Compared to wild type rats, trpc4 knockout rats show reduced cocaine induced impulsivity without effects of cocaine on reversal learning.

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Abstract

Previous research in our lab showed the deletion of the *trpc4* gene produced a brain-wide elimination of TRPC4 channels, including a subpopulation of TRPC4-bearing dopamine neurons in the ventral tegmental area (VTA). That research found the *trpc4* knockout (KO), compared to normal (WT) rats, exhibited reduced cocaine self-administration and reduced cell firing rates in VTA dopamine neurons, with no differences in simple or complex reversal learning [1]. We have found that cocaine administration produces a greater dose-dependent increase in early responding in WT than KO rats. We also report cocaine administration does not differentially affect or disrupt KO or WT rats' performance of a Y-maze reversal task, where running toward the lighted alley is reversed to running to the unlighted alley. However, we found that *d*-amphetamine produced a dose dependent increase in errors for both KO and WT rats. These results replicate the finding that KO and WT rats do not differ in learning a reversal task. Importantly, these results also show that the reduction in cocaine self-administration and increased impulsivity on the DRL task in KO compared to WT rats are specific to those tasks and do not represent a more general disruption of learning or performance. The finding that amphetamine but not cocaine disrupts performance in both KO and WT rats suggests a possible selective role for TRPC4 channels in impulse dependent dopamine pathways. Taken collectively, these findings suggest a potentially important role for the TRPC4 channels in cocaine addiction and dopamine disorders.

Introduction

- The TRPC4 channel is one of the two most abundant TRPC channel subtypes found in the adult mammalian brain, but until recently, its functional and behavioral role was unknown.
- Previous findings indicate that *trpc4* is highly expressed in corticolimbic regions, which receive extensive input from dopamine (DA) neurons in the ventral tegmental area (VTA) and are associated with the brain's reward and emotion circuitry. This expression pattern, along with its ability to regulate neuronal excitability, suggests the possibility that TRPC4 channels are important for learning and memory and in motivated behaviors.
- To identify a function for TRPC4 channels we compared the behavior of rats with a genetic knockout of the *trpc4* gene (*trpc4* KO) to wild-type (WT) controls in two experiments:
  - **Experiment 1**: Past research in our lab revealed *trcp4* KO rats showed diminished self-administration of cocaine compared to WT rats. Here, we sought to examine other behaviors associated with cocaine addiction, such as impulsivity. We tested the effects of acute cocaine administration on early response errors (impulsivity) made by *trpc4* KO and WT rats during a differential reinforcement of low rate (DRL) reinforcement schedule.
  - **Experiment 2:** We also examined the effects of cocaine and *d*-amphetamine on a Y-maze reversal learning task, on which we have shown comparable performance of *trpc4* KO and WT rats in the past.

Experiment 1: Effects of acute cocaine on impulsivity

Methods

- 18 *trpc4* KO and WT Blue Spruce Hooded rats were maintained on 23 hours of water deprivation and shaped to press a lever for 4-sec access to a water dipper. Following three days of continuous reinforcement of lever pressing, the DRL schedule was initiated with gradual increases in "t" seconds for the DRL delay from DRL 3-sec to DRL-14 seconds depending on the rat's performance. Early responses before t-seconds placed the rat in a 1-second blackout of the chamber and reset the t-second DRL clock after the blackout.
- Once the final DRL performance was achieved, the rats were given intraperitoneal injections of saline, or cocaine doses (15, 10, or 5 mg/kg) 15 minutes before the start of a training session. The number of seconds before each error for each rat was compiled into one second bins across each treatment condition. All data was collected using Coulbourn operant chambers.

Experiment 1 Cont.

Results

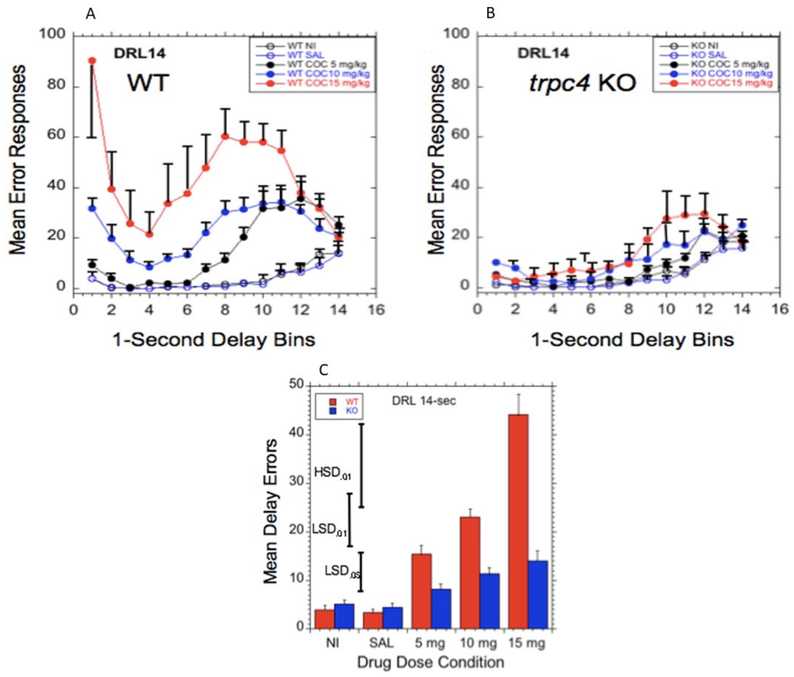
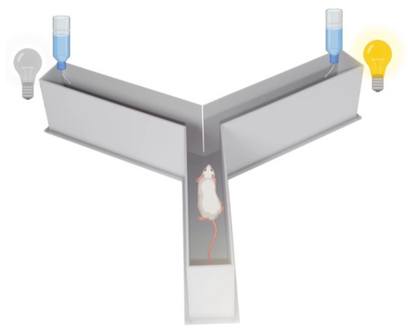


Figure 1: A distribution of mean error responses emitted by five WT rats (A) and five *trpc4* KO rats (B) during DRL 14-sec training sessions during no injection, saline and cocaine (15, 10 and 5 mg/kg i.p.) injections. Panels A and B represent the significant ($p<0.0001$) Dose by Delay interaction at levels of genotype. The errors are shown as the number of seconds before the first error that resets the DRL clock. Note the substantial decrease in the cocaine effect for the *trpc4* KO rats. Panel C shows the significant ($p<0.0001$) Dose by Genotype interaction. Internal error bars represent 1 standard error of the mean, external error bars are for LSD and HSD. These data show a robust dose dependent effect of cocaine on delay errors for WT with only a marginal effect on *trpc4* KO rats.

Experiment 2: Effects of acute cocaine and amphetamine on complex reversal learning

Methods

- 12 *trpc4* KO and WT Blue Spruce Hooded rats were maintained on 23 hours of water deprivation and shaped to respond to a lighted water dipper, and then allowed to run through the Y-maze for 10 daily sessions. After fully entering an alley other than the starting alley, the door closed behind the rat. A correct choice resulted in access to a 4-s activation of the water dipper. An incorrect choice resulted in no water and a 20-s time out.
- Once performance of this task reached 90% for five consecutive days, the task was reversed so running to the unlighted alley was reinforced. When performance on the reversal task reached 90% correct for five days, the rats were given intraperitoneal injections of saline, cocaine (15, 10, or 5 mg/kg), or *d*-amphetamine (3, 2, or 1 mg/kg), 15 minutes before the start of a training session. At least two days of retraining occurred between injections. Both correct and incorrect choices were recorded and used to calculate the percent correct choices for each session. All data was collected using Coulbourn maze systems.



Experiment 2 Cont.

Results

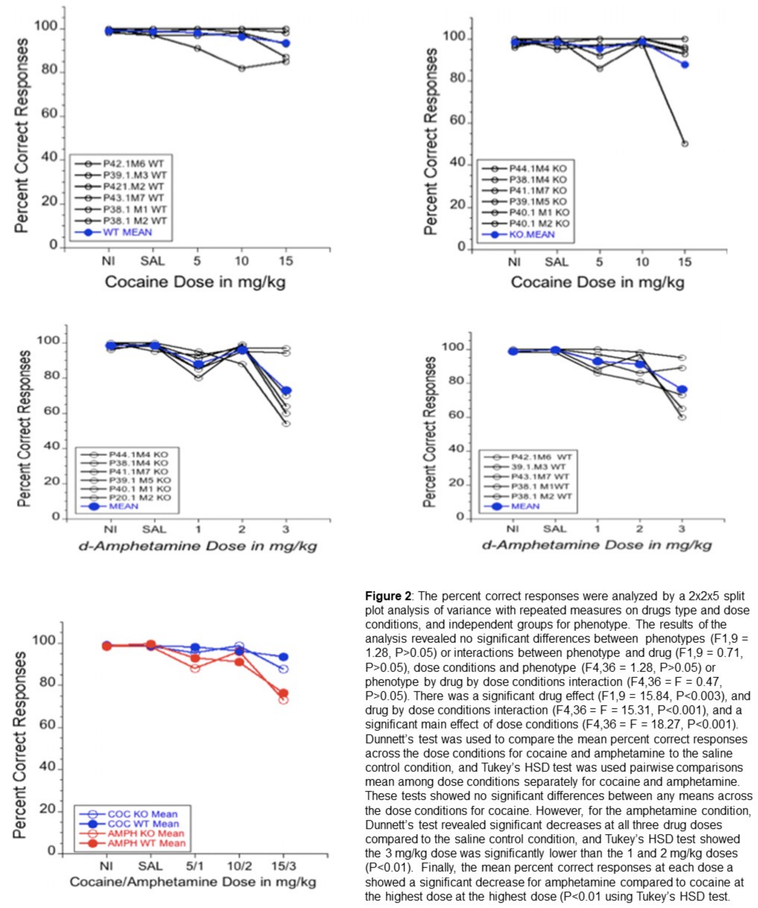


Figure 2: The percent correct responses were analyzed by a 2x2x5 split plot analysis of variance with repeated measures on drugs type and dose conditions, and independent groups for phenotype. The results of the analysis revealed no significant differences between phenotypes ($F1,9 = 1.28$, $P>0.05$) or interactions between phenotype and drug ($F1,9 = 0.71$, $P>0.05$), dose conditions and phenotype ($F4,36 = 1.28$, $P>0.05$) or phenotype by drug by dose conditions interaction ($F4,36 = F = 0.47$, $P>0.05$). There was a significant drug effect ($F1,9 = 15.84$, $P<0.003$), and drug by dose conditions interaction ($F4,36 = F = 15.31$, $P<0.001$), and a significant main effect of dose conditions ($F4,36 = F = 18.27$, $P<0.001$). Dunnett's test was used to compare the mean percent correct responses across the dose conditions for cocaine and amphetamine to the saline control condition, and Tukey's HSD test was used pairwise comparisons mean among dose conditions separately for cocaine and amphetamine. These tests showed no significant differences between any means across the dose conditions for cocaine. However, for the amphetamine condition, Dunnett's test revealed significant decreases at all three drug doses compared to the saline control condition, and Tukey's HSD test showed the 3 mg/kg dose was significantly lower than the 1 and 2 mg/kg doses ($P<0.01$). Finally, the mean percent correct responses at each dose a showed a significant decrease for amphetamine compared to cocaine at the highest dose at the highest dose ($P<0.01$ using Tukey's HSD test.

Conclusion

- In this experiment we found no differences between *trpc4* KO and WT rats in their baseline learning or performance on the DRL 14-sec reinforcement schedules and Y-maze reversal. This is consistent with the extensive evidence reported in earlier experiments that deletion of the *trpc4* gene has no effect on simple or complex learning for natural reinforcers like food and water.
- The finding that Y-maze performance in WT rats was not disrupted by cocaine but was disrupted by *d*-amphetamine is confirmed by this experiment. However, here we saw no differential effects on *trpc4* KO and WT rats, suggesting the effects of cocaine are specific to drug-seeking and impulsivity.
- The finding that cocaine produced significantly greater increases in impulsivity in WT compared to *trpc4* KO rats, is further evidence for a unique role of the TRPC4 channels in cocaine reinforcement and addiction.
- This demonstrates a broader effect of cocaine on TRPC4 channels, controlling behaviors motivated by natural reinforcers, compared to our earlier self-administration findings where cocaine was the reinforcer.
- These data further demonstrate a novel role for the TRPC4 channel regulating the effects of cocaine on behavior and support our premise that functional TRPC4 expression may be an important model for investigating cocaine addiction and a variety of dopamine disorders. Accordingly, the development of novel pharmacological approaches selectively targeting the subpopulation of dopaminergic neurons that contain TRPC4 channels may be an effective way to treat cocaine addiction, without interference with learning and performance of behaviors motivated by natural reinforcers.

Literature Cited

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