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Individual differences in elevated plus-maze exploration predicted higher ethanol consumption and preference in outbred mice

Amine Bahi *

Department of Anatomy, Tawam Medical Campus, CMHS, United Arab Emirates University, Al Ain, United Arab Emirates

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ABSTRACT

Psychiatric illnesses, such as anxiety, are highly comorbid with drug use disorders in general and alcohol abuse in particular. Unfortunately, the causal role of anxiety in ethanol addiction is still unclear. We asked the question whether high anxiety predicts predilection of mice to voluntarily consume more alcohol than water. In the current study, we used the voluntary alcohol intake in two bottle choice drinking paradigm to explore whether high anxiety predicts higher alcohol preference and intake in outbred Tuck-Ordinary "TO" mice. To this end, mice were tested for their anxiety-like behavior using the elevated plus maze, open field and the marble burying test prior to voluntary continuous access to increasing concentrations of alcohol solutions. To assess their taste discrimination, mice had access to saccharin and quinine solutions. Results showed that compared to low-anxious mice (LAM), high-anxious mice (HAM) showed greater consumption and preference for ethanol but not for saccharin and quinine suggesting alterations in the rewarding effects of alcohol. Taken together, these findings suggest a correlative link between trait anxiety and the behavioral responses to ethanol.

1. Introduction

The socioeconomic impact of anxiety and addiction disorders on both patients and the community is not negligible. For a person with such illnesses, quality of life is considerably reduced; professional, social, and personal relationships are all affected. Thus, the comorbidity of anxiety disorders and drug abuse has been investigated in a number of epidemiologic studies. Several reports showed positive correlation between anxiety and drug use disorders like ethanol (Forsyth et al., 2003; Staiger et al., 2011).

Briefly, and in relation to ethanol use disorders and alcoholism, it has been shown that high rates of anxiety disorders have been found in patients suffering from alcohol dependence suggesting that alcoholic subjects with anxiety represent a more severe subgroup of patients (Loas et al., 2002; Segui et al., 2001). A retrospective study indicated a strong relationship between bipolar disorders and alcohol abuse comorbidity in patients with social phobia (Hahesy et al., 2002; Perugi et al., 2002). Baseline and 4-year follow-up data from adolescents and young adults aged 14 to 24 years carried out in Munich has shown that social phobia significantly predicts the onsets of regular and hazardous use of alcohol. Also, panic attacks significantly predict the onsets of ethanol use and abuse as well as the persistence of combined abuse and dependence (Zimmermann et al., 2003). Hence, several more studies have shown positive correlation between increased-alcohol consumption and anxiety in humans (Brown et al., 1995; Dawson et al., 2005; Kuntsche et al., 2009; Linsky et al., 1985; Nesic and Duka, 2006; Stevens et al., 2008). In summary, the available data indicate that comorbid anxiety/alcohol use disorders may involve more complicated and severe approach when treating individuals presenting symptoms of one disorder.

Similar positive correlation between ethanol intake and anxiety-like behavior was reported also as in laboratory animals (Boyce-Rustay et al., 2007; Lopez et al., 2011; Ploj et al., 2003; Sanna et al., 2011; Thorsell et al., 2005). However, attempts to model the effects of stress-induced anxiety on ethanol intake have yielded contrasting results. For example, upon induced anxiety chronic but not acute stress exposure (foot shock), adolescent mice acquired significant ethanol-induced place preference (CPP) trained with 2 g/kg alcohol that did not produce CPP under non-stress conditions (Song et al., 2007). Also, exposure to a mild stressor produced an increase in ethanol consumption (Minnick et al., 1995). In rats, a robust increase in ethanol intake and preference was observed after one-day and 7 days chronic overcrowding anxiety stressed groups (Nagaraja and Jeganathan, 2003). In contrast, social defeat stress exposure significantly decreased alcohol intake in home cage drinking and rate of alcohol reinforcements in operant conditioning in rats (van Erp and Miczek, 2001). Also in mice, other results showed that swim stress produced a significant decrease in ethanol

Abbreviations: AA, Alko alcohol; ANA, Alko non-alcohol; CPP, Conditioned-Place Preference; EPM, Elevated plus maze; EtOH, Ethanol; HAD, High Alcohol Drinking; HAM, High Anxiety Mice; LAD, Low Alcohol Drinking; LAM, Low Anxiety Mice; MBT, Marble burying test; sNP, Sardinian Non-Preferring; sP, Sardinian Preferring; TO mice, Tuck-Ordinary mice.

* Department of Anatomy, College of Medicine & Health Sciences, United Arab Emirates University, PO Box 17666, Al Ain, United Arab Emirates. Tel.: +971 3 7137 516; fax: +971 3 7672 033.

E-mail address: amine.bahi@uaeu.ac.ae.

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consumption (Boyce-Rustay et al., 2008). More importantly, using the multivariate concentric square field (MCSF) test to provoke exploration and behaviors associated with risk assessment and risk taking in a novel environment, it has been reported very recently that Alko alcohol (AA) rats displayed higher risk taking and lower anxiety-like behavior compared to Alko non-alcohol (ANA) rats (Roman et al., 2012). In contrast, higher anxiety-related behavior in the open field EPM test was observed in Sardinian alcohol-preferring (sP) compared to non-preferring (sNP) rats (Roman et al., 2012) as well as in Indiana alcohol-preferring (P) compared to non-preferring (NP) rats (Pandey et al., 2005; Stewart et al., 1993). Finally, Hwang and co-workers have shown that P rats exhibited higher anxiety than NP rats (Hwang et al., 2004) but anxiety levels were similar between high alcohol-drinking (HAD) and low alcohol-drinking (HAD) rats (Badia-Elder et al., 2003; Hwang et al., 2004). Together, these data raise questions about anxiety-like behavior and the incentive motivation for alcohol in rodents.

However, the role of anxiety in prompting excessive alcohol consumption and preference has not been extensively evaluated. Using elevated plus maze test (EPM),open field and marble burying tests (MBT), high and low-anxious mice (HAM and LAM respectively) were identified and tested for their response to voluntary alcohol intake in a two-bottle choice drinking procedure.

2. Materials & methods

2.1. Animals

Tuck-Ordinary (TO) outbred mice were purchased from Harlan Olac (Bicester, UK) and bred in the local central animal facility of the College of Medicine and Health Sciences. Adult male mice (n = 50) weighing 25–30 g were housed in standard Plexiglas cages for one week before the experimental procedure started. All mice were kept under standard laboratory conditions (12/12 h light–dark cycle, lights off at 6 pm, 22 °C, 55% relative humidity) with free access to standard mouse chow diet (National Feed and Flour Production and Marketing Company LLC, Abu Dhabi, UAE) and tap water presented in two bottles. All animal care and use were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (publication no. 85-23, revised 1985). The procedures were approved by the local Institutional Animal Research Ethics Committee (approval no. A1-12). Efforts were made to minimize the number of animals used.

2.2. Drugs

For the two bottle choice drinking experiments, absolute ethanol obtained from Panreac Quimica SAU (Barcelona Spain) was diluted to 2.5%, 5%, 10% or 20% (*v*/*v*) solution in tap water. For taste neophobia discrimination, saccharin was prepared as a 0.03% or 0.06% (*w*/*v*) solution in tap water. Quinine hemisulfate salt monohydrate was prepared as a 30 or 60 μM solution in tap water. Both tastants were purchased from Sigma-Aldrich (MO, USA).

2.3. Elevated plus-maze (EPM) test and selection of high and low-anxious mice

The EPM test was carried out as described previously (Bahi and Dreyer, 2012b; Bahi et al., 2009). Briefly, the apparatus consists of four arms (6 cm wide, 40 cm long) and was elevated 40 cm above the floor. Two opposing arms were enclosed with high black-painted walls. The experiment was conducted for 5 min, with initial mouse placement facing an open arm. Each plus-maze trial was manually scored. The amount of time spent with head and forepaws on the open arm, closed arm of the maze as well as the number of entries into each arm was manually recorded. Consequently, the lower and upper quartiles of the population were selected as high- (HAM; n = 8) and low-anxious mice (LAM; n = 8). Thus, a total of 16 mice were selected to undergo the other anxiety-like behavior tests and voluntary alcohol consumption in a two-bottle choice setup.

2.4. Open field behavior

The open field was a 32 × 32-cm white Plexiglas arena, surrounded by 20-cm high walls. The floor of the arena was marked into 64 equal squares by black lines and the central sixteen squares were defined as the center area. Mice were put in the center of the arena, and allowed to explore freely for 5 min. Line-crossing (defined as at least three paws in a square) was used as measure of locomotor activity. The time spent in the center of the arena was manually recorded and used as measure of anxiety.

2.5. Marble burying test (MBT)

We used the MBT as described previously (Bahi and Dreyer, 2012b). Briefly, each mouse was placed in a cage containing 20 marbles, evenly spaced (1 cm apart) on top of bedding. After 15 min, the number of marbles buried and the total duration of digging bouts were manually recorded for each animal. A marble was considered buried if at least two-thirds of its surface area was covered in sawdust.

2.6. Two-bottle choice voluntary ethanol and saccharin/quinine consumption

This procedure was done as described previously (Bahi and Dreyer, 2012a, 2012c; Bahi et al., 2012). Briefly, mice were given a choice between ethanol (2.5%, *w*/*v*) and water and had continuous access to both 10 ml pipettes with fixed stainless steel drinking spouts. Ethanol concentration was increased from 2.5% to 20% (2.5%, 5%, 10%, and 20%, *w*/*v*) with 4-day access at each concentration. Every day, the fluid consumption was recorded and pipette sides switched. Mice were weighed every four days when ethanol solution was raised. One week after the ethanol self-administration procedure, the same mice were tested for saccharin (0.03% and 0.06%, *w*/*v*) and quinine (30 and 60 μM, *w*/*v*) consumption and preference using the same two-bottle choice protocol.

2.7. Data presentation and statistics

For the EPM, open field and MBT data were analyzed using a one-way ANOVA. For the two bottle choice drinking tests (ethanol, saccharin and quinine), data were analyzed by one-way repeated measures ANOVA with anxiety as the between subject factor (LAM, HAM) and concentration as the within subject factor. The α value was set at $p < 0.05$. None of the tested mice were excluded from the statistical analysis. All statistical analyses were performed with the software package SPSS (version 19.0).

3. Results

3.1. Anxiety-like behaviors in an elevated plus maze

To examine the effects of innate anxiety on ethanol-related behaviors, mice were subjected to an EPM test. As shown in Fig. 1, LAM and HAM cohorts were selected as the upper and lower quartiles, respectively, of a population, based on the percentage time spent in the EPM open arms. The scatter plots of the data from the original screen of the mice on the EPM are depicted in Fig. 1A. One way ANOVA revealed that HAM (n = 8), compared with LAM group (n = 8), showed reduced time spent on the open arm (OA) of the maze ($F_{(1,14)} = 758.811$, $p = 0.000$) (Fig. 1B). In addition, the percentage (Fig. 1C) and the number (Fig. 1D) of entries were significantly lower in HAM ($F_{(1,14)} = 10.124$, $p = 0.007$; $F_{(1,14)} = 26.640$, $p = 0.000$ respectively). Importantly, no significant changes were found in the number of closed arm entries ($F_{(1,14)} = 0.068$, $p = 0.798$) (Fig. 1E), indicating that locomotor activity