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Ethanol Effects on Multiple Fixed Interval, Fixed Ratio Responding in Mice with Deletions of the Serotonin Transporter Gene

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Abstract

Serotonin transporter (SERT) knock out (KO) mice self-administer less ethanol than either heterozygous (HET) or wild type (WT) mice; however the mechanistic basis for this difference remains unclear. Here we examine the possibility that ethanol more readily decreases responding in KO mice, thereby limiting ethanol self-administration. To examine whether KO mice were more sensitive to the response-decreasing effects of ethanol, we administered ethanol (0.2 to 3.2 g/kg) to mice responding under a multiple fixed-ratio (FR) 30-response, fixed-interval (FI) 300-sec schedule of milk presentation. Ethanol decreased responding similarly in all three genotypes. FR responding tended to be decreased at lower doses than FI responding. The decreased level of ethanol self-administration in SERT KO mice is not explained by an increased sensitivity to the response-decreasing effects of ethanol in KO mice, as sensitivity to the response decreasing effects of ethanol was similar in the KO, HET and WT mice.

Keywords

operant; ethanol; mouse; serotonin transporter

Introduction

Extracellular serotonin levels are thought to be lower in those with alcoholism compared to those without alcoholism (Sellers et al 1992). Extracellular serotonin levels are largely regulated by the serotonin transporter (SERT) (Blakely & Bauman 2000) and genetic inactivation of SERT increases extracellular serotonin levels (Mathews et al., 2004; Shen et

al., 2004). If increased alcohol intake is related to reduced levels of extracellular serotonin, then SERT knockout (KO) mice might consume less alcohol than wild type (WT) mice. We recently observed this to be the case (Lamb and Daws, 2013). As shown in Figure 1, an economic analysis shows that reduced self-administration by KO mice is due to reduced unconstrained demand. However, the reduction, was not due to different sensitivities to the cost of ethanol, *i.e.*, elasticity, as increasing the response requirements for ethanol produced proportionally similar decreases in consumption across genotypes. Moreover, reduced unconstrained demand for ethanol in SERT KO mice did not result from a general lack of motivation or ability to respond, as they responded at greater levels for milk, relative to ethanol. Thus, SERT KO mice had a relatively specific decrease in unconstrained demand for ethanol.

Unconstrained demand reflects the level of consumption obtained when price does not limit consumption. Thus, unconstrained demand might be lower in SERT KO mice relative to WT mice because of an increased sensitivity to ethanol effects that limit ethanol consumption. For instance, SERT KO mice may require less ethanol to become sated, to encounter adverse effects that limit further drinking, and/or to become so intoxicated they can not respond further. The latter possibility is perhaps the most unambiguous to test and was the focus of the present study. This notion is supported by clinical studies reporting that a low level of response to certain ethanol effects is associated with problem drinking (Morean & Corbin, 2010). Those with a family history of alcoholism, or who drink heavily, are at greater risk for development of problem drinking and are less sensitive to ethanol-induced body sway and sedation (Schukitt, 1988; Schuckit & Smith, 1996; King et al 2011). Thus, to examine if ethanol had greater response-impairing effects in SERT KO mice that might limit their unconstrained demand for ethanol, we examined the effects of ethanol on KO, heterozygous (HET), and WT mice responding under a multiple fixed-interval, fixed-ratio (mult FI FR) schedule.

Methods

Subjects

Twenty-four male mice were used: 8 of each SERT genotype. Ultimately, only 4 KO, 5 HET and 6 WT met training criteria. Mice were derived from an in-house colony established from breeding pairs generously provided by Dr. Dennis Murphy (NIMH) in 1999 (see Bengel et al., 1998). SERT KO, HET or WT mice on a congenic C57BL/6J background were littermates derived from HET $\times$ HET matings and were raised and housed together in same-sex groups from weaning until the start of this experiment, when they were housed singly in a temperature- and humidity-controlled vivarium under a 12 h light/dark cycle (lights on 0600 h). Water was freely available except during experimental sessions. Food was limited to 2.5 g rodent chow each day. The procedures used were approved by the local institutional animal care and use committee and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Apparatus

Experiments were conducted in operant chambers (Med Associates, St. Albans, VT) with two head-entry detectors and associated dipper mechanisms, with a yellow LED light at the rear of the entry detector. One detector was associated with the FI schedule and one with the FR schedule. Dippers delivered evaporated milk (Sunshine Vitamin D Evaporated Milk, Minot Co., Gahanana, OH) diluted 1:1 with tap water in a volume of 0.01 ml. Operant chambers were housed inside large fan equipped sound-attenuating chambers.

Procedure

Initially, mice were trained to nose poke each detector. During an overnight training session, both detectors were operative and LED stimuli were lit on both sides. Each response raised the dipper; when one dipper was raised, both LEDs were extinguished and the other dipper could not be raised. All mice responded on both options at the end of training. Mice were then trained on a multiple fixed-ratio 30 fixed-interval 300 s schedule of milk delivery. Counterbalancing of the side associated with each schedule within each genotype was maximized.

Sessions began with the FR. The associated LED and the houselight were illuminated. Completion of a fixed number of responses extinguished the LED and raised the dipper for 10 s. After the dipper was lowered, all stimuli in the chamber were turned off for a 30-s blackout. After the blackout, the houselight and FI associated LED were illuminated. The first response occurring following the end of the interval extinguished the LED and raised the dipper for 10 s. Once the dipper lowered, all stimuli were extinguished for 30-s. FR and FI alternated until each schedule was experienced ten times. Initially, the FR was set to 1 and the FI to 10 s. Over a few days, the FR was increased to 30 and the FI to 300 s. While initially sessions were conducted every weekday, sessions were ultimately conducted on Mondays, Wednesdays and Fridays as behavior was more stable under these conditions.

Ethanol

Ethanol was injected i.p. 10 min before sessions, in a concentration of 5% (w/v). Doses of 0.2, 0.4, 0.8, 1.6, and 3.2 g/kg were tested. Doses of 0.2 to 1.6 g/kg were given in ascending and then descending order. Doses of 3.2 g/kg were given twice following this sequence. Ethanol was administered on Mondays and Fridays and saline on Wednesday.

Analysis

Effects of ethanol on responding, expressed as a percent of control, were analyzed using ANOVA with Huyn-Feldt corrections. Differences in control values were also analyzed using ANOVA.

Results

Control Responding

Mean response-rates under the FR were 1.29 (SD: 1.20), 1.15 (0.48), and 1.58 (0.58) responses/sec in KO, HET and WT respectively. These were not significantly different ($F(2,12)=0.47$, NS). Response rates under the FI were 0.351 (0.215), 0.457 (0.255), and

0.694 (0.241) in KO, HET, and WT; these rates did not differ significantly ($F(2,12)=2.75$, NS).

Effects of Ethanol

Ethanol dose-dependently and similarly decreased responding in SERT KO, HET & WT mice under both the FR (Figure 2, left panel: genotype $F(2,48)=1.28$, NS; dose $F(4,48)=43.5$, $p<0.01$; dose X genotype $F(8,48)=0.80$, NS) and the FI (Figure 2, right panel: genotype $F(2,48)=0.45$, NS; dose $F(4,48)=73.4$, $p<0.01$; dose X genotype $F(8,48)=1.23$, NS).

FR responding was decreased slightly more by ethanol than FI responding (genotype $F(2,108)=1.09$, NS; schedule $F(1,108)=3.46$, $p=0.07$; dose $F(4,108)=108.3$, $p<0.01$; schedule X dose $F(4,108)=3.47$, $p<0.01$; genotype X schedule $F(2,108)=0.33$, NS; genotype X dose $F(8,108)=0.96$, NS; genotype X dose X schedule $F(8,108)=1.03$, NS). Specifically, 1.6 g/kg ethanol decreased FR responding more than FI responding (paired t-test: $t(28)=3.65$, $p<0.01$).

Discussion

The effects of ethanol on response rate under either the FR 30 or FI 300 s were similar among genotypes, indicating that differences in sensitivity to the effects of ethanol effects on responding cannot account for differences in unconstrained ethanol demand among SERT KO, HET, & WT mice (Lamb & Daws, 2013).

Thus, other ethanol effects must limit ethanol consumption. Cunningham (1994) suggested that hypothermia induced by ethanol might limit its consumption. However, the hypothermic effects of ethanol are similar among SERT genotypes (Boyce-Rustay et al 2006). SERT KO mice are more sensitive to the sedative effects of ethanol, having longer sleep times and somewhat greater motor incoordination (Boyce-Rustay et al 2006; Daws et al 2006). Greater sensitivity to the motor impairing effects of ethanol would presumably work by decreasing the ability of KO mice to respond for ethanol, which the present study indicates is unlikely to be the case.

Thus, effects of ethanol studied to date do not explain the lower unconstrained demand for ethanol in SERT KO compared to HET or WT mice. SERT KO mice do appear to less readily acquire operant tasks than HET or WT mice. Both Sanders et al (2007) and Trigo (2007) report lower breakpoints for food or water in SERT KO mice responding under a progressive ratio schedule, and in this study fewer KO mice acquired stable performance. However, these differences disappear with continued training and mice in this study that acquired stable responding did not differ in response rate across genotype. Further, SERT KO mice in our previous study showed large increases in responding when switched from responding for ethanol to responding for milk. Thus, differences in inherent capabilities to respond do not appear to be an adequate explanation for differences in unconstrained ethanol demand across genotypes.

Based on available data, it appears that reduced unconstrained ethanol demand in SERT KO mice is attributable to effects not yet identified, but may include greater sensitivity to

ethanol satiation or to adverse effects of ethanol other than hypothermia. Future studies should investigate these possibilities, as better understanding the role serotonin plays in limiting ethanol consumption will help us to better understand the role serotonin plays in alcoholism.

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Figure 1.

Mean unconstrained demand for ethanol calculated from individual data of the mice used in the study of Lamb & Daws (2013). Points represent the mean Q0 value calculated according to Hursh & Silberberg (2008). Bars represent standard errors of the means. The WT value is significantly greater than the SERT KO value ($t(12)=2.9$, $P<0.05$).

Figure 2.

The effects of ethanol on responding under a multiple Fixed Ratio (FR) 30 response (triangles), Fixed Interval (FI) 300 sec (circles) schedule of presentation of 0.01 ml 50% evaporated milk, in SERT KO mice (open symbols), SERT HET mice (gray symbols) or SERT WT mice (black symbols). Responding under the FR schedule is shown in the left panel and responding under the FI schedule is shown in the right panel. The vertical axis shows responding as a percent of each mouse's control rate of responding and the horizontal axes show ethanol dose in g/kg on a log2 scale. The effects of ethanol did not differ across the three genotypes.