Brief Report

neuroscience

Sociability is decreased following deletion of the *trpc4* gene

Kristin C. Rasmus[1], Jun-Gang Wang[1], Andrew L. Varnell[1], Eric M. Ostertag[2], John P. Christianson[1] & Donald C. Cooper[1]*

Shyness and social anxiety are predominant features of some psychiatric disorders including autism, schizophrenia, anxiety and depression. Understanding the cellular and molecular determinants of sociability may reveal therapeutic approaches to treat individuals with these disorders and improve their quality of life. Previous experiments from our laboratory have identified selective mRNA and protein expression of a nonselective cation channel known as the canonical transient receptor potential channel 4 (TRPC4s) in brain regions implicated in emotional regulation and anxiety. TRPC4 is highly expressed in the corticolimbic regions of the mammalian brain. We hypothesized that robust corticolimbic expression of TRPC4 may regulate the brain's response to emotion and anxiety resulting in changes in social interaction. Here we test *trpc4* gene knockout rats in a model of social anxiety/interaction. We found that the Trpc4 knockout animals spent significantly less time exploring a juvenile intruder rat compared to their wild-type counterparts and Sprague-Dawley (SD) rats. Furthermore, Trpc4 wild-type (Fisher 344) rats explored the juvenile significantly less than the SD rats. These findings indicate that the *trpc4* gene plays a role in modulating cellular excitability in specific regions of the brain associated sociality and/or anxiety.

This study examines the role of the canonical transient receptor potential channel 4 (TRPC4) in regulating social interaction behavior using rats lacking the *trpc4* gene. We have previously found TRPC4 mRNA to be predominantly expressed in brain regions associated with memory, motivation, stress and anxiety[1,2]. Furthermore, TRPCs have been suggested to be important cation channels involved in modulating cellular excitability. To establish a role for TRPC4 channels in social behavior, we knocked out the *trpc4* gene using the Sleeping Beauty transposon gene trap system and measured social interaction. We hypothesized that *trpc4* gene deletion would disrupt social interaction in the knockout rats compared to their wild-type controls. In addition, we examined whether the wild-type (Fisher 344) strain differed from the Sprague-Dawley (SD) strain.

RESULTS

TRPC4 Knock-out Rats

The Sleeping Beauty (SB) gene-trap transposon method was used to create the Trpc4 knock-out animals[4]. The SB method uses cut-and-paste transposable elements to generate heritable loss-of-function mutations. Figure **1a** shows the location of the *trpc4* gene on the rat genome and where the transposon was inserted. By inserting the SB transposon into the first intron of the *trpc4* gene, the full-length protein product is completely eliminated. Using primers for the Trpc4 knock-out and wild-type alleles, we were able to confirm the deletion using PCR and gel electrophoresis (Fig **1b**).

Social Exploration Trials

Social interaction tests were developed 25 years ago and have since been reliable in assessing anxiety-like behaviors in rats and mice[5]. On average, healthy naïve rats will explore for about 80 seconds per 3-minute trial[1]. Results from our social exploration trials (Fig. **2a**) show that Trpc4 knock-out rats exhibited the least amount of exploratory behaviors with a mean exploration time of 54 seconds. This is significantly less than Trpc4 wild-type rats, who explored for 81.63 seconds on average, and SD rats, with a mean exploration time of 93.19 sec. Additionally, the mean exploration time of Trpc4 wild-type rats is significantly less than the SD rats.

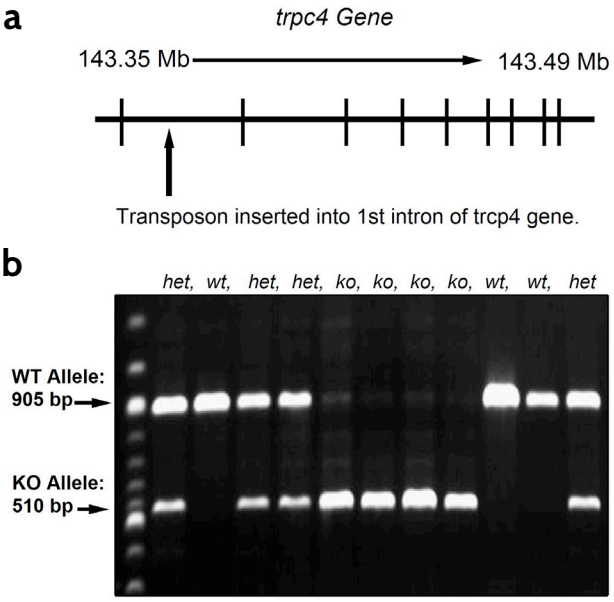

Fig. 1: **a.** Schema of the *trpc4* gene in the rat genome and the Sleeping Beauty (SB) gene knock-out system. The *trpc4* gene is located on chromosome 2 of the rat genome, between 143.35 Mb and 143.49 Mb. The Sleeping Beauty transposon was inserted into the first intron of *trpc4*, therefore creating creating a complete knockout of the full-length protein **b.** Ethidium bromide-stained agarose gel visualizing the 905 bp marker for the WT allele and the 510 bp marker for the *trpc4* KO allele. To genotype the animals, a 1.5% agarose gel electrophoresis was used.

[1] Center for Neuroscience, Department of Neuroscience, University of Colorado at Boulder, Boulder Colorado 80303, USA
[2] Transposagen Biopharmaceuticals, Inc Lexington, KY 40506
*Correspondence should be sent to D.Cooper@Colorado.edu

DISCUSSION

We have identified *trpc4* as a gene implicated in regulating social exploration and/or social anxiety. This is the first evidence showing the involvement of the *trpc4* gene in social interaction and anxiety. The TRPC channels are a group of non-selective cation channels that have been shown to play a pivotal role in intracellular Ca^{2+} regulation, which in turn regulates several critical processes including synaptic plasticity, axonal outgrowth and persistent action potential activity in the cortex[1]. The mammalian TRPC channels are made up of seven members (TRPC1-7); however, we have previously identified TRPC4 and 5 as the two predominant TRPC channels in the brain[3].

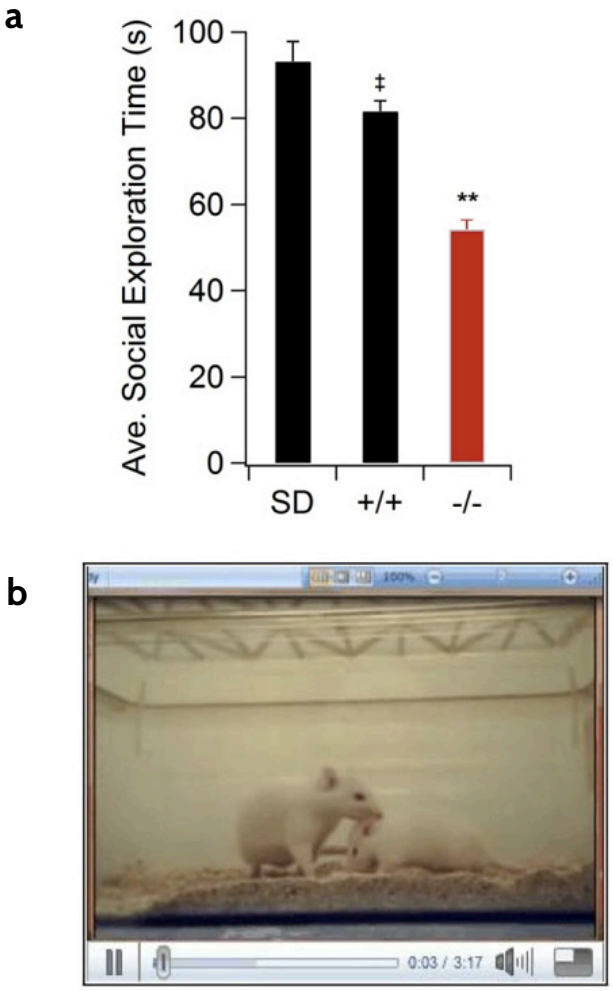

Fig 2: **a.** Bar graph of the mean (+SEM) social exploration time (s) for Sprague-Dawley (SD), Trpc4 wild-type (+/+) and Trpc4 knock-out (-/-) adult rats. Juvenile rats were introduced into the home-cage of the adult rat for 3 minute trials. An observer recorded how much time each adult rat spent exploring the juvenile rat. Exploratory behaviors include: sniffing, grooming, pinning and sparring. The SD rats (n=8) were the most exploratory. The (+/+) rats (n=15) explored significantly less than the SD rats (‡$p<0.05$). The (-/-) rats (n=12) explored significantly less than both the (+/+) and SD rats (**$p<0.001$).
b. Screenshot of an adult Trpc4 KO rat with the juvenile rat stimulus. A video of this social exploration trial can be found on Neuro-Cloud at http://www.Neuro-Cloud.net/nature-precedings/rasmus/

TRPC4 is highly expressed in the corticolimbic system which has been implicated in regulating emotions and social anxiety. Specifically, some of the most concentrated TRPC4 expression is in the lateral septum (LS) and lateral habenula (LHb), which have been implicated in emotional responses, anxiety, stress and social bonding[1]. Multiple studies have shown that there is increased neural activity in the LS in animals exposed to anxiety-provoking situations[1]. The LS receives input from both the amygdala and prefrontal cortex, in addition to strong glutamatergic inputs from the hippocampus, all of which contain moderate to high TRPC4 expression. The LHb neurons project to brain regions that are rich in dopamine including the ventral tegmental area (VTA), which controls reward-related behavior and plays a role in social anxiety and depression[6]. In general, excitation of LHb neurons causes inhibition of dopamine neurons in the VTA in rats. Increased activity in the LHb neurons is observed in the learned helplessness model of depression in which unpredictable and inescapable stress is used to model depression and induce social anxiety.

METHODS

Animals

All adult rats were between 55 and 75 days old at the time of testing. Juveniles were between 28 and 32 days old at the time of testing and were used as stimuli for the social exploration tests. Adult male Sprague-Dawley rats and juvenile male rats of the same strain and source were used in all SD trials. Adult male Trpc4 knock-out rats and their wild-type Fischer 344 littermates and juveniles also of the same strain and source were used in all Trpc4 trials. Trpc4 knock-out rats were generated using the Sleeping Beauty transposon system[4]. Adult rats were housed in groups of two/cage, while juvenile rats were housed in groups of four/cage. All experiments were conducted in accordance with guidelines of the Institutional Animal Care and Use Committees at University of Colorado at Boulder.

For detailed genotyping methods, a video of the socail exploration test and methods describing the social interaction test please see http:// www.Neuro-Cloud.net/nature-precedings/rasmus/.

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/rasmus/

AUTHOR CONTRIBUTIONS

J.C. and D.C.C. designed the experiments. J.G.W. carried out the experiments, and recorded and analyzed the data. K.C.R., A.L.V. and D.C.C. wrote and prepared the manuscript.

ACKNOWLEDGMENTS

This work was supported by National Institute on Drug Abuse grant R01-DA24040 (to D.C.C.), NIDA K award K-01DA017750 (to D.C.C.).

Submitted online to Nature Precedings

1.In Neuro-cloud.net. Retrived Aug 31, 2011 from, http://www.Neuro-Cloud.net/nature-precedings/rasmus/
2. Kessler RC, et al. *Arch of Gen Psych*, **62**(6):617-27. (2005)
3. Fowler MA, et al. *PLoS ONE* **2**(6): e573. doi:10.1371/journal.pone.0000573
4. Geurts AM, et al. *BMC Biotechnol.* **6**:30. (2006)
5. File, SE, Seth, P. *Eur J Pharmacol* **463**:35–53 (2003)
6. Li, Bo, et al. *Nature* **470**; (7335):535-9 (2011)