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Performance of the trial-unique, delayed non-matching-to-location (TUNL) task depends on AMPA/Kainate, but not NMDA, ionotropic glutamate receptors in the rat posterior parietal cortex

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ABSTRACT

Working memory (WM), the capacity for short-term storage and manipulation of small quantities of information, depends on fronto-parietal circuits. However, the function of the posterior parietal cortex (PPC) in WM has gone relatively understudied in rodents. Recent evidence calls into question whether the PPC is necessary for all forms of WM. Thus, the present experiment examined the role of the rat PPC in the Trial-Unique Non-matching-to-Location (TUNL) task, a touchscreen-based visuospatial WM task that relies on the rat medial prefrontal cortex (mPFC). Temporary inactivation of the PPC caused by bilateral infusions of muscimol and baclofen significantly impaired accuracy and increased the number of correction trials performed, indicating that the PPC is necessary for performance of TUNL. Additionally, we investigated the effects of blocking NMDA or non-NMDA parietal ionotropic glutamate receptors on TUNL and found that, in contrast to the prefrontal cortex, NMDA receptors in the PPC are not necessary for TUNL performance, whereas blockade of AMPA/Kainate receptors significantly impaired accuracy. These results indicate that performance of the TUNL task depends on the PPC but that NMDA receptor signaling within this brain area is not necessary for intact performance.

1. Introduction

Working memory (WM) is the ability to temporarily store small quantities of information for use or manipulation and is likely mediated by a distributed network of brain areas including a fronto-parietal circuit involved in attention and executive control (D'Esposito & Postle, 2015; Eriksson, Vogel, Lansner, Bergström, & Nyberg, 2015). The neural correlates of WM have been understudied in rodents relative to primates and reveal a less consistent story with respect to the role played by the parietal cortex (PC). In primates, the PC has been consistently shown to participate in WM tasks (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey, Devinsky, Doyle, Golfinos, & Curtis, 2016; Öztekin, Mcelree, Staresina, & Davachi, 2009; Ravizza, Delgado, Chein, Becker, & Fiez, 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004). In rodents, however, disruption of the PC has led to inconsistent results. Tasks involving spatial stimuli seem to engage the PC (Espina-Marchant et al., 2006; McDaniel, Compton, & Smith, 1994) whereas tasks with non-spatial stimuli are independent of the PC (Kolb, Buhrmann, McDonald, & Sutherland, 1994; Scott, Zabder, Greba, & Howland, 2018).

In a recent paper, we demonstrated that the odor span task (OST), a non-spatial olfactory WM task (Dudchenko, Talpos, Young, & Baxter, 2013), does not rely on the posterior parietal cortex (PPC) in rats (Scott et al., 2018), a finding contrary to the view that the PPC is critical for WM performance. We hypothesized that the PC in both rodents and primates plays a sensory modality-specific role in WM that has been overlooked due to the heavy emphasis in primate research on visual or visuospatial WM tasks (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey et al., 2016; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004). Hence, further investigation is required to delineate the role of the rodent PPC in WM.

In the present experiment, we investigated role of the rat PPC in the Trial-Unique Non-matching-to-Location task (TUNL), a visuospatial WM task for rodents (Dudchenko et al., 2013; Hvoslef-Eide et al., 2015). The TUNL task offers several advantages over other WM tasks typically used in rodents. It is fully automated, allowing the rapid administration of large numbers of trials with minimized bias or

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variability introduced by heavy experimenter interaction with animals. Additionally, it closely mimics human visuospatial WM testing in order to provide high construct and predictive validity (Hvoslef-Eide et al., 2015). The stimuli are also presented at varying degrees of physical separation in TUNL, thereby varying the level of difficulty from trial to trial which may engage hippocampal-dependent pattern separation processes (McAllister, Saksida, & Bussey, 2013). Performance of TUNL relies on the rodent mPFC (Davies, Hurtubise, Greba, & Howland, 2017; Kim et al., 2015; McAllister et al., 2013) in line with studies in human and non-human primates.

In addition to investigating the role of the PPC in visuospatial WM with temporary inactivations, we investigated the relative contributions of NMDA and non-NMDA ionotropic glutamate receptors in the PC. WM performance relies heavily on NMDA receptors in the PFC (Davies, Greba, & Howland, 2013; MacQueen, Bullard, & Galizio, 2011; Wang et al., 2013) and the TUNL task specifically requires NMDA receptor activation in the mPFC (Davies et al., 2017; Hurtubise et al., 2017; Kumar, Olley, Steckler, & Talpos, 2015). The role of NMDA receptors in the PPC for WM has received very little study, but some research suggests that NMDA receptors in the rat PC are not necessary for short-term retention in a one trial step-down inhibitory avoidance task (Izquierdo et al., 1997, 1998). Thus, we were interested in assessing the involvement of PPC ionotropic glutamate receptors in the TUNL task.

2. Methods

2.1. Subjects

Fifteen male Long Evans rats (Charles River Laboratories, Kingston, NY) weighing 300–500 g were used in the experiment. Rats were individually housed in standard ventilated cages and kept on a 12-hour light/dark cycle (lights on at 0700). Rats were maintained at 85–90% of their free-feeding weight with water 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. Behavioural training

2.2.1. Training Apparatus

An illustration of the training apparatus for TUNL is shown in Fig. 1B. Training in TUNL was conducted within 8 touchscreen-equipped operant conditioning chambers controlled by ABET II Touch software (Lafayette Instruments, Lafayette, IN) as we have used previously (Davies et al., 2017; Roebuck, Liu, Lins, Scott, & Howland, 2018). Briefly, each chamber was housed within a sound-attenuated, vented box, with live video feed. Touchscreens were fitted with an interchangeable mask which obscured the screen except for the response windows. For TUNL, response windows consisted of an array of 14 2 × 2 cm square holes in a 7 × 2 arrangement. The response windows sat above a spring-loaded response shelf requiring the animal to stand when responding.

2.2.2. Touchscreen Habituation and Pretraining

All habituation, pretraining, and training stages were conducted according to protocols developed by Lafayette, and previous experiments conducted in our lab (Davies et al., 2017; Hurtubise et al., 2017). All stages of TUNL occurred once daily, 5 days per week. Behavioral training began with several days of habituation, which first involved 1 h in the touchscreen room with all equipment on. On days 2 and 3 of habituation, rats were placed in the touchscreen chambers for 30 min with reward pellets (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; BioServ, NJ, USA) placed in the food dispenser.

Following habituation, animals progressed through 4 pretraining stages upon reaching intermediate criterion. The first pretraining stage was Initial Touch where one square is illuminated pseudorandomly.

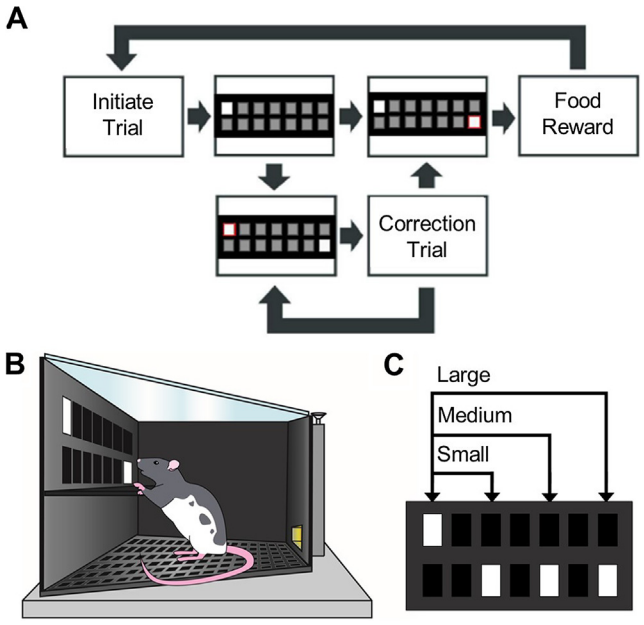

Fig. 1. (A) Schematic showing the general progression of a TUNL trial. (B) A representation of the interior of a touchscreen chamber used in the experiment. (C) A schematic representation of example stimulus distances used in TUNL.

Correct touches during this stage were followed by illumination of the food port and delivery of 3 reward pellets. If the animal failed to contact the illuminated square within 30 s, the food port was illuminated, and a single reward pellet dispensed. Each trial was separated by a 20 s inter-trial interval (ITI) which was consistent across all stages. Criterion for Initial Touch was completion of 100 selection trials in 1 h. Following Initial Touch, animals progressed to Must Touch on the next training day. Must Touch was administered similarly except that the rat must touch the illuminated square to receive a single reward pellet. There was no reward if the animal did not contact the illuminated square and the trial continued until a response was recorded. Criterion for Must Touch was completion of 100 selection trials in 1 h. Must Initiate followed and added the requirement that the animal nose poke the food port to initiate each trial. Criterion for Must Initiate is 100 selection trials in 1 h. The final pretraining stage, Punish Incorrect, was administered similarly to previous stages wherein animals must initiate by nose poking the food port and correctly touch the illuminated square to receive a food reward. However, in Punish Incorrect, incorrect touches were punished by a 5 s timeout and illumination of the house lights. Following the timeout period, a 'correction trial' began where the animal must repeat the same trial configuration until it was successfully completed, at which point the animal was rewarded with a food pellet and a selection trial was completed. Criterion for Punish Incorrect was completion of 100 selection trials in 1 h, with > 80% accuracy for two consecutive days. Accuracy was computed from the initial presentation of a trial only. Correction trials did not count toward accuracy or the number of completed selection trials.

2.2.3. TUNL Task Acquisition

Following pretraining, animals began TUNL task acquisition (Fig. 1A and C). TUNL is a non-matching to sample task consisting of multiple trials. Trials were composed of a sample phase and a test phase. During the sample phase, one of the 14 squares was illuminated and the animal was required to touch the square. After the lit square was touched, there was a delay period (2 s in Initial TUNL, 6 s in Full TUNL), before the test phase began. During the test phase, the same square from the sample phase was illuminated, as well as a new separate square. The animal was required to touch the new square to complete the trial and receive a food reward. The distance between the

sample stimuli and test stimuli varied (ranging from 2 to 6 squares apart). These distances were categorized as Large (least challenging), Medium, and Small (most challenging). If the sample stimuli were incorrectly chosen during the test phase, the animal was punished with a 5 s timeout and illumination of the house lights. This also triggered a correction trial where the animal had to repeat the same trial configuration until it was successfully completed, at which point the animal was rewarded with a food pellet and a selection trial was completed. Accuracy was computed from the initial presentation of a trial only. Correction trials did not count toward accuracy or the number of completed selection trials.

Acquisition of TUNL was completed in 2 stages: Initial TUNL and Full TUNL. During Initial TUNL, animals were trained for 1 h daily until they were able to complete 42 selection trials in 40 min with a 2 s delay between the sample and test phase. After completing initial TUNL, animals were moved to Full TUNL where the maximum is 84 selection trials in 1 h, with a 6 s delay between the sample and test phase. Before undergoing intracranial surgeries, rats were trained to a criterion of at least 75% accuracy at large distances for 2 consecutive days. On infusion days, rats were tested using the same Full TUNL protocol as above, including correction trials. Although the inclusion of correction trials makes for a less pure assessment of memory performance, it confers the major benefit of simultaneously measuring behavioural flexibility (Hurtubise et al., 2017; Kumar et al., 2015; Lins & Howland, 2016; Lins, Phillips, & Howland, 2015). Additionally, accuracy on test phases is calculated only from rats' first attempt at choosing the correct test stimulus, so correction trials do not influence rats' accuracy score.

2.3. Surgery and intracranial infusions

Intracranial cannulae implantation as well as the basic infusion procedure were conducted in the same manner described in Scott et al. (2018). Briefly, rats were bilaterally implanted with 2 23-gauge stainless steel guide cannulae per hemisphere aimed at the PPC (AP −4.0 mm, ML ± 2.2 and 3.4, DV −0.2 from brain surface). On infusion days, rats received infusions of combined muscimol and baclofen (M/B; each 0.5 mg/mL, mixed together in a 1/1 ratio (Davies, Molder, Greba, & Howland, 2013; Scott et al., 2018)), the competitive NMDA antagonist AP5 (5.90 mg/mL (Bett et al., 2013)), the competitive AMPA/Kainate receptor antagonist CNQX (disodium salt; 828.36 μg/mL (Bett et al., 2013)), or vehicle (0.9% physiological saline) in counterbalanced order over 4 consecutive days of testing. Drugs were infused through 30-gauge stainless steel needles connected to PE50 tubing using Hamilton syringes and a microinfusion pump (Harvard Apparatus, Holliston, MA, USA). Needles were lowered 1 mm past the end of the guide cannuale. Each drug was infused at 0.5 μL/min to a final infusion volume of 0.5 μL per site and a total of 1 μL per hemisphere. Infusions were given 20–60 min before rats were placed in the touchscreen chambers.

2.4. Perfusions and histology

Following the conclusion of testing, rats were perfused intracardially with 0.1 M PBS followed by 30% formalin. Brains were removed and post-fixed in 30% formalin and cryoprotected in 0.1% sodium azide/30% sucrose until brains were no longer buoyant in the cryoprotectant solution. Brains were sectioned at 40 μm on a freezing sliding microtome (Leica Biosystems, Concord, ON). Sections were mounted to glass slides and assessed for cannulae placement with the help of a rat brain atlas (Paxinos & Watson, 2006).

2.5. Statistical analyses

All data were collected automatically and are presented as group means +/- SEM. Analyses consisted of several measures of task performance: selection trial completion (number of completed selection trials in a session), percent accuracy on initial test stimuli presentation, correction trial completion (number of correction trials in a session), total trial completion (number of completed selection trials + correction trials in a session), correct response latency (latency to respond during the test phase for correct choices), incorrect response latency (latency to respond during the test phase for incorrect choices), and reward collection latency (latency from reward dispensation to reward collection) which were all obtained using the in-software analysis tools included in Abet Touch II. Each drug condition was separately compared to Saline because some rats did not complete all drug conditions (fewer rats were administered CNQX) meaning that a combined within-subjects analysis was not possible. Percent accuracy was analyzed in a 2x3 mixed-design factorial ANOVA with a Drug factor with 2 repeated measures levels and a Distance factor with 3 independent levels representing large, medium, and small stimulus separations.

The number of selection trials completed, the number of correction trials, and the total number of trials (including both selection and correction trials) were each compared between Saline and drug conditions in within-subjects t-tests. Latency for correct choices and latency for incorrect choices were compared between Saline and Drug in 2x2 mixed-design ANOVAs with a repeated measures Drug factor and an independent Trial Outcome factor (correct versus incorrect choice), and latency for reward collection were also compared between Saline and Drug conditions in within-subjects t-tests.

3. Results

3.1. Histology

Approximate placements of the infusion needles, as well as a representative photomicrograph of the infusion sites, are depicted in Fig. 2. All 15 rats had infusion sites that were deemed acceptable. All rats had some damage to the superficial layers of the cortex proximal to the location of the guide cannulae.

3.2. Percent accuracy

Percent accuracy for all 3 drug conditions are shown in Fig. 3. One rat was removed from the M/B analysis due to excessive motor impairment and response latency after M/B infusions while another rat was euthanized due to illness before completing all drug conditions and was not included in the CNQX or AP5 conditions. Additionally, a smaller subset of the total sample was administered CNQX. Thus, the final sample sizes for each drug condition were n = 14 for M/B, n = 10 for CNQX, and n = 14 for AP5.

A 2x3 mixed design ANOVA comparing Saline with M/B infusions revealed a main effect of Drug ($F_{(1,39)} = 21.47$, $p < .0001$) indicating that rats had significantly impaired accuracy following inactivation of the PPC. There was also a significant interaction of Drug by Distance ($F_{(2,39)} = 6.08$, $p < .01$), but no main effect of Distance ($F_{(2,39)} = 2.45$, $p = .10$). Fisher's LSD post-hoc tests revealed that percent accuracy was significantly reduced at large ($p < .05$) and medium ($p < .0001$) distances but not at small distances ($p = .67$). To test for a possible floor effect at small distances that might obscure differences between drug conditions, we performed single-sample t-tests comparing rats' accuracy to chance (50%) which revealed that rats still performed significantly better than chance in the Saline ($t_{(13)} = 7.22$, $p < .0001$, 2-tailed) and M/B ($t_{(13)} = 4.86$, $p < .001$, 2-tailed) conditions.

CNQX infusions also yielded a significant main effect of Drug ($F_{(1,27)} = 9.98$, $p < .005$) indicating a significant impairment in accuracy following blockade of AMPA/Kainate receptors in the PPC. There was also a significant interaction of Drug by Distance ($F_{(2,27)} = 5.15$, $p < .05$), but no main effect of Distance ($F_{(2,27)} = 1.60$, $p = .22$). Fisher's LSD post-hoc tests revealed that percent accuracy was reduced at medium distances ($p < .001$) but not at large ($p = .35$) or small ($p = .90$) distances. Single-sample t-tests were again run to test for a

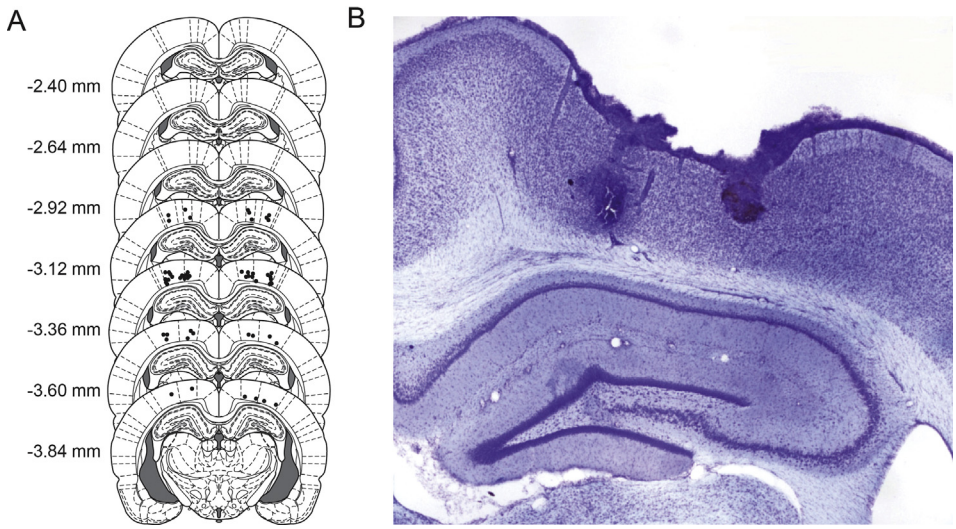

Fig. 2. (A) Diagram showing approximate locations of infusion sites. Images were adapted from Paxinos and Watson (2006). (B) Photomicrograph of a representative cresyl violet-stained section showing the locations of infusions.

floor effect at small distances and revealed that rats performed significantly better than chance in the Saline ($t_{(9)} = 7.11$, $p < .0001$, 2-tailed) and CNQX ($t_{(9)} = 4.20$, $p < .01$) conditions.

In contrast, infusions of AP5 exhibited no main effect of Drug ($F_{(1,39)} = 0.04$, $p = .85$) or any interaction ($F_{(2,39)} = 0.57$, $p = .57$), indicating that blockade of NMDA receptors in the PPC did not affect accuracy. However, a significant main effect of Distance was found ($F_{(2,39)} = 18.91$, $p < .0001$). Fisher's LSD post-hoc tests revealed that rats in both conditions had significantly lower accuracy at small distances than at large ($p < .001$) and medium ($p < .0001$) distances with no difference in accuracy between large and medium distances ($p = .11$).

3.3. Trial completion

Trial completion for all 3 Drug conditions and Saline is shown in Fig. 4. A within-subjects *t*-test revealed a significant increase in the number of correction trials performed in the M/B condition ($t_{(13)} = 3.30p < .01$), indicating an increase in perseverative behaviour. Additional within-subject *t*-tests revealed no difference between Saline and M/B in the number of selection trials completed ($t_{(13)} = -1.99$, $p = .07$) or the total number of trials completed ($t_{(13)} = -0.16$, $p = .88$). Infusions of CNQX, by contrast, did not increase correction trials relative to Saline ($t_{(9)} = 0.64$, $p = .54$), nor did it affect completion of selection trials ($t_{(9)} = -0.95$, $p = .37$) or the total number of trials ($t_{(9)} = -0.46$, $p = .66$). Infusions of AP5 had no effect on correction trials ($t_{(13)} = 0.71$, $p = .49$), selection trials ($t_{(13)} = -0.27$, $p = .79$), or total trials ($t_{(13)} = 0.14$, $p = .89$).

3.4. Response latency

Response latency for all 3 drug conditions and Saline is shown in Fig. 5. A 2 × 2 mixed design ANOVA on latencies for correct versus incorrect choices after M/B infusions revealed no main effect of Drug ($F_{(1,26)} = 1.48$, $p = .24$), Trial Outcome ($F_{(1,26)} = 2.09$, $p = .16$), or any interaction ($F_{(1,26)} = 0.01$, $p = .93$), suggesting that PPC inactivation did not affect rats' speed of responding during test phases. However, a small but significant increase in reward collection latency was observed after M/B infusions ($t_{(13)} = 3.94$, $p < .01$). Similarly, infusions of CNQX also yielded no main effect of Drug ($F_{(1,18)} = 0.57$, $p = .46$), Trial Outcome ($F_{(1,18)} = 2.40$, $p = .14$), or any interaction ($F_{(1,18)} = 0.002$, $p = .97$) but, unlike M/B infusions, did not cause a change in reward collection latency ($t_{(9)} = -0.24$, $p = .81$). Infusions of AP5 produced no main effect of Drug ($F_{(1,26)} = 0.77$, $p = .39$), Trial Outcome ($F_{(1,26)} = 1.39$, $p = .25$), or any interaction ($F_{(1,26)} = 0.38$, $p = .54$), or difference in reward collection latency ($t_{(26)} = 0.26$, $p = .80$).

4. Discussion

The present experiment aimed to determine whether the rodent PPC plays a role in the TUNL task, a rodent model of visuospatial WM. After PPC inactivation with M/B or AMPA/Kainate blockade with CNQX, rats performed with significantly impaired accuracy. Thus, the present results indicate that the rat PPC is necessary for intact performance of the

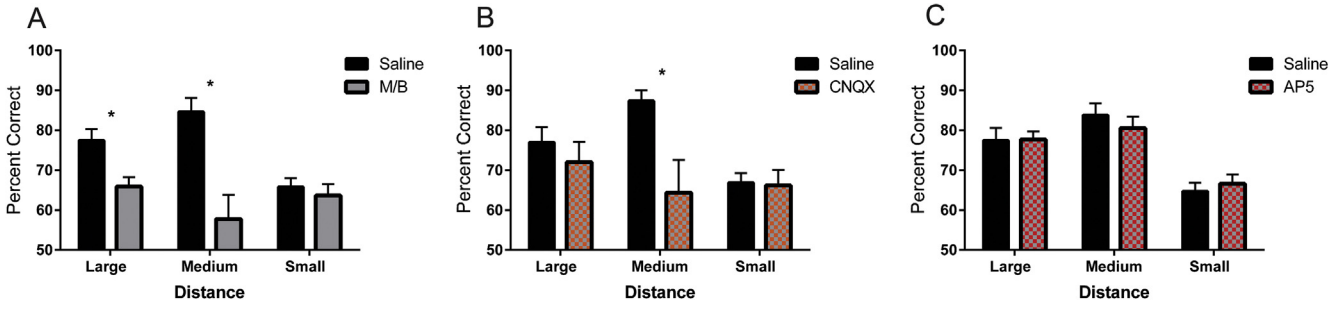

Fig. 3. (A) Percent accuracy (Mean ± SEM) in TUNL after Saline or M/B infusions. Accuracy was significantly reduced by M/B infusions at large and medium distances, indicating that intact PC function is necessary for TUNL. (B) Percent accuracy (Mean ± SEM) in TUNL after Saline or CNQX infusions. Accuracy was significantly reduced at medium distances, indicating that AMPA/Kainate receptors are necessary for intact TUNL performance. (C) Percent accuracy (Mean ± SEM) in TUNL after Saline or AP5 infusions. Accuracy was completely unaffected by AP5, indicating that TUNL performance is independent of NMDA signaling in the PC.

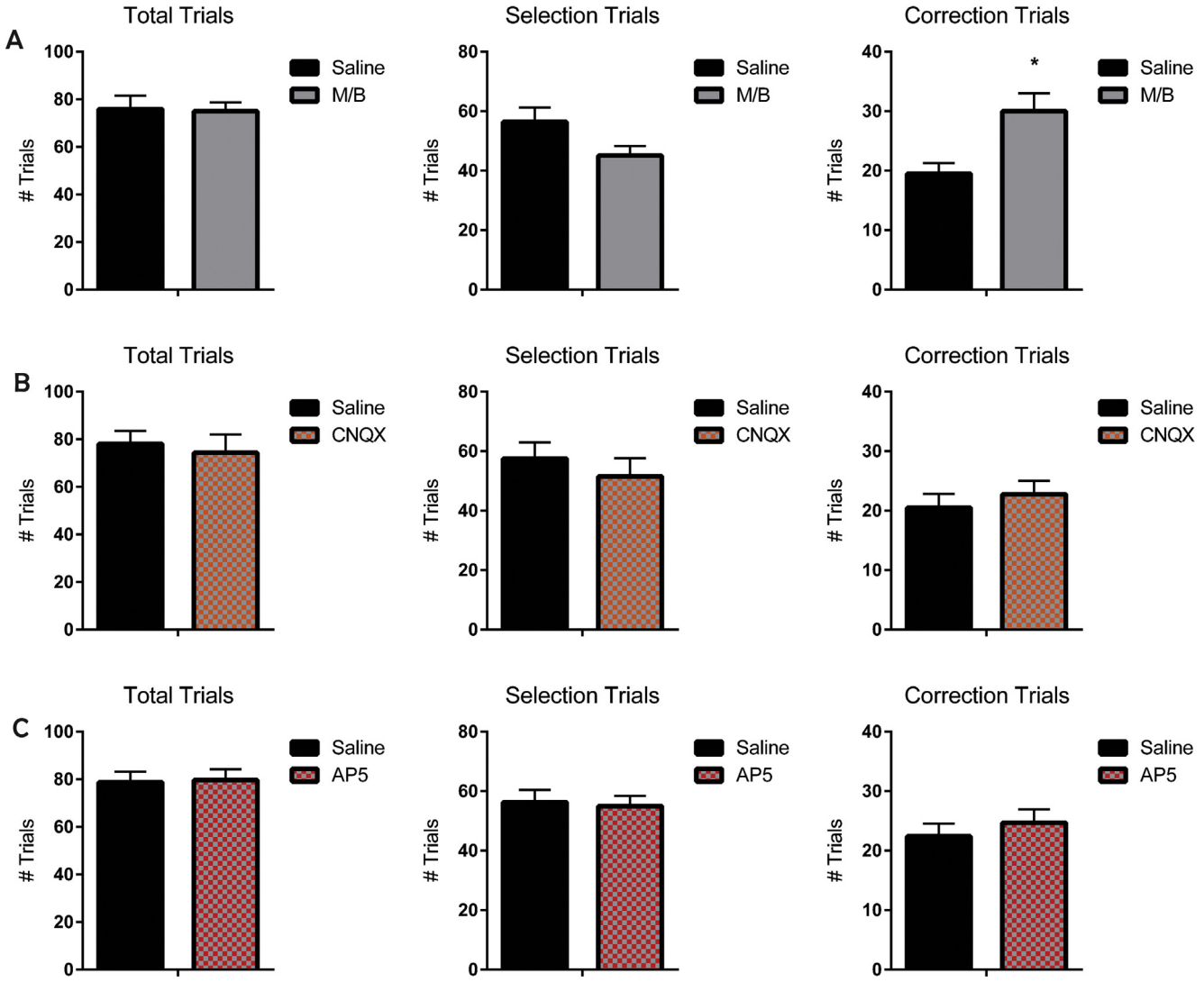

Fig. 4. (A) Mean (± SEM) number of completed trials in TUNL after Saline or M/B infusions. Neither the number of selection trials or the number of total trials completed were affected by M/B. However, M/B caused a significant increase in the number of correction trials performed, indicating a possible impairment in behavioral flexibility. (B) Mean (± SEM) number of completed trials in TUNL after Saline or CNQX infusions. CNQX had no effect on selection, correction, or total trials completed. (C) Mean (± SEM) number of completed trials in TUNL after Saline or AP5 infusions. AP5 had no effect on selection, correction, or total trials completed.

TUNL task. The results are similar to previous studies which have demonstrated an involvement of the rodent PPC in spatial WM tasks including a multiple T-maze task (McDaniel et al., 1994) and the Olton 4 × 4 maze (Espina-Marchant et al., 2006). Additionally, they are in agreement with the ample evidence from primates that the PPC is involved in visuospatial WM (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey et al., 2016; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004). In contrast, disruption of the rat PPC during WM tasks using odours (Scott et al., 2018) or objects (Kolb et al., 1994) does not cause impairments. Overall, the combined findings strongly suggest that the PPC plays a sensory modality-specific role in WM that includes visual/visuospatial stimuli. Indeed, the PPC is a part of the dorsal visual stream (Cooper & O'Sullivan, 2016), which is involved in the perception of the location of objects in space. Recent evidence also confirms that the rodent PPC is connected to auditory and tactile sensory areas (Hovde, Gianatti, Witter, & Whitlock, 2018) although manipulation of the PPC has been shown to affect the visual modality more than the auditory modality (Licata et al., 2017). The PC is also a crucial part of the frontoparietal attention network along with the PFC that has gained significant recent interest for its role in the executive control of WM and attention (Fiebelkorn, Pinsk, & Kastner, 2018; Johnson et al., 2017; Murray, Jaramillo, & Wang, 2017; Wallis, Stokes, Cousijn, Woolrich, & Nobre, 2015).

The impaired accuracy that we observed after infusing M/B or CNQX was specific to large and medium stimulus distances. Rats performed small distances with less accuracy than medium or large distances, although the main effect of Distance only reached significance in the AP5 comparison. The lack of an effect of M/B and CNQX appears to reflect a drop-off in performance in the Saline condition at these distances. Under all drug conditions, however, rats still performed with accuracy significantly better than chance, making it unlikely that this was a floor effect. Rather, it appears that M/B and CNQX caused rats to perform with mostly equally poor accuracy across all stimulus distances while performance in the Saline and AP5 conditions benefited from the less-challenging large and medium stimulus distances and that our manipulations, while causing significant impairment, still spared some intact performance. This pattern of results is similar to Talpos, McTighe, Dias, Saksida, and Bussey (2010) who found that, using a 6-s delay (the same as the present experiment), hippocampal lesions only resulted in a

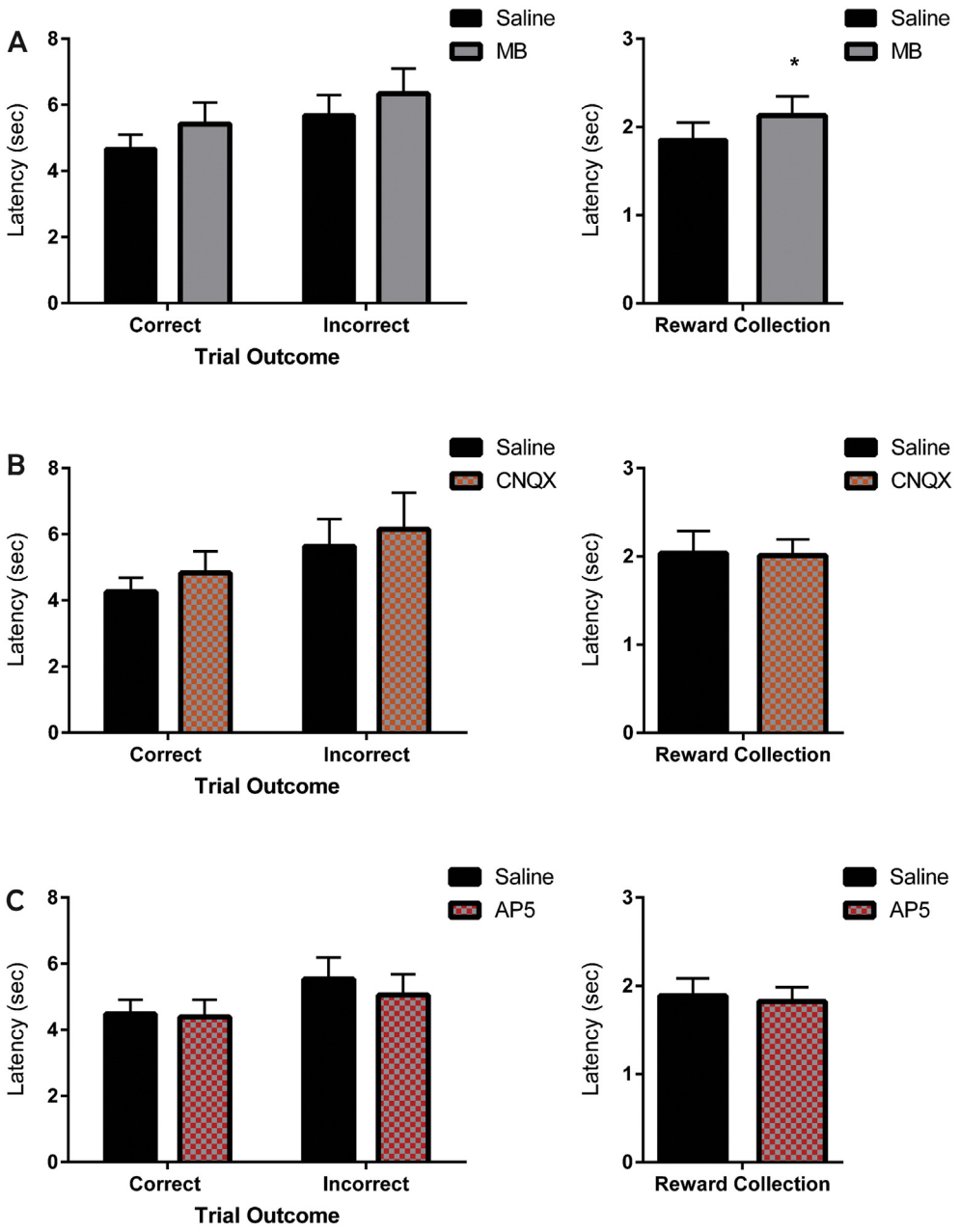

Fig. 5. (A) Mean (± SEM) latency in TUNL after Saline or M/B infusions. Neither correct choice latency or incorrect choice latency were affected by M/B. However, M/B caused a small but statistically significant increase in reward collection latency which may be related to the PPC's role in task-reward associations. (B) Mean (± SEM) latency in TUNL after Saline or CNQX infusions. CNQX had no effect on latency. (C) Mean (± SEM) latency in TUNL after Saline or AP5 infusions. AP5 had no effect on latency.

difference in percent accuracy at large and medium distances, with control performance dropping to the level of lesion performance at small distances.

An additional dimension to our accuracy results is pattern separation. The TUNL task, in addition to measuring visuospatial WM, has been used to measure hippocampal-dependent pattern separation (Talpos et al., 2010). Specifically, rats are inferred to have impaired pattern separation when they are more severely impaired at small distances than large distances. There is also some evidence from humans that the parietal cortex participates in pattern separation (Pidgeon & Morcom, 2016). The present CNQX results could be argued to partially fit this pattern given that rats are impaired at medium distances but not at large distances (although there is no drug effect at minimum distances). Conversely, the impairment from M/B does not satisfy this criterion as rats are impaired at large and medium distances. Hence, AMPA/Kainate blockade within the PPC may have a specific effect on pattern separation, leaving memory intact on trials with more distinct choices, whereas complete inactivation with M/B causes a more global impairment in accuracy.

In addition to impairing accuracy, other impairments were observed after infusions of M/B. Correction trials were significantly increased, indicating that rats perseverated more on incorrect choices, an effect which has previously been used to infer impaired behavioral flexibility (Hurtubise et al., 2017; Kumar et al., 2015; Lins & Howland, 2016; Lins et al., 2015). This finding is in general agreement with previous research that has shown that lesions of the rodent PPC can impair attentional set-shifting (Fox, Barense, & Baxter, 2003), indicating that the PPC plays some role in mediating behavioral flexibility. Of note, the inclusion of correction trials during the tests, while providing a measure of behavioural flexibility, may have contributed some bias to the results

in that they comprise a practice element that rats may benefit more from under control conditions. However, given that the TUNL task is trial-unique, this practice would ostensibly introduce a bias in rule acquisition rather than accuracy on individual trials as accuracy was only computed from rats' first choice on a set of test stimuli. Another interesting finding was the increased reward collection latency following M/B infusions. Although the difference is extremely small (~0.3 sec), previous research has shown that the human PPC contains representations of task-reward associations (Wisniewski, Reverberi, Momennejad, Kahnt, & Haynes, 2015) meaning that this finding could be interpreted as an impairment in task-reward association.

Interpretation of WM impairments can be confounded if changes in latency are found along with changes in accuracy. Longer latencies may fatigue the maintenance mechanisms of WM and impair performance even if the given manipulation has no direct effect on WM accuracy or capacity. Despite the increased reward collection latency following infusions of M/B, we did not observe significant increases in latency for correct or incorrect choices during test phases, so the reduction in accuracy after M/B infusions likely had little to do with increased latency. Additionally, there was no significant reduction in the total number of trials rats completed after M/B infusions compared with Saline infusions. We therefore deem it unlikely that the results of M/B infusions are confounded by locomotor deficits, as rats evidently completed trials with the same efficiency after M/B or Saline.

Interestingly, the impairment caused by CNQX infusions was specific to accuracy and was not associated with alterations in latency or correction trials. This result may simply reflect a less severe impairment due to our chosen CNQX dose. We deliberately used a CNQX dose similar to what has been previously used in experiments dissociating NMDA from non-NMDA signaling (Bett et al., 2013). However, other groups have used considerably higher CNQX doses when comparing NMDA and non-NMDA signaling (Agrawal & Fehlings, 1997; Barker & Warburton, 2011). Alternatively, it is possible that this apparent dissociation reflects the pharmacological differences between M/B and CNQX. While CNQX would primarily exert its effects post-synaptically as the majority of AMPA receptors are located post-synaptically (Feligioni, Holman, Haglerod, Davanger, & Henley, 2006; Fujiyama et al., 2004), the baclofen in our M/B mixture would presumably bind to GABAb receptors on the presynaptic terminals of afferent connections arising from other areas of cortex or from neuromodulator-secreting nuclei and inhibit neurotransmitter release (Misgeld, Bijakt, & Jarolimek, 1995). For example, it has been demonstrated that norepinephrine signaling in the human PPC is involved in task switching under memory load (Wolff, Mückschel, Ziemssen, & Beste, 2018) which could be said to be similar to the rats in the present experiment having to switch their response during correction trials.

The present findings confirm a role for the PPC in TUNL, but do not indicate during which epochs of the task (i.e. sample, delay, test, and reward collection) it is critically engaged. The impairment we found could reflect the disruption of stimulus encoding/delay period maintenance (Christophel, Cichy, Hebart, & Haynes, 2015; Christophel, Hebart, & Haynes, 2012; Ester, Sprague, & Serences, 2015; Vogel & Machizawa, 2004) or retrieval (Berryhill & Olson, 2008; Öztekin et al., 2009). Further experimentation with more temporally precise methods will be necessarily to clarify this issue. Additionally, we did not include variable delay periods in this experiment. Thus, we cannot rule out the possibility that performance would still be impaired by our manipulations under short delays or no delay, which would reflect an impairment in choice accuracy rather than WM maintenance *per se*. Therefore, although the present results fit well with the literature showing a role for the PPC in visuospatial WM (Brigadoi et al., 2017; Champod & Petrides, 2007; Curtis, 2006; Mackey et al., 2016; Öztekin et al., 2009; Ravizza et al., 2004; van Asselen et al., 2006; Vogel & Machizawa, 2004), we cannot rule out the possibility that our findings reflect impairments in processes other than WM maintenance.

The lack of an effect of blocking NMDA receptors is interesting given that major models of WM require NMDA receptor signaling (specifically NR2B-containing NMDA receptors) for maintenance of delay period persistent activity (Wang et al., 2013; Wang, 1999). Additionally, a considerable body of research, including from our own lab, has found that systemic or PFC-specific blockade of NMDA receptors impairs WM (Arnsten & Jin, 2014; Davies et al., 2017; Hurtubise et al., 2017; Monaco, Gulchina, & Gao, 2015; Wang et al., 2013; but see Auger & Floresco, 2017). NMDA receptors have been localized in the rat PPC using binding assays (Bean, Zheng, Patel, & Monaghan, 2006) demonstrating the presence of available AP5 binding sites within the PPC. Hence, it is unlikely that the null effect in our experiment is the result of our drug treatment lacking a pharmacological target. Additionally, we used a high dose of AP5 (Davies et al., 2017). These results are similar to previous findings using a step-down inhibitory avoidance task that showed NMDA receptors in the PPC not to be necessary for "short-term" memory, but rather to become engaged after longer intervals following training (Izquierdo et al., 1997, 1998).

5. Conclusions

The present experiment showed that inactivation of the PPC and intra-PPC AMPA/Kainate blockade impaired performance in the TUNL task, a visuospatial WM task for rodents. In contrast, blockade of NMDA receptors in the PPC with AP5 did not impair TUNL, whereas CNQX infusions impaired performance, suggesting that, unlike in the PFC, WM function in the PPC relies only on non-NMDA ionotropic glutamate receptors. The results may have implications for models of WM involving NMDA receptors as a fundamental mechanism for maintaining stimulus representations over a delay (Wang et al., 2013; Wang, 1999). These findings, along with previous research (Izquierdo et al., 1997, 1998), suggest that this involvement of NMDA receptors in WM does not extend to all areas of the cortex, and that NMDA receptor function in the PPC is not necessary to support visuospatial WM.

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References

Agrawal, S. K., & Fehlings, M. G. (1997). Role of NMDA and non-NMDA ionotropic glutamate receptors in traumatic spinal cord axonal injury. *The Journal of Neuroscience, 17*(3), 1055–1063.

Arnsten, A. F. T., & Jin, L. E. (2014). Molecular influences on working memory circuits in dorsolateral prefrontal cortex. Progress in Molecular Biology and Translational Science (1st ed., Vol. 122). Elsevier Inc.

Auger, M. L., & Floresco, S. B. (2017). Prefrontal cortical GABAergic and NMDA glutamatergic regulation of delayed responding. *Neuropharmacology, 113*(Pt A), 10–20. https://doi.org/10.1016/j.neuropharm.2016.09.022 (Epub 2016 Sep 24).

Barker, G. R. I., & Warburton, E. C. (2011). Evaluating the neural basis of temporal order memory for visual stimuli in the rat. *European Journal of Neuroscience, 33*, 705–716.

Bean, L., Zheng, H., Patel, K. P., & Monaghan, D. T. (2006). Regional variations in NMDA receptor downregulation in streptozotocin-diabetic rat brain. *Brain Research,* 217–222.

Berryhill, M. E., & Olson, I. R. (2008). Is the posterior parietal lobe involved in working memory retrieval? Evidence from patients with bilateral parietal lobe damage. *Neuropsychologia, 46*, 1775–1786.

Bett, D., Stevenson, C. H., Shires, K. L., Smith, M. T., Martin, S. J., Dudchenko, P. A., & Wood, E. R. (2013). The postsubiculum and spatial learning: The role of postsubicular synaptic activity and synaptic plasticity in hippocampal place cell, object, and object-location memory. *The Journal of Neuroscience, 33*(16), 6928–6943.

Brigadoi, S., Cutini, S., Meconi, F., Castellaro, M., Sessa, P., Marangon, M., ... Dell'Acqua, R. (2017). On the role of the inferior intraparietal sulcus in visual working memory for lateralized single-feature objects. *Journal of Cognitive Neuroscience, 29*(2), 337–351.

Champod, A. S., & Petrides, M. (2007). Dissociable roles of the posterior parietal and the prefrontal cortex in manipulation and monitoring processes. *Proceedings of the National Academy of Sciences, 104*(37), 14837–14842.

Christophel, T. B., Cichy, R. M., Hebart, M. N., & Haynes, J. D. (2015). Parietal and early visual cortices encode working memory content across mental transformations. *NeuroImage, 106*, 198–206. https://doi.org/10.1016/j.neuroimage.2014.11.018.
Christophel, T. B., Hebart, M. N., & Haynes, J. (2012). Decoding the contents of visual short-term memory from human visual and parietal cortex. *The Journal of Neuroscience, 32*(38), 12983–12989.
Cooper, S. A., & O'Sullivan, M. (2016). Here, there and everywhere: Higher visual function and the dorsal visual stream. *Practical Neurology, 16*, 176–183.
Curtis, C. E. (2006). Prefrontal and parietal contributions to spatial working memory. *Neuroscience, 139*(1), 173–180.
D'Esposito, M., & Postle, B. R. (2015). The cognitive neuroscience of working memory. *Annual Review of Psychology, 66*(1), 115–142.
Davies, D. A., Greba, Q., & Howland, J. G. (2013). GluN2B-containing NMDA receptors and AMPA receptors in medial prefrontal cortex are necessary for odor span in rats. *Frontiers in Behavioral Neuroscience, 7*, 1–8.
Davies, D. A., Hurtubise, J. L., Greba, Q., & Howland, J. G. (2017). Medial prefrontal cortex and dorsomedial striatum are necessary for the trial-unique, delayed non-matching-to-location (TUNL) task in rats: Role of NMDA receptors. *Learning and Memory, 24*(6), 262–267.
Davies, D. A., Molder, J. J., Greba, Q., & Howland, J. G. (2013). Inactivation of medial prefrontal cortex or acute stress impairs odor span in rats. *Learning & Memory, 20*(12), 665–669.
Dudchenko, P. A., Talpos, J. C., Young, J. W., & Baxter, M. G. (2013). Animal models of working memory: A review of tasks that might be used in screening drug treatments for the memory impairments found in schizophrenia. *Neuroscience and Biobehavioral Reviews, 37*(9), 2111–2124.
Eriksson, J., Vogel, E. K., Lansner, A., Bergström, F., & Nyberg, L. (2015). Neurocognitive architecture of working memory. *Neuron, 88*(1), 33–46.
Espina-Marchant, P., Pinto-Hamuy, T., Bustamante, D., Morales, P., Robles, L., & Herrera-marschitz, M. (2006). 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*, 601–609.
Ester, E. F., Sprague, T. C., & Serences, J. T. (2015). Parietal and frontal cortex encode stimulus-specific mnemonic representations during visual working memory. *Neuron, 87*(4), 893–905.
Feligioni, M., Holman, D., Haglerod, C., Davanger, S., & Henley, J. M. (2006). Ultrastructural localisation and differential agonist-induced regulation of AMPA and kainate receptors present at the presynaptic active zone and postsynaptic density. *Journal of Neurochemistry, 99*(2), 549–560 (Epub 2006 Aug 11).
Fiebelkorn, I. C., Pinsk, M. A., & Kastner, S. (2018). A dynamic interplay within the frontoparietal underlies rhythmic spatial attention. *Neuron, 99*(4), 842–853.
Fox, M. T., Barense, M. D., & Baxter, M. G. (2003). Perceptual attentional set-shifting is impaired in rats with neurotoxic lesions of posterior parietal cortex. *The Journal of Neuroscience, 23*(2), 676–681.
Fujiyama, F., Kuramoto, E., Okamoto, K., Hioki, H., Furuta, T., Zhou, L., ... Kaneko, T. (2004). Presynaptic localization of an AMPA-type glutamate receptor in corticostriatal and thalamostriatal axon terminals. *European Journal of Neuroscience, 20*, 3322–3330.
Hovde, K., Gianatti, M., Witter, M. P., & Whitlock, J. R. (2018). Architecture and organization of mouse posterior parietal cortex relative to extrastriate areas. *European Journal of Neuroscience*. https://doi.org/10.1111/ejn.14280 (Epub ahead of print).
Hurtubise, J. L., Marks, W. N., Davies, D. A., Catton, J. K., Baker, G. B., & Howland, J. G. (2017). MK-801-induced impairments on the trial-unique, delayed nonmatching-to-location task in rats: Effects of acute sodium nitroprusside. *Psychopharmacology, 234*, 211–222.
Hvoslef-Eide, M., Mar, A. C., Nilsson, S. R. O., Alsiö, J., Heath, C. J., Saksida, L. M., ... Bussey, T. J. (2015). The NEWMEDS rodent touchscreen test battery for cognition relevant to schizophrenia. *Psychopharmacology, 232*(21–22), 3853–3872.
Izquierdo, I. A., Izquierdo, L. A., Barros, D. M., Mello e Souza, T., De Souza, M. M., Quevedo, J., ... Medina, J. H. (1998). Differential involvement of cortical receptor mechanisms in working, short-term and long-term memory. *Behavioural Pharmacology, 9*, 421–427.
Izquierdo, I. A., Quillfeldt, J. A., Zanatta, M. S., Quevedo, J., Schaeffer, E., Schmitz, P. K., & Medina, J. H. (1997). Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats. *European Journal of Neuroscience, 9*(4), 786–793.
Johnson, E. L., Dewar, C. D., Solbakk, A. K., Endestad, T., Meling, T. R., & Knight, R. T. (2017). Bidirectional frontoparietal oscillatory systems support working memory. *Current Biology, 27*(12), 1829–1835.
Kim, C. H., Romberg, C., Hvoslef-Eide, M., Oomen, C. A., Mar, A. C., Heath, C. J., ... Saksida, L. M. (2015). Trial-unique, delayed nonmatching-to-location (TUNL) touchscreen testing for mice: Sensitivity to dorsal hippocampal dysfunction. *Psychopharmacology, 232*(21–22), 3935–3945.
Kolb, B., Buhrmann, K., McDonald, R., & Sutherland, R. J. (1994). Dissociation of the medial prefrontal, posterior parietal, and posterior temporal cortex for spatial navigation and recognition memory in the rat. *Cerebral Cortex, 4*(6), 664–680.
Kumar, G., Olley, J., Steckler, T., & Talpos, J. C. (2015). Dissociable effects of NR2A and NR2B NMDA receptor antagonism on cognitive flexibility but not pattern separation. *Psychopharmacology, 232*(21–22), 3991–4003.
Licata, A. M., Kaufman, M. T., Raposo, D., Ryan, M. B., Sheppard, J. P., & Churchland, A. K. (2017). Posterior parietal cortex guides visual decisions in rats. *The Journal of Neuroscience, 37*(19), 0105–117.
Lins, B. R., & Howland, J. G. (2016). Effects of the metabotropic glutamate receptor 5 positive allosteric modulator CDPPB on rats tested with the paired associates learning task in touchscreen-equipped operant conditioning chambers. *Behavioural Brain Research, 301*, 152–160.
Lins, B. R., Phillips, A. G., & Howland, J. G. (2015). Effects of D- and L-govadine on the disruption of touchscreen object-location paired associates learning in rats by acute MK-801 treatment. *Psychopharmacology, 232*(23), 4371–4382.
Mackey, W. E., Devinsky, O., Doyle, W. K., Golfinos, J. G., & Curtis, C. E. (2016). Human parietal cortex lesions impact the precision of spatial working memory. *Journal of Neurophysiology, 116*, 1049–1054.
MacQueen, D. A., Bullard, L., & Galizio, M. (2011). Effects of dizocilpine (MK801) on olfactory span in rats. *Neurobiology of Learning and Memory, 95*(1), 57–63.
McAllister, K. A. L., Saksida, L. M., & Bussey, T. J. (2013). Dissociation between memory retention across a delay and pattern separation following medial prefrontal cortex lesions in the touchscreen TUNL task. *Neurobiology of Learning and Memory, 101*, 120–126.
McDaniel, W. F., Compton, D. M., & Smith, S. R. (1994). Spatial learning following posterior parietal or hippocampal lesions. *Neuroreport, 5*, 1713–1717.
Misgeld, U., Bijakt, M., & Jarolimek, W. (1995). A physiological role for GABAb receptors and the effects of baclofen in the mammalian central nervous system. *Progress in Neurobiology, 46*, 423–462.
Monaco, S. A., Gulchina, Y., & Gao, W. J. (2015). NR2B subunit in the prefrontal cortex: A double-edged sword for working memory function and psychiatric disorders. *Neuroscience and Biobehavioral Reviews, 56*, 127–138.
Murray, J. D., Jaramillo, J., & Wang, X.-J. (2017). Working memory and decision-making in a frontoparietal circuit model. *The Journal of Neuroscience, 37*(50), 12167–12186.
Öztekin, I., Mcelree, B., Staresina, B. P., & Davachi, L. (2009). Working memory retrieval: Contributions of the left prefrontal cortex, the left parietal cortex, and the hippocampus. *Journal of Cognitive Neuroscience, 21*(3), 581–593.
Paxinos, G., & Watson, C. (2006). *The rat brain in stereotaxic coordinates* (6th ed.). Academic Press.
Pidgeon, L. M., & Morcom, A. M. (2016). Cortical pattern separation and item-specific memory encoding. *Neuropsychologia, 85*(May), 256–271.
Ravizza, S. M., Delgado, M. R., Chein, J. M., Becker, J. T., & Fiez, J. A. (2004). Functional dissociations within the inferior parietal cortex in verbal working memory. *NeuroImage, 22*, 562–573.
Roebuck, A. J., Liu, M. C., Lins, B. R., Scott, G. A., & Howland, J. G. (2018). Acute stress, but not corticosterone, facilitates acquisition of paired associates learning in rats using touchscreen-equipped operant conditioning chambers. *Behavioural Brain Research, 348*, 139–149. https://doi.org/10.1016/j.bbr.2018.04.027.
Scott, G. A., Zabder, N. K., Greba, Q., & Howland, J. G. (2018). Performance of the odour span task is not impaired following inactivations of parietal cortex in rats. *Behavioural Brain Research, 341*, 181–188.
Talpos, J. C., McTighe, S. M., Dias, R., Saksida, L. M., & Bussey, T. J. (2010). Trial-unique, delayed nonmatching-to-location (TUNL): A novel, highly hippocampus-dependent automated touchscreen test of location memory and pattern separation. *Neurobiology of Learning and Memory, 94*(3), 341–352.
van Asselen, M., Kessels, R. P. C., Neggers, S. F. W., Kappelle, L. J., Frijns, C. J. M., & Postma, A. (2006). Brain areas involved in spatial working memory. *Neuropsychologia, 44*, 1185–1194.
Vogel, E. K., & Machizawa, M. G. (2004). Neural activity predicts individual differences in visual working memory capacity. *Nature, 428*(6984), 748–751.
Wallis, G., Stokes, M. G., Cousijn, H., Woolrich, M., & Nobre, A. C. (2015). Frontoparietal and cingulo-opercular networks play dissociable roles in control of working memory. *Journal of Cognitive Neuroscience, 27*(10), 2019–2034.
Wang, M., Yang, Y., Wang, C.-J., Gamo, N. J., Jin, L. E., Mazer, J. A., ... Arnsten, A. F. T. (2013). NMDA receptors subserve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. *Neuron, 77*(4), 736–749.
Wang, X.-J. (1999). Synaptic basis of cortical persistent activity: The importance of NMDA receptors to working memory. *The Journal of Neuroscience, 19*(21), 9587–9603.
Wisniewski, D., Reverberi, C., Momennejad, I., Kahnt, T., & Haynes, J. (2015). The role of the parietal cortex in the representation of task – Reward associations. *The Journal of Neuroscience, 35*(36), 12355–12365.
Wolff, N., Mückschel, M., Ziemssen, T., & Beste, C. (2018). The role of phasic norepinephrine modulations during task switching: Evidence for specific effects in parietal areas. *Brain Structure and Function, 223*, 925–940.