Brief Report

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Deletion of the *trpc4* gene and its role in simple and complex strategic learning

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The TRPC4 ion channel is expressed extensively in corticolimbic and a subpopulation of midbrain dopamine neurons. While TRPC4 knockout (KO) rats exhibit reduced sociability and social exploration, little is known about the role of TRPC4 in motivation and learning. To identify a function for TRPC4 channels in learning processes we tested TRPC4 KO and normal wild type (WT) rats. TRPC4 KO and WT rats exhibited no differences in Y-maze learning or simple discrimination learning. Furthermore, on a more complex serial reversal shift task designed to assess strategic learning where the reward and non-reward cues were repeatedly reversed between training sessions both TRPC4 KO and WT rats performed equally well. Finally, we found no performance differences when using a conditional reversal shift task where a tone signals the reversal of reward and non-reward cues within sessions. These data suggest that although TRPC4 channels may play a role in social interaction/anxiety they exert a minimal role in simple and complex strategic learning.

The TRPC4 channel is a nonselective cation channel that is widely expressed in lateral septum, hippocampus and prefrontal cortex (PFC). These areas receive extensive input from dopamine (DA) neurons originating in the ventral tegmental area (VTA)[1]. These DA neurons are involved in the modulation of reward systems and stress. We have reported the TRPC4 protein is localized selectively in a subpopulation of dopamine neurons in the VTA[2]. In a previous experiment we found reduced social interaction in TRPC4 KO compared to wild type (WT) rats[3]. Here we examined the behavioral effects of TRPC4 ion channels by comparing learning in TRPC4 KO and WT rats on simple Y-maze learning and complex strategic learning using serial reversal shift and conditional reversal shift paradigms using natural water reward.

RESULTS

Y-Maze Learning

TRPC4 KO and WT rats were trained to run to the lighted arm of a Y-maze for water reward. The learning curves for both male and female TRPC4 KO and WT rats across ten days of training are presented in **Figure 1**. All groups reached asymptotic performance by the fifth day with no differences between KO and WT rats on any session. Deletion of the TRPC4 protein had no apparent effect on the rate of acquisition or maintenance on this simple learning task**.**

Complex Strategic Learning

For complex learning we chose a serial reversal shift paradigm (SRS) where S+ and S- are repeatedly reversed between blocks of training. Figure 2 shows the gradual acquisition of a multiple VR6 (EXT) lever pressing discrimination task with a light on as S+ and Light off as S- in block 1. By serially reversing S+ and S- the rat learns to transition quickly at the beginning of each block.

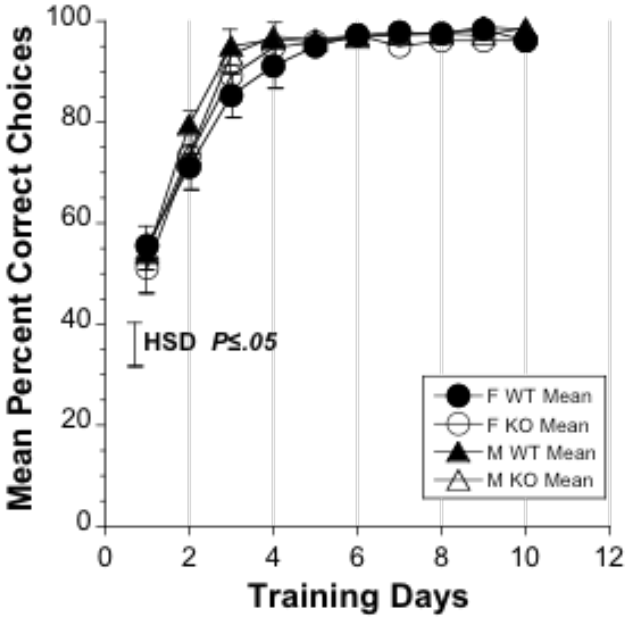

Figure 1: Wild-type and TRPC4 knockout rats show no difference in Y-Maze Discrimination Learning. Rats maintained on 23-hrs water deprivation were shaped to respond to the water dipper, and then allowed to run daily through the Y-maze toward a lighted dipper, with the lighted position randomly alternated across 60 daily trials. A 2x2x12 split plot ANOVA revealed a significant effect of training days ($P < 0.001$), but no significant differences in rate of acquisition or asymptotic performance. While the female rats made more errors (88.5% correct) than male rats (90.6% correct) over the sessions ($P < 0.03$) there were no differences between TRPC4 KO and WT rats ($P > 0.71$) and none of the interactions approached significance. The error bars on the first four days are for Tukey's HSD test, with correction for unequal n, and error rate per training day.

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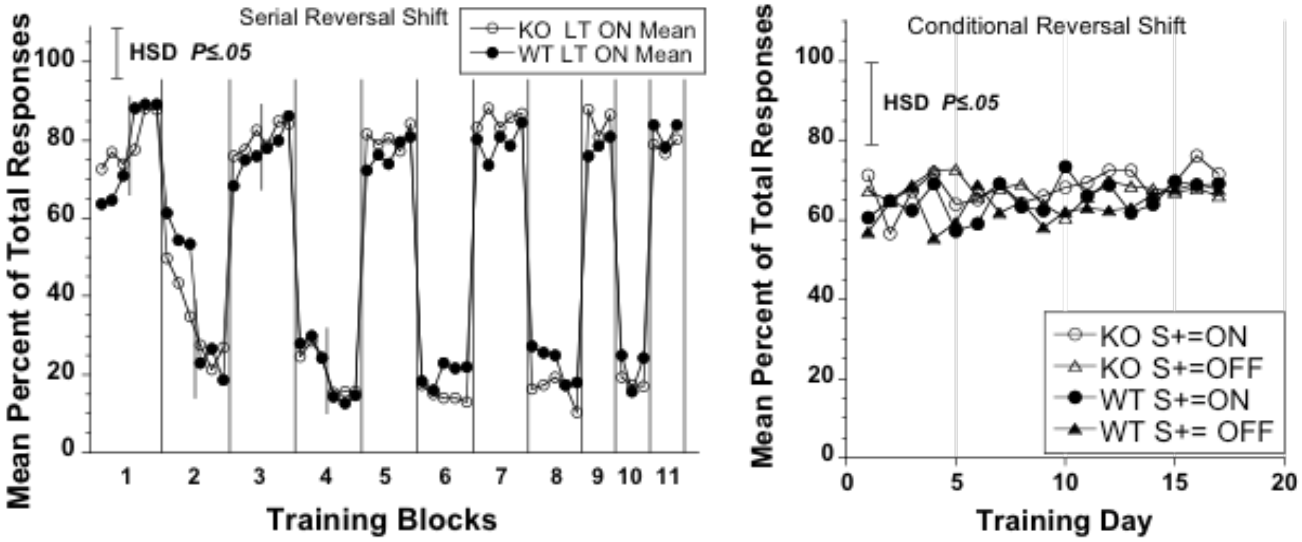

Figure 2: TRPC4 KO and WT rat performance on a serial reversal shift paradigm and conditional reversal shift task.
TRPC4 KO (n=9) and WT (n=12) rats were trained on a simple discrimination task to lever press for water reinforcement (VR-6 schedule) when a light over the lever was on (S+) and not respond (extinction) when the light was off (S-). S+ and S- were repeatedly reversed between sequential blocks. Four 5-day blocks and three 3-day blocks followed four blocks of 14 days. The figure shows the first and last 3 days of each 14 day block and every day for the remaining blocks. Across the blocks both groups acquired the reversal strategy equally well ($P < 0.001$) However, there were no differences between TRPC4 KO and WT rats ($P > 0.90$) or sex ($P > 0.40$) and no significant interactions. On the within session conditional reversal shift (CRS) a 2kHz, 70 dB tone signaled S+ was light on and S- was light off, while the absence of the tone signaled the reversal of S+ and S-. Overall performance was substantially reduced from about 79% correct responses in the serial reversal shift (SRF) to about 68% correct responses in the CRS. There were no significant differences between TRPC4 KO and WT rats during the CRS ($P>0.49$) or sex differences ($P>0.49$) and there were no significant interactions. The error bars are for Tukey's HSD test.

Since we found no differences between KO and WT rats on this task we progressed to a more difficult conditional reversal shift (CRS) task where S+ and S- were reversed contingent upon a tonal cue within session. We found no differences between TRPC4 and KO rats performance on this task.

DISCUSSION

Although the deleted *trpc4* gene is associated with decreased sociability, we found no differences between TRPC4 KO and WT rats in rate of acquisition or asymptotic performance on any paradigm from simple Y-maze learning to complex CRS learning. These data provide an important benchmark for assessing behavioral pharmacological effects in TRPC4 KO and WT rats. Performance differences in response to agonists and antagonists would be attributable to non-associative factors since the TRPC4 KO rats did not display learning or performance deficits in these experiments.

METHODS

The procedures for generating and genotyping the Fischer 344 TRPC4 KO and WT rats and genotyping procedures have been previously reported[3]. The rats were approximately 3 months old at the onset of the experiments and individually housed with 23 hours of water deprivation between sessions that were conducted five days per week. Experiments were conducted in fully automated Coulbourn Y-maze and operant chamber systems. All experiments were approved by the Institutional Animal Care and Use Committee at Drake University. A detailed explaintion of the methods and recordings can be found at http://www.Neuro-Cloud.net/nature-precedings/klipec.

PROGRESS AND COLLABORATIONS

To see up to date progress on this project or if you are interested in contributing to this project visit: http://www.Neuro-Cloud.net/nature-precedings/klipec.

AUTHOR CONTRIBUTIONS

W.D.K. designed the experiments. P.N., B.D., C.W., K.W., J.S., M.C., A.D. AND L.S. conducted the experiments. W.D.K. and D.C.C. wrote the paper.

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