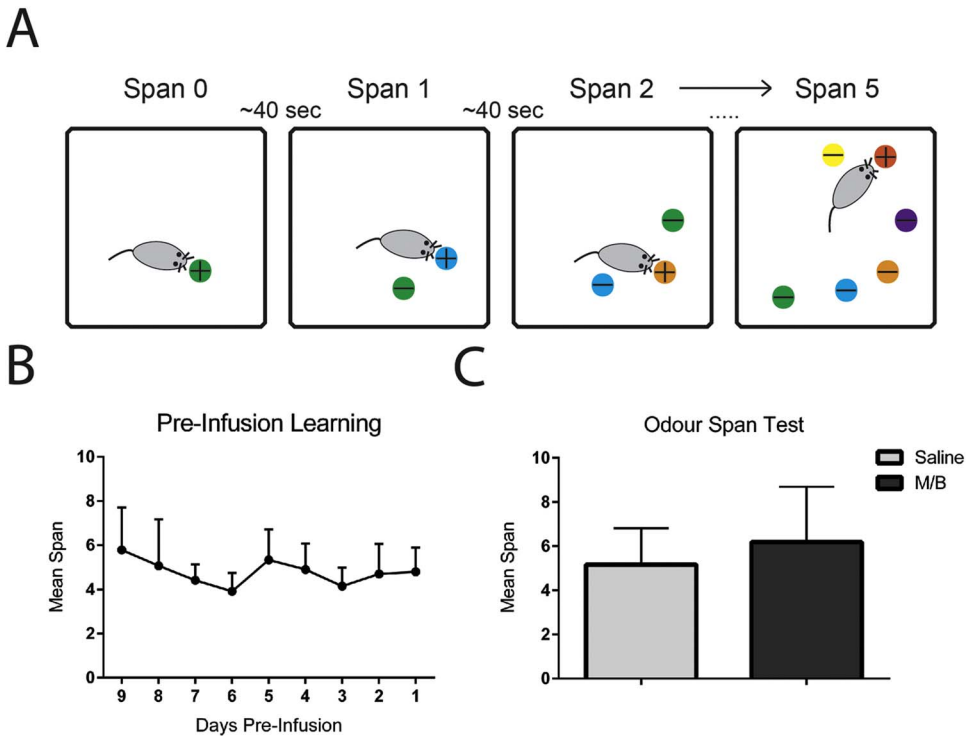

Fig. 2. A) A schematic illustrating the basic learning procedure taking place in the OST; B) Mean (± SEM) OST performance of all rats during the 9 days of training before infusion (due to rats receiving different amounts of training, only the final 9 days of training before infusions represent the learning data of every rat). Group performance was stable across these training days, although the performance of individual rats tended to fluctuate; C) Mean (+SEM) OST performance of rats with either Saline or M/B infusions in the posterior parietal cortex. Mean span was unaffected by infusions of M/B, suggesting that memory capacity in the OST does not depend on the PC.

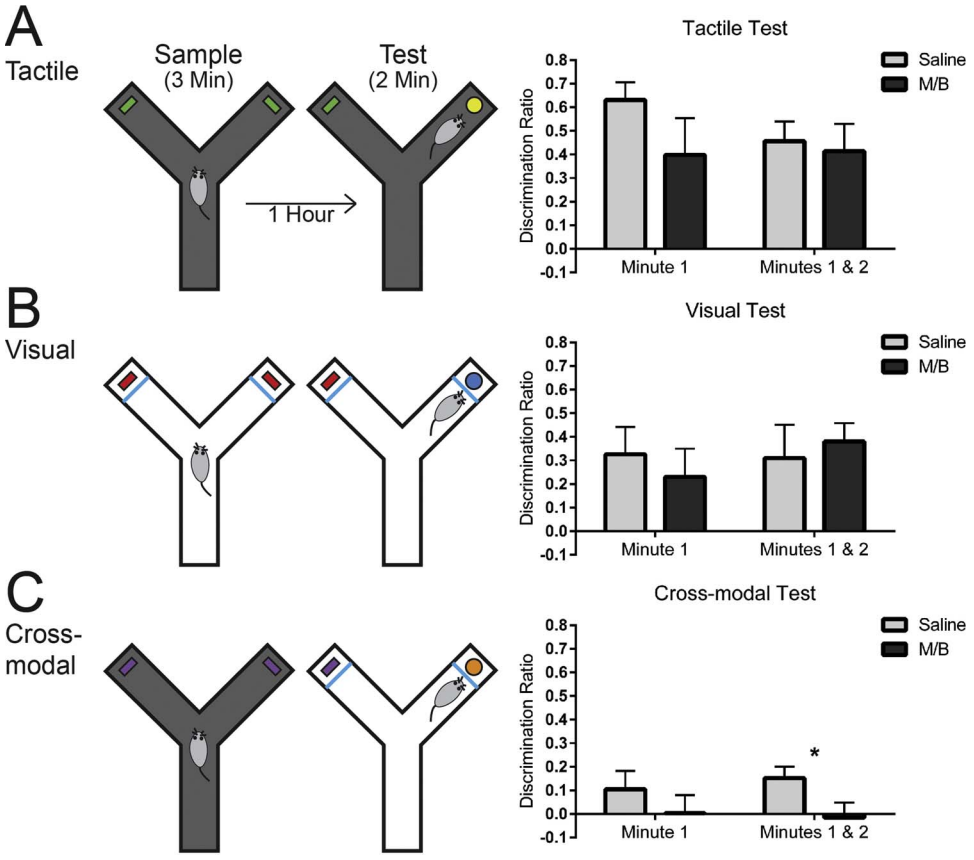

Fig. 3. A) A schematic illustrating the behavioural procedure for Tactile OR and behavioural performance (Mean DR ± SEM). Rats in both conditions showed a significant novel object preference across the duration of the test and there were no group differences B) A schematic illustrating the behavioural procedure for Visual OR and behavioural performance (Mean DR ± SEM). Rats in the Saline condition showed a significant novel object preference only during Minute 1 of the test, whereas M/B rats showed a significant novel object preference over both minutes of the test, but not during Minute 1 specifically. No group differences were observed. C) A schematic illustrating the behavioural procedure for Cross-modal OR and behavioural performance (Mean DR ± SEM). Rats in the Saline condition showed a significant novel object preference across the duration of the test, but not during Minute 1 specifically, whereas rats in the M/B condition showed no novel object preference. Additionally, a significant group difference was found where rats in the M/B condition showed a significantly lower DR across the duration of the test than in the Saline condition.

Table 1
Mean (SEM) Total Exploration Time During CMOR Sample Phases (s).

Infusion	Tactile	Visual	Cross-modal
Saline	36.14 (5.22)	7.92 (0.58)	35.11 (4.21)
M/B	25.66 (4.80)	9.58 (0.48)	34.19 (3.71)

Table 2
Mean (SEM) Total Exploration Time During CMOR Test Phases (s).

Infusion		Tactile	Visual	Cross-modal
Saline	*Minute 1*	18.89 (2.70)	1.86 (0.39)	3.39 (0.41)
	Minutes 1 & 2	32.17 (4.03)	3.63 (0.63)	5.31 (0.55)
M/B	*Minute 1*	12.66 (3.43)	3.60 (0.48)	3.84 (0.48)
	Minutes 1 & 2	21.69 (4.59)	5.46 (0.65)	5.93 (0.62)

modal tests ($t_{(33)} = 0.74$, $p = .46$). The analysis of the DRs from test phases is described below.

3.3.1. Tactile object recognition

The mean DRs for Saline and M/B conditions is shown in Fig. 3A. Single sample t-tests (2-tailed) revealed that a significant DR for the Saline condition in Minute 1 ($t_{(11)} = 8.37$, $p < .05$) and Minutes 1 & 2 ($t_{(11)} = 5.47$, $p < .05$), and the M/B condition in Minute 1 ($t_{(7)} = 2.55$, $p < .05$) and Minutes 1 & 2 ($t_{(7)} = 3.59$, $p < .05$), indicating that rats explored the novel object significantly more than the familiar object in both infusion conditions. An independent *t*-test was conducted on the DRs between Saline and M/B rats and showed no significant group difference on Minute 1 ($t_{(18)} = 1.49$, $p = .15$, 2-tailed) or Minutes 1 & 2 ($t_{(18)} = 0.31$, $p = .76$, 2-tailed), indicating that infusions of M/B into the PC had no effect on Tactile OR.

3.3.2. Visual object recognition

The mean DR for Saline and M/B conditions is shown in Fig. 3B. Single sample *t*-tests (2-tailed) revealed that a significant DR for the Saline condition in Minute 1 ($t_{(5)} = 2.80$, $p < .05$) but not Minutes 1 & 2 ($t_{(7)} = 2.25$, $p = .06$). In the M/B condition, a significant DR was found in Minutes 1 & 2 ($t_{(9)} = 4.83$, $p < .05$), but not in Minute 1 ($t_{(8)} = 1.93$, $p = .09$), indicating that rats explored the novel object more than the familiar object in at least part of the test in both conditions. An independent *t*-test was conducted on the DRs between Saline and M/B rats and showed no significant group difference in Minute 1 ($t_{(13)} = 0.55$, $p = .59$, 2-tailed) or Minutes 1 & 2 ($t_{(16)} = 0.40$, $p = .70$, 2-tailed).

3.3.3. Cross-modal object recognition

The mean DR for Saline and M/B conditions is shown in Fig. 3C. Single sample *t*-tests (2-tailed) revealed that significant DRs in the Saline condition for Minutes 1 & 2 ($t_{(16)} = 3.10$, $p < .05$), but not Minute 1 ($t_{(16)} = 1.36$, $p = .19$). In the M/B condition, no significant DR was found in Minute 1 ($t_{(17)} = 0.05$, $p = .96$) or Minutes 1 & 2 ($t_{(17)} = 0.24$, $p = .81$), indicating that in the M/B condition, rats did not explore the novel object significantly more than the familiar object. An independent t-test was chosen to compare Saline and M/B due to the fact that some rats were excluded unequally from one drug condition or the other and the analysis could therefore not be run as a true repeated measures design. A significant difference in the DRs was found between Saline and M/B condition on Minutes 1 & 2 ($t_{(33)} = 2.07$, $p < .05$, 2-tailed), indicating that infusions of M/B significantly impaired cross-modal OR across the whole duration of the test, but not Minute 1 ($t_{(33)} = 0.93$, $p = .36$, 2-tailed), although rats in the Saline condition did not exhibit a significant novel object preference during this minute of the test.

4. Discussion

The present experiment showed that temporary inactivations of the PC in rats did not disrupt span capacity in the OST, whereas the same inactivation procedure impaired performance in CMOR. Although previous studies have investigated the role of the rat PC in working memory [12–14], this was the first study, to our knowledge, that tested the role of the rat PC in mediating WM capacity for multiple non-spatial items. These results suggest that performance of the OST is independent of the PC in rats. Our findings provide evidence that the PC may not be a critical brain structure mediating WM capacity with all types of stimuli, which is surprising given that the PC is frequently implicated in WM in humans and non-human primates in many studies [3–9].

The present results are also in contrast to previous experiments in which the PC was disrupted in rats during performance of a WM task [12–14]. Importantly, McDaniel et al. [12] used a multiple T-maze and Espina-Marchant et al. used an Olton 4 × 4 maze, both WM procedures that are spatial in nature, and found impairments in WM. Kolb et al. tested rats in two DNMS tasks, the Aggleton DNMS [35] and the Mumby DNMS [36], both of which are tests of non-spatial working memory. Rats with lesions to the PC performed normally in both tasks. However, the present experiment differs from Kolb et al. in a critical way in that the OST measures a greater range of WM capacity than DMNS. Even given this difference, we did not find any effect of PC inactivations, indicating that even when the load on WM capacity is increased, the PC does not play a critical role in mediating it.

Of note, the mean spans achieved by the rats in the current study were lower than previously observed in our lab [18,26]. Importantly, we used a procedure adapted from MacQueen, Bullard, & Galizio [28] to administer the OST that requires the rats to flip scented plastic lids off cups, rather than dig in bowls of scented sand used previously in our laboratory [18,26]. Nonetheless, we are confident that the spans are high enough to have revealed an impairment from our manipulation and that the present results do not appear to be an issue of floor effects. In the saline condition, rats performed significantly better than a hypothetical mean span of 0, and indeed, half of the rats performed better under the M/B condition than the Saline condition, with one of the rats achieving a span of 23 (the maximum possible score) after M/B infusions. Performance was also stable across 9 days before infusions began, and performance following saline infusions did not differ from performance on the day before infusions.

A caveat to our interpretation of the span data is that we considered only mean span as a measure of capacity in the OST, whereas other authors have considered percent accuracy or “longest run” (the largest span achieved by a rat within a session) as measures of OST performance [37,38]. It is therefore possible that deficits in some other such metric of OST performance may be detected after PC disruption. What is clear from our interpretation, however, is that mean WM capacity in the OST – arguably the most basic metric for performance - does not depend on an intact PC.

We used CMOR as a positive control to verify that our inactivations sufficiently disrupted PC function. This task assesses the ability of rats to use multisensory integration to recognize objects [22–25], and relies in part on the PC [23]. The present findings showed an impairment in the cross-modal phase of CMOR after inactivations of the PC, in line with previous findings [23], and confirm the efficacy of our inactivations. However, in contrast to these previous findings, infusions of M/B did not impair either the Tactile or Visual phases of the task. An additional caveat with respect to our cross-modal test is that the Saline DR, despite being significantly above chance across both minutes of the test, is noticeably lower than controls in previously published papers [22–24], which may be a result of damage to the PC caused by the implantation of the 4 guide cannulae. Nonetheless, we observed that rats in the M/B condition showed no novel object preference during the cross-modal test and were significantly impaired relative to the Saline condition, providing evidence that our PC inactivations significantly affected behaviour.

It should also be noted that our null finding with respect to the PC and WM capacity pertains only to performance after extensive OST training. The OST and CMOR differ in that OST requires weeks of behavioural training, whereas CMOR is a spontaneous task involving no skill or rule learning on the part of the rat. The findings, therefore do not preclude a role of the PC in initial learning of task-related rules in the OST. There is some evidence from human neuroimaging studies that the PC is engaged during initial learning of task rules [39,40]. It is therefore possible that manipulations of the PC during early shaping of the task would impair acquisition. However, this would likely be an impairment specifically related to the non-matching *rule* rather than an impairment in maintaining task stimuli.

The lack of involvement of the PC in the OST may reflect functional differences between the rodent and primate PC. In contrast to findings with primates [41], rats with lesions to the PC do not appear impaired in various tests of attention [31,42–45] but see [46]. The OST in humans also depends on the hippocampus [47] whereas it does not in rats [15]. However, it is noteworthy that a spatial span task [15] and other spatial [48] and object-based tasks [49] for measuring memory capacity involve the hippocampus in rats. Hence, there may be evolutionary differences in the circuitry underlying the OST, including the relative importance of the PC for attention, between primates and rodents which, in light of the present results, could extend to WM capacity.

A more likely reason for the discrepancy between the present results and the majority of previous literature on the role of the PC in WM is the type of stimuli used. Human and non-human primate studies have predominantly used visuospatial or phonological stimuli [3–10]. The PC is well-known as a major component of the dorsal visual processing stream [50]. The evidence of involvement of the human PC in olfactory processing is mixed. Although a few neuroimaging studies find activation of parts of the PC during olfactory tasks [51–53], patients with lesions to the PC are not impaired in odour identification [54]. Rather, olfactory stimuli are processed in the piriform, entorhinal, and amygdalar cortices [55,56]. If the items held in WM are non-spatial olfactory stimuli, as they were in the present study, the PC may simply not be engaged. Hence, the present results may reflect that the PC performs a sensory role in visual or visuospatial WM, and is not involved in non-spatial olfactory WM. This interpretation is bolstered by results from other labs showing evidence for sensory modality-specific processing in the PC [30,57].

5. Conclusions

Previous studies of the role of the rat PC in WM have found involvement of the PC in tests of spatial WM [12,13] but not non-spatial WM [14]. Importantly, these studies have not investigated the role of the PC in WM in a non-spatial test requiring WM capacity for multiple items. The present study found no disruption of performance in the OST by PC inactivations with combined muscimol/baclofen, whereas cross-modal object recognition was impaired by the same manipulation. These results suggest that WM in rats, at least for non-spatial olfactory stimuli, is independent of the PC. The discrepancy between the present findings and the findings in humans and non-human primates may be a result of an evolutionary difference between primates and rodents in the type of information processing in the PC, or a reflection of differences in the types of sensory stimuli used in primate studies and the present study.

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References

[1] J. Eriksson, E.K. Vogel, A. Lansner, F. Bergström, L. Nyberg, Neurocognitive architecture of working memory, Neuron 88 (2015) 33–46, http://dx.doi.org/10.1016/j.neuron.2015.09.020.

[2] M. D'Esposito, B.R. Postle, The cognitive neuroscience of working memory, Annu. Rev. Psychol. 66 (2015) 115–142, http://dx.doi.org/10.1146/annurev-psych-010814-015031.

[3] E.K. Vogel, M.G. Machizawa, Neural activity predicts individual differences in visual working memory capacity, Nature 428 (2004) 748–751, http://dx.doi.org/10.1038/nature02447.

[4] S. Brigadoi, S. Cutini, F. Meconi, M. Castellaro, P. Sessa, M. Marangon, A. Bertoldo, P. Jolicoer, R. Dell'Acqua, On the role of the inferior intraparietal sulcus in visual working memory for lateralized single-feature objects, J. Cogn. Neurosci. (2016) 1–15, http://dx.doi.org/10.1162/jocn.

[5] I. Öztekin, B. Mcelree, B.P. Staresina, L. Davachi, Working memory retrieval: contributions of the left prefrontal cortex, the left parietal cortex, and the hippocampus, J. Cogn. Neurosci. 21 (2009) 581–593, http://dx.doi.org/10.1162/jocn.2008.21016.

[6] S.M. Ravizza, M.R. Delgado, J.M. Chein, J.T. Becker, J.A. Fiez, Functional dissociations within the inferior parietal cortex in verbal working memory, Neuroimage 22 (2004) 562–573, http://dx.doi.org/10.1016/j.neuroimage.2004.01.039.

[7] A.S. Champod, M. Petrides, Dissociable roles of the posterior parietal and the prefrontal cortex in manipulation and monitoring processes, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 14837–14842.

[8] M. van Asselen, R.P.C. Kessels, S.F.W. Neggers, L.J. Kappelle, C.J.M. Frijns, A. Postma, Brain areas involved in spatial working memory, Neuropsychologia 44 (2006) 1185–1194, http://dx.doi.org/10.1016/j.neuropsychologia.2005.10.005.

[9] C.E. Curtis, Prefrontal and parietal contributions to spatial working memory, Neuroscience 139 (2006) 173–180, http://dx.doi.org/10.1016/j.neuroscience.2005.04.070.

[10] W.E. Mackey, O. Devinsky, W.K. Doyle, J.G. Golfinos, C.E. Curtis, Human parietal cortex lesions impact the precision of spatial working memory, J. Neurophysiol. 116 (3) (2016) 1049–1054, http://dx.doi.org/10.1152/jn.00380.2016.

[11] D.J. Bucci, Posterior parietal cortex: an interface between attention and learning? Neurobiol. Learn. Mem. 91 (2009) 114–120, http://dx.doi.org/10.1016/j.nlm.2008.07.004.

[12] W.F. McDaniel, D.M. Compton, S.R. Smith, Spatial learning following posterior parietal or hippocampal lesions, Neuroreport 5 (1994) 1713–1717.

[13] P. Espina-Marchant, T. Pinto-Hamuy, D. Bustamante, P. Morales, L. Robles, M. Herrera-marschitz, Spatial cognition and memory: a reversible lesion with lidocaine into the anteromedial/posterior parietal cortex (AM/PPC) affects differently working and long-term memory on two foraging tasks, Biol. Res. 39 (2006) 601–609.

[14] B. Kolb, K. Buhrmann, R. Mcdonald, R.J. Sutherland, Dissociation of the medial prefrontal, posterior parietal, and posterior temporal cortex for spatial navigation and recognition memory in the rat, Cereb. Cortex 4 (1994) 664–680, http://dx.doi.org/10.1093/cercor/4.6.664.

[15] P.A. Dudchenko, E.R. Wood, H. Eichenbaum, Neurotoxic hippocampal lesions have no effect on odor span and little effect on odor recognition memory but produce significant impairments on spatial span, recognition, and alternation, J. Neurosci. 20 (2000) 2964–2977 http://www.ncbi.nlm.nih.gov/pubmed/10751449.

[16] P.A. Dudchenko, J.C. Talpos, J.W. Young, M.G. Baxter, Animal models of working memory: a review of tasks that might be used in screening drug treatments for the memory impairments found in schizophrenia, Neurosci. Biobehav. Rev. 37 (2013) 2111–2124, http://dx.doi.org/10.1016/j.neubiorev.2012.03.003.

[17] H. Moore, M.A. Geyer, C.S. Carter, D.M. Barch, Harnessing cognitive neuroscience to develop new treatments for improving cognition in schizophrenia: CNTRICS selected cognitive paradigms for animal models, Neurosci. Biobehav. Rev. 37 (2013) 2087–2091, http://dx.doi.org/10.1038/jid.2014.371.

[18] D.A. Davies, J.J. Molder, Q. Greba, J.G. Howland, Inactivation of medial prefrontal cortex or acute stress impairs odor span in rats, Learn. Mem. 20 (2013) 665–669, http://dx.doi.org/10.1101/lm.032243.113.

[19] D.A. Davies, Q. Greba, J.C. Selk, J.K. Catton, L.D. Baillie, S.J. Mulligan, J.G. Howland, Interactions between medial prefrontal cortex and dorsomedial striatum are necessary for odor span capacity in rats: role of GluN2B-containing NMDA receptors, Learn. Mem. 24 (2017) 524–531, http://dx.doi.org/10.1101/lm.045419.117.24.

[20] S. Funahashi, C.J. Bruce, P.S. Goldman-Rakic, Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex, J. Neurophysiol. 61 (1989) 331–349, http://dx.doi.org/10.1016/j.neuron.2012.12.039.

[21] P.J. Olesen, H. Westerberg, T. Klingberg, Increased prefrontal and parietal activity after training of working memory, Nat. Neurosci. 7 (2004) 75–79, http://dx.doi.org/10.1038/nn1165.

[22] W.N. Marks, S.M. Cain, T.P. Snutch, J.G. Howland, The T-type calcium channel antagonist Z944 rescues impairments in crossmodal and visual recognition memory in genetic absence epilepsy rats from Strasbourg, Neurobiol. Dis. 94 (2016) 106–115, http://dx.doi.org/10.1016/j.nbd.2016.06.001.

[23] B.D. Winters, J.M. Reid, A distributed cortical representation underlies crossmodal object recognition in rats, J. Neurosci. 30 (2010) 6253–6261, http://dx.doi.org/10.1523/JNEUROSCI.6073-09.2010.

[24] J.M. Reid, D.L. Jacklin, B.D. Winters, Crossmodal object recognition in rats with and without multimodal object pre-exposure: no effect of hippocampal lesions, Neurobiol. Learn. Mem. 98 (2012) 311–319, http://dx.doi.org/10.1016/j.nlm.2012.09.001.

[25] J.M. Cloke, D.L. Jacklin, B.D. Winters, The neural bases of crossmodal object recognition in non-human primates and rodents: a review, Behav. Brain Res. 285 (2015) 118–130, http://dx.doi.org/10.1016/j.bbr.2014.09.039.

[26] D.A. Davies, Q. Greba, J.G. Howland, GluN2B-containing NMDA receptors and AMPA receptors in medial prefrontal cortex are necessary for odor span in rats, Front. Behav. Neurosci. 7 (2013) 183, http://dx.doi.org/10.3389/fnbeh.2013.00183.

[27] B.G. Murray, D.A. Davies, J.J. Molder, J.G. Howland, Maternal immune activation during pregnancy in rats impairs working memory capacity of the offspring, Neurobiol. Learn. Mem. 141 (2017), http://dx.doi.org/10.1016/j.nlm.2017.04.005.

[28] D.A. MacQueen, L. Bullard, M. Galizio, Effects of dizocilpine (MK801) on olfactory span in rats, Neurobiol. Learn. Mem. 95 (2011) 57–63, http://dx.doi.org/10.1016/j.nlm.2010.11.004.

[29] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 6th ed., Academic Press, 2006.

[30] A.M. Licata, M.T. Kaufman, D. Raposo, M.B. Ryan, J.P. Sheppard, A.K. Churchland, Posterior parietal cortex guides visual decisions in rats, J. Neurosci. 37 (2017) 105–117, http://dx.doi.org/10.1523/JNEUROSCI.0105-17.2017.

[31] A.L. Rosner, G. Mittleman, Visuospatial attention in the rat and posterior parietal cortex lesions, Behav. Brain Res. 79 (1996) 69–77 http://www.ncbi.nlm.nih.gov/pubmed/8883818.

[32] A.A. Wilber, B.J. Clark, A.J. Demecha, L. Mesina, J.M. Vos, B.L. McNaughton, Cortical connectivity maps reveal anatomically distinct areas in the parietal cortex of the rat, Front. Neural Circuits 8 (2014) 146, http://dx.doi.org/10.3389/fncir.2014.00146.

[33] I. Izquierdo, J.A. Quillfeldt, M.S. Zanatta, J. Quevedo, E. Schaeffer, P.K. Schmitz, J.H. Medina, Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats, Eur. J. 9 (1997) 786–793 (Accessed 28 November, 2013), http://onlinelibrary.wiley.com/doi/10.1111/j.1460-9568.1997.tb01427.x/full.

[34] S. Sangha, P.D. Robinson, Q. Greba, D.A. Davies, J.G. Howland, Alterations in reward, fear and safety cue discrimination after inactivation of the rat prelimbic and infralimbic cortices, Neuropsychopharmacology 39 (2014) 2405–2413, http://dx.doi.org/10.1038/npp.2014.89.

[35] J.P. Aggleton, One-trial object recognition by rats, Q. J. Exp. Psychol. Sect. B 37 (1985) 279–294, http://dx.doi.org/10.1080/14640748508401171.

[36] D.G. Mumby, J.P.J. Pinel, E.R. Wood, Nonrecurring-items delayed nonmatching-to-sample in rats: a new paradigm for testing nonspatial working memory, Psychobiology 18 (1990) 321–326, http://dx.doi.org/10.3758/BF03327250.

[37] M. Galizio, M. Deal, A. Hawkey, L.B. April, Working memory in the odor span task: effects of chlordiazepoxide, dizocilpine (MK801), morphine, and scopolamine, Psychopharmacology (Berl.) 225 (2013) 397–406, http://dx.doi.org/10.1007/s00213-012-2825-7.

[38] M. Galizio, B. April, M. Deal, A. Hawkey, D. Panoz-Brown, A. Prichard, K. Bruce, Behavioral pharmacology of the odor span task: effects of flunitrazepam, ketamine, methamphetamine and methylphenidate, J. Exp. Anal. Behav. 106 (2016) 173–194, http://dx.doi.org/10.1002/jeab.224.

[39] H. Ruge, U. Wolfensteller, Distinct contributions of lateral orbito-frontal cortex, striatum, and fronto-parietal network regions for rule encoding and control of memory-based implementation during instructed reversal learning, Neuroimage 125 (2016) 1–12, http://dx.doi.org/10.1016/j.neuroimage.2015.10.005.

[40] I. Dumontheil, R. Thompson, J. Duncan, Assembly and use of new task rules in fronto-parietal cortex, J. Cogn. Neurosci. 23 (2011) 168–182, http://dx.doi.org/10.1162/jocn.2010.21439.

[41] T.J. Buschman, E.K. Miller, Top-down versus bottom-up control of attention in the prefrontal and posterior parietal cortices, Science 315 (2007) 1860–1862, http://dx.doi.org/10.1126/science.1138071.

[42] J. Broussard, M. Sarter, B.S. Givens, Neuronal correlates of signal detection in the posterior parietal cortex of rats performing a sustained attention task, Neuroscience. 143 (2006) 407–417, http://dx.doi.org/10.1016/j.neuroscience.2006.08.030.

[43] J.-M. Maddux, E.C. Kerfoot, S. Chatterjee, P.C. Holland, Dissociation of attention in learning and action: effects of lesions of the amygdala central nucleus, medial prefrontal cortex, and posterior parietal cortex, Behav. Neurosci. 121 (2007) 63–79, http://dx.doi.org/10.1016/j.pestbp.2011.02.012.Investigations.

[44] J.L. Muir, B.J. Everitt, T.W. Robbins, The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task, Cereb. Cortex 6 (1996) 470–481, http://dx.doi.org/10.1093/cercor/6.3.470.

[45] N.M. Ward, V.J. Brown, Deficits in response initiation, but not attention, following excitotoxic lesions of posterior parietal cortex in the rat, Brain Res. 775 (1997) 81–90, http://dx.doi.org/10.1016/S0006-8993(97)00915-3.

[46] M.T. Fox, M.D. Barense, M.G. Baxter, Perceptual attentional set-shifting is impaired in rats with neurotoxic lesions of posterior parietal cortex, J. Neurosci. 23 (2003) 676–681.

[47] D.A. Levy, J.R. Manns, R.O. Hopkins, J.J. Gold, N.J. Broadbent, L.R. Squire, Impaired visual and odor recognition memory span in patients with hippocampal lesions, Learn. Mem. 10 (2003) 531–536, http://dx.doi.org/10.1101/lm.66703.

[48] L. Olivito, P. Saccone, V. Perri, J.L. Bachman, P. Fragapane, A. Mele, R.L. Huganir, E. De Leonibus, Phosphorylation of the AMPA receptor GluA1 subunit regulates memory load capacity, Brain Struct. Funct. 221 (2016) 591–603, http://dx.doi.org/10.1007/s00429-014-0927-1.

[49] M. Sugita, K. Yamada, N. Iguchi, Y. Ichitani, Hippocampal NMDA receptors are involved in rats' spontaneous object recognition only under high memory load condition, Brain Res. 1624 (2015) 370–379, http://dx.doi.org/10.1016/j.brainres.

2015.08.001.
[50] S.A. Cooper, M. O'Sullivan, Here, there and everywhere: higher visual function and the dorsal visual stream, Pract. Neurol. 16 (3) (2016) 176–183, http://dx.doi.org/10.1136/practneurol-2015-001168.
[51] P. Karunanayaka, P.J. Eslinger, J.-L. Wang, C.W. Weitekamp, S. Molitoris, K.M. Gates, P.C.M. Molenaar, Q.X. Yang, Networks involved in olfaction and their dynamics using independent component analysis and unified structural equation modeling, Hum. Brain Mapp. 35 (2014) 2055–2072, http://dx.doi.org/10.1002/hbm.22312.
[52] I. Savic, B. Gulyas, M. Larsson, P. Roland, Olfactory functions are mediated by parallel and hierarchical processing, Neuron 26 (2000) 735–745, http://dx.doi.org/10.1016/S0896-6273(00)81209-X.
[53] J. Frasnelli, J.N. Lundström, V. Schöpf, S. Negoias, T. Hummel, F. Lepore, Dual processing streams in chemosensory perception, Front. Hum. Neurosci. 6 (2012) 1–9, http://dx.doi.org/10.3389/fnhum.2012.00288.
[54] M. Jones-Gotman, R.J. Zatorre, Olfactory identification deficits in patients with focal cerebral excision, Neuropsychologia 26 (1988) 387–400, http://dx.doi.org/10.1016/0028-3932(88)90093-0.
[55] J. Chapuis, Y. Cohen, X. He, Z. Zhang, S. Jin, F. Xu, D.A. Wilson, Lateral entorhinal modulation of piriform cortical activity and fine odor discrimination, J. Neurosci. 33 (2013) 13449–13459, http://dx.doi.org/10.1523/JNEUROSCI.1387-13.2013.
[56] K. Miyamichi, F. Amat, F. Moussavi, C. Wang, I. Wickersham, N.R. Wall, H. Taniguchi, B. Tasic, Z.J. Huang, Z. He, E.M. Callaway, M.A. Horowitz, L. Luo, Cortical representations of olfactory input by trans-synaptic tracing, Nature 472 (2011) 191–196, http://dx.doi.org/10.1038/nature09714.
[57] L. Pisella, N. Berberovic, J.B. Mattingley, Impaired working memory for location but not for colour or shape in visual neglect: a comparison of parietal and non-parietal lesions, Cortex 40 (2004) 379–390, http://dx.doi.org/10.1016/S0010-9452(08)70132-1.