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Effect of different stressors on voluntary ethanol intake in ethanol-dependent and nondependent C57BL/6J mice

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ABSTRACT

Several animal models have evaluated the effect of stress on voluntary ethanol intake with mixed results. The experiments reported here examined the effects of different stressors on voluntary ethanol consumption in dependent and nondependent adult male C57BL/6J mice. In Experiment 1, restraint, forced swim, and social defeat stress procedures all tended to reduce ethanol intake in nondependent mice regardless of whether the stress experience occurred 1 h or 4 h prior to ethanol access. The reduction in ethanol consumption was most robust following restraint stress. Experiment 2 examined the effects of forced swim stress and social defeat stress on drinking in a dependence model that involved repeated cycles of chronic intermittent ethanol (CIE) exposure. Repeated exposure to forced swim stress prior to intervening test drinking periods that followed repeated cycles of CIE exposure further increased ethanol consumption in CIE-exposed mice while not altering intake in nondependent mice. In contrast, repeated exposure to the social defeat stressor in a similar manner reduced ethanol consumption in CIE-exposed mice while not altering drinking in nondependent mice. Results from Experiment 3 confirmed this selective effect of forced swim stress increasing ethanol consumption in mice with a history of CIE exposure, and also demonstrated that enhanced drinking is only observed when the forced swim stressor is administered during each test drinking week, but not if it is applied only during the final test week. Collectively, these studies point to a unique interaction between repeated stress experience and CIE exposure, and also suggest that such an effect depends on the nature of the stressor. Future studies will need to further explore the generalizability of these results, as well as mechanisms underlying the ability of forced swim stress to selectively further enhance ethanol consumption in dependent (CIE-exposed) mice but not alter intake in nondependent animals.

Introduction

Stress has been extensively implicated as a factor that may lead to ethanol (alcohol) drinking and induce relapse in abstinent alcoholics. Studies involving animal models and clinical investigations have indicated that the relationship between stress and ethanol consumption is complex and depends on a large number of variables (Becker, Lopez, & Doremus-Fitzwater, 2011; Spanagel, Noori, & Heilig, 2014). From a clinical perspective, the tension-reduction hypothesis postulates that people seek to consume ethanol for its sedative or anxiolytic effects (Brady & Sonne, 1999; Cappell & Greeley, 1987; Pohorecky, 1991; Sayette, 1999; Uhart & Wand, 2009). Further, as many individuals suffering with alcohol-use disorder (AUD) invariably experience several episodes of abstinence, stress associated with withdrawal may perpetuate excessive drinking motivated by attempts to reduce or avoid withdrawal-related distress (Becker, 2008, 2013, 2014; Heilig, Egli, Crabbe, & Becker, 2010; Koob, 2003, 2014; Koob & Le Moal, 2008). On the other hand, ethanol is known to serve as a stressor itself. For example, alcoholics undergoing treatment to maintain abstinence often present with alterations in hypothalamic-pituitary-adrenal axis (HPA) function (Adinoff et al., 1996; Costa et al., 1996; Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000; Marchesi, Chiodera, Ampollini, Volpi, & Coiro, 1997; Uhart & Wand, 2009). These alterations in baseline HPA axis activity along with enhanced reactivity to stress may trigger relapse and further augment drinking. Alcoholics frequently report that

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they resumed ethanol drinking after a stressful episode, and changes in the HPA axis have been related to ethanol craving (O'Malley, Krishnan-Sarin, Farren, Sinha, & Kreek, 2002; Sinha & O'Malley, 1999).

The preclinical literature concerning ethanol-stress interactions consists of numerous studies with mixed results. Some studies indicate that stress increases ethanol intake while others indicate that ethanol intake is reduced or unaltered by stress experience (reviewed by Becker et al., 2011; Spanagel et al., 2014). Methodological differences among the studies likely account for the variety of outcomes – many of these studies differ in the nature of stressor used, schedule of stress administration in relation to ethanol access, or amount/duration of prior ethanol intake (Becker et al., 2011; Pohorecky, 1990; Sillaber & Henniger, 2004; Spanagel et al., 2014).

Several preclinical studies have evaluated the effect of stress on relapse using operant conditioning models of reinstatement. In this case, stress is demonstrated to induce ethanol-seeking behavior when rodents are tested under extinction conditions (Lê & Shaham, 2002; Lê et al., 1999, 1998). Stress has also been shown to enhance cue-induced reinstatement in both rats and mice (Liu & Weiss, 2002). Furthermore, and of relevance for the present study, the effect of stress on ethanol-seeking behavior is magnified in ethanol-dependent rats compared to nondependent animals (Gehlert et al., 2007; Liu & Weiss, 2002; Sommer et al., 2008). In these studies, it may be argued that ethanol withdrawal-related stress enhances the effect of an acute stressor to trigger ethanol seeking. However, it is important to note that in these reinstatement studies, animals are not provided the opportunity to consume ethanol following stress exposure (i.e., testing is conducted under extinction conditions). Indeed, little is known about the effect of stress on voluntary ethanol intake in ethanol-dependent rodents. Numerous studies conducted with rats (Brown, Jackson, & Stephens, 1998; Gilpin, Richardson, Lumeng, & Koob, 2008; Gilpin et al., 2009; O'Dell, Roberts, Smith, & Koob, 2004; Roberts, Cole, & Koob, 1996; Roberts, Heyser, Cole, Griffin, & Koob, 2000; Valdez et al., 2002) and mice (Becker & Lopez, 2004; Finn et al., 2007; Griffin, Lopez, & Becker, 2009; Griffin, Lopez, Yanke, Middaugh, & Becker, 2009; Lopez & Becker, 2005) have shown increases in ethanol intake in subjects that experienced repeated episodes of chronic ethanol intoxication followed by periods of withdrawal. The studies presented in the current report aim to evaluate the effect of stress on ethanol consumption within the context of dependence. Using a mouse model of dependence and relapse drinking, the hypothesis guiding these experiments is that ethanol-dependent mice will not only exhibit the expected increase in ethanol intake relative to nondependent mice, but they also will be more sensitive to the ability of stress to further increase voluntary ethanol consumption.

Methods

Subjects

Adult male C57BL/6 mice purchased from Jackson Laboratories (Bar Harbor, ME) were individually housed with free access to food (Harlan Teklad, Madison, WI) and tap water throughout all phases of the experiments. Body weights were recorded weekly during ethanol drinking periods or daily during chronic intermittent ethanol (CIE) or air inhalation exposure (detailed below). In the experiment involving social defeat stress, adult male CD1 mice from Charles River Laboratories (Raleigh, NC) served as aggressors (described below). Mice were housed in a temperature- and humidity-controlled animal facility under a modified 12-h light/dark cycle (lights on at 2:00 AM). All procedures were approved by the Institutional Animal Care and Use Committee and followed the NIH Guide for the Care and Use of Laboratory Animals (8th edition, National Research Council, 2011).

Study designs

Experiment 1: effect of exposure to different stressors on voluntary ethanol intake in nondependent mice

Mice ($n = 9–10$/group) were allowed to drink ethanol (15% v/v versus water) in their home cage using a limited-access (2 h/day) procedure described below. After 6 weeks of voluntary ethanol intake (baseline), mice were separated into seven groups balanced on the basis of intake level during the last baseline week. Mice in the no-stress (noSTS) control group continued to drink ethanol as during baseline weeks. The rest of the groups were exposed to restraint stress (RS), social defeat (SD), or forced swim (FS) either 1 or 4 h before access to ethanol in their home cages for 5 consecutive days. These time intervals were chosen to evaluate ethanol intake near peak stress-induced physiological changes or at a more remote time point when the acute effects of stress, such as elevated corticosterone levels and reduced locomotor activity had subsided (Cabib, Kempf, Schleef, Mele, & Puglisi-Allegra, 1988; Hare, Beierle, Toufexis, Hammack, & Falls, 2014; Patchev & Patchev, 2006). The following week, all mice resumed ethanol intake without exposure to stress.

Experiment 2: effect of exposure to different stressors on voluntary ethanol intake in ethanol-dependent and nondependent mice

Mice ($n = 7–12$/group) were allowed to drink ethanol in their home cages under limited-access (2 h/day) conditions. Once stable baseline intake was observed, mice were separated into CIE-exposed (dependent) and air-exposed (nondependent) groups, and these groups were further separated into three stress conditions: no-stress (noSTS), social defeat (SD), and forced swim (FS). Mice were separated into these groups based on their intake level during the last week of baseline intake. Seventy-two hours after each CIE (or air) exposure cycle, stress procedures were administered 4 h before each daily drinking test session. This schedule of CIE (or air) exposure followed by 5 days of ethanol intake after stress or no stress exposure was repeated four times (Test cycles 1–4). Details regarding CIE (or air) exposure and stress procedures are presented below.

Experiment 3: effect of forced swim stress on voluntary ethanol intake in ethanol-dependent and nondependent mice

This experiment followed similar procedures described for Experiment 2. Once stable baseline ethanol intake under limited-access conditions was established, mice ($n = 8–10$/group) were separated into CIE and air-control (CTL) groups, and further separated on the basis of stress condition. One group of CIE and CTL mice (FS4 groups) received forced swim (FS) stress exposure 4 h prior to test drinking sessions that followed each of the four weekly CIE (or air) exposure cycles. A second cohort of CIE and CTL mice (FS1 groups) received FS stress 4 h prior to drinking sessions, but only following the last (4th) CIE/air exposure cycle. The remaining CIE and CTL mice (noSTS groups) did not receive FS stress at any time during the study.

Limited-access ethanol drinking procedure

Mice had access to ethanol 5 days/week (Mon–Fri) starting at 30 min before the start of the dark cycle (1:30 PM). Two 15-mL graduated tubes containing either 15% v/v ethanol or tap water