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Research report

Alcohol hangover: Type and time-extension of motor function impairments

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HIGHLIGHTS

- Motor performance and exploratory activity are impaired during alcohol hangover.
- Ataxia and slow locomotion are observed for more than 10 h after hangover onset.
- Motor and exploratory impairments last between 16 and 20 h after hangover onset.

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ABSTRACT

Alcohol hangover is defined as the unpleasant next-day state following an evening of excessive alcohol consumption. Hangover begins when ethanol is absent in plasma and is characterized by physical and psychological symptoms. During hangover cognitive functions and subjective capacities are affected along with inefficiency, reduced productivity, absenteeism, driving impairments, poor academic achievement and reductions in motor coordination. The aim of this work was to study the type and length of motor and exploratory functions from the beginning to the end of the alcohol hangover. Male Swiss mice were injected i.p. either with saline (control group) or with ethanol (3.8 g/kg BW) (hangover group). Motor performance, walking deficiency, motor strength, locomotion and exploratory activity were evaluated at a basal point (ZT0) and every 2 h up to 20 h after blood alcohol levels were close to zero (hangover onset). Motor performance was 80% decreased at the onset of hangover ($p < 0.001$). Hangover mice exhibited a reduced motor performance during the next 16 h ($p < 0.01$). Motor function was recovered 20 h after hangover onset. Hangover mice displayed walking deficiencies from the beginning to 16 h after hangover onset ($p < 0.05$). Moreover, mice suffering from a hangover, exhibited a significant decrease in neuromuscular strength during 16 h ($p < 0.001$). Averaged speed and total distance traveled in the open field test and the exploratory activity on T-maze and hole board tests were reduced during 16 h after hangover onset ($p < 0.05$). Our findings demonstrate a time-extension between 16 to 20 h for hangover motor and exploratory impairments. As a whole, this study shows the long lasting effects of alcohol hangover.

1. Introduction

Alcohol hangover (AH) is a physiological state described as the unpleasant next-day effect following an evening of excessive alcohol consumption in humans [35]. Hangover begins when ethanol (EtOH) is absent in plasma and is characterized by a cluster of physical and psychological symptoms which include headaches, nausea, diarrhea, fatigue and tremors combined with decreased occupational, cognitive and/or visuospatial skills [16,18,39] with substantial individual, social and economical consequences [6]. It is widely recognized that during AH cognitive functions and subjective capacities are affected along with inefficiency, reduced productivity and even absenteeism in the workplace, driving impairments, poor academic achievement and reductions in motor coordination [28,36]. In addition, Rohsenow et al. [29] has verified that a poor neurocognitive performance which affects safety-sensitive occupations correlates with the next morning of heavy drinking events. Despite this, it is very difficult to clearly define the symptoms of the hangover experience. In fact, early studies indicate that even when general feeling is unpleasant, human behavior and motor functions are more or less uncontrolled [40]. Moreover, it was confirmed that AH compromises the psychomotor performance being fatigue the most

Abbreviations: AH, alcohol hangover; CNS, Central Nervous System; FSL, forelimb stride length; HSL, hindlimb stride length; MD, maximum difference of stride length; ZT, Zeitgeber time.

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commonly reported sign which could persist 12 h after consumption [21].

In the case of experimental animals, studies on AH have been developed in order to provide insights into the physiological and behavioral changes that occur in the period immediately after ethanol intoxication [12,13]. Indeed, hypo-activity [10], fluctuations in body temperature, anxiety-like behavior [42], reduced wheel running activity and pain perception impairments were demonstrated to take place during the hangover state [3,33]. Other researchers reported a decreased in locomotor activity during AH in adult rodents tested on the elevated plus maze [20]. Likewise, we have previously demonstrated reduction in motor performance at the beginning of AH in mice [17] establishing also an association between this motor impairments with changes in brain cortex energetic metabolism [4]. Together with this, previous studies confirmed the negative after-effect of acute ethanol intoxication in open field locomotion and wheel running activity a day after drug exposure [31]. Also, Gauvin et al. [14] have shown that rats injected intraperitoneally (i.p.) with high doses of ethanol (3–4 g/kg) displayed an anxiety-like behavior when tested 9–18 h after acute ethanol challenge. Moreover, some reports have demonstrated that, 18 h after the acute administration of EtOH (4 g/kg, i.p.), adult male rats present a reduced exploration into the open arms in the elevated plus maze [9]. The evidence presented here together with the convergent findings from naturalistic methodology in humans and the experimental investigations firmly suggest motor and effective impairments during alcohol induced hangover.

According to Penning et al. [27] there is no hypothetical model explaining the pathology of AH or an effective and available animal representation to study this state. Moreover, it was well established that the main reason for the absence of an effective cure is that limited research has been dedicated to elucidate the pathology of AH [19]. Although several studies have been performed to evaluate locomotion and other motor functions during AH in experimental animals, the majority of them focused only on the beginning or a middle time-point. Even though the mechanisms are not well understood, the aftermath of alcohol use may also adversely affect performance for many hours after consumption. Furthermore, to our knowledge there are no previous researches which considered the type and length of motor impairments together with a possible influence of light changes during a complete episode of AH throughout a day. Researchers specialized in hangover effects state that assessing the duration for hangover symptoms could provide important descriptive information, such as an indicator of the burden imposed by hangovers in daily life or the period of risk for "hair-of-the-dog" drinking [37]. This last refers to the situation by which alcohol is consumed with the aim of lessening the effects of a hangover. Taking all together into account, the aim of this work was to study different kind of behavioral parameters from the beginning to the end of the AH in mice and thus establish the duration of the possible motor function impairments.

2. Materials and methods

2.1. Animals

A total of 120 from three cohorts of male Swiss mice (*Mus musculus*) weighing 30–40 g were acquired from the School of Pharmacy and Biochemistry, Universidad de Buenos Aires, and housed in a soundproof room under conditions of controlled temperature (22 ± 2 °C) and humidity, with a 12-h light/dark cycle. Standard rat chow and tap water were provided *ad libitum*.

Animal handling, treatment and experimental procedures were reviewed in accordance with the guidelines of the National Institutes of Health (NIH) and with Regulation 6344/96 of Argentina's National Drug, Food and Medical Technology Administration (ANMAT). Moreover, the present study had the legal ethical accreditation from Ethics Committee for Laboratory Animal Handling of the School of Medicine from Universidad de Buenos Aires where the protocol was performed. All efforts were made to minimize suffering and reduce the number of animals used.

Table 1
Blood alcohol concentration during and after EtOH treatment.

Time after i.p. injection (m)	BAC (mg/dl)
60	318.15 ± 15.33
180	246.21 ± 14.64**
360	9.67 ± 1.81***

Blood alcohol concentration (BAC) in male Swiss mice was measured 60, 180 and 360 min after acute i.p. ethanol injection. Values are expressed as mean ± SEM ($n = 9$ each group). Independent samples t-test.
** $p < 0.01$ significantly different from BAC at 60 m.
*** $p < 0.001$ significantly different from BAC at 60 m.

2.2. Experimental procedure

Animals received i.p. injections of 15% EtOH at a dose of 3.8 g/kg. Ethanol dose was previously applied in alcohol-induced hangover animal models [3,10]. Control mice received saline i.p. injections. Three mice from each group were decapitated 60, 180 or 360 min after the injection. Blood was collected from the trunk and blood alcohol concentration (BAC) was measured by gas chromatography (Hospital Británico, Buenos Aires, Argentina) to determine the animals' response to ethanol and the onset of hangover. Experiments were conducted in the morning (9:00 a.m.). The criteria used to establish onset of alcohol hangover was when BAC was less than or equal to 10% of the maximum value reached (Table 1). Behavioral tests were carried out at a basal point that matched with lights onset (ZT0) and every 2 h up to 20 h after alcohol hangover onset (ZT3 of the following day) (see Fig. 1). Specific time points were selected to evaluate different behavioral parameters an hour before and after lights turning on and off. Motor performance, walking deficiency, motor strength, locomotion and exploratory activity were evaluated using a battery of different behavioral tests. During experimental procedures, test boxes or the apparatus used for behavioral studies were cleaned with 10% EtOH after every individual test session to prevent the next mouse from being influenced by the odors deposited in the urine and feces of the previous mouse. Testing was conducted during the mouse's normal lights-on sleeping time.

2.3. Behavioral assessments

2.3.1. Tightrope test

Motor coordination was evaluated with a modified tightrope test [2]. Briefly, the procedure consisted in placing the animal on the middle of a 60 cm long horizontal rope suspended 30 cm above the floor and time was recorded until the animal either reached the end of the rope or fell down during a period of 60 s. A score was assigned accordingly: animals reaching the end of the rope in ≤6 s were given 1 point and an additional point was given for every additional 6 s needed to complete the test. Animals that stayed on the rope for 60 sec without reaching the end were given 11 points. When mice fell down, while test was running, 11 points were assigned and 1 extra point was added for every 6 s before the test ending time (60 s). The test evaluates the motor performance of the animal as a mean of its intrinsic neuromuscular coordination. For this work, results were shown as a percentage of the motor performance which was calculated considering the maximum score for the test (20 points) and the score reached for each animal.

2.3.2. Footprint pattern

Ataxia and gait abnormalities were studied by mice' footprint pattern [5]. The forepaws and hindpaws were dipped in red and blue nontoxic paint respectively to maximize the sensitivity of the footprint analysis. The mouse was then placed at the brightly lit end of a tunnel, which was dark at its far end. The bottom surface of the tunnel was lined with white paper. Tunnel dimensions used were 10 cm wide × 50 cm long × 10 cm high. The mouse walked down the tunnel, leaving a set of red and blue footprints on the white paper. The paper was then removed and the footprint pattern analyzed. Forelimb and hindlimb stride length (FSL and HSL respectively), right and left overlap and the maximum difference in stride length (MD) provide measures of the ability of the mouse to walk in a straight line, with regular, even steps. Particularly, stride length is the mean of the forelimb or hindlimb strides, overlap is the mean of the right and left overlaps and the maximum difference in stride length is the distance of the shortest stride subtracted from the distance of the longest stride. Ataxic gait is represented by highly variable stride length, an increase in overlap distances and over the variability in stride length [1].

2.3.3. Hanging wire

Neuromuscular abnormalities were detected by the evaluation of balance and grip strength in a hanging wire [30]. A standard wire cage lid was used. The perimeter was masked by duct tape to prevent the mice from walking off the edge. The hanging wire test was performed by placing the mouse on the top of a wire cage lid. The lid was lightly shaken three times to cause the mouse to grip the wires, and then the lid was turned upside down. The upside-down lid was hold at a height approximately 20 cm above the cage litter. The latency to fall off the wire lid was quantified. Normal mice can hang upside down for at least one minute. A 60-s cut-off time was used for the every test session.