The Separate or Concurrent Effects of Methylphenidate and Alcohol on Acquisition and Retention of the Morris Water Maze in Adolescent Rats

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Background & Rationale

- Within the last two decades there has been an increase in Attention Deficit Hyperactivity Disorder (ADHD) diagnoses and, consequently, a rise in psychostimulant prescriptions for methylphenidate (Ritalin) and dextro-amphetamine (Adderall). Due to increased availability, there is a greater likelihood that adolescents recreationally use these compounds and experience pharmacological interactions between prescription stimulants and alcohol (Barrett, Darredeau, & Pihl, 2006; Bezman, Genel, Goldman, & Slanetz, 1998; Zetterqvist, Asherson, Halldner, & Lanstrom, 2012).
- While alcohol's capacity to impair learning and memory has been well documented in the Morris Water Maze (MWM) (Markwiese et al., 1998), few studies have characterized methylphenidate's impact on MWM performance in adolescent rats. However, recent work suggests that methylphenidate (1 mg/kg) does not change spatial learning and memory in the MWM (Zeise et al., 2007).

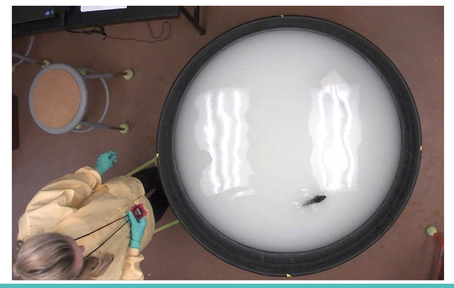

Figure 1: Subject during trial in Morris Water Maze.

Questions

In a rat model of adolescent drug use:

- Does methylphenidate (2 mg/kg) change acquisition and retention of spatial information in the Morris Water Maze?
- Does alcohol (2 g/kg) change acquisition and retention of spatial information in the Morris Water Maze?
- Does concurrently administered methylphenidate (2 mg/kg) and alcohol (2 g/kg) change spatial learning and memory in the Morris Water Maze?

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Methods

- *Subjects* – 32 adolescent (P30-P34) male, Long-Evans hooded rats were used.
- *Drugs* – Eight rats were assigned to one of four separate drug treatment conditions. All animals received two injections (1 cc/kg), the first 50 mins and second 30 mins prior to training. The methylphenidate (MPH) group (2.0 mg/kg, I.P.) was injected with MPH 50 mins prior to training and saline 30 mins prior to training . The ethanol (EtOH) group (2.0 g/kg, I.P.) was injected with EtOH 30 mins prior to training and saline 50 mins prior to training. The saline group received two injections, the first 50 mins and the second 30 mins prior to training.
- *Acquisition MWM (Day 1-6)* – The pool was made opaque using non-toxic, white paint and the platform was submerged in the NE quadrant during each of the six training days. Each training day consisted of four daily swim trials, and rats were released from one of four different start locations (block randomized, N, S, E, W). Maximum trial duration was 60 secs, and rats were allowed to remain on the platform for 10 secs. The experiment was video recorded and scored, allowing analysis of latency and errors. Error scores were calculated by establishing Whishaw Corridors between the start location and platform; an error was recorded when swim paths exited the established corridor.
- *Retention MWM (Day 7)* – The submerged platform was removed from the NE quadrant and a single, 60 sec, drug-free retention test was conducted. Time swam in the NE quadrant was analyzed to asses memory for the distinctive spatial location.

Results

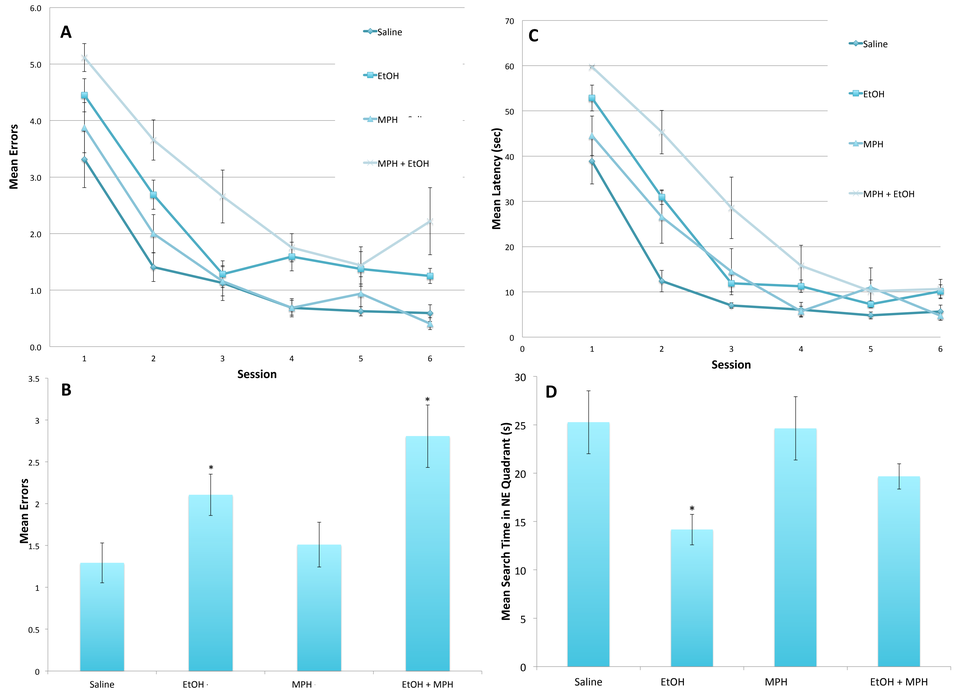

Figure 2. Mean errors and mean latency plotted by day for each drug treatment condition during acquisition (**A** and **C**, respectively). Mean errors for each treatment condition during acquisition depicting the drug main effect (**B**). Mean time swimming in the NE quadrant for each drug treatment condition during the retention test (**D**).

Results continued

Acquisition

Latency (Figure C)

A 4 (saline, MPH, EtOH, MPH+EtOH) x 6 (Day 1-6) mixed, factorial ANOVA was used to evaluate main effects, between groups and repeated measures, and the interaction. Main effects were revealed between drug treatment groups ($F(3, 28) = 9.60$, $p < .001$) and across acquisition days ($F(5, 140) = 146.27$, $p < .001$). A unique effect of drug treatment conditions across days of training was found ($F(15, 140) = 3.57$, $p < .001$). Subsequent post hoc tests showed the MPH+EtOH group performed poorly compared to the other drug treatment conditions on days 1, 2, 3 and 6. Additionally, the EtOH group performed poorly on days 2, 5 and 6 when compared to saline and MPH treated groups. Saline and MPH treated animals performed similarly well.

Errors (Figure A,B)

A 4 (saline, MPH, EtOH, MPH+EtOH) x 6 (Day 1-6) mixed, factorial ANOVA was used as above. Main effects were revealed between drug treatment groups ($F(3, 28) = 19.58$, $p < .001$), across acquisition days ($F(5, 140) = 69.31$, $p < .001$), but no interaction was observed ($F(15, 140) = 1.07$, $p = .39$). Post hoc analyses of the drug treatment main effect revealed that saline and MPH treated groups performed similarly well. However, EtOH treated animals performed more poorly than saline and MPH groups, and the EtOH+MPH group was more impaired than the EtOH group.

Retention

Time Spent in NE Quadrant (Figure D)

A one way ANOVA (saline, MPH, EtOH, MPH + EtOH) revealed a significant difference between groups ($F(3,31) = 4.17$, $p < .05$). Subsequent post hoc tests showed that saline, MPH and MPH+EtOH groups performed similarly well. However, the EtOH treated group was impaired when compared to both saline and MPH groups.

Discussion

While all groups showed significant improvement across acquisition days , unique effects of drug treatment were observed on latency measures, and MPH+EtOH groups performed quite poorly on select acquisition days when compared to all other groups (saline, MPH, EtOH). Additionally, EtOH alone treated animals differed from saline and MPH groups on select training days. Similar outcomes were observed on the error measure, and the MPH+EtOH group was especially impaired in spatial navigation when compared to all other groups. Additionally, EtOH alone impaired spatial navigation when compared to saline and MPH groups. In sum, MPH+EtOH appeared to compromise acquisition of the MWM even more so than EtOH alone. Finally, the retention test showed that the EtOH group was significantly impaired relative to both saline and MPH groups and suggested that EtOH compromised spatial memory processes. While not statistically significant, the EtOH+MPH group curiously appeared to perform better than the EtOH alone group indicating that MPH, combined with EtOH, may attenuate the memory impairment.

Future Directions

- Replicate the experiment with rodent models of ADHD
- Evaluate drug effects on swim speed to assess drug-induced changes in motor performance
- Explore potential state-dependent properties of these compounds in the MWM