



DATA ARTICLE

Cocaine self-administration in mice with forebrain knock-down of *trpc5* ion channels [v1; ref status: indexed, <http://f1000r.es/pb>]

Matthew B Pomrenze¹, Michael V Baratta¹, Kristin C Rasmus¹, Brian A Cadle¹, Shinya Nakamura¹, Lutz Birnbaumer², Donald C Cooper¹

¹Institute for Behavioral Genetics, University of Colorado, Boulder, CO, 80309, USA

²National Institute of Environmental Health Science, National Institute of Health, Research Triangle Park, NC, 27709, USA

v1 **First Published:** 15 Feb 2013, 2:53 (doi: 10.12688/f1000research.2-53.v1)
Latest Published: 15 Feb 2013, 2:53 (doi: 10.12688/f1000research.2-53.v1)

Abstract

Canonical transient receptor potential (TRPC) channels are a family of non-selective cation channels that play a crucial role in modulating neuronal excitability due to their involvement in intracellular Ca²⁺ regulation and dendritic growth. TRPC5 channels a) are one of the two most prevalent TRPC channels in the adult rodent brain; b) are densely expressed in deep layer pyramidal neurons of the prefrontal cortex (PFC); and c) modulate neuronal persistent activity necessary for working memory and attention. In order to evaluate the causal role of TRPC5 in motivation/reward-related behaviors, conditional forebrain TRPC5 knock-down (*trpc5*-KD) mice were generated and trained to nose-poke for intravenous cocaine. Here we present a data set containing the first 6 days of saline or cocaine self-administration in wild type (WT) and *trpc5*-KD mice. In addition, we also present a data set showing the dose-response to cocaine after both groups had achieved similar levels of cocaine self-administration. Compared to WT mice, *trpc5*-KD mice exhibited an apparent increase in self-administration on the first day of cocaine testing without prior operant training. There were no apparent differences between WT and *trpc5*-KD mice for saline responding on the first day of training. Both groups showed similar dose-response sensitivity to cocaine after several days of achieving similar levels of cocaine intake.

Article Status Summary

Referee Responses

Referees	1	2
v1 published 15 Feb 2013	 report 1	 report

1 Simon Barak Caine, McLean Hospital
USA

2 Micky Marinelli, Rosalind Franklin
University of Medicine and Science USA

Latest Comments

No Comments Yet

Corresponding author: Donald C Cooper (d.cooper@colorado.edu)

How to cite this article: Pomrenze MB, Baratta MV, Rasmus KC *et al.* (2013) Cocaine self-administration in mice with forebrain knock-down of *trpc5* ion channels [v1; ref status: indexed, <http://f1000r.es/pb>] *F1000Research* 2013, 2:53 (doi: 10.12688/f1000research.2-53.v1)

Copyright: © 2013 Pomrenze MB *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

Grant information: This work was supported by R01 DA24040 (DCC); T32 DA017637 (MVB); Z01 ES101684 (LB).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: No competing interests were disclosed.

First Published: 15 Feb 2013, 2:53 (doi: 10.12688/f1000research.2-53.v1)

First Indexed: 07 Mar 2013, 2:53 (doi: 10.12688/f1000research.2-53.v1)

Introduction

The prefrontal cortex (PFC) supports higher order cognitive functions, such as decision-making, reasoning, and working memory¹. PFC functioning is impaired in cocaine addicts, which is manifest in their inability to make proper decisions when presented with challenges in their environment^{2,3}. Genetic and environmental factors can influence PFC excitability⁴⁻⁶, and repeated cocaine alters the excitability of the PFC by biasing neurons towards strong inputs, such as those associated with drug cues, which may diminish cognitive function⁷. Understanding the mechanism underlying how PFC excitability influences the behavioral responses to psychostimulants is fundamental to learning how to reverse these maladaptive alterations in order to treat addiction.

Canonical transient receptor potential (TRPC) channels are a family of non-selective cation channels that play a crucial role in modulating neuronal excitability due to their involvement in intracellular Ca²⁺ regulation⁸. The TRPC5 isoform has been shown to play a role in dendritic growth and arborization through CaMKII-mediated mechanisms throughout the brain^{9,10}, as well as the expression of fear conditioning in the amygdala¹¹. TRPC5 channels a) are one of the two most prevalent TRPC channels in the adult rodent brain¹²; b) are densely expressed in deep layer pyramidal neurons of the PFC¹²; and c) modulate neuronal persistent activity necessary for working memory and attention¹³.

Since deep-layer pyramidal neurons of the PFC are known to project to limbic structures that subservise reward, such as the ventral tegmental area, nucleus accumbens, amygdala, laterodorsal tegmentum¹⁴, and the rostromedial tegmental nucleus¹⁵, TRPC5 channels may influence the ability of cortical networks to exert inhibitory control over these structures. Consequently, motivational and drug reward-seeking behaviors may be affected. In the present data sets, we gathered data from mice that lack functional TRPC5 channels in their forebrain CaMKII-expressing pyramidal neurons to measure their cocaine self-administration behavior as an index of cocaine reward.

Materials and methods

Subjects

19 adult (25–30 g) male C3H mice were group-housed until surgery. Mice were maintained in a reverse 12 hr light:dark cycle (lights off at 7:00 am) with access to food and water *ad libitum*. Using the cre-lox system, forebrain specific knock-down of *trpc5* was achieved by crossing floxed *trpc5* mice with mice that express Cre recombinase under the control of the α CaMKII promoter, where Cre transgene expression was restricted to excitatory neurons in the forebrain. Breeding pairs of the floxed *trpc5* mice were initially obtained from Dr. Lutz Birnbaumer at the NIEHS and bred at the Institute for Behavioral Genetics. CaMKII-Cre mice were obtained from UTSW Medical Center, Dallas. All procedures were approved by the Institute for Animal Care and Use Committee of the University of Colorado Boulder.

Surgery

Prior to behavioral experimentation, mice were anaesthetized with a cocktail of 80 mg/mL ketamine and 6 mg/mL xylazine (Sigma Aldrich) and implanted with intravenous catheters as previously described¹⁶. Chronically implanted custom catheters consist of Silastic tubing that is affixed to 23-gauge steel tubing bent at a right

angle and inserted into a plastic hypodermic needle hub bound to a circular polyurethane backmount and surgical mesh. Catheters were steam autoclaved and rinsed with 70% ethanol prior to surgery. The skin area on the back and above the jugular vein were shaved and prepared with Betadine scrub, 70% ethanol, and 1% Zephiran to prevent infection. Following incisions and exposure of the right external jugular vein, catheter tubing was channeled subcutaneously from the back out the chest above the exposed vein. Catheter tubing was inserted approximately 7 mm into the vein and secured to the vein and surrounding tissue with sterile suture. Following successful insertion and jugular attachment, the incisions were sutured, stapled, and fixed with Vetbond (3M). The neck of the needle hub contains the 23-gauge tubing that remains capped when animals are not connected to the intravenous self-administration apparatus. Catheters were flushed with heparinized pyrogen-free sterile physiological saline *daily* to detect resistance to flow and patency. If animals' nose-poking behavior deviated by >20% of mean responding, catheter integrity and access to the jugular vein was examined using 10 mg/kg Sodium Brevital. If animals did not exhibit sedation within 3 seconds they were omitted from the study. Requests for the customized mouse catheter system used in this study should be directed to <http://neuro-cloud.net/nature-precedings/pomrenze>¹⁶.

Self-administration

Seven days following catheter implantation mice (Saline - *trpc5*-KD ($n = 8$), *trpc5*-WT ($n = 8$); Cocaine - *trpc5*-KD ($n = 9$), *trpc5*-WT ($n = 10$)) were individually housed in self-administration operant chambers that contain two identical nose-poke portals (active and inactive). For acquisition and maintenance of cocaine (unit dose = 0.75 mg/kg/infusion; compounded in pyrogen-free sterile physiological saline; NIDA) self-administration, mice received continuous reinforcement (fixed-ratio 1) of cocaine paired with a 10-second LED illumination and 10-second time-out following a nose-poke into the "active" portal. Inactive portals yielded no consequence. All studies were done without prior operant training. Infusions of 50 μ L were delivered over a 4-second time period. For the data set presented mice were exposed to a 3-hr saline self-administration pretest on the first day. Subsequent cocaine daily 3-hr sessions continued until stable responding (> 20 infusions, < 20% variability in number of infusions across three daily sessions, > 70% discriminative responding in "active" portal vs "inactive" portal) was achieved in both groups. All genotypes were blind to the investigators.

Dose-response

After acquisition of cocaine self-administration and stable maintenance for ≥ 6 days, mice (*trpc5*-KD ($n = 9$), *trpc5*-WT ($n = 10$)) were challenged with a dose-response schedule of varying unit doses (0.05, 0.1, 0.75, and 2.0 mg/kg/infusion) of cocaine (one unit dose per session). The mean number of cocaine infusions at each unit dose was determined during two separate consecutive sessions.

Results

All mice were exposed to the self-administration chambers to nose-poke for light and saline on their first day of training to establish their baseline levels of exploratory behavior between treatment groups. Cocaine replaced saline thereafter and mice were trained to self-administer cocaine (0.75 mg/kg/infusion) without prior operant training for natural rewards (food pellets). Mice acquired cocaine

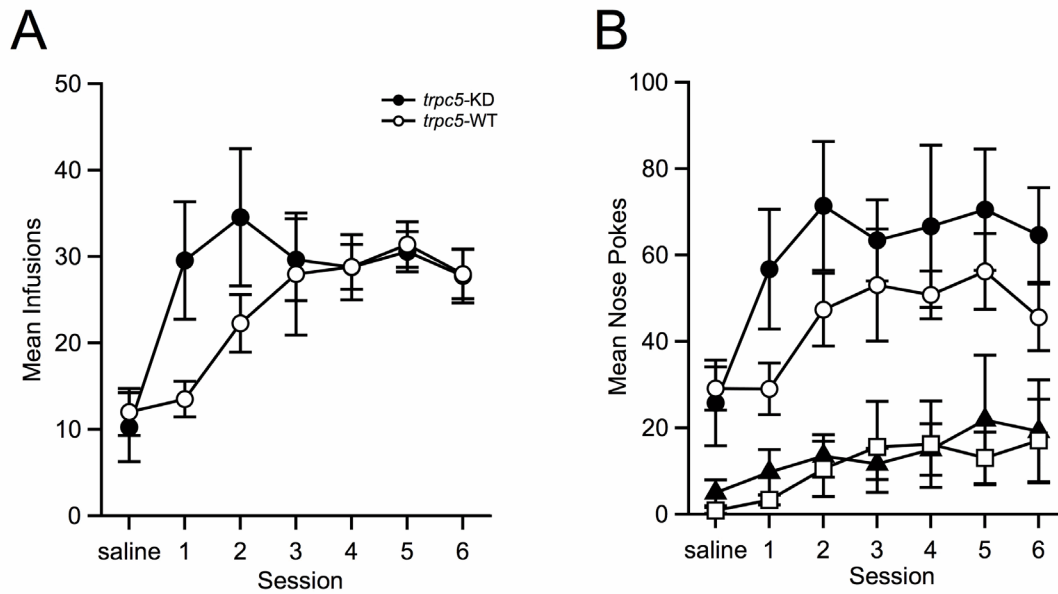


Figure 1. A. Mean (\pm SEM) number of saline (1 session) and cocaine (6 sessions) infusions. Saline responding is similar between genotypes, yet *trpc5*-KD mice exhibit an increased responding on day 1 for cocaine. Saline-*trpc5*-KD ($n = 8$) versus Saline-*trpc5*-WT ($n = 8$), Cocaine-*trpc5*-KD ($n = 9$) versus Cocaine-*trpc5*-WT ($n = 10$). **B.** Mean (\pm SEM) number of saline (1 session) and cocaine (6 sessions) nose-poking in active (rewarding) and inactive (non-reinforced) portals. Nose-poking for saline is similar between genotypes, yet *trpc5*-KD poking is significantly elevated on day 1 of responding for cocaine and continues throughout the sessions. Black circles represent *trpc5*-KD active poking, open circles represent *trpc5*-WT active poking, triangles represent *trpc5*-KD inactive poking, squares represent *trpc5*-WT inactive poking.

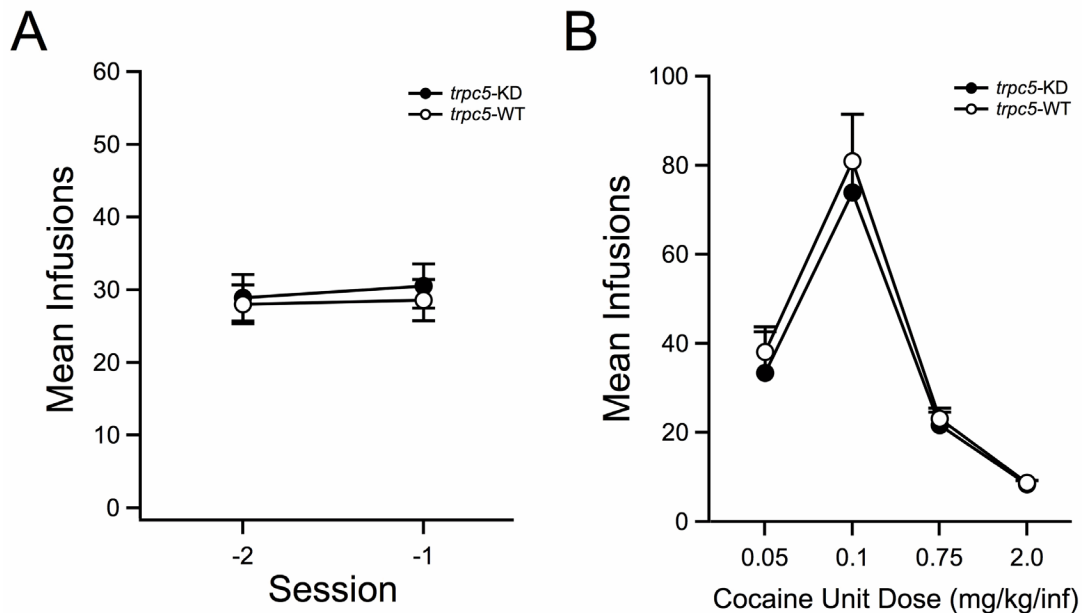


Figure 2. A. Mean (\pm SEM) number of infusions for the last two self-administration sessions prior to the dose-response challenge are similar between *trpc5*-KD and WT mice. Cocaine-*trpc5*-KD ($n = 9$) versus Cocaine-*trpc5*-WT ($n = 10$). **B.** Mean (\pm SEM) number of infusions for 2 sessions at each indicated cocaine dose. Dose-response curves reveal similar responding to varying unit doses of cocaine between genotypes.

self-administration by meeting our stated criteria and subsequently exhibited stable self-administration maintenance for ≥ 6 days. WT and *trpc5*-KD mice demonstrated identical cocaine responding during the maintenance phase. The *trpc5*-KD mice reached the criteria by the first session and continued stable responding for the duration of the experiments, whereas WT took several days to catch up to KD responding. WT and *trpc5*-KD mice exhibited similar responding for saline, yet infusions earned by *trpc5*-KD surpassed WT for cocaine on the first session (Figure 1a). The mouse *trpc5*-KD nose-poking showed a similar pattern on the first session, surpassing WT nose-pokes (Figure 1b).

After the maintenance phase of self-administration training, separate groups of mice were taken through a dose-response. The sessions leading up to the dose response tests demonstrate similar responding between genotypes (Figure 2a). Dose-response functions demonstrated no difference between genotypes (Figure 2b).

Number of infusions and active/inactive nose-pokes for saline or first 6 days of cocaine

1 Data File

<http://dx.doi.org/10.6084/m9.figshare.156843>

Cocaine infusions earned before and during the dose-response challenge

1 Data File

<http://dx.doi.org/10.6084/m9.figshare.156844>

Author contributions

MBP, MVB, and DCC designed the experiments, summarized the data, and wrote the manuscript. KCR conducted the mouse genotyping. BAC constructed the customized self-administration system and contributed to the conceptual design. SN programmed the custom Labview scripts for self-administration and contributed to the conceptual design and contributed to the revision of the manuscript. MBP and MVB performed the experiments and contributed to the conceptual design and contributed to the revision of the manuscript. LB designed and generated the floxed *trpc5* mice and contributed to the conceptual design and contributed to the revision of the manuscript. DCC revised the manuscript for critical intellectual content. All authors approve this manuscript for publication.

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by R01 DA24040 (DCC); T32 DA017637 (MVB); Z01 ES101684 (LB).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

The authors would like to thank Dr. Melissa Fowler for her contributions to the TRPC5 project, including the characterization of TRPC5 expression in the rodent brain and the development of mRNA probes. We also thank Sam Dolzani for insightful commentary and suggestions for surgical and behavioral procedures.

References

- Kesner RP, Churchwell JC: **An analysis of rat prefrontal cortex in mediating executive function.** *Neurobiol Learn Mem.* 2011; **96**(3): 417–31.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Trantham H, Szumlanski KK, McFarland K, *et al.*: **Repeated cocaine administration alters the electrophysiological properties of prefrontal cortical neurons.** *Neuroscience.* 2002; **113**(4): 749–53.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Goldstein RZ, Volkow ND: **Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex.** *Am J Psychiatry.* 2002; **159**(10): 1642–52.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Uhl GR, Drgon T, Jhonson C, *et al.*: **Molecular genetics of addiction and related heritable phenotypes: genome-wide association approaches identify “connectivity constellation” and drug target genes with pleiotropic effects.** *Ann N Y Acad Sci.* 2008; **1141**: 318–81.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Haile CN, Kosten TR, Kosten TA: **Genetics of dopamine and its contribution to cocaine addiction.** *Behav Genet.* 2007; **37**(1): 119–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Niwa M, Yan Y, Nabeshima T: **Genes and molecules that can potentiate or attenuate psychostimulant dependence: relevance of data from animal models to human addiction.** *Ann N Y Acad Sci.* 2008; **1141**: 76–95.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kalivas PW, Volkow N, Seamans J: **Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission.** *Neuron.* 2005; **45**(5): 647–50.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Montell C: **The TRP superfamily of cation channels.** *Sci STKE.* 2005; (272): re3.
[PubMed Abstract](#) | [Publisher Full Text](#)
- He Z, Jia C, Feng S, *et al.*: **TRPC5 channel is the mediator of neurotrophin-3 in regulating dendritic growth via CamKII α in rat hippocampal neurons.** *J Neurosci.* 2012; **32**(27): 9383–95.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Puram SV, Riccio A, Koirala S, *et al.*: **A TRPC5-regulated calcium signaling pathway controls dendrite patterning in the mammalian brain.** *Genes Dev.* 2011; **25**(24): 2659–73.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Riccio A, Li Y, Moon J, *et al.*: **Essential role for TRPC5 in amygdala function and fear-related behavior.** *Cell.* 2009; **137**(4): 761–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Fowler MA, Sidiropoulou K, Ozkan ED, *et al.*: **Cortic limbic expression of TRPC4 and TRPC5 channels in the rodent brain.** *PLoS One.* 2007; **2**(6): e573.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sidiropoulou K, Lu FM, Fowler MA, *et al.*: **Dopamine modulates an mGluR5-mediated depolarization underlying prefrontal persistent activity.** *Nat Neurosci.* 2009; **12**(2): 190–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sesack SR, Carr DB, Omelchenko N, *et al.*: **Anatomical substrates for glutamate-dopamine interactions: Evidence for specificity of connections and extrasynaptic actions.** *Ann N Y Acad Sci.* 2003; **1003**: 36–52.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Barrot M, Sesack SR, Georges F, *et al.*: **Braking dopamine systems: A new GABA master structure for mesolimbic and nigrostriatal functions.** *J Neurosci.* 2012; **32**(41): 14094–101.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Pomrenze MB, Baratta MV, Cadle BA, *et al.*: **Cocaine self-administration in the mouse: A low-cost, chronic catheter preparation.** *Nature Precedings.* 2012.
[Publisher Full Text](#)

Current Referee Status:



Referee Responses for Version 1



Micky Marinelli

Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

Approved: 07 March 2013

Referee Report: 07 March 2013

This is an interesting study, showing the role of TRPC5 channels in the forebrain, on cocaine self-administration. The experiments are described well. The dose-response curve is convincing, as it shows the characteristic inverted-U-shaped curve. In line with the journal's guidelines, no statistical results and/or interpretations are provided for any result. However, this makes it difficult to assess the reliability of data, so my comments address this concern.

An analysis of drug intake using repeated measures ANOVA with genotype as between-subject factor and days (cocaine 1-6) as the repeated measure does not yield any significant effects (effect of genotype, main effect of days, or interaction genotype x days). Only a t test on day 1 yields a significant genotype effect. I therefore understand that the authors refer to an "apparent" initial increase in drug intake (abstract). It would be important to replicate this finding in the future, to ascertain that it is a true result, rather than variability in initial intake across mice.

An analysis of active/inactive hole discrimination using ANOVA with genotype as between-subject factor and days (cocaine 1-6) and hole (active/inactive) as the repeated measure yields a trend for an interaction genotype x hole ($p=0.068$). It therefore appears that the -/- might have better discrimination. This could be reported. The authors could also consider if there is insufficient power for this difference to be significant.

The animals were trained first with saline. Could this have influenced their subsequent intake, and could the initial difference in intake (day 1 of cocaine) be the consequence of differences in the ability to switch across genotypes?

From the data, it appears that not all mice were tested for initial saline intake. Could this have influenced the results?

The authors should describe (methods) how they ran the dose-response curve (descending doses? Random doses? Etc...)

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.



Simon Barak Caine

Alcohol & Drug Abuse Research Center, McLean Hospital, Belmont, MA, USA

Approved: 22 February 2013

Referee Report: 22 February 2013

A nicely conducted study. Note three important reservations regarding interpretation and conclusions:

- Acquisition studies are notorious for variability, and differences between genotypes were observed only on Day 1 of acquisition and throughout the large majority of the study were identical for the two genotypes;
- A slight hyperactivity especially under reinforced conditions is a fairly common observation with a variety of manipulations, and nose pokes in Figure 1B are suggestive of such a phenomenon (i.e., higher nose pokes during timeouts as well as during reinforcer availability in the active nose poke hole for KD mice than for WT);
- There are no data addressing whether similar results would be obtained with other reinforcers, such as food.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

1 Comment

Author Response

Donald Cooper, University of Colorado, Boulder, USA

Posted: 27 Feb 2013

We thank Dr. Caine for his helpful critique of our Data article. One goal we had for publishing this Data article in F1000Research was to quickly share some of our ongoing behavioral datasets in order to encourage collaboration with others in the field. Our ultimate goal is to publish these datasets together with more complete analysis and conclusions as well as other work from our lab or new collaborators. Because we intend on publishing these datasets with additional data and more complete analyses and conclusions we kept the analyses and conclusions here to a minimum.

Reviewer Concern: Acquisition studies are notorious for variability.

We certainly agree. To address this issue we took the following approach.

1. The study was conducted with the experimenter blind to the genotype.
2. We reported 9 knock-down and 10 wild-type male age-matched and litter-mate controls that made it through our lengthy testing procedure including dose-response and progressive ratio test (not reported). We have done further analysis on mice that self-administered on Day 1 of cocaine acquisition but whose catheters failed during the next 3 weeks of testing. This brought our numbers up to 12 knock-down and 12 wild-type animals. The Day 1 cocaine means are now $trpc5$ KD = 27.75 with a standard deviation of 5.3 and wild-type=14.1 with a standard deviation of 2.0. This gives an effect size of 3.39 and a statistical power estimate at 1.0. For behavioral studies a

statistical power greater than 0.8 is considered good. So we are confident that there is a difference between groups on Day 1 of cocaine acquisition that is not due to chance alone although ANOVA and posthoc tests are needed and will be presented in a future complete research article.

Reviewer Concern: The issue of hyperactivity to a reinforcer

Nose poking for light in mice is a behavior that is easy to learn because of the naturally reinforcing property of light activation triggered by a beam break in the nose poke hole. To address the issue of general hyperactivity:

1. We reported nose poke responding for light and found no differences between genotypes on Day 1 of acquisition (Figure 1). Active nose pokes were higher than inactive nose pokes indicating discrimination in both groups.
2. There were no differences between genotypes in the inactive nose poke port on Day 1 of cocaine acquisition, a general measure of exploratory activity.
3. Other studies in the laboratory have looked at locomotor activation to i.p injections of a locomotor activating dose of cocaine at 10 mg/kg and found no differences between genotypes (data not shown). In addition, a subthreshold dose of 5 mg/kg cocaine or saline also show no differences between genotypes (data not shown). This suggest no group differences in cocaine-induced locomotor activation in response to an initial exposure to cocaine.
4. The 4 second duration cocaine infusion time and the 10 second time out likely explains the increase in active nose pokes compared to the number of infusions. However, the ratio of active pokes to infusions was only about 2. Both groups had similar (1.5 -2 active pokes/infusion) differences at steady-state responding (days 3-6) once acquisition criteria was reached. Future analysis will include a within session analysis of nose pokes to determine if the trpc5 KD mice showed differential nose-poke behavior during the 3 hour session (e.g. more nose pokes/infusion during the first part or last part of the session). Furthermore, we plan to examine the ratio of active pokes to infusions across the entire dose response to see if the ratio is dose-dependent. We will include this data set in a revision of the current Data Article.

Reviewer Concern: Would we observe similar results with other reinforcers?

Although we tested light as an alternative reinforcer it is a very good question whether other consumable natural reinforcers like sucrose would be similar to cocaine on Day 1. These experiments are underway and will be reported with expanded analysis in a future Research Article together with cocaine conditioned place preference, progressive ratio responding and locomotor activity. We welcome any new suggestions from other reviewers or the community at large!

Competing Interests: No competing interests were disclosed.