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Research paper

Increased ethanol consumption despite taste aversion in mice with a human tryptophan hydroxylase 2 loss of function mutation

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HIGHLIGHTS

- Mice with a loss of function mutation in Tph2 self administer ethanol under aversive conditions.
- Mice with a human Tph2 loss of function mutation may be a valuable model for ethanol use disorder.
- Loss of function mutations affecting Tph2 may confer enhanced susceptibility to ethanol use disorder.

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ABSTRACT

Polymorphisms in the gene encoding the brain serotonin synthesis enzyme Tph2 have been identified in mental illnesses, with co-morbidity of substance use disorder. However, little is known about the impact of Tph2 gene variants on addiction. Mice expressing a human Tph2 loss of function variant were used to investigate consequences of aversive conditions on ethanol intake. Mice were familiarized either with ethanol or a solution containing both ethanol and the bittering agent quinine. Effect of familiarization to ethanol or an ethanol–quinine solution was then evaluated using a two-bottles preference test in Tph2-KI and control littermates. Mice from both genotypes displayed similar levels of ethanol consumption and quinine avoidance when habituated to ethanol alone. In contrast, addition of quinine to ethanol during the familiarization period resulted in a reduction of avoidance for the quinine–ethanol solution only in mutant mice. These results indicate that loss of function mutation in Tph2 results in greater motivation for ethanol consumption under aversive conditions and may confer enhanced sensitivity to alcohol use disorder.

1. Introduction

Serotonin (5-HT) is believed to modulate susceptibility to pathological drinking [1,2]. Drugs acting or 5-HT receptors or affecting extracellular 5-HT levels have been shown to modulate ethanol intake in animal models [3,4]. Also, polymorphisms in 5-HT related genes such as 5-HT transporter and 5-HT1B receptor have been associated with alcoholism [5,6]. Tryptophan hydroxylase 2 (Tph2) is the rate-limiting enzyme for adult brain 5-HT synthesis [7,8]. Humans carrying a G1463A loss of function single nucleotide polymorphism in the *TPH2* gene often have family history of substance use disorder (SUD) with anxiety and depression [9]. Additional polymorphisms in *TPH2* may also play a role in regulating alcohol consumption [10]. Furthermore, knockin mice expressing a R439H mutant of Tph2 corresponding to this human polymorphism display increased basal ethanol consumption as well as stronger preference for an ethanol/sucrose cocktail in a two-bottles test, thus suggesting that these mice may represent a good model to study alcohol use disorder (AUD) [4]. Yet, increased ethanol consumption in mice does not necessarily represent addictive behavior. Indeed, addiction is often defined as an engagement in rewarding stimuli, despite adverse consequences [11]. Here we examined the impact of an aversive stimulus, the bittering agent quinine, on ethanol intake in mice expressing the R439H mutant Tph2.

2. Material and methods

Homozygote (HO) Tph2 R439H knockin mice (Tph2-KI) have been described previously and display about 80% reduction of

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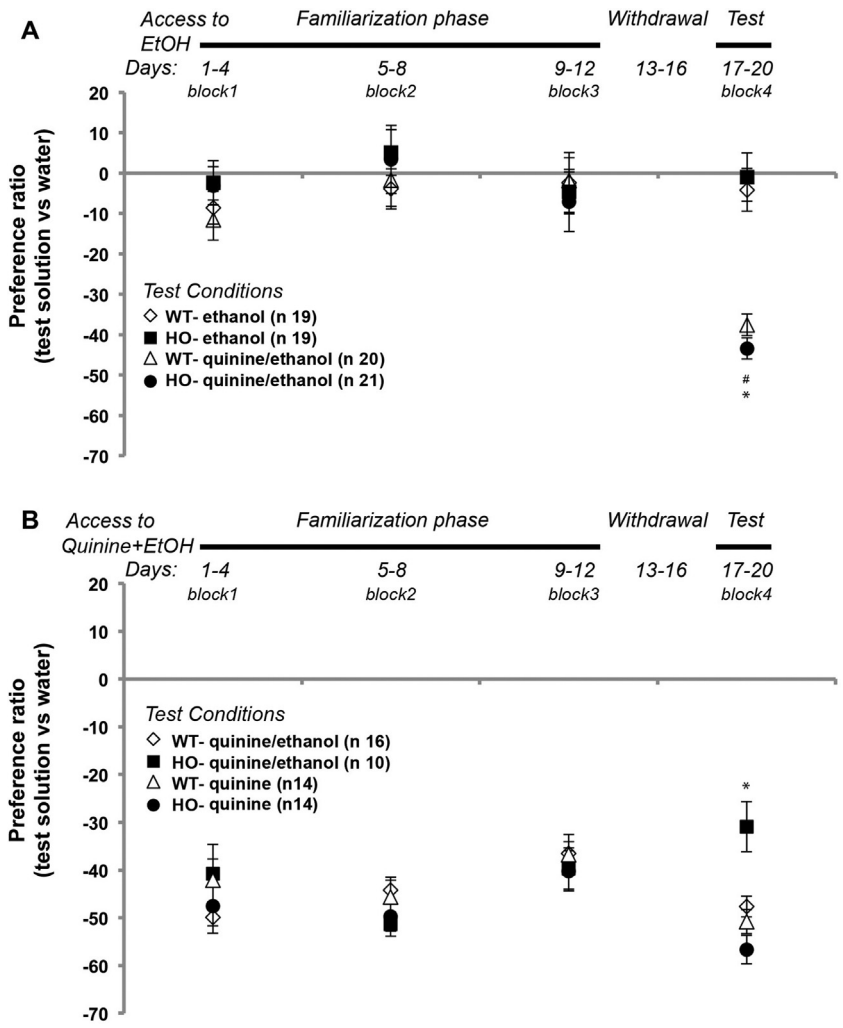

Fig. 1. Drinking preferences in two-bottles tests following familiarization to ethanol or quinine ethanol solutions.
(A) Post-withdrawal preference test following familiarization with ethanol solutions (means ± S.E.M.). * = $p < .05$ between and # = $p < .05$ between HO for the two test solution. Symbols in legend represent genotypes and the solution used for the post-withdrawal test.◊: WT mice tested with the ethanol solution. ■: HO mice tested with the ethanol solution. △: WT mice tested with the quinine ethanol solution. ●: HO mice tested with the quinine ethanol solution.
(B) Post-withdrawal preference test following familiarization with quinine–ethanol solutions (means ± S.E.M.). * $p < .05$ between WT and HO for the ethanol–quinine test condition. Symbols in legend represent genotypes and the solution used for the post-withdrawal test. ◊: WT mice tested with the quinine ethanol solution. ■: HO mice tested with the quinine ethanol solution. △: WT mice tested with the quinine solution. ●: HO mice tested with the quinine solution.

brain 5-HT synthesis as compared to control wild type littermates (WT) [12]. All mice were individually housed in an air-conditioned, humidity-controlled room in which temperature was kept between 22 and 23 °C. They were maintained on a 12–12 h light–dark cycle (lights on at 7AM). Mice HO and control WT littermates used in behavioral tests were aged 3–5 months. Groups were composed of mice from both sexes, in approximately equal number (±one mouse). Food and water were always available ad libitum. All procedures were conducted in accordance with the Canadian Council on Animal Care guidelines and approved by the Laval University Animal Care Committee.

Tph2-KI mice have previously been shown to have normal olfactory sensitivity, sucrose preference and quinine avoidance as compared to WT littermates [13], thus supporting the use of quinine as an aversive agent. Ethanol preference was access using a short-term protocol which allowed to detect different levels of ethanol preference in mice [14]. Relatively short tests are well-suited to assess ethanol preference in mice because mice tend to avoid unpalatable solutions and to choose palatable ones within minutes of first exposure [15]. In addition, 4-day tests were shown to be sufficient to detect differences between genotypes [16].

The ethanol preference tests were series of two-bottle preference tests in which one bottle contained water, whereas the other contained different ethanol solutions. Positions of the bottles were switched every 24 h. Bottles were weighed daily at the same hour to measure consumption. The first part of each version of the test was a 12 days familiarization phase, in which the ethanol (Alcool Global, Société des Alcools du Québec, QC) content of the bottle was gradually raised each 4 days from 3, to 6 to 10% (v/v) in water. Alternatively, ethanol solutions were complemented with .025% (v/v) quinine (Sigma–Aldrich, Oakville, ON), while water bottles remained exempt of this aversive agent. Mice then underwent a 4 days withdrawal during which two bottles containing only water were available. Following this period, preference/aversion of mice for water as compared to ethanol (10%v/v), quinine (.025% v/v) or an ethanol quinine combination was evaluated for one or several blocks of 4 days.

3. Results

In a first set of tests, (Fig. 1A) mice from both genotypes were familiarized to ethanol alone and their preference/avoidance