Medial prefrontal cortex and dorsomedial striatum are necessary for the trial-unique, delayed nonmatching-to-location (TUNL) task in rats: role of NMDA receptors

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The trial-unique, delayed nonmatching-to-location (TUNL) task is a recently developed behavioral task that measures spatial working memory and a form of pattern separation in touchscreen-equipped operant conditioning chambers. Limited information exists regarding the neurotransmitters and neural substrates involved in the task. The present experiments tested the effects of systemic and intracranial injections of NMDA receptor antagonists on the TUNL task. After training, male Long Evans rats systemically injected with the competitive NMDA receptor antagonist CPP (10 mg/kg) had impaired accuracy regardless of the degree of stimuli separation or length of delay between the sample and test phases. Injections of Ro 25-6981 (6 or 10 mg/kg), an antagonist selective for GluN2B subunit-containing NMDA receptors, did not affect accuracy on the task. Direct infusion of the competitive NMDA receptor antagonist AP5 into mPFC or dmSTR reduced overall accuracy on the TUNL task. These results demonstrate that TUNL task performance depends on NMDA receptors within the mPFC and dmSTR.

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with saline. Brains were then removed, stored in a 10% formalin—

2010; Baker and Ragozzino 2014). After testing, rats were perfused

was infused bilaterally into mPFC or dmSTR (Winters et al. 

min following brain infusions. AP5 (1.0 mg/kg) was injected (i.p.) was infused into mPFC or dmSTR (AP+ + 0.80 mm; ML + 2.20; DV-3.40) using previously described procedures (Davies et al. 2013a,b). One rat died during surgery and another rat did not learn the task adequately and was excluded from testing. Therefore, 14 rats received brain infusions and were tested on the TUNL task with trials lasting 1 min and the infusion needles remained in place for 1 min after the infusion to allow diffusion of the drug. Rats were tested 5 min following brain infusions. APS (1.0 μL of a 30 mM solution) was infused bilaterally into mPFC or dmSTR (Winters et al. 2010; Baker and Ragozzino 2014). After testing, rats were perfused with saline. Brains were then removed, stored in a 10% formalin—

Similar to previous studies that used NMDA receptor antagonists (Kumar et al. 2015; Hurtubise et al. 2017), systemic NMDA receptor blockade with CPP impaired accuracy (Fig. 1A; t(7) = 3.52, P = 0.01) regardless of separation (Fig. 1B; main effect of treatment: F(1,7) = 14.37, P = 0.01) or delay (Fig. 1C; main effect of treatment: F(1,7) = 8.71, P = 0.02). Analyses of treatment, stimuli separation, and delay revealed a main effect of treatment (Fig. 1D; F(1,7) = 14.37, P = 0.01), delay (F(1,7) = 7.13, P = 0.03), and reward collection.

Figure 1. Performance of rats in the TUNL task following CPP (10 mg/kg) or vehicle (Veh) treatment. Overall accuracy (percent correct) on selection trials was reduced following CPP treatment (A) regardless of stimuli separation (B) or delay (C). (D) When trials were broken down by both stimuli separation and delay, significant main effects of treatment and delay emerged (see Results for details). (E) CPP treatment did not affect the number of selection trials but significantly increased the number of correction trials (F). (G,H) CPP treatment did not affect the number of total trials (selection trials + correction trials) or the reward latency, correct response latency, or incorrect response latency. Values are plotted as mean ± standard error of the mean. Asterisks indicate a significant effect of treatment.
Infusions of AP5 into mPFC impaired accuracy (Fig. 3A; $t_{(13)} = 3.56, P = 0.01$). When trials were analyzed according to separation (Fig. 3B), significant main effects of stimuli separation ($F_{(1,13)} = 52.59, P < 0.001$) and treatment ($F_{(1,13)} = 5.83, P = 0.03$) were noted with no interaction. In addition, intra-mPFC AP5 infusions reduced selection trials (Fig. 3C; $t_{(13)} = 4.92, P < 0.001$) and total trials (Fig. 3E; $t_{(13)} = 3.02, P = 0.01$). The number of correction trials was not significantly influenced by AP5 infusions into mPFC (Fig. 3D). Latencies were unaffected by AP5 infusions into mPFC (Fig. 2F). AP5 infused into dmSTR impaired overall accuracy (Fig. 3A; $t_{(13)} = 2.16, P = 0.049$). A main effect of stimuli separation (Fig. 3B; $F_{(1,13)} = 14.46, P = 0.002$) was observed on days and an interaction between stimuli separation and delay ($F_{(1,7)} = 6.58, P = 0.04$) but no main effect of stimuli separation or other interactions. CPP increased correction trials (Fig. 1F; $t_{(7)} = -3.18, P = 0.02$) but did not affect the number of selection trials (Fig. 1E), total trials (Fig. 1G), or latencies (Fig. 1H). Ro 25-6981 did not affect accuracy when trials were considered separately by stimuli separation and delay. Ro 25-6981 treatment did not affect the number of selection trials (Fig. 2E), although Ro 25-6981 (10 mg/kg) increased correction trials relative to Veh treatment ($t_{(13)} = 3.66, P < 0.05$). Both doses of Ro 25-6981 increased total trials relative to Veh (Fig. 2G; $F_{(2,30)} = 6.28, P = 0.01$; post hoc $P < 0.05$). Latencies were unaffected by Ro 25-6981 (Fig. 2H).

**Figure 2.** Performance of rats in the TUNL task following Ro 25-6981 (Ro Low = 6 mg/kg or Ro High = 10 mg/kg) or vehicle (Veh) treatment. Ro 25-6981 did not affect accuracy (percent correct) on selection trials (A) regardless of stimuli separation (B) or delay (C). (D) Ro 25-6981 did not affect accuracy when trials were considered separately by stimuli separation and delay. Ro 25-6981 treatment did not affect the number of selection trials (E) but increased correction trials relative to Veh treatment (F). (G) Ro 25-6981 (Ro Low and Ro High) increased total trials relative to Veh treatment. (H) Ro 25-6981 did not affect reward response latency, correct response latency, or incorrect response latency. Values are plotted as mean ± standard error of the mean. Asterisks indicate a significant effect of treatment.

**Figure 3.** Performance of rats in the TUNL task following APS (1 µL of a 30 mM solution) or vehicle (Veh) infusions into mPFC or dmSTR. (A) Accuracy (percent correct) on selection trials was reduced following APS infusions into either site. (B) Accuracy on large and small separations was reduced with APS infusions into mPFC but not dmSTR. (C) APS infusions reduced the number of selection trials regardless of infusion site. (D) APS infused into dmSTR, but not mPFC, increased correction trials relative to Veh treatment. (E) APS treatment in mPFC, but not dmSTR, reduced the number of total trials (selection trials + correction trials). (F) APS did not affect reward latency, correct response latency, or incorrect response latency. (G) Representative infusion sites in the mPFC and dmSTR. Values are plotted as mean ± standard error of the mean. Circles show the locations of the ventral aspect of the guide cannulae and crosses show location of the infusion site. Numbers refer to the anterior–posterior location of plates relative to bregma. (H) A photomicrograph of representative placements from rats included in the present experiment (mPFC: top; dmSTR: bottom). Asterisks indicate a significant effect of treatment.
when dmSTR infusions were performed, although main effects of treatment \((F_{1,13} = 3.67, P = 0.078)\) and the treatment by separation interaction were not significant. Infusions of APS into dmSTR also decreased selection trials (Fig. 3C; \(t_{13} = 2.86, P = 0.01\)) and increased the number of correction trials (Fig. 3D; \(t_{13} = 2.67, P = 0.02\)). Total trials (Fig. 3E) and latencies (Fig. 3F) were unaffected by APS infusions into dmSTR.

The present results show that systemic administration of CPP, but not Ro 25-6981, impairs accuracy on the TUNL task. These results confirm previous findings with MK-801 that TUNL is NMDA receptor dependent (Kumar et al. 2015; Hurtubise et al. 2017). In the present study and Hurtubise et al. (2017), correction trials were increased following NMDA receptor blockade. These results are consistent with other studies showing that broad NMDA receptor antagonists disrupt working memory in a variety of tasks (Li et al. 1997; Doyle et al. 1998; Moghaddam and Adams 1998; Aura and Riekkinen Jr. 1999; MacQueen et al. 2011; Rushforth et al. 2011; Smith et al. 2011; Davies et al. 2013a; Galizio et al. 2013). Interestingly, Ro 25-6981 increased correction trials in the present experiment without significant effects on task accuracy. In Kumar et al. (2015), no effects were found of the GluN2B-containing NMDA receptor antagonist CP 101-606 on the TUNL task, although correction trials were not used. Ro 25-6981 also failed to have effects in other working memory studies using operant delayed-match-to-position tasks (Doyle et al. 1998; Smith et al. 2011). However, systemic blockade of GluN2B-containing NMDA receptors impaired working memory capacity on the odor span task (Davies et al. 2013a) and GluN2B-containing NMDA receptors in monkey prefrontal cortex are important for the persistent neural firing observed during the delay phase of a working memory task (Wang et al. 2013). GluN2B-containing NMDA receptors on adult-born granule cells within the dentate gyrus also contribute to contextual discrimination in similar environments, a form of pattern separation (Kheirbek et al. 2012). In the current experiment, there was not a significant separation by treatment interaction following Ro 25-6981 suggesting that GluN2B subunit-containing NMDA receptors do not contribute to spatial pattern separation in the TUNL task. Kumar et al. (2015) showed similar findings for spatial pattern separation in the TUNL task using CP 101-606.

Local infusions of APS into either mPFC or dmSTR also impaired task accuracy. To the best of our knowledge, these findings are novel. Although the role of the mPFC in TUNL has been described previously (McAllister et al. 2013), the importance of NMDA receptors in mPFC has not been shown. In addition, no manipulations of dmSTR during TUNL have been reported. As the dmSTR receives substantial glutamatergic projections from the prefrontal cortex, it is logical to expect its involvement in the TUNL task. Others have shown that dmSTR is involved in working memory as lesions of the dmSTR impair working memory in a delayed-match-to-sample task (DeCoteau et al. 2004; Kesner and Gilbert 2006) and a t-maze task (Moussa et al. 2011). Smith-Roe et al. (1999) found impaired performance in a working memory task on the radial arm maze when NMDA receptors were blocked within dmSTR. NMDA receptor blockade with APS in the prelimbic cortex or dmSTR also impaired the ability to switch a response choice for an entire trial block in a behavioral flexibility task (Baker and Ragozzo 2014).

The face validity of the TUNL task is high relative to the CANTAB visual spatial working memory task. Human patients with PFC lesions are impaired on the CANTAB spatial working memory task (Chase et al. 2008), and in bipolar patients, enlarged caudate volumes are correlated with poorer performance (Kozicky et al. 2013). Therefore, PFC and striatum appear essential for visual spatial working memory. Our experiments provided back translation, from humans to rodents, and neurotransmitter specificity since we show that NMDA receptors within the mPFC and dmSTR are necessary for TUNL performance. Therefore, increasing the activation of NMDA receptors in the prefrontal cortex and striatum may result in new therapeutics for disorders such as schizophrenia, which are associated with frontal-striatal dysfunction and working memory impairment (Pantelis et al. 1997).

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