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Dose-dependent effects of alcohol administration on behavioral profiles in the MCSF test

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ABSTRACT

The acute effects of alcohol administration are age-, dose-, time- and task-dependent. Although generally considered to be a sedative drug, alcohol has both stimulatory and depressant effects on behavior, depending on dose and time. Alcohol-induced motor activating effects are consistently shown in mice but rarely demonstrated in adult, outbred rats using conventional behavioral tests. The aim of the present experiment was to study acute alcohol-induced effects on behavioral profiles in a more complex environment using the novel multivariate concentric square field™ (MCSF) test, designed for assessing different behaviors in the same trial including locomotor activity. Adult male Wistar rats (Sca:WI) were administered one intraperitoneal (i.p.) injection of alcohol (0.0 g/kg, 0.5 g/kg, 1.0 g/kg, or 1.5 g/kg) 5 min prior to the 30-min MCSF test. The two highest doses induced marked motor-suppressing effects. A significant interaction between group and time was found in general activity when comparing rats exposed to alcohol at 0.0 g/kg and 0.5 g/kg. In contrast to the 0.0 g/kg dose that increased the activity over time, animals administered the low dose (0.5 g/kg) demonstrated an initial high activity followed by a decline over time. No indications for acute alcohol-induced anxiolytic-like effects were found. The multivariate setting in the MCSF test appears to be sensitive for detecting motor-activating effects of low doses of alcohol as well as reduced locomotion at doses lower than in other behavioral tasks. The detection of subtle changes in behavior across time and dose is important for understanding alcohol-induced effects. This approach may be useful in evaluating alcohol doses that correspond to different degrees of intoxication in humans.

Introduction

There is little doubt that the pleasant subjective effects of alcohol reinforce drinking of alcoholic beverages and play a significant role in the development of alcohol use disorders (AUDs). Understanding the factors promoting vulnerability to problematic alcohol consumption and AUDs is important (Hendler, Ramchandani, Gilman, & Hommer, 2013; Koob & Volkow, 2010; Spanagel, 2009). One of these factors is the hedonic nature of the first experience with the drug. Individuals who perceive the drug as more rewarding and less aversive may be at higher risk for AUDs (Hendler et al., 2013; Schuckit, Smith, & Kalmijn, 2004).

Although generally considered to be a sedative or depressant drug, alcohol has a biphasic effect with both stimulatory and depressant effects on behavior depending on dose and time (Brabant, Guarnieri, & Quertemont, 2014; Lewis & June, 1990; Pohorecky, 1977). In rodents, alcohol-induced locomotor activation has been considered an index of the appetitive, rewarding effects of the drug (Brabant et al., 2014; Camarini et al., 2010), and there are studies indicating positive relationships between locomotor activation and alcohol preference (Waller, Murphy, McBride, Lumeng, & Li, 1986). However, the alcohol-induced acute motor activation appears to be context- (Lewis & June, 1990; Pohorecky, 1977) and age-dependent with more pronounced effects during adolescence (Acevedo, Pautassi, Spear, & Spear, 2013). In addition, while more consistently shown in mice (Brabant et al., 2014; Camarini et al., 2010; Quoilin, Didone, Tirelli, & Quertemont, 2012), the acute motor-activating effects of alcohol are rarely seen in adult outbred, non-selected rats (Brabant et al., 2014; Chuck, McLaughlin, Arizzi-LaFrance, Salamone, & Correa, 2006;

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Criswell et al., 1994; Linakis & Cunningham, 1979; Masur, Oliveira de Souza, & Zwicker, 1986). Another effect commonly reported for alcohol is anxiolysis, which for some individuals may drive drug use and contribute to the development of AUDs. Using conventional tests for interpretation of anxiety-like behavior, a number of studies have demonstrated anxiolytic properties of alcohol, both after acute administration as well as when voluntarily consumed (Hall, Huang, Fong, Pert, & Linnoila, 1998; Pohorecky, Patel, & Roberts, 1989; Spanagel et al., 1995).

The aim of the present study was to evaluate the acute effects of alcohol administration in rats in a more complex environment using the novel multivariate concentric square field™ (MCSF) test. The MCSF provides several areas for the animal to explore including sheltered, open, and elevated areas, a hole-board device, and areas with different illumination. The multivariate design of the MCSF test allows investigation of a broader behavioral profile including general activity, exploration, risk assessment, risk taking, and shelter-seeking behavior in a single trial (Meyerson, Augustsson, Berg, & Roman, 2006; Meyerson, Jurek, & Roman, 2013; Roman & Colombo, 2009). Compared to conventional behavioral tests, the animals have a free choice of where to stay in areas of different qualities in the MCSF test, which could give valuable information about acute alcohol-induced effects.

Material and methods

Animals and housing

Twenty-four outbred, adult male Wistar rats (Sca:WI; Scanbur BK AB, Sollentuna, Sweden) with a mean (±SEM) body weight of 534 ± 7 g were used. The rats were housed three per cage in transparent polysulfone cages (59 × 38 × 20 cm) containing wood chip bedding and paper sheets for enrichment purposes. The animals were maintained on standard pellet food (R36 Labfor; Lantmännen, Kimstad, Sweden) and water *ad libitum*. They were housed in a temperature- (22 ± 0.7 °C) and humidity-controlled (55 ± 7%) environment on a reversed 12-h light/dark cycle with lights off at 7:00 A.M. All animal experiments were approved by the Uppsala Animal Ethical Committee and followed the guidelines of the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS1998: 56) and the European Communities Council Directive (86/609/EEC).

Experimental procedures

To allow adjustments to the reversed light/dark cycle, the rats were undisturbed for 2 weeks after arrival from the supplier. The rats were handled during the week prior to testing. The handling procedure consisted of individual handling, weighing, and adaptation to the transportation bucket, which was used to take the animals from the home cage to the test arena. Ethanol (Solveco Ethanol A 96%; Solveco AB, Rosersberg, Sweden) was dissolved in physiological saline (15% v/v) and rats were intraperitoneally (i.p.) injected at doses of 0.0 g/kg ($n = 6$), 0.5 g/kg ($n = 6$), 1.0 g/kg ($n = 6$), or 1.5 g/kg ($n = 6$) in a maximum volume of 1.25 mL/100 g of body weight 5 min prior to the start of the MCSF test. The doses were chosen based on previous reports in the literature using a variety of tests for assessment of alcohol-induced effects (Bell, McKinzie, Murphy, & McBride, 2000; Brabant et al., 2014; Chuck et al., 2006; Criswell et al., 1994; Lê & Israel, 1994; Linakis & Cunningham, 1979; Masur et al., 1986).

The multivariate concentric square field™ (MCSF)

The MCSF test has been described in detail elsewhere (Meyerson et al., 2006, 2013; Roman & Colombo, 2009). The animal to be tested was placed in the center facing the wall between the center and bridge and allowed to explore the arena for 30 min. The MCSF test was carried out during the dark period of the light/dark cycle. After each session, the arena was wiped with 10% ethanol solution and allowed to dry before the next animal was tested. The light conditions (lx) in the MCSF arena were as follows: dark corner room: <1; center, corridors and hurdle: 10–20; slope: approximately 50; and bridge: 600–650.

The behavior was recorded by a video camera placed above the arena. The number of fecal boli, urinations, and number of head dips into the hole board on the hurdle was noted after each trial. The number of rearings and groomings was scored by direct observation from an adjacent room. A blinded observer scored the behavior manually using the program Score 3.3 (Soldis, Uppsala, Sweden). Latency (L, s) to first visit, and frequency (F) and duration (D, s) of visits to each zone were registered. The following parameters were calculated: duration per frequency (D/F, s), total activity (sum of all frequencies), total corridors (sum of frequencies, durations, and durations/frequency, respectively, to the corridors), percentage duration (%D), percentage frequency (%F), slope/bridge interval, and risk/shelter index (explained in the Supplementary Table). Ethovision version 2.3 (Noldus Information Technology Inc., Wageningen, The Netherlands) was used for automatic tracking of mean velocity (cm/s) and total distance (cm) traveled. An operational categorization of the various parameters generated from the MCSF with regard to function (i.e., general activity, exploratory activity, risk assessment, risk taking, and shelter seeking) is used in the interpretation of results. In addition, a rank-order procedure referred to as the trend analysis is used (Meyerson et al., 2013).

Statistical analyses

The nonparametric Kruskal–Wallis test followed by the Mann–Whitney U test was used for intergroup comparisons of specific descriptive behavioral parameters because most of the data did not show a normal distribution according to the Shapiro–Wilk's W test. When animals did not enter a zone, the latency measure was considered to be missing and occurrence of visits were analyzed using the Chi-square test. The Friedman test was used for analysis of total activity, distance moved, and rearing during the six 5-min periods in the MCSF, followed by the Wilcoxon matched-pairs test where appropriate.

A rank-order procedure (Meyerson et al., 2013) was used for analysis of performance in the MCSF. This analysis uses the scored parameters and ranks all individuals against each other. The rank values are then summed into a sum rank for the functional categories general activity (total activity, i.e., sum of all frequencies; frequency total corridors, i.e., sum of visits to the corridors, duration per frequency total corridors*, frequency center, distance arena), exploratory activity (duration total corridors*, center* and hurdle, rearing), risk assessment (frequency, duration, duration per frequency slope), risk taking (frequency, duration, duration per frequency bridge and central circle), and shelter seeking (frequency, duration, duration per frequency dark corner room). Values for parameters marked with * are inverted in order to reflect a correct meaning of the rank value. The results from the trend analysis were analyzed using Analysis of Variance (ANOVA) followed by the Fisher's Least Significant Difference (LSD) *post hoc* test, and analysis over time was performed using repeated-measures ANOVA followed by the Fisher's LSD *post hoc* test.

Differences were considered statistically significant at $p < 0.05$. Statistica 12.0 (StatSoft Inc., Tulsa, OK) was used for the statistical analyses.