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Research report

# The effect of constant darkness and circadian resynchronization on the recovery of alcohol hangover

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## HIGHLIGHTS

- Darkness is effective to reduce the recovery time for the impairments due to hangover.
- Synchronized clock is involved in the recovery of deleterious effect of hangover.
- Circadian clock was found to be involved in the recovery of hangover symptoms.

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## ABSTRACT

Alcohol hangover (AH) is a particular state after binge-like drinking. AH begins when ethanol is absent in plasma and is characterized by a cluster of physical and psychological symptoms. Alcohol disrupts circadian patterns of behavioral and physiological parameters; however, the involvement of circadian clock on the recovery of AH was not explored. Our aim was to study the effect of continuous darkness and the possible involvement of the circadian clock in the recovery time of neuromuscular impairment and anxiety related-behavior due to AH. Male Swiss mice were habituated to 12:12 L:D or continuous darkness. Each group was injected i.p. either with saline (control group) or with ethanol (3.8 g/kg BW) (hangover group). Motor performance and anxiety phenotype 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). A third group was subjected to a phase advance during which a hangover episode was induced and behavioral tests were carried out for each group of treatment and resynchronization day. Constant darkness resulted to be in a faster recovery of both motor and anxiety impairments in AH compared with the recovery pattern observed under normal light–dark conditions. Mice suffering from a phase shift exhibited behavioral disruptions due to both AH and phase advance. Results indicated that a synchronized circadian clock is necessary for an adequate recovery of alcohol hangover symptoms.



## 1. Introduction

Excessive alcohol consumption has a variety of serious consequences on health. The different effects are widespread, altering numerous physiological, endocrine and behavioral functions. A particular state after binge-like drinking is defined as alcohol hangover (AH). In this sense, AH is described in humans as a physiological state which involves the unpleasant next-day effect following an evening of excessive alcohol consumption [1]. Hangover begins when ethanol 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 [2–4] with substantial individual, social and economical consequences [5]. Together with this, previous research work described for experimental animals, hypo-activity [6], fluctuations in body temperature, anxiety-like behavior [7] and reduced wheel running activity [8,9].

We previously reported that hangover induced serious motor and affective impairment which persist several hours (16–20 h)

*Abbreviations:* %FEO, proportion of entrance into open arms; %TSO, proportion of time spent in open arms; AH, alcohol hangover; BD, basal day; CT, circadian time; EMP, elevated-plus maze; LD, light–dark; RD, resynchronization day; TE, total number of entries; ZT, Zeitgeber time.

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after acute ethanol exposure [10,11]. In addition, we have demonstrated an association between this motor impairments and changes in brain cortex energetic metabolism [12]. Our data join to other research work that demonstrated that hangover symptoms in both humans and animals models are time- and dose-dependent [13–15]. These evidences were obtained by experimental models of AH or binge-like drinking pattern when animals were tested under normal light–dark conditions.

The effects of alcohol consumption on circadian rhythms were widely studied. In fact, it is known that the normal circadian patterns of a variety of behavioral and physiological parameters are disrupted by alcohol administration, ingestion, and/or withdrawal syndrome [16]. For instance, in laboratory rats and mice as in human subjects, alcohol administration alters the circadian response related to locomotor activity, body temperature [17], sleep [18], food intake [19] and the secretion of the stress related hormones [20,21]. A low blood alcohol content of 0.1% reached after the consumption of several alcoholic drinks (about 6–8 beverages) could lead to impaired clock synchronization [22]. Also, a previous work demonstrated that acute ethanol can inhibit photic phase shifts in hamsters [23]. Although the effects of alcohol consumption on circadian rhythms and its associated physiological functions were extensively studied, the involvement of circadian clock and the effect of changes in photoperiod on the recovery of AH were not explored. Moreover, it was stated that individual differences in circadian rhythmicity (i.e. circadian typology) and sleep factors should be taken into account as they influence performances and may interact with hangover effects [24]. Furthermore, it was hypothesized that alcohol-induced dysrhythmia could be one of cause for hangover syndrome [25]. Taking all together into account, the aim of this work was to study motor function and affective behavior during AH in order to achieve several goals: (1) to determine the time course of neuromuscular impairment and anxiety related-behavior under continuous darkness; (2) to compare the recovery of AH under normal light–dark conditions between constant darkness and (3) to establish the possible involvement of the circadian clock on the behavioral recovery at the onset of AH.

## 2. Materials and methods

### 2.1. Animals

A total of 150 from six 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 ($22 \pm 2\,^{\circ}C$) 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 (USA) 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.

### 2.2. Experimental procedure

Animals received intraperitoneal (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 [6,9]. Control mice received saline i.p. injections. The onset of alcohol hangover was previously determined being six hours after ethanol administration. This time point matched when blood alcohol concentration was less than or equal to 10% of the maximum value reached for the dose of ethanol used [26]. Three different experiments regarding photoperiod conditions were carried out as follows.

#### 2.2.1. Experiment I: time extension of motor function impairments and anxiety-like behavior during alcohol hangover

A total of 40 animals were habituated to a photoperiod of 12:12 h light/dark cycle. 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, Experiment I). Animals were randomly assigned to saline or ethanol treatment before baseline tests ($n = 10$ per treatment and for each behavioral task). Each subject was tested every two hours in only one behavioral test avoiding multiple tasks for animals groups. Control groups (saline treatment) let observe in- and between-group differences due to time-course (photoperiod), acute treatments and carry-over effects.

#### 2.2.2. Experiment II: effect of constant darkness on the time extension of motor function impairments and anxiety-like phenotype during alcohol hangover

A total of 40 animals were transferred to constant darkness. Following the same schedule for Experiment I, behavioral tests were carried out at a basal point that matched with the subjective lights onset (CT0) and every 2 h up to 20 h after alcohol hangover onset (CT3 of the following day) (see Fig. 1, Experiment II). Animals were randomly assigned to saline or ethanol treatment before baseline tests ($n = 10$ per treatment and for each behavioral task). Each subject was tested every two hours in only one behavioral test avoiding multiple tasks for animals groups. Control groups (saline treatment) let observe in- and between-group differences due to time-course (photoperiod), acute treatments and carry-over effects.

#### 2.2.3. Experiment III: effect of phase shift on the motor impairment and anxiety-like behavior at the onset of alcohol hangover

A total of 70 animals were habituated to a photoperiod of 12:12 h light/dark cycle. All mice were transferred to a different room where a phase shift was introduced as shown in Fig. 6. Animals experienced a 5 h phase advance which changed the photoperiod conditions to a new 12:12 h light/dark cycle (lights off at 2:00 p.m.) and also resulted in a shortening of the active phase during the night. It was previously reported that resynchronization after a phase shift requires a day for each hour of advance of the LD cycle [27]. Thus, five days of resynchronization was necessary to establish the new circadian rhythm. The involvement of circadian clock on the effect of alcohol hangover was evaluated by testing motor and anxiety-like behavior across the resynchronization period. For that propose, animals were randomly divided in five groups ($n = 14$) and a hangover episode was induced for each group and resynchronization day. Considering this methodology, for each resynchronization day, a group of 14 mice ($n = 7$, controls; $n = 7$, treated with alcohol) were evaluated in two different behavioral test. Thus, animals were divided as RD 1 (resynchronization day 1), RD 2, RD 3, RD 4 and RD 5. In addition, to ensure that basal level of the behavioral parameters were similar to that obtained for Experiment I and II, all animals which were divided in the five groups mentioned above, were previously tested before the phase shift; thus, basal level for each group was obtained (BD 1, BD 2, BD 3, BD 4 and BD 5; being BD, basal day). Behavioral tests were carried out at the onset of alcohol hangover which matched with lights off at 2:00 p.m.