Deletion of the testosterone-sensitive TRPM8 Ca++ channel disrupts measures of anxiety and depression in mice: Implications for dopamine

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Background

- Sex differences are a signature feature of anxiety, depression and substance use, yet the identification of neurobiological mechanism to account for this dimorphism has not been meaningfully identified (Altemus et al., 2014; Bangasser & Cuarenta, 2021; Santos-Toscano et al., 2023).
- The recent finding that the Transient Receptor Potential Melastatin-8 (TRPM8) cation channel is a high affinity target for testosterone suggests a mechanism by which androgens can rapidly influence central nervous system activity (Asuthkar et al., 2015).
- In addition to its well-established role in peripheral cold sensation (Bautista et al., 2007), TRPM8 is highly expressed in the central nervous system, including the hypothalamus, and limbic and cortical regions relevant to motivated behavior.
- Male mice lacking the TRPM8 gene exhibit disrupted sexual behavior, including increased mounting, indiscriminate approach, and a prolonged latency to satiety. These behaviors were linked to altered dopamine (DA) neuron activity in the ventral tegmental area (Mohandass et al., 2020). DA exerts an essential role in a variety of motivated behaviors, including response to novelty and reinforcement learning, and is critically implicated in substance use disorders and depression.

Hypothesis

It is possible that the effects of TRPM8 deletion on sexual reward will translate to more global reward processes. Therefore, we subjected TRPM8 null mutant mice to a battery of tests that are sensitive to DA and/or are established rodent models of anxiety, depression, and substance abuse. We hypothesize that male mice lacking the TRPM8 gene will exhibit impairment on these measures compared to wildtype mice.

Methods

Animals: Female and male C57BL/6J WT and TRPM8$^{-/-}$ (#008198) mice (12 weeks of age) were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were group housed by sex at the University of Illinois College of Medicine in Peoria with their conspecifics on a 12:12 light/dark cycle under constant temperature and humidity and with food and water available *ad libitum.*

Behavioral Measures

Locomotion in Activity Frame (AF): Mice were individually housed in AF for 48 h. with food and water access *ad libitum* to assess spontaneous activity. Locomotion was measured by infrared beam breaks using SmartFrame hardware and MotorMonitor software (Kinder Scientific, Chula Vista, CA).

Sucrose 2-Bottle Preference: To assess sensitivity to a natural reward, mice were subjected to a two bottle preference test: tap water or sucrose solution. Bottles were weighed every 24 h. to determine liquid consumption, and bottle placements were alternated daily to avoid place preference. Each sucrose concentration (.1%, .5%, 1%) was available for 7 days beginning with the lowest concentration. A Preference Index was calculated as the average sucrose consumption across the last 2-day period divided by the total volume consumed.

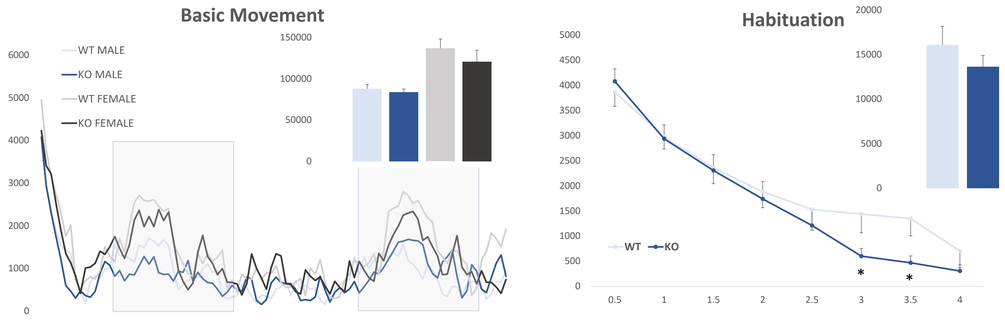
Light/Dark Box: To assess the response to an angiogenic stimulus, mice were placed in the center of light/dark chamber (91.5x46x47 cm) with freedom to explore either side for 10 min. The number of center crosses and time spent on either light or dark sides were recorded.

Forced Swim: To assess behavioral despair, mice were placed in a circular glass chamber (20x 27.5cm) with water 7 cm high at 23-25°C for 6 min. Latency to immobility and total active swim time from min 2-6 were recorded.

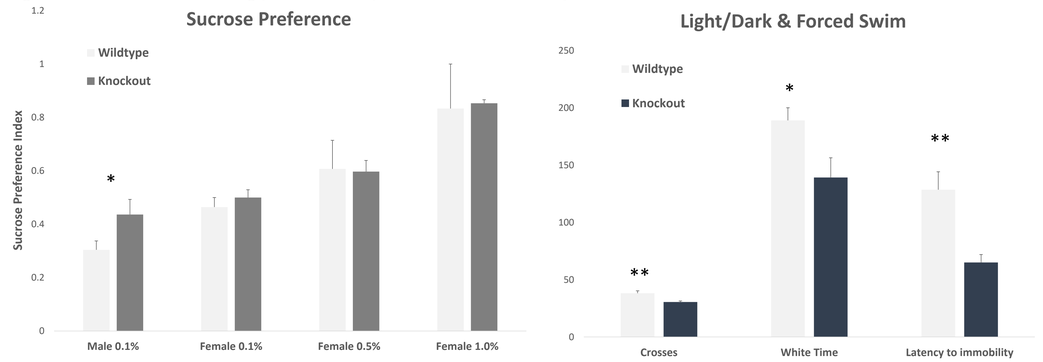
Amphetamine Administration: Mice were placed in AF and allowed to habituate for 20 min prior to an injection of physiological saline (3ml/kg body weight, s.c.). After 20 minutes, mice were injected with either 1.0 mg/kg (Day 1) or 3.0 mg/kg (Days 2-6), and activity was recorded for an additional 70 minutes.

All procedures were performed in compliance with an approved IACUC protocol. Statistical analyses were perfomed using SPSS using either ANOVA or Student's t tests.

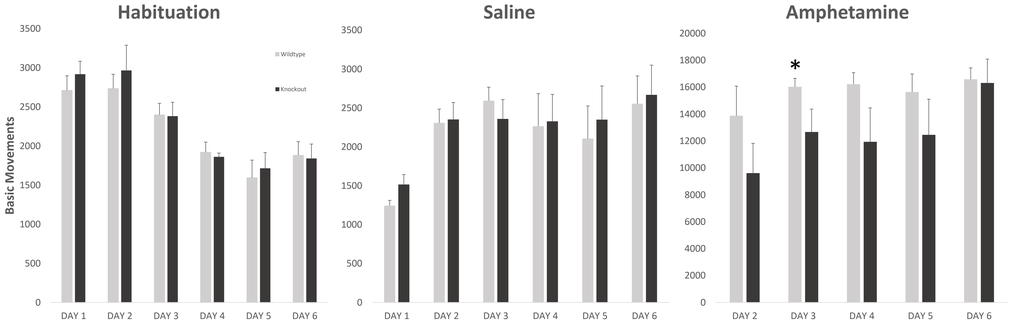
Results



Spontaneous Locomotor Activity: As expected, female mice were more active than male mice across the 48 h test (F (1,26) = 16.35, $p < 0.001$; Left). There were no differences between TRPM8 +/+ and -/- mice. Inset represents cumulative activity totals. Shaded area denotes dark cycle. DA plays a role in habituating to a novel environment. Male TRPM8 -/- mice exhibited a trend towards increased latency to habituate (F (1,12) = 1.60, p = 0.2; Right). Asterisks denote statistically significant differences among planned comparisons. Error bars depict SEM.



Sucrose Preference: Male TRPM8 deficient mice exhibited a preference for a low concentration of sucrose (0.1%) compared to male wildtype mice (t (10) = 2.01, $p < 0.05$; d = 1.00). In contrast, deletion of the TRPM8 gene had no impact on sucrose preference among females across three different concentrations (Left). **Light Dark Test**: During the 10 minute test, male mice lacking the TRPM8 gene made fewer crosses between the light and dark side (t (10) = 3.34, $p < 0.005$), and spent statistically significantly less time in the light side of the chamber (t (10) = 2.44, $p < 0.05$). **Forced Swim Test:** TRPM8 -/- mice exhibited a statistically significant decrease in the latency to immobility, a measure of behavioral despair, compared to wildtype mice (t (10) = 3.70, $p < 0.005$; right). Error bars depict SEM.



Repeated Amphetamine: On Day 1 of testing, mice were allowed 40 minutes to acclimate to the test environment (Habituation), prior to an injection of physiological Saline (3 ml/kg body weight, i.p.) prior to an injection of a low dose of Amphetamine (1.0 mg/kg). Procedures were identical for 5 subsequent days, except the dose of amphetamine was increased (3.0 mg/kg). Data analysis indicate no differences between groups during habituation or in response to saline across 6 days of testing. However, RMANOVA revealed a statistically significant main effect of test day (F (5,65) = 157, $p <$ 0.001). Error bars depict SEM.

Discussion

- Deletion of the TRPM8 receptor did reliably influence spontaneous locomotor activity among either male or female mice. However, there was a trend for male TRPM8 null mutant mice to habituate to the test environment faster than wildtype mice. **These findings suggest that DA is not altered under basal conditions.**
- Male TRPM8 knockout mice exhibited a preference for a low concentration of sucrose; this effect was not observed among female mice. In contrast, deletion of the TRPM8 receptor resulted in decreased activity in the light-dark test, and reduced time in the anxiogenic light side of the chamber. These mice also exhibited a decreased latency to immobility in the forced swim test. Together, these findings suggest that **TRPM8 may play a protective role in the etiology of anxiety and depression**.
- Male TRPM8 knockout mice exhibited a diminished response to amphetamine compared to wildtype, suggesting that **TRPM8 may be involved in substance use disorders.**
- Future research seeks to clarify the relationship of testosterone, TRPM8 and DA.

Acknowledgements

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