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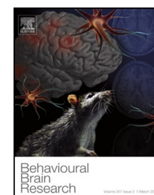

Research report

Loss of the *trpc4* gene is associated with a reduction in cocaine self-administration and reduced spontaneous ventral tegmental area dopamine neuronal activity, without deficits in learning for natural rewards

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HIGHLIGHTS

- We report a brain wide genetic knockout of TRPC4 channels in rats.
- *trpc4* KO and WT rats exhibit no differences in learning and performance for food or water.
- Compared to WT rats, *trpc4* KO rats show reduced cocaine self-administration.
- Within the VTA, TRPC4 channels are selectively expressed in a subset of DA neurons in WT rats.
- Compared to WT controls, *trpc4* KO rats show decreased spontaneous firing rates in VTA DA neurons.

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ABSTRACT

Among the canonical transient receptor potential (TRPC) channels, the TRPC4 non-selective cation channel is one of the most abundantly expressed subtypes within mammalian corticolimbic brain regions, but its functional and behavioral role is unknown. To identify a function for TRPC4 channels we compared the performance of rats with a genetic knockout of the *trpc4* gene (*trpc4* KO) to wild-type (WT) controls on the acquisition of simple and complex learning for natural rewards, and on cocaine self-administration (SA). Despite the abundant distribution of TRPC4 channels through the corticolimbic brain regions, we found *trpc4* KO rats exhibited normal learning in Y-maze and complex reversal shift paradigms. However, a deficit was observed in cocaine SA in the *trpc4* KO group, which infused significantly less cocaine than WT controls despite displaying normal sucrose SA. Given the important role of ventral tegmental area (VTA) dopamine neurons in cocaine SA, we hypothesized that TRPC4 channels may regulate basal dopamine neuron excitability. Double-immunolabeling showed a selective expression of TRPC4 channels in a subpopulation of putative dopamine neurons in the VTA. *Ex vivo* recordings of spontaneous VTA dopamine neuronal activity from acute brain slices revealed fewer cells with high-frequency firing rates in *trpc4* KO rats compared to WT controls. Since deletion of the *trpc4* gene does not impair learning involving natural rewards, but reduces cocaine SA, these data demonstrate a potentially novel role for TRPC4 channels in dopamine systems and may offer a new pharmacological target for more effective treatment of a variety of dopamine disorders.

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1. Introduction

Canonical transient receptor potential (TRPC) channels are a group of non-selective cation channels that have recently gained attention due to their involvement in neuronal excitability. This family of channels consists of 7 members (TRPC1-7) that can be activated in response to Gq protein-coupled receptor activation [1]. Stimulation of Gq protein-coupled receptors activates phospholipase C Beta (PLCβ) producing elevations in inositol triphosphate (IP_3) and intracellular Ca^{2+} [2]. TRPC channels contain three calmodulin sites and an IP_3 site on the C-terminus of each subunit [3,4]. Thus, intracellular signaling resulting from Gq protein-coupled receptors can enhance the activity of TRPC channels [1]. These properties allow TRPC channels to play a pivotal role in responding to intracellular Ca^{2+} signaling, thereby affecting neuronal excitability. TRPC4 channels are colocalized with mGluR1 receptors [5]. Activation of mGluR1 receptors produce prolonged depolarizing potentials in lateral septal neurons and this effect is eliminated in *trpc4* KO mice and rats [5]. Seizure-induced neuronal cell death is greatly reduced in *trpc1/4* double-knockout mice in the pyramidal cell layers of the hippocampus and in the lateral septum suggesting an important role for TRPC4 channels in facilitating neuronal excitability [5].

The TRPC4 channel is one of the two most abundant TRPC channel subtypes found in the adult mammalian brain [6]. Previous findings indicate that trpc4 mRNA is highly expressed in corticolimbic regions including the lateral septum, hippocampus, prefrontal cortex (PFC), and the amygdala [5,6]. These regions 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 interesting possibility that TRPC4 channels are important for learning and memory and in motivated behaviors.

In the present study we used a combination of behavioral, immunohistochemical and electrophysiological approaches to assess the functional role of the TRPC4 channels. To test simple learning with natural rewards, we compared the performance of *trpc4* knockout (*trpc4* KO) and wild-type (WT) rats using a Y-maze discrimination task with water reward and lever pressing acquisition with sucrose reward. To compare *trpc4* KO and WT rats' performance on more complex learning, we used a serial reversal shift paradigm using water reinforcement, where the reward and non-reward cues switched between sessions, and a conditional reversal shift paradigm where a tone cue signaled the switching of reward cues within sessions. To assess the role of TRPC4 channels in modulating cocaine infusion we compared *trpc4* KO and WT rats' performance during a cocaine self-administration (SA) paradigm. Finally, we explored the functioning of TRPC4 channels in the VTA by directly measuring cellular excitability changes in *trpc4* KO compared to WT rats.

2. Materials and methods

2.1. Animals

We used *trpc4* KO and WT Fischer 344 rats (Transposagen, Lexington, KY) in all of the experiments in these studies except the cocaine SA. Fisher 344 *trpc4* KO rats were generated using the Sleeping Beauty transposon system [7]. Because of the potential for strain differences affecting cocaine SA, we used *trpc4* KO and WT rats from both the Fisher 344 strain and a Long Evans (LE) hybrid strain we created. To introduce the knockout to the LE strain, LE WT females were backcrossed with Fisher 344 *trpc4* KO males to generate heterozygous LE x Fisher 344 hybrid breeders (LE hybrid), and then

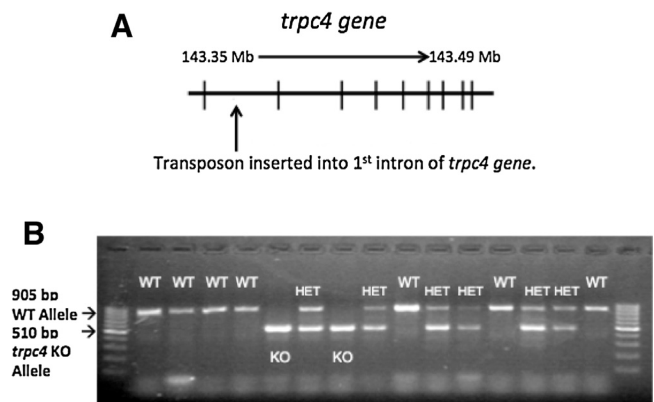

Fig. 1. (A) Schema of the *trpc4* gene in the rat genome and the Sleeping Beauty (SB) gene knock-out system. The *trpc4* gene is located on chromosome 2 of the rat genome, between 143.35 Mb and 143.49 Mb. The Sleeping Beauty transposon was inserted into the first intron of *trpc4*, therefore creating a complete knock-out of the coding sequence. (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.

the F1 generation was crossed back to the LE background for 3 successive generations. In order to maximize the yield of *trpc4* KO and WT rats, the litters were not culled. The rats used in these experiments were at least eight-weeks old (250–300 g), maintained on a 12 h light-darks cycle, and always run during the light cycle, except for the sucrose and cocaine SA that were run in the dark cycle. Although the genotypic ratios were essentially Mendalian across the series of experiments, the proportion of female rats was greater than male rats in the early generations. This was observed with both *trpc4* KO and WT rats and did not appear to be linked to the genetic manipulation of the *trpc4* KO. Litters ranged from 3 to 18 pups with an average litter size of about 8 pups. With many small litters, genotypic variations between litters made forming *trpc4* KO and WT littermate pairs difficult. For each experiment, rats were sampled from all available litters, with no more than two male or female rats of the same genotype sampled from a single litter. All experiments were conducted in accordance with guidelines of the Institutional Animal Care and Use Committees at Drake University, the University of Colorado at Boulder, and the University of St. Thomas.

2.2. Animal genotyping and quantitative PCR

Three polymerase chain reaction (PCR) primers were designed and used as 20–24 oligonucleotide sequences (Eurofins MWG Operon, Ebersberg, Germany) to genotype rats. Reactions were carried out using Choice Taq Blue DNA polymerase (Denville Scientific Inc., Metuchen, NJ) in either a Techne Touchgene thermal cycler (Techne, Minneapolis, MN) or a BioRad C1000 thermal cycler. Ethidium bromide-stained agarose gels were photographed with a Kodak Gel Logic 200 UV transilluminator imager (Carestream Health Inc., Rochester, NY).

2.3. *In situ hybridization and emulsion autoradiography*

In situ hybridization (ISH) and emulsion autoradiography experiments were carried out by Cytochem Inc. (Montreal, QC Canada). S^{35}-labeled cRNA antisense and sense probes were freshly prepared. Rat sections were hybridized overnight at 55 °C in 50% deionized formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, 10 nM $NaPO_4$, 10% dextran sulfate, 1× Denhardt's, 50 μg/ml total yeast RNA, and 50–80,000 cpm/μl S^{35}-labeled probes. The tissues were then subjected to stringent washing at 65 °C in 50%

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