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Ethanol induces second-order aversive conditioning in adolescent and adult rats

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Abstract

Alcohol abuse and dependence are considered public health problems, with an etiological onset often occurring during late childhood and adolescence, and understanding age-related differences in ethanol sensitivity is important. Low to moderate ethanol doses (0.5 and 2.0 g/kg, intragastrically [i.g.]) induce single-trial, appetitive second-order place conditioning (SOC) in adolescent, but not adult, rats. Recent studies have demonstrated that adolescents may be less sensitive than adults to the aversive properties of ethanol, reflected by conditioned taste aversion. The present study assessed the aversive motivational effects of high-dose ethanol (3.0 and 3.25 g/kg, i.g., for adolescents and adults, respectively) using SOC. Experiment 1 revealed similar blood and brain ethanol levels in adolescent and adult rats given 3.0 and 3.25 g/kg ethanol, respectively. In Experiment 2, animals received ethanol or vehicle paired with intraoral pulses of sucrose (conditioned stimulus 1 [CS1]). After one, two, or three conditioning trials, the rats were presented with the CS1 while in a distinctive chamber (CS2). When tested for CS2 preference, ethanol-treated animals exhibited reduced preference for the CS2 compared with controls. This result, indicative of ethanol-mediated aversive place conditioning, was similar for adolescents and adults; for females and males; and after one, two, or three training trials. In conjunction with previous results, the present study showed that, in adolescent rats subjected to SOC, ethanol's hedonic effects vary from appetitive to aversive as the ethanol dose increases. Adolescent and adult animals appear to perceive the postingestive effects of high-dose ethanol as similarly aversive when assessed by SOC.

Keywords: Adolescence; Adulthood; Ethanol; Reinforcement; Second-order conditioning

Introduction

Early initiation of alcohol consumption is associated with a greater likelihood of developing alcohol abuse and dependence (''early debut effect''; Pedersen and Skrondal, 1998). This relationship is not linear, nor is it necessarily causal. Alcohol initiation at certain developmental stages is critically important to determine the pattern of alcohol consumption at adulthood. Specifically, the risk of alcohol abuse and dependence is greater when the onset of alcohol intake occurs during early adolescence (13–14 years old; Anthony and Petronis, 1995). These findings have strong public health implications, particularly when viewed in conjunction with the fact that alcohol intake usually begins during adolescence, with 28% of underage drinkers in the United States having started at age 13 years (Johnston and O'Malley, 2007).

The use of animal models has identified factors that could help explain the avidity for alcohol during adolescence and the enduring consequences of such consumption. Ethanol intake in adolescent (postnatal days [PD] 28–42) and late-adolescent animals (until approximately PD55 or so; Spear, 2000) surpasses that observed in older animals (Doremus et al., 2005). Adolescents are also more sensitive than adults to the facilitating effects of low-dose ethanol on social behavior but are less sensitive to the disruptive effects that higher ethanol doses have on social behavior (Varlinskaya and Spear, 2002). Intriguingly, adolescents are remarkably resistant to several acute effects of ethanol (e.g., motor incoordination, hypothermia, narcosis; Spear, 2004; White et al., 2002) that normally should serve to preclude further engagement in alcohol intake.

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The motivational effects of ethanol are critical in the modulation of drug seeking and self-administration (Cunningham et al., 2000). Adult rats readily detect an aversive component derived from alcohol intoxication. For example, they reject a taste that has been previously paired with ethanol's effects (conditioned taste aversion [CTA]; Davies and Parker, 1990). In contrast, evidence of the expression of ethanol-mediated conditioned preferences in adult rats has proven problematic. Unlike mice, rats tend to avoid locations or textures that signal the drug (conditioned place aversion; Cunningham et al., 1993). Some intriguing data suggest that adolescent rats may exhibit differential sensitivity to ethanol's motivational effects compared with their more mature counterparts. Philpot et al. (2003) found ethanol-induced conditioned place preference (CPP) at PD25 (0.2 g/kg) and late in adolescence (PD45, 0.5 and 1 g/kg, intraperitoneally [i.p.]), whereas a trend toward conditioned aversion was found in young adults (PD60).

A variation of the CPP procedure has provided another venue for the analysis of ethanol-mediated motivational learning. In this preparation, described as second-order conditioning (SOC; Molina et al., 2006, 2007), a gustatory stimulus (e.g., water or sucrose, conditioned stimulus 1 [CS1]) is paired with ethanol's pharmacological effects. Animals are then stimulated with the CS1 while placed in a visually and tactually distinctive chamber (CS2). Preference or aversion toward the CS2 is then assessed in a choice procedure (CS2 vs. CS novel). In other words, ethanol's motivational effects are assessed not through direct responsiveness to the taste CS1 but rather by assessing whether the ethanol-paired taste can transfer motivational information to the CS2.

The use of SOC proved as a valuable tool for detecting appetitive effects of ethanol in infant and adolescent rats (Molina et al., 2006, 2007; Pautassi et al., 2008b). The preweanling, 14-day-old rats were given pairings of an intraoral CS and either the early or late (5–15 min or 30–45 min postintubation, respectively) effects of intragastric (i.g.) administration of a low dose of ethanol (0.5 g/kg) or the early effects of a moderate dose (2 g/kg). This resulted in the gustatory CS becoming a positive second-order reinforcer. Interestingly, aversions emerged when the CS1 was paired with 2.0 g/kg, 30–45 min postadministration (Molina et al., 2006, 2007). A subsequent study assessed ethanol-mediated, one-trial SOC in adolescent and adult rats (PD32 and PD70, respectively; Pautassi et al., 2008b). The CS1 (a sucrose taste) was delivered through a surgically implanted catheter 5–15 min or 30–45 min after ethanol administration (0.5 or 2.0 g/kg, i.g.). The CS1 then acted as an appetitive second-order reinforcer in the adolescents, mediating the expression of CPP, which was particularly strong when the CS1 was originally paired with 2.0 g/kg ethanol. The adult rats did not exhibit changes in tactile preferences, thus suggesting the absence of ethanol-mediated learning. These results suggest greater sensitivity to ethanol's appetitive effects in adolescent than in adult rats assessed by SOC (Pautassi et al., 2008b). In a follow-up study, the second-order appetitive conditioning in adolescents was blocked after treatment with naloxone, a general opioid antagonist (Pautassi et al., 2010).

The previous studies underscore an important advantage of SOC, namely, it can be used with minimal modification across ontogeny. Because of inherent developmental changes, the ontogeny of ethanol reinforcement has been studied through different tests for infant (Pautassi et al., 2002); adolescent (Ristuccia and Spear, 2008); and adult (Bienkowski et al., 1999) subjects. The development of SOC has provided a single benchmark to study ethanol's motivational effects. SOC has also proven useful to detect "silent" (i.e., not detectable through first-order conditioning) associations in young rats. A study conducted with 4-day-old rats revealed a lack of aversion for an odor CS previously paired with lithium chloride (LiCl), an emetic, nonaddictive substance. The conditioned aversion was observed, however, after pups were provided subsequent second-order pairings between the odor and a novel texture (Miller et al., 1990). The SOC procedure may be more likely to reveal these seemingly elusive associations, because it minimizes the effects of conditioned responses often emitted in the presence of the CS after first-order conditioning—conditioned behaviors that may compete with the target response used to reflect conditioning. This property of SOC may be particularly valuable for analyzing ethanol-mediated place conditioning, given previous studies revealing first-order, motor conditioned responses in response to a taste CS previously paired with ethanol (Molina et al., 2006; Pautassi et al., 2008b).

Age-specific predisposition in terms of sensitivity to ethanol's motivational effects may render adolescents at risk of ethanol-related problems. Adolescents may be more sensitive to ethanol's appetitive effects (Pautassi et al., 2008b) but less sensitive to the aversive consequences of ethanol. The latter effects are easily observed in both adult and infant rats, particularly at doses greater than or equal to 2.0 g/kg. Adolescent rats are less susceptible to CTA induced by psychoactive drugs (e.g., cocaine, amphetamine, and nicotine) and also by LiCl (Schramm-Sapyta et al., 2006). Less is known, however, about ethanol's ability to induce aversive learning in adolescent rats. A recent series of studies (Anderson et al., 2008; Varlinskaya and Spear, 2008; Vetter-O'Hagen et al., 2009) assessed age- and sex-related differences in terms of ethanol-mediated CTA in adult and adolescent rats (PD32 and PD74, respectively). Ethanol-induced CTA was evident in the adolescents but at higher doses than those in adults. The older animals showed CTA at i.p. doses of 1.0 and 1.5 g/kg, whereas CTA in adolescents was evident only at 2.0 g/kg. These results suggest that, when assessed by CTA, adolescents may be less sensitive than their older counterparts to the aversive properties of ethanol. To date, responsiveness to the aversive properties of ethanol as a function of age and sex has only been studied using the