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Chronic swim stress alters sensitivity to acute behavioral effects of ethanol in mice

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Abstract

Epidemiological data support a strong link between stress, stress-related disorders and risk for alcoholism. However, precisely how stress might impact sensitivity to the intoxicating effects of ethanol or the willingness to voluntary consume ethanol remains unclear. The present study assessed the effects of daily exposure to forced swim stress on subsequent sensitivity to the sedative/hypnotic, hypothermic, ataxic (measured using accelerating rotarod), and anxiolytic-like (measured using elevated plus-maze) effects of ethanol, and ethanol consumption and preference in a two-bottle choice paradigm, in male C57BL/6J mice. Stress effects on the sedative/hypnotic effects of the barbiturate pentobarbital were also tested. Results showed that chronic (fourteen days) but not acute (one or three days) swim stress significantly potentiated the sedative/hypnotic and hypothermic effects of 4 g/kg, but not 3 g/kg, ethanol. The sedative/hypnotic effects of pentobarbital were attenuated by chronic swim stress. Irrespective of chronicity, swim stress did not alter the ataxic or anxiolytic-like effects of ethanol, or alter ethanol self-administration either during or after stress. These data provide further evidence that stress alters the intoxicating effects of high doses of ethanol in a behaviorally selective manner.
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1. Introduction

There is considerable comorbidity between mood disorders and alcoholism [1,2]. Individuals with a history of stress and mood disorders such as anxiety and depression have approximately three times the risk for developing an alcohol-related disorder, and alcoholics with a co-morbid mood disorder tend to drink more heavily and have a poorer prognosis [3–6]. These epidemiological data have lent support to the notion that mood abnormalities represent a major risk factor for alcoholism, possibly because individuals abuse alcohol (ethanol) for its 'anti-stress' or 'anti-tension' properties [2,7,8]. The association between stress, mood disturbances and alcoholism appears to be complex. For example, while a history of adverse life events positively correlates with increased rates of alcoholism, a proportion of individuals actually exhibit higher than normal rates of abstinence following stress [9–16]. These data suggest that individual differences, possibly at the levels of modifying psychosocial, biological and genetic factors, determine the relationship between stress and alcoholism.

In this context, a number of studies have shown that individuals with a family history of alcoholism (family history positive, FHP) are less sensitive to certain behavioral effects of ethanol than family history negatives (FHN), apparently due to increased acute functional tolerance to the drug's effects [17,18]. Moreover, some studies show that FHP's also exhibit greater hypothalamic–pituitary–adrenal (HPA)-axis activation than FHN's following stress or ethanol challenge [19–25]. In addition, FHP's show more pronounced neuroendocrine and autonomic responses to ethanol itself than FHN's [26–28].

While these findings suggest that decreased sensitivity to ethanol and increased risk for alcoholism is associated with abnormal stress responsivity, how stress might impact sensitivity to ethanol intoxication has not been fully elucidated. Previous studies have shown that non-alcoholics exposed to laboratory

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stressors report increased feelings of sedation in response to ethanol challenge [29,30]. In rodents, while stress reinstates ethanol-seeking behavior after periods of abstinence [31,32], the effects of various types of stress on voluntary self-administration have been inconsistent [33–44]. Moreover, to-date there have been relatively few studies examining the effects of stress on sensitivity to the acute intoxicating effects of ethanol in rodents, with existing studies again report conflicting results [45–52].

The aim of the present study was to further examine the effects of stress on sensitivity to ethanol-related behaviors including self-administration in mice. Forced swim stress was employed as a stressor. Swim stress was used because it is a simple and readily replicable procedure and variants of this test are commonly used to assay depression-related behaviors in rats and mice [53]. Previous studies have also shown that swim stress can increase ethanol self-administration in mice [54–56]. In the present study, C57BL/6J mice were subjected to either acute or chronic swim stress and subsequently tested for ethanol-induced sedation/hypnosis, hypothermia, ataxia in the accelerating rotarod, and anxiety-like behavior in the elevated plus-maze. The effects of stress on ethanol consumption were measured using a standard two-bottle free choice paradigm.

2. Animals, materials and methods

2.1. Ethics

All experimental procedures were approved by the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use Committee, and followed the National Institute of Health guidelines outlined in 'Using Animals in Intramural Research.'

2.2. Animals

Subjects were male C57BL/6J mice obtained from The Jackson Laboratory (Bar Harbor, ME) and aged 10–15 weeks at the time of testing. Mice were housed in groups of 3–5 (except for the voluntary ethanol consumption experiment) in a temperature- and humidity-controlled vivarium under a 12 h light/dark cycle (lights on 0600 h). Separate cohorts of experimentally-naïve mice were used for each behavioral (and neuroendocrine) assay. The number of animals used in each experiment is shown in the corresponding table and figures legends. Ethanol doses were chosen to elicit an appropriate behavioral response based on previous studies [57].

With the exception of the voluntary ethanol consumption experiment, mice were given a 1 h acclimation period in the test room prior to testing, and apparatuses were cleaned with 70% v/v ethanol and dried between subjects.

2.3. Forced swim stress

Mice were stressed by being placed into a transparent Plexiglas cylinder (20 cm diameter) filled halfway with water for 10 min, as previously described [58]. Water temperature was 24±1 °C; in line with that typically used for studies employing the forced swim test as an assay for depression-related behavior and which likely produces a mild hypothermia [53]. A schematic representation of the procedure used to assess swim stress effects on acute sensitivity to ethanol is depicted in Fig. 1a, and on ethanol consumption in Fig. 7a. Unless otherwise indicated, mice were stressed daily for 0, 1, 3, or 14 days and then tested for ethanol-related behaviors 24 h later.

2.4. Behavioral and neuroendocrine responses to forced swim stress

To test for changes in depression-related behavior with repeated swim stress exposures, mice were observed for the presence/absence of immobility (cessation of limb movements except minor involuntary movements of the hind limbs) every

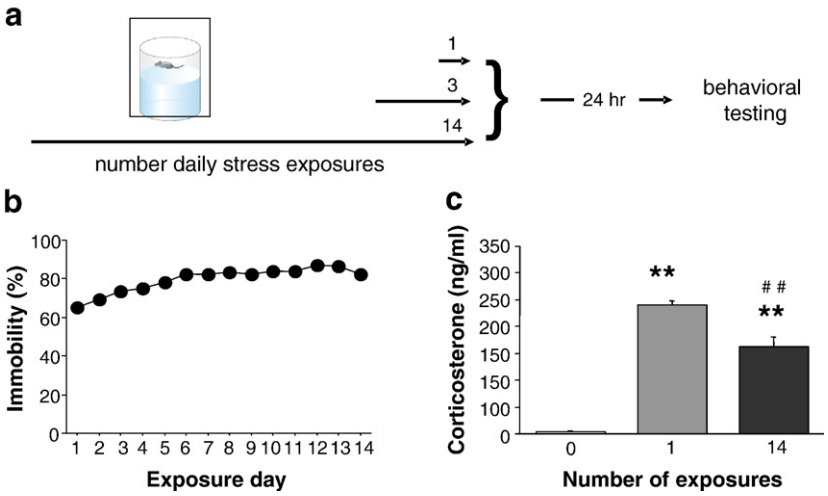

Fig. 1. Procedure for assessing effects of swim stress on ethanol-related behaviors, and behavioral and neuroendocrine responses to repeated swim stress. (a) Effects of swim stress on ethanol-related behaviors were measured 24 h after 0, 1, 3 or 14 daily exposures. (b) Repeated daily exposure to swim stress produced a progressive increase in percent time immobile, a measure of 'depression-related' behavior ($n=16$). (c) Plasma corticosterone levels were elevated following either 1 or 14 days of stress exposure and significantly greater following 1 than 14 exposures ($n=4$–7/stress condition). **$P<0.01$ vs. 0; ##$P<0.01$ vs. 1. Data in this figure as well as in Figs. 2 –7 are Means±SEM.