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Research report

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

Analía G. Karadayian*, Rodolfo A. Cutrera

Laboratory of Neurobiology and Rhythms, Department of Physiology, School of Medicine, Universidad de Buenos Aires, Buenos Aires, Argentina

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.

* Corresponding author at: Laboratory of Neurobiology and Rhythms, Department of Physiology, School of Medicine, Universidad de Buenos Aires, Paraguay 2155, C1121ABG, Buenos Aires, Argentina. Tel.: +5411 4950 9500.
E-mail addresses: analiakaradayian@conicet.gov.ar, analia11@hotmail.com (A.G. Karadayian).

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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.

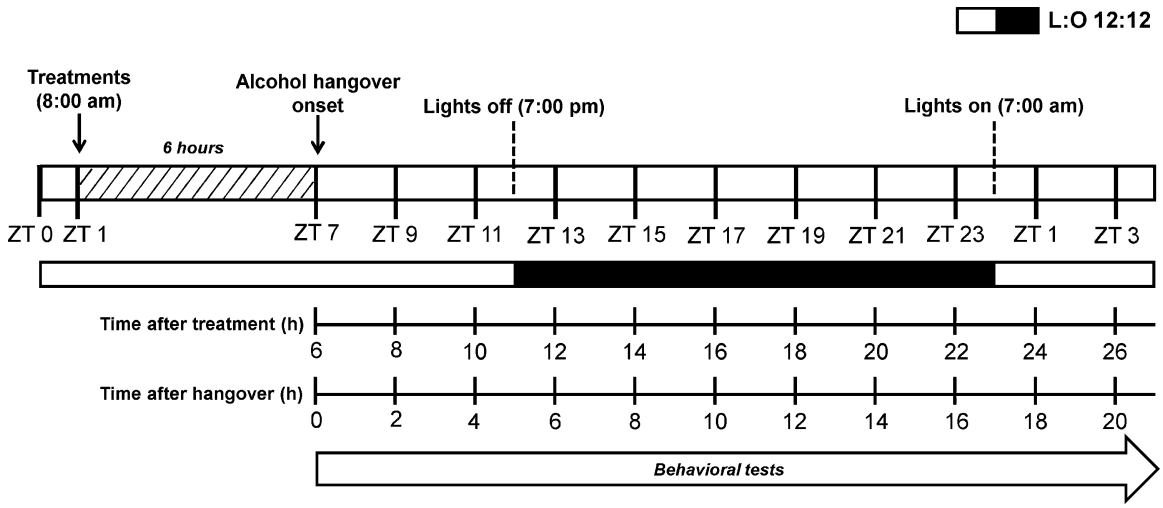

Fig. 1. Timeline and experiments. Male mice received intraperitoneal treatment with saline or ethanol at a dose of 3.8 mg/kg or an equivalent of normal saline at 8:00 am. Behavioral tests were performed before and 6 h after treatment when alcohol hangover began. ZT: Zeitgeber time; ZT12: 7:00 pm.

2.3.4. Open field test

Motor function and locomotion were evaluated by the open field test [8]. The test box consisted of a 60 × 60 cm square arena surrounded by a 50 cm high wall divided in two zones: center (30% of the entire area) and periphery. The apparatus (made of Plexiglas) was elevated 80 cm off the floor level. Mice were individually tested in the open field during a 5 min session. At the onset of the each session, mice were placed at the center of the apparatus and its movement throughout the duration of each trial was recorded and analyzed by the video tracking system ANY Maze (Stoelting Co., Wood Dale, Illinois). For the purpose of this work, open field activity was studied by the total distance traveled (m) and the averaged speed (m/s) of active walking, excluding periods of rest.

2.3.5. T-maze exploration

Spontaneous exploratory behavior was tested in a T-shaped wooden maze (110 cm × 60 cm of transverse and longitudinal arms, respectively) as previously described [24]. The mouse was placed on the base of the longitudinal arm and it was allowed to freely explore the maze for 20 s. The proportion of animals that reach three arms' intersection with both hind legs was evaluated [15,23,24].

2.3.6. Hole board exploration

Exploratory activity was assessed by the hole board test [32], 1998). The test apparatus consisted in a black wooden platform (60 cm × 60 cm) raised to a height of 10 cm from the floor of a transparent Plexiglas box (60 cm × 60 cm × 50 cm). The platform consisted of 16 equivalent circular holes (2.5 cm diameter). Mice were individually placed in the center and their behavior was recorded for 5 min. Measures included the latency to the first head poke (s) and the number of head pokes. Mice behavior was recorded and analyzed by the video tracking system ANY Maze (Stoelting Co., Wood Dale, Illinois).

2.4. Statistical analysis

Results are presented as means ± SEM. Before each analysis, test variables were checked for normality, so all data were evaluated by the Kolmogorov–Smirnov test to follow a posterior parametric or nonparametric statistical analysis. Data obtain from behavioral test were analyzed using the unpaired independent Student's *t*-test to analyze the significance of differences between hangover and control groups. In-group differences were examined by repeated-measures two-factor ANOVA. In all the cases, the statistical software used was SPSS (version 13.0) and a difference was considered statistically significant when $p < 0.05$. The proportions of success obtained by the T-maze test were analyzed by the Chi-Square (χ^2) test.

3. Results

Different behavioral tests were assessed to study motor functions during hangover.

3.1. Motor performance by the tightrope test (Fig. 2)

At the onset of hangover (ZT7), mice average motor performance was almost 80% lower compared either with controls ($p < 0.001$) or

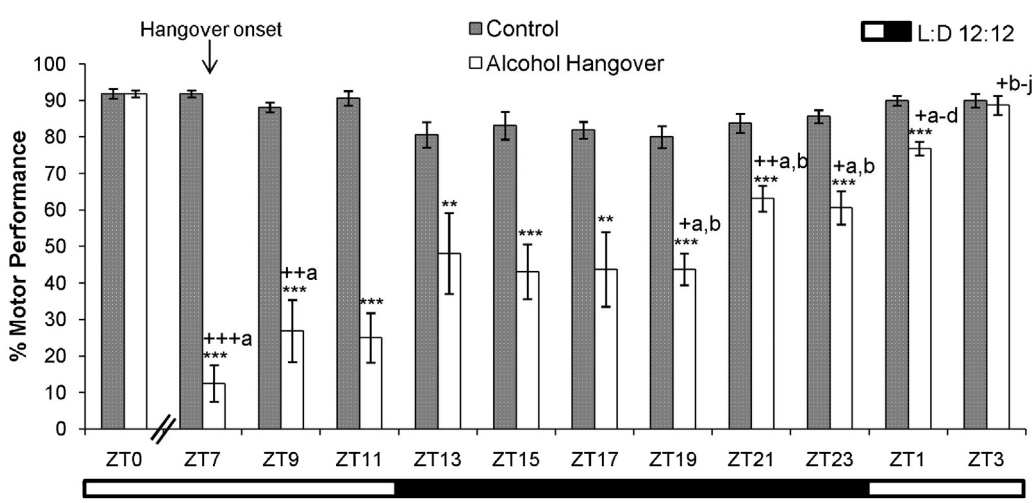

Fig. 2. Motor performance on the tightrope test during alcohol hangover. Values are expressed as mean ± SEM ($n = 10$ each group). ZT: Zeitgeber time; L:D; light:dark. Unpaired independent samples Student's *t*-test was used ($^{**}p < 0.01$, $^{***}p < 0.001$) for intergroup differences. Repeated-measures two-factor ANOVA was used ($^{++}p < 0.01$, $^{+++}p < 0.001$) for in-group difference. Letters indicate the time point of the comparison as follows: a: ZT0, b: ZT7, c: ZT9, d: ZT11, e: ZT13, f: ZT15, g: ZT17, h: ZT19, i: ZT21, j: ZT23 and k: ZT1. Bars shading indicate group: gray, control; white, alcohol hangover.

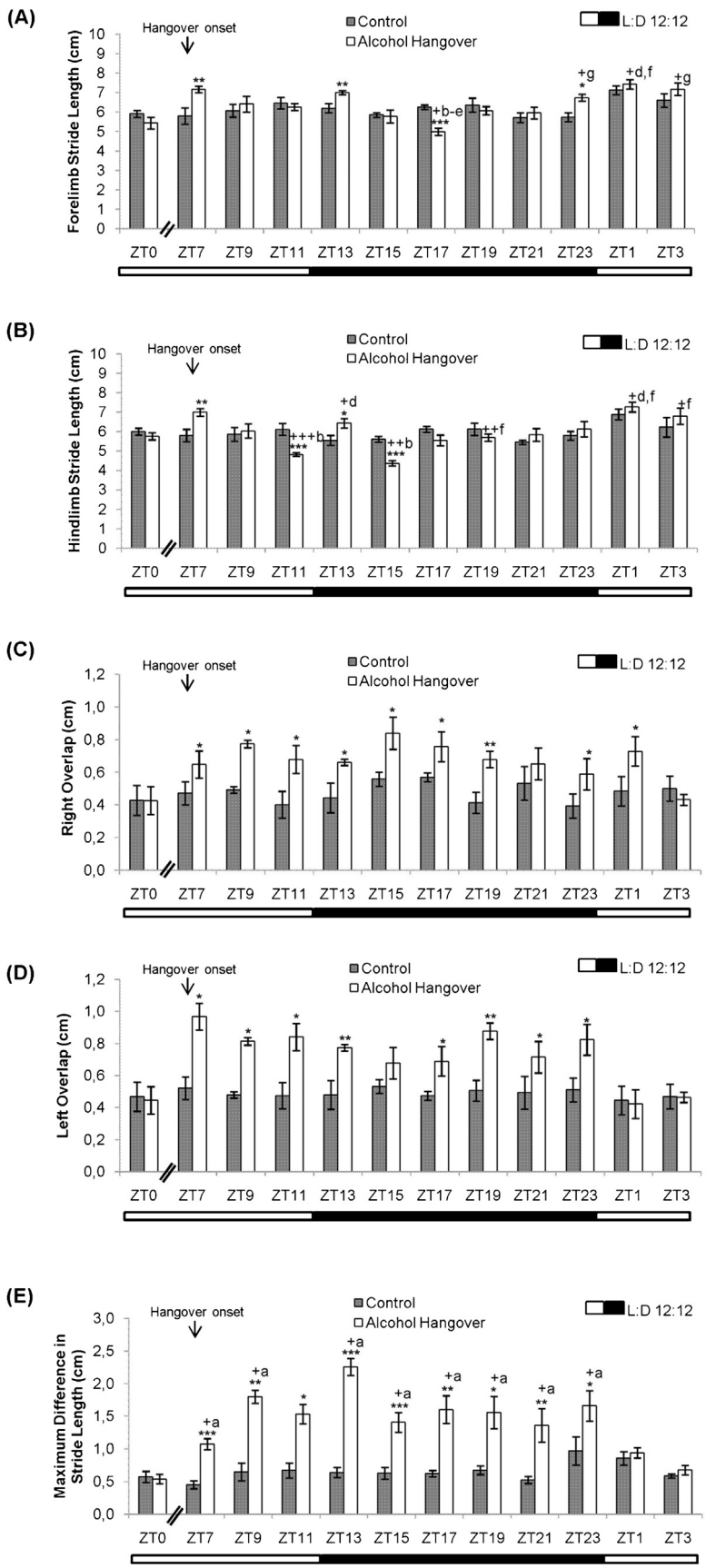

Fig. 3. Footprint pattern during alcohol hangover. Values are expressed as mean ± SEM ($n = 10$ each group). ZT: Zeitgeber time; L:D; light:dark. Student's *t*-test was used for intergroup differences ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Repeated-measures two-factor ANOVA was used for in-group difference ($^{+}p < 0.05$, $^{++}p < 0.01$; $^{+++}p < 0.001$). (A) Forelimb stride length, (B) hindlimb stride length, (C) right overlap, (D) left overlap and (E) maximum difference in stride length. Letters indicate the time point of the comparison as follows: a: ZT0; b: ZT7; c: ZT9; d: ZT11; e: ZT13; f: ZT15; g: ZT17; h: ZT19; i: ZT21; j: ZT23 and k: ZT1. Bars shading indicate group: gray, control; white, alcohol hangover.

its basal point (ZT0) ($p < 0.001$). During the next 16 h, hangover mice exhibited a reduced motor performance compared with controls ($p < 0.01$ for ZT13 and ZT17; $p < 0.001$ for ZT9, ZT11, ZT15 and ZT19 to ZT1). Motor coordination was not significantly different from control mice 20 h after hangover onset (ZT3). Control mice did not show any significant differences in motor performance during the evaluation period (20 h).

3.2. Walking deficiency by footprint pattern analysis (Figs. 3 and 4)

At the beginning of alcohol hangover, animals exhibited an increase in forelimb (FSL) and hindlimb stride length (HSL) compared with controls ($p < 0.01$). Also, FSL showed increases at ZT13 and ZT23 compared with controls ($p < 0.05$) (Fig. 3A). No differences were observed at ZT9, ZT11, ZT15, ZT19 and ZT21 in FSL. A significant increment was observed in hangover mice ZT1 and ZT3 ($p < 0.05$, in-group differences). At ZT17, FSL was significantly reduced in hangover group compared with controls ($p < 0.001$). Hangover mice exhibited a significant reduction in HSL at ZT11 and ZT15 compared with controls or same group at the onset of alcohol hangover ($p < 0.001$, Fig. 3B). At the end of evaluation (ZT1 and ZT3), HSL was significantly higher in hangover mice compared with same group at ZT11 and ZT15). Overlap was also measured along footprint pattern as the distance between right and left footprint (Fig. 3C and D). Hangover mice presented a significant increment in right and left overlap from ZT7 to ZT23 ($p < 0.05$). No significant differences were observed at ZT3 compared with control mice for both footprint overlap. The maximum difference in stride length (MD) was calculated as the distance of the shortest stride subtracted from the distance of the longest stride (Fig. 3E). At the onset of alcohol hangover, mice showed a significant increase in MD compared with controls ($p < 0.001$). This increment was presented along the evaluation up to ZT23. No significant differences were observed at ZT1 and ZT3 in hangover animals. Control group did not showed any significant differences in stride length across the experiment.

Representative images of footprint patterns from control and hangover groups at ZT7 (6 h after treatment) are presented in Fig. 4. Control mice had a narrow-based stance with steady close proximity forelimb (red color) and hindlimb footprints (blue color). In contrast, hangover mice footprints were featured with markedly wide-based stance, variable stride lengths and separated forelimb and hindlimb prints.

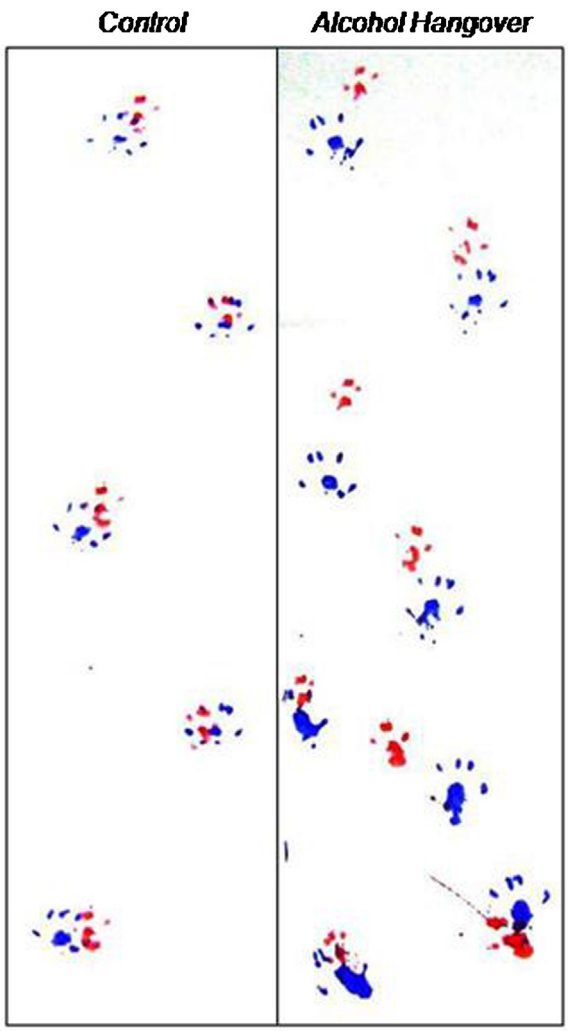

Fig. 4. Footprint pattern during alcohol hangover. Representative images of footprint pattern from control and hangover groups at ZT7 (6 h after treatment). Red and blue footprints correspond to forelimbs and hindlimbs respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Balance and grip strength by the hanging wire (Fig. 5)

Animals exhibited changes in neuromuscular strength during alcohol hangover. At the onset of this state, mice showed a significant reduction of neuromuscular strength given by a decrease in the latency of fall after turning the cage compared with controls ($p < 0.001$). This decrease is kept throughout the next 16 h after the onset of hangover. Control mice did not show any change across the experiment. The complete recovery of neuromuscular strength in hangover mice was evidenced at ZT1 (18 h after hangover onset).

3.4. Motor function and locomotion by the open field test (Fig. 6)

Different changes are observed in the distance traveled (Fig. 6A) and average speed (Fig. 6B) throughout the whole experiment. Both motor parameters significant decreased at ZT7 ($p < 0.001$), ZT9 ($p < 0.001$) and ZT11 ($p < 0.05$) in hangover mice compared with controls. During the night period, hangover mice exhibited a slight increase in the distance traveled and average speed. In the same way, control animals presented an increase in both variables being significantly different from what was achieved by hangover mice ($p < 0.05$). No significant differences were observed between controls and hangover mice from ZT23 and ZT1 for distance traveled and average speed respectively.

3.5. Spontaneous exploratory activity by the T-maze test (Table 2)

At alcohol hangover onset, mice showed a 62.5% decrease in the proportion of mice that successfully completed the trial compared with controls ($\chi^2 = 2.25$, df = 1, $p = 0.043$). Hangover animals exhibited a reduced exploratory activity during the behavioral evaluation that ranged between 50% and 87% ($p < 0.05$ compared with controls). At ZT3 no significant differences were observed between control and hangover groups ($\chi^2 = 2.24$, df = 1, $p = 0.134$).

3.6. Exploratory tendency by the hole board test (Fig. 7)

The latency to first head poke (Fig. 7A) and the number of head pokes (Fig. 7B) by control and ethanol-treated mice were assessed by the hole board test in order to evaluate the exploratory tendency during the alcohol hangover. Control mice exhibited a significant reduced latency to the first head poke during the night period between ZT15 and ZT21 compared with the early hours of the light period ($p < 0.05$, in-group differences). Hangover mice showed

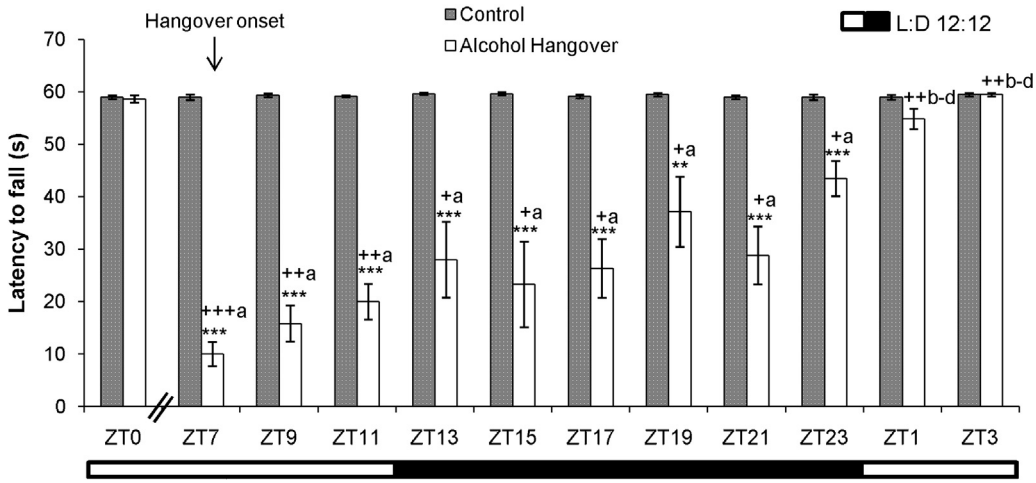

Fig. 5. Motor strength during alcohol hangover. Values expressed as mean ± SEM ($n = 10$ each group) represent the latency to fall (s) after turning the cage. ZT: Zeitgeber time; L:D; light:dark. Student's *t*-test was used for intergroup differences ($^{**}p < 0.01$, $^{***}p < 0.001$). Repeated-measures two-factor ANOVA was used for in-group difference ($^{++}p < 0.01$; $^{+++}p < 0.001$). Letters indicate the time point of the comparison as follows: a: ZT0; b: ZT7; c: ZT9; d: ZT11; e: ZT13; f: ZT15; g: ZT17; h: ZT19; i: ZT21; j: ZT23 and k: ZT1. Bars shading indicate group: gray, control; white, alcohol hangover.

an increased in the latency to first head poke from ZT7 to ZT23 ($p < 0.001$ compared with controls). No significant differences were observed between controls and hangover mice at ZT1 and ZT3, 18 and 20 h after hangover onset respectively. In addition to this, the number of head pokes was found to be significantly reduced in hangover mice compared with controls ($p < 0.01$). In this case, saline-treated animals did not display any significant in-group difference throughout the evaluation time. Different from what was observed for the latency to first head poke, ethanol-treated mice showed no difference in the number of head pokes compared with control at ZT23, 16 h after hangover onset.

4. Discussion

It is well known that the abuse of alcohol consumption, either acute or chronic, has a variety of serious consequences on health. The different effects are wide-spread, altering numerous physiological, endocrine and behavioral functions. However, less is known about a state that exists after excessive alcohol consumption in a short time period: the AH. The key finding to emerge from this study is that AH is a transient pathophysiological state that compromises exploratory and motor function impairments for at least 18 h from the time alcohol disappears from blood.

Table 2
Spontaneous exploratory activity during alcohol hangover.

Time point	% of Success		χ^2 (df)	*p*
	Control	Hangover		
ZT 0	100.0	100.0	7,64 (1)	0.054
ZT 7	100.0	37.5	2,25 (1)	0.043
ZT 9	62.5	37.5	12,30 (1)	0.004
ZT 11	87.5	75.0	6,25 (1)	0.012
ZT 13	100.0	50.0	4,00 (1)	0.046
ZT 15	100.0	75.0	9,00 (1)	0.003
ZT 17	100.0	87.5	12,25 (1)	0.000
ZT 19	100.0	75.0	9,00 (1)	0.003
ZT 21	100.0	75.0	9,00 (1)	0.003
ZT 23	100.0	87.5	12,25 (1)	0.000
ZT 1	100.0	87.5	12,00 (1)	0.000
ZT 3	100.0	100.0	2,24 (1)	0.134

Spontaneous exploratory activity was evaluated by T-maze test as described in Section 2. Values represent % of success achieved by male mice from control and hangover groups. Comparisons by Chi-square (χ^2) test, difference statistically significant when $p < 0.05$.

As previously observed, motor coordination decreases in male mice during ethanol hangover together with changes in brain cortex energetic metabolism [4,17]. In humans, it is well known that hangover presents several unpleasant physical and psychological symptoms [18,39]. However, there are no previous studies which considered the sort and length of motor impairments. In order to establish the type and time extension of exploratory and motor signs of AH, different behavioral parameters were studied at multiple times during 26 h after ethanol exposure. Experimental protocol was designed specifically to evaluate the possible changes due to both AH and the combined effects of light changes (see Section 2.2.).

Animals suffered from AH exhibited a significant reduction in the neuromuscular coordination which took place up to 20 h after hangover onset. York and Regan [41] demonstrated that 6, 12 and 16 h after an alcohol intoxication episode, hypothermia, free operant activity and motor performance were impaired in female Long-Evans hooded rats. Together with this, Ling et al. [22] reported the impact of AH on driving and flying where motor coordination is one of the most affected skills together with others like bad cognitive performance, poor sleep quality and concentration problems. Recently, Penning et al. [26] affirmed that reductions in motor coordination are observed during AH. In addition to impairments in motor coordination, it was established that BAC higher than 100 mg/dl provokes ataxia symptoms [38]. The results obtained from footprint test showed a significant reduction both for hindlimb and forelimb stride length at the onset of AH in our mice. Moreover, animals presented a significant loss of gait stability and walking deficiencies for 18 h after AH onset. Likewise, hangover mice experienced a 60–75% significant deficit in motor strength during 16 h after AH onset.

Results from open field test showed a significant decrease in the distance and averaged speed from the onset of AH to 22 h after exposure. As expected, control mice exhibited an increase in the distance and averaged speed in the open field test during the night period [11]. Moreover, an increment in locomotion was observed during the night period in the hangover group; however, this was still significant lower than controls. Thus, locomotor activity was slowed during AH. This result is in agreement with [20] who demonstrated a decrease in locomotor activity during AH evaluated by the elevated-plus maze and open field test. In addition, [31] observed a 24 h after-effect in rats by an increase in body temperature and vocalizations and a decrease in ambulation in the open field test. This confirmed and extended previous

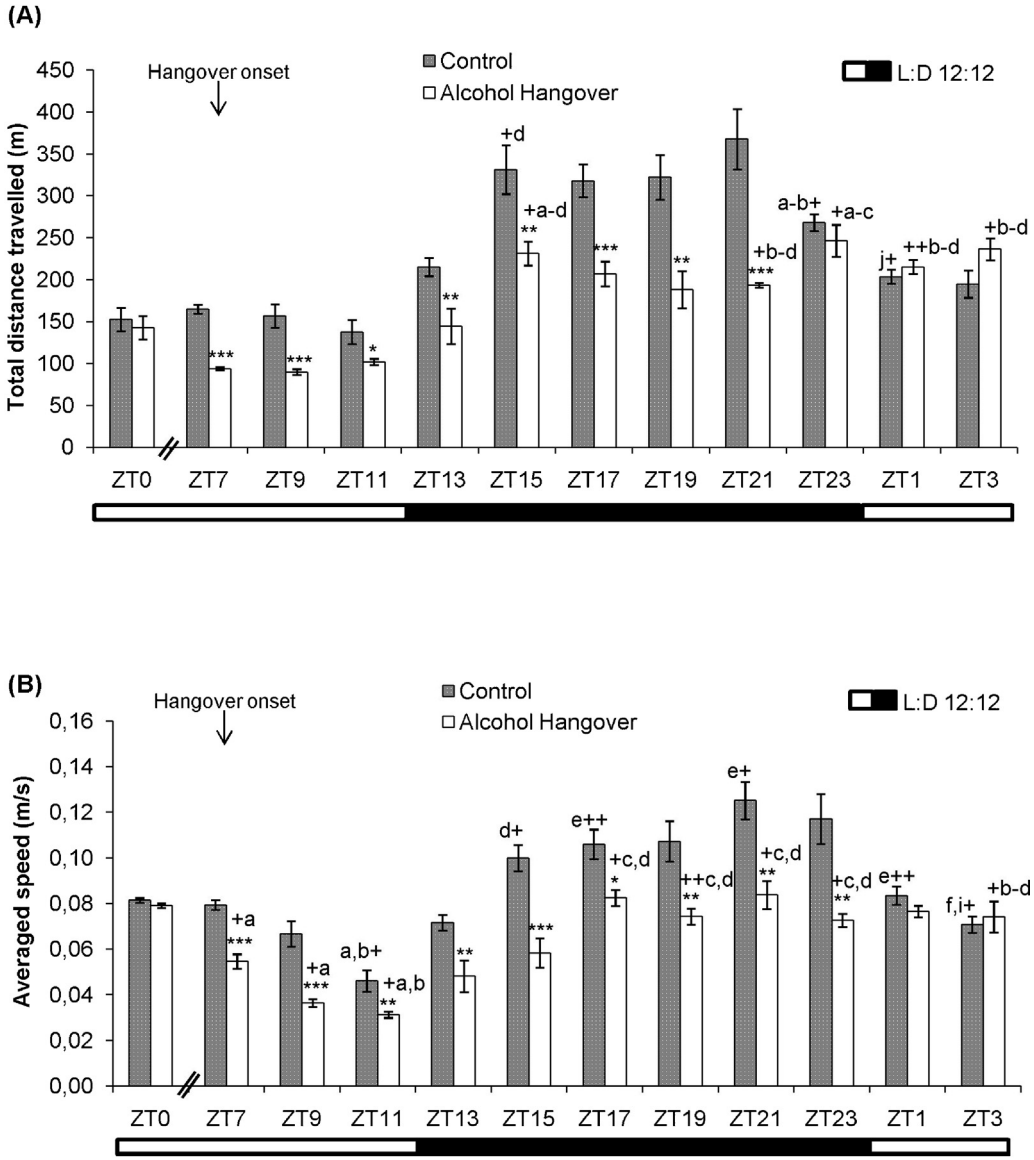

Fig. 6. Open field locomotion during alcohol hangover. Values are expressed as mean ± SEM ($n = 10$ each group). ZT: Zeitgeber time; L:D; light:dark. Student's t-test was used for intergroup differences ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Repeated-measures two-factor ANOVA was used for in-group difference ($^{+}p < 0.05$; $^{++}p < 0.01$). (A) Total distance traveled (cm) and (B) averaged speed (m/s). Letters indicate the time point of the comparison as follows: a: ZT0; b: ZT7; c: ZT9; d: ZT11; e: ZT13; f: ZT15; g: ZT17; h: ZT19; i: ZT21; j: ZT23 and k: ZT1. Bars shading indicate group: gray, control; white, alcohol hangover.

findings of after-effects of acute alcohol intoxication in rats with a time course similar to hangover in humans. When exploratory activity was examined a 62.5% decrease of spontaneous exploration together with a significant decrease in the exploratory latencies and hole-visits was observed at the onset of the hangover. The impairments in exploration lasted for 18 h. Although this last result could be interpreted as a sign of apathetic behavior seen in humans during the hangover, the decrease of exploratory activity may be due to two factors: the reduction in locomotion as observed in the open field test or a decrease in motivation. If the latter, it should be necessary to evaluate possible changes in the affective behavior during AH; however, this is beyond the scope of this paper and will probably be evaluated in a future work.

One of the most important aspects in the study of the alcohol hangover is the duration of the ethanol after-effects. There are few assessments of AH extension. One of the first studies conducted in healthy volunteers was done by [40] who demonstrated that the maximal hangover occurred 12–14 h after the initiation of drinking and it could last for at least 20 h. This study, however, was assessed taking into account the metabolic effects due to alcohol oxidation and the volunteers subjective symptoms. What is more, the study of Ylikarhi et al. [40] was designed considering AH onset when BAC started to decline. At the moment, a number of studies and reviews agree that hangover starts when BAC is close to zero because otherwise it would be probably to overlap the effects due to alcohol intoxication with the real signs of alcohol hangover [25]. Here, we observed the manifestation of motor and exploratory impairments as a result of a longer lasting effect of alcohol that persists beyond the metabolic cycle of the substance or the light–dark period. Our results indicate that further than metabolic and hormonal changes due to alcohol oxidation, hangover impairs various behavioral parameters which could be probable attributable to at least two facts: the pharmacological effect of alcohol or the immune activity both of them acting on the Central Nervous System (CNS). Supporting the first hypothesis, our previous results demonstrated that hangover caused a severe mitochondrial dysfunction in brain cortex at least at the onset of this state [4]. On the other hand, current scientific evidences propose that changes in

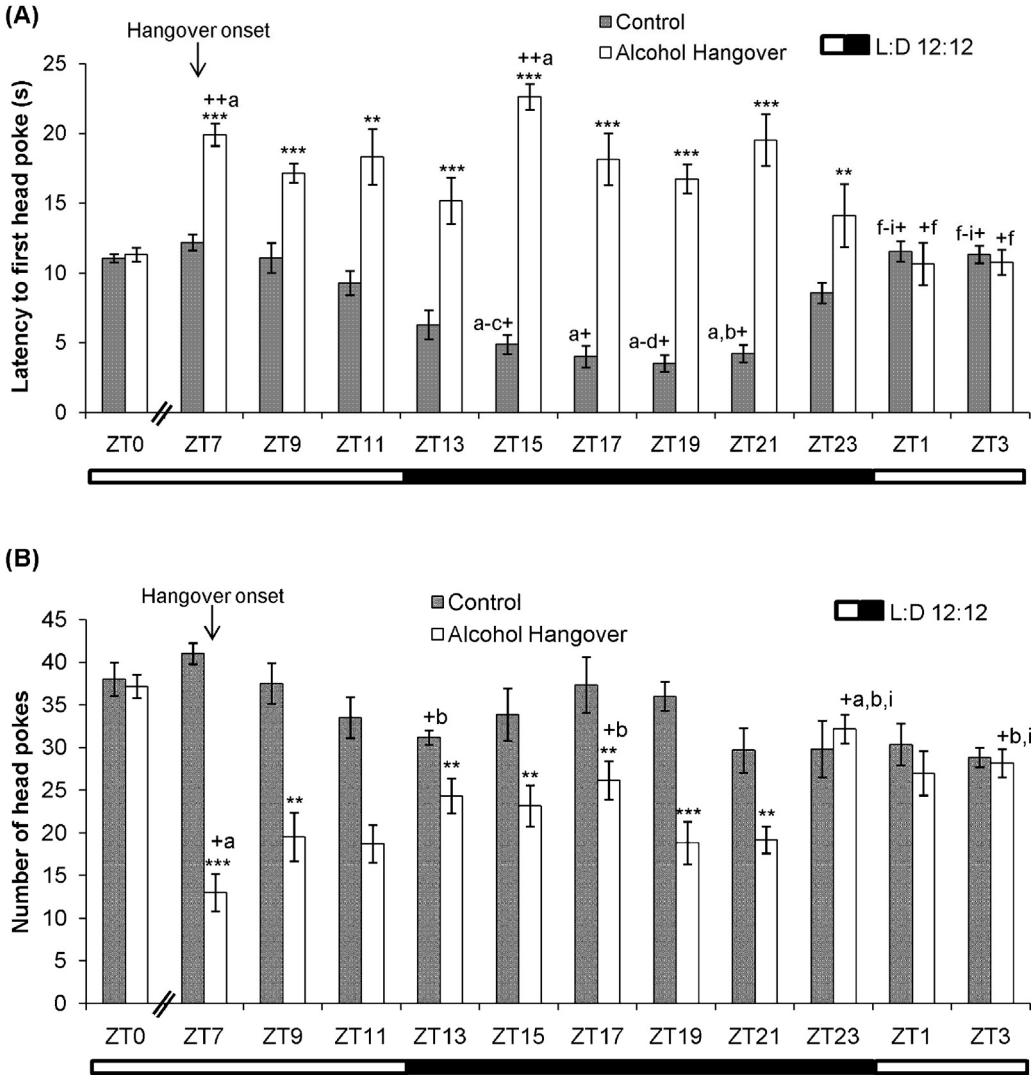

Fig. 7. Hole board exploration during alcohol hangover. Values are expressed as mean ± SEM ($n = 10$ each group). ZT: Zeitgeber time; L:D; light:dark. Student's *t*-test was used for intergroup differences ($^{**}p < 0.01$, $^{***}p < 0.001$). Repeated-measures two-factor ANOVA was used for in-group difference ($^{+}p < 0.05$; $^{++}p < 0.01$). (A) Latency to the first head poke (s) and (B) number of head pokes. Letters indicate the time point of the comparison as follows: a: ZT0; b: ZT7; c: ZT9; d: ZT11; e: ZT13; f: ZT15; g: ZT17; h: ZT19; i: ZT21; j: ZT23 and k: ZT1. Bars shading indicate group: gray, control; white, alcohol hangover.

immune system parameters could also be significantly related to the presence and severity of alcohol hangover. Indeed, it was demonstrated that immune activity on the CNS through the release of cytokines takes place during AH [34]. In this sense, cerebral cytokines are involved in sickness behavior which includes weakness, disability to concentrate, decreased appetite, reduced activity, sleepiness, and loss of interest in usual activities [7]. In humans, the same symptoms are all commonly reported during alcohol hangover. On the other hand, a potential influence of light changes on hangover effects was observed in the different behavioral test. In this sense, our unpublished results suggest that hangover symptoms could be mitigated when animals are subjected to free running by continuous darkness. Indeed, it was observed that hangover mice recovered their basal neuromuscular coordination and spontaneous exploratory pattern in the open field test in half of the time compared with same test at normal photoperiod conditions. We think that light changes could positive or negative interfere with the recovery of the hangover state; however, this needs to be explored in detail in the future. As a whole, we consider that the results obtained in this study could contribute to the knowledge of hangover pathophysiological state and to the proposal for a 'standard' criterion for hangover impairments.

5. Conclusion

During alcohol hangover there is a decrease in neuromuscular coordination, motor strength and locomotion together with gait variability and slowness in exploratory activity. In addition, our findings demonstrate a time-extension between 16 and 20 h for hangover motor and exploratory impairments. As a whole, this study shows the long lasting effects of alcohol hangover.

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References

[1] Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, et al. Atm-deficient mice: a paradigm of ataxia telangiectasia. Cell 1996;86:159–71.
[2] Boveris A, Navarro A. Systemic and mitochondrial adaptive responses to moderate exercise in rodents. Free Radical Biology and Medicine 2008;44:224–9.

[3] Brasser SM, Spear NE. Physiological and behavioral effects of acute ethanol hangover in juvenile, adolescent, and adult rats. Behavioral Neuroscience 2002;116:305–20.
[4] Bustamante J, Karadayian AG, Lores-Arnaiz S, Cutrera RA. Alterations of motor performance and brain cortex mitochondrial function during ethanol hangover. Alcohol 2012;46:473–9.
[5] Carter RJ, Morton J, Dunnett SB. Motor coordination and balance in rodents. Current Protocols in Neuroscience 2001:12.
[6] Crofton J. Extent and costs of alcohol problems in employment: a review of British data. Alcohol 1987;22:321–5.
[7] Dantzer R, Bluthé RM, Layé S, Bret-Dibat JL, Parnet P, Kelley KW. Cytokines and sickness behavior. Annals of the New York Academy of Sciences 1998;840:586–90.
[8] Dietrich MO, Mantese CE, Anjos GD, Souza DO, Farina M. Motor impairment induced by oral exposure to methylmercury in adult mice. Environmental Toxicology and Pharmacology 2005;19:169–75.
[9] Doremus TL, Brunell SC, Varlinskaya EI, Spear LP. Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. Pharmacology Biochemistry and Behavior 2003;75:411–8.
[10] Doremus-Fitzwater TL, Spear LP. Developmental differences in acute ethanol withdrawal in adolescent and adult rats. Alcoholism, Clinical and Experimental Research 2007;31:1516–27.
[11] Froy O. The circadian clock and metabolism. Clinical Science 2011;120:65–72.
[12] Gauvin DV, Briscoe RJ, Baird TJ, Vallett M, Holloway FA. The paradoxical hedonic valence of acute ethanol withdrawal (hangover) states in rats: place and taste conditioning. Alcohol 1997;14:261–8.
[13] Gauvin DV, Goulden KL, Holloway FA. State-dependent stimulus control: cueing attributes of ethanol "hangover" in rats. Alcoholism, Clinical and Experimental Research 1993;17:1210–4.
[14] Gauvin DV, Youngblood BD, Holloway FA. The discriminative stimulus properties of acute ethanol withdrawal (hangover) in rats. Alcoholism, Clinical and Experimental Research 1992;16:336–41.
[15] Guayerbas N, Puerto M, Hernanz A, Miquel J, De la Fuente M. Thiolic antioxidant supplementation of the diet reverses age-related behavioural dysfunction in prematurely ageing mice. Pharmacology Biochemistry and Behavior 2005;80:45–51.
[16] Howland J, Rohsenow DJ, Allensworth-Davies D, Greece J, Almeida A, Minsky SJ, et al. The incidence and severity of hangover the morning after moderate alcohol intoxication. Addiction 2008;103:758–65.
[17] Karadayian AG, Mac Laughlin MA, Cutrera RA. Estrogen blocks the protective action of melatonin in a behavioral model of ethanol-induced hangover in mice. Physiology and Behavior 2012;107:181–6.
[18] Kim DJ, Yoon SJ, Lee HP, Choi BM, Go HJ. The effects of alcohol hangover on cognitive functions in healthy subjects. International Journal of Neuroscience 2003;113:581–94.
[19] Kruisselbrink LD, Martin KL, Megeney M, Fowles JR, Murphy RJ. Physical and psychomotor functioning of females the morning after consuming low to moderate quantities of beer. Journal of Studies on Alcohol 2006;67:416–20.
[20] Lal H, Prather PL, Rezazadeh SM. Anxiogenic behavior in rats during acute and protracted ethanol withdrawal: reversal by buspirone. Alcohol 1991;8:467–71.
[21] Lemon J, Chesher G, Fox A, Greeley J, Nabke C. Investigation of the "hangover" effects of an acute dose of alcohol on psychomotor performance. Alcoholism, Clinical and Experimental Research 1993;17:665–8.
[22] Ling J, Stephens R, Heffernan TM. Cognitive and psychomotor performance during alcohol hangover. Current Drug Abuse Reviews 2010;3:80–7.
[23] Navarro A, Sanchez Del Pino MJ, Gomez C, Peralta JL, Boveris A. Behavioral dysfunction, brain oxidative stress, and impaired mitochondrial electron transfer in aging mice. AJP Regulatory, Integrative and Comparative Physiology 2002;282:R985–92.
[24] Pallares ME, Scacchi Bernasconi PA, Feleder C, Cutrera RA. Effects of prenatal stress on motor performance and anxiety behavior in Swiss mice. Physiology and Behavior 2007;92:951–6.
[25] Penning R, McKinney A, Bus LD, Olivier B, Slot K, Verster JC. Measurement of alcohol hangover severity: development of the Alcohol Hangover Severity Scale (AHSS). Psychopharmacology 2013;225:803–10.
[26] Penning R, McKinney A, Verster JC. Alcohol hangover symptoms and their contribution to the overall hangover severity. Alcohol 2012;47:248–52.
[27] Penning R, van Nuland M, Fliervoet LA, Olivier B, Verster JC. The pathology of alcohol hangover. Current Drug Abuse Reviews 2010;3:68–75.
[28] Prat G, Adan A, Perez-Pamies M, Sanchez-Turet M. Neurocognitive effects of alcohol hangover. Addictive Behaviors 2008;33:15–23.
[29] Rohsenow DJ, Howland J, Arnedt JT, Almeida AB, Greece J, Minsky S, et al. Intoxication with bourbon versus vodka: effects on hangover, sleep, and next-day neurocognitive performance in young adults. Alcoholism, Clinical and Experimental Research 2010;34:509–18.
[30] Sango K, McDonald MP, Crawley JN, Mack ML, Tifft CJ, Skop E, et al. Mice lacking both subunits of lysosomal beta-hexosaminidase display gangliosidosis and mucopolysaccharidosis. Nature Genetics 1996;14:348–52.
[31] Sinclair JD, Gustafsson K. Behavioral changes in rats on the day after acute ethanol intoxication. Alcohol 1987;4:503–7.
[32] van Gaalen MM, Steckler T. Behavioural analysis of four mouse strains in an anxiety test battery. Behavioural Brain Research 2000;115:95–106.
[33] Varlinskaya EI, Spear LP. Acute ethanol withdrawal (hangover) and social behavior in adolescent and adult male and female Sprague-Dawley rats. Alcoholism, Clinical and Experimental Research 2004;28:40–50.
[34] Verster JC. The alcohol hangover—a puzzling phenomenon. Alcohol and Alcoholism 2008;43:124–6.
[35] Verster JC. The "hair of the dog": a useful hangover remedy or a predictor of future problem drinking? Current Drug Abuse Reviews 2009;2:1–4.
[36] Verster JC, Penning R. Treatment and prevention of alcohol hangover. Current Drug Abuse Reviews 2010;3:103–9.
[37] Verster JC, Stephens R, Penning R, Rohsenow D, McGeary J, Levy D, et al. The alcohol hangover research group consensus statement on best practice in alcohol hangover research. Current Drug Abuse Reviews 2010;3:116–26.
[38] Vonghia L, Leggio L, Ferrulli A, Bertini M, Gasbarrini G, Addolorato G. Acute alcohol intoxication. European Journal of Internal Medicine 2008;19:561–7.
[39] Wiese JG, Shlipak MG, Browner WS. The alcohol hangover. Annals of Internal Medicine 2000;132:897–902.
[40] Ylikahri RH, Huttunen MO, Eriksson CJ, Nikkilä EA. Metabolic studies on the pathogenesis of hangover. European Journal of Clinical Investigation 1974;4:93–100.
[41] York JL, Regan SG. After-effects of acute alcohol intoxication. Alcohol 1988;5:403–7.
[42] Zhang Z, Morse AC, Koob GF, Schulteis G. Dose- and time-dependent expression of anxiety-like behavior in the elevated plus-maze during withdrawal from acute and repeated intermittent ethanol intoxication in rats. Alcoholism, Clinical and Experimental Research 2007;31:1811–9.