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The effects of alcohol on anxiety-like, depression-like, and social behavior immediately and a day after binge drinking

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ABSTRACT

The aim of the present study was to determine the effects of binge drinking on anxiety-like, depression-like, and social behavior. The participation of the corticotropin-releasing factor (CRF) receptors (CRF1 and CRF2) in these effects was also investigated. Therefore, male C57BL/6 mice were exposed to drinking in the dark, a classical animal model for binge drinking, and treated intracerebroventricularly (icv) with selective CRF1 antagonist antalarmin or selective CRF2 antagonist astressin$_2$B, immediately or 24 h after binge drinking. After 30 min, the animals were investigated in an elevated plus-maze test and a forced swim test for anxiety-like and depression-like signs, respectively. In addition, mice were tested in a three-chamber social interaction arena for sociability and preference for social novelty. Immediately after binge drinking, mice exposed to alcohol expressed anxiolytic and antidepressant effects, which were reduced by astressin$_2$B, but not antalarmin. Moreover, mice exposed to alcohol showed increased sociability and preference for social novelty immediately after binge drinking. In contrast, 24 h after binge drinking mice exposed to alcohol presented anxiety-like and depression-like signs, which were reversed by antalarmin, but not astressin$_2$B. However, mice exposed to alcohol did not show any significant change in social interaction after 24 h. The present study demonstrates that alcohol exerts different effects on anxiety-like, depression-like, and social behavior immediately and a day after binge drinking, and that the anxiolytic and antidepressant effects produced by binge drinking are mediated by CRF2, whereas the anxiety-like and depression-like signs observed the next day are promoted by CRF1.

Introduction

Binge drinking is defined as consuming a large amount of alcohol in a short period of time (Chung, Creswell, Bachrach, Clark, & Martin, 2018). A large amount of alcohol refers to five or more alcoholic drinks in men and four or more alcoholic drinks in women that, by definition, brings their blood alcohol concentration (BAC) to 0.08 g/dL within 2 h, and is commonly associated with acute impairment in motor coordination and cognitive functioning (Chung et al., 2018). Hangover is a temporary state described as the unpleasant next-day effects after binge drinking (van Schrojenstein Lantman, van de Loo, Mackus, & Verster, 2016). This state usually emerges after a single episode of heavy drinking when BAC approaches zero and is associated with a combination of physical signs, such as ataxia, locomotor and exploratory dysfunctions, and affective symptoms, such as fear, anxiety, and depression (van Schrojenstein Lantman et al., 2016). Alcoholism is best resembled by alternating episodes of binge drinking and hangover (Koob, 2013, 2014). Individuals who regularly engage in episodic heavy drinking do not entirely meet the diagnostic criteria for alcoholism; however, repeated cycles of binge drinking that emerge during adolescence are an important risk factor for development of alcohol addiction in adulthood (Koob, 2013, 2014). Furthermore, repeated episodes of binge drinking may elicit persistent negative affect, including anxiety and depression (Jimenez Chavez et al., 2022; Lee, Coehlo, McGregor, Waltermire, & Szumlinski, 2015; Lee, Coehlo, Solton, & Szumlinski, 2017; Olney, Marshall, & Thiele, 2018), and alteration of social behavior

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similar to that described during acute alcohol withdrawal (Kent, Butler, & Wood, 2014; Wood, Knoll, & Levitt, 2015). Nevertheless, anxiety, depression, and hangover are usually attributed to humans. In the present study we used male C57BL/6 mice, hereby we refer to these terms as anxiety-like and depression-like signs, and hangover-like symptoms.

The aim of the present study was to determine the effects of binge drinking on anxiety-like, depression-like, and social behavior. For this purpose, male C57BL/6 mice were exposed to drinking in the dark, a classic method to investigate binge drinking in animals (Rhodes, Best, Belknap, Finn, & Crabbe, 2005). Corticotropin-releasing factor (CRF) and its receptors (CRF1 and CRF2) have been involved in the pathogenesis of anxiety and depression (Reul & Holsboer, 2002), and various aspects of social behavior (Bagosi, Czébely-Lénárt et al., 2017; Bagosi, Karasz, et al., 2017). Therefore, the participation of the CRF receptors (CRF1 and CRF2) in these effects was also investigated. In order to do so, first the dark–light cycle of the mice was inverted for 14 days and then their water bottles were replaced by bottles of 20% alcohol for 4 days (2 h on the first, second, and third day, and 4 h on the fourth day). On the 4th day, immediately after binge drinking, or on the 5th day, 24 h after binge drinking, mice were treated intracerebroventricularly (icv) with selective CRF1 antagonist antalarmin or selective CRF2 antagonist astressin$_2$B. After 30 min, the animals were investigated in an elevated plus-maze test and a forced swim test for signs of anxiety and depression, respectively. In addition, mice were tested in a three-chamber social interaction arena for sociability and preference for social novelty.

Materials and methods

Animals

Male C57BL/6 mice (Charles River Laboratories Ltd., Hungary) 6 weeks old, weighing 18–24 g, were used. The mice were kept in their home cages at a constant temperature on a standard illumination schedule with 12-h light and 12-h dark periods (lights on from 6:00 PM to 6:00 AM). Commercial food and tap water were available *ad libitum*. To minimize the effects of non-specific stress the mice were handled daily. All tests were performed between 9:00 AM to 12:00 noon. The animals were treated in accordance with the ARRIVE guidelines and the experiments were carried out in accordance with the EU Directive 2010/63/EU for animal experiments.

Surgery

The mice were implanted with a stainless-steel Luer cannula, aimed at the right lateral cerebral ventricle under anesthesia with 60 mg/kg pentobarbital sodium (Euthanasol, CEVA-Phylaxia Ltd., Hungary). The stereotaxic coordinates were 0.5 mm lateral and 0.5 mm posterior from the bregma and 3 mm deep from the dural surface according to the stereotaxic atlas of the mouse brain (Paxinos & Franklin, 2004). Cannulas were secured to the skull with Ferrobond instant glue (Ferrokémia Ltd., Hungary) and they were closed by a metal string between injections. Before the experiments, the mice were allowed to recover for 5 days after the surgery. After the experiments, 4 μL of dye methylene blue (Reanal Ltd., Hungary) at 1 g/100 mL concentration was injected through the cannula to identify the site of injection. Animals without the dye in the lateral cerebral ventricle were discarded.

Drinking in the dark

The mice were exposed to drinking in the dark, a classic animal model for binge drinking (Rhodes et al., 2005). First, *the* dark–light cycle of the mice was inverted for 14 days, and then their water bottles were replaced by bottles of 20% alcohol (Reanal Ltd., Hungary) for 4 days (2 h on the first, second, and third day, and 4 h on the fourth day).

Treatment

On the 4th day (immediately after binge drinking) or on the 5th day (24 h after binge drinking) mice were treated icv with the selective CRF1 antagonist antalarmin (Sigma–Aldrich Ltd., Hungary) or the selective CRF2 antagonist astressin$_2$B (Sigma–Aldrich Ltd., Hungary). The mice were assigned randomly for one of the treatments on the 4th day or the 5th day; they were not exposed to more than one icv administration in two consecutive days. The dose of antalarmin was 0.1 μg/2 μL, and that of astressin$_2$B was 1 μg/2 μL. As in our previous experiments these doses have been already been proven to effectively block the neuroendocrine stress response, without altering the social behavior of animals (Bagosi, Czebely-Lenart et al., 2017; Bagosi, Karasz, et al., 2017). After 30 min the animals were investigated in an elevated plus-maze test or a forced swim test for signs of anxiety or depression, respectively. In parallel, a three-chamber social interaction test was also performed, in order to investigate the sociability and the preference for social novelty of mice. Also, the mice were assigned randomly for one of the behavioral tests; they were not exposed consequently to the elevated plus-maze, forced swim, or social interaction test.

Elevated plus-maze test

The mice were investigated in an elevated plus-maze test described first by Lister (1987). The elevated plus-maze apparatus consists of a plus-shaped wooden platform elevated at 40 cm from the floor, made up of four opposing arms of 30 cm × 5 cm. Two of the opposing arms are enclosed by 15-cm high side and end walls (closed arms), whereas the other two arms have no walls (open arms). The room where the behavioral tests were performed had been darkened, with only the central area of the elevated plus-maze illuminated with a lamp 50 cm from the platform, having an LED bulb of 3.5 W that produces 2230 lumens. The principle of the test is that open arms are more fear-provoking than the closed arms, and the ratio of the time spent in open vs. closed arms, or the ratio of the entries into open vs. closed arms, reflects the relative safety of closed arms, as compared with the relative danger of open arms. Each mouse was placed in the central area of 5 cm × 5 cm of the maze, facing one of the open arms. For a 5-min period, two parameters were recorded by an observer sitting 100 cm from the center of the plus-maze: 1) the number of entries into the open arms relative to the total number of entries, and 2) the time spent in the open arms relative to the total time. All parameters were expressed as percentages. The platform of the apparatus was cleaned with sodium hypochlorite solution (HIP-TOM Ltd., Hungary) between the subjects.

Forced swim test

The mice were also investigated in a forced swim test described first by Porsolt and co-workers (Porsolt, Bertin, & Jalfre, 1977). The forced swim apparatus consists of a plexiglass cylinder of 40-cm height and 12-cm diameter positioned on a table. The cylinder

was half-filled with water maintained at 25 ± 1 °C. The principle of the test is that in such a situation, from which they cannot escape, animals rapidly became immobile, that is, floating in an upright position and making only small movements to keep their heads above water. Meanwhile, their attempts to escape the cylinder by climbing or swimming may decrease or cease eventually. Each mouse was placed individually into the water. For a 5-min period the following parameters were recorded by an observer sitting 100 cm from the table: the time that mice spent with swimming and climbing the walls, in their attempt to remain at the surface and escape the cylinder, respectively, and the time spent with immobility or floating. All parameters were expressed in time units, a time unit consisting of 5 s. The water from the cylinder was completely changed between the subjects.

Three-chamber social interaction test

The mice were also tested in a social interaction arena invented by Crawley and co-workers (Crawley et al., 2007). The arena is represented by a rectangular plexiglass box divided into three chambers, each chamber having the dimensions of 19 × 45 × 25 cm. The right and left chambers could be isolated from the middle one by using two dividing plexiglass walls. Two identical, wire cup-like cages of 10 × 17 cm with removable lids large enough to hold a single mouse were placed vertically inside the apparatus, one in each side chamber. Each cage was composed of metal wires to allow for air exchange between the interior and exterior of the cylinder but was small enough to prevent direct physical (aggressive or sexual) interactions between the animal on the inside with the animal on the outside. Two types of tests were performed: the first test was meant to measure the sociability, whereas the second test was meant to measure the preference for social novelty of the mice (Kaidanovich-Beilin, Lipina, Vukobradovic, Roder, & Woodgett, 2011). In the first test, the tested mouse was first habituated with the middle chamber for 5 min and then allowed to explore the remaining chambers for another 5 min. Then, a stranger male mouse in a cage was placed into one of the chambers and an empty cage was put into the other chamber. The principle of the first test is based on the observation that a wild-type mouse enters and spends more time in interaction with the stranger mouse over a foreign object (e.g., empty cage), indicative of intact sociability. In the second test, a stranger male mouse in a cage was placed into one of the chambers and the male mouse that was the stranger in the previous test (now considered familiarized) in a cage was placed into the opposite chamber. The principle of this second test is based on the assessment that a wild-type mouse enters and spends more time in interaction with the stranger mouse over the familiarized one, indicating a natural preference for social novelty. In both tests the following parameters were recorded by an observer sitting 200 cm from the box for two 5-min periods: the number of entries into the chamber relative to the total number of entries, and the time of interaction with the stranger relative to the total time of interaction. The number of entries was counted when both the head and the four paws of the tested mouse had entered into the chamber. The time of interaction was measured when the tested mouse was at least 3 cm from the cage. The floors and the walls of the arena were cleaned with sodium hypochlorite solution (HIP-TOM Ltd., Hungary) between the tests.

Blood alcohol concentration (BAC) measurement

In our study the amount of alcohol consumed was calculated by bottle weight each day, and BAC was determined only on the 4th day (immediately after binge drinking) and on the 5th day (24 h after binge drinking) for each mouse. The mice were decapitated, and trunk blood was collected after the behavioral tests. Ethanol was determined from the plasma obtained by centrifugation of the trunk blood, immediately after sample collection by commercially available enzymatic kit (Ref. No. 03183777 190, Roche Diagnostics, Mannheim, Germany) on a cobas c502 analyzer (Roche Diagnostics). The sensitivity of the assay was 10.1 mg/dL (0.01 g/dL). Based on previous experiments, drinking in the dark results in alcohol intakes between 3.5 and 5.0 g/kg alcohol (Thiele, Crabbe, & Boehm, 2014; Thiele & Navarro, 2014). The intake of this amount of alcohol should produce a BAC of 0.08 g/dL within 2 h in C57BL/6 mice (Thiele et al., 2014; Thiele & Navarro, 2014). However, in the present experiments, mice did not always reach the alcohol level that is characteristic for binge drinking; therefore, mice with BAC lower than 0.08 g/dL were excluded from the statistical analysis.

Statistical analysis

Statistical analysis of the results was performed by analysis of variance (GraphPad Prism, GraphPad Software Inc., United States). The differences between groups were determined by one-way ANOVA, followed by Tukey's *post hoc* test for pair-wise comparisons. The probability level of 0.05 or less was accepted as indicating a statistically significant difference.

Results

On the 4th day (immediately after binge drinking), the number of entries into and the time spent in the open arms of the elevated plus-maze increased significantly in mice exposed to alcohol, compared to the control mice (Fig. 1). Consequently, mice exposed to alcohol spent significantly more time with swimming and climbing, and significantly less time with floating in the water, when compared to the control mice (Fig. 2). These anxiolytic and antidepressant effects of alcohol were decreased significantly by astressin$_2$B, but not the antalarmin (Figs. 1 and 2). The number of entries to the stranger was not affected in the social interaction tests, but mice exposed to alcohol spent significantly more time with the stranger in both social interaction tests, when compared to the control (Figs. 3 and 4). These signs of enhanced sociability and preference for social novelty were reduced significantly by antalarmin, but not astressin$_2$B (Figs. 3 and 4). The results of the statistical analysis were summarized in a separate table for each test (Tables 1–4). The results of the BAC measurements were also summarized in a table (Table 5). The BACs of the mice exposed to binge drinking were 0.10 ± 0.02 g/dL.

On the 5th day (24 h after binge drinking), the number of entries into and the time spent in the open arms were decreased significantly in mice exposed previously to alcohol, compared to the control (Fig. 1). Also, mice exposed previously to alcohol spent significantly less time with swimming and climbing, and significantly more time with floating in the water, when compared to the control mice (Fig. 2). These signs of anxiety and depression were reversed significantly by antalarmin, but not astressin$_2$B (Figs. 1 and 2). However, previous exposure to alcohol did not affect the number of entries to or the time spent with the stranger in either of the social interaction tests (Figs. 3 and 4). Accordingly, neither antalarmin nor astressin$_2$B did influence considerably the sociability and preference for social novelty of the mice (Figs. 3 and 4). The results of the statistical analysis were summarized in a separate table for each test (Tables 1–4). The results of the BAC measurements were also summarized in a table (Table 5). As we previously mentioned, mice did not always reach the alcohol level that is characteristic for binge drinking; therefore, mice with BACs lower than 0.08 g/dL were excluded from the statistical analysis.

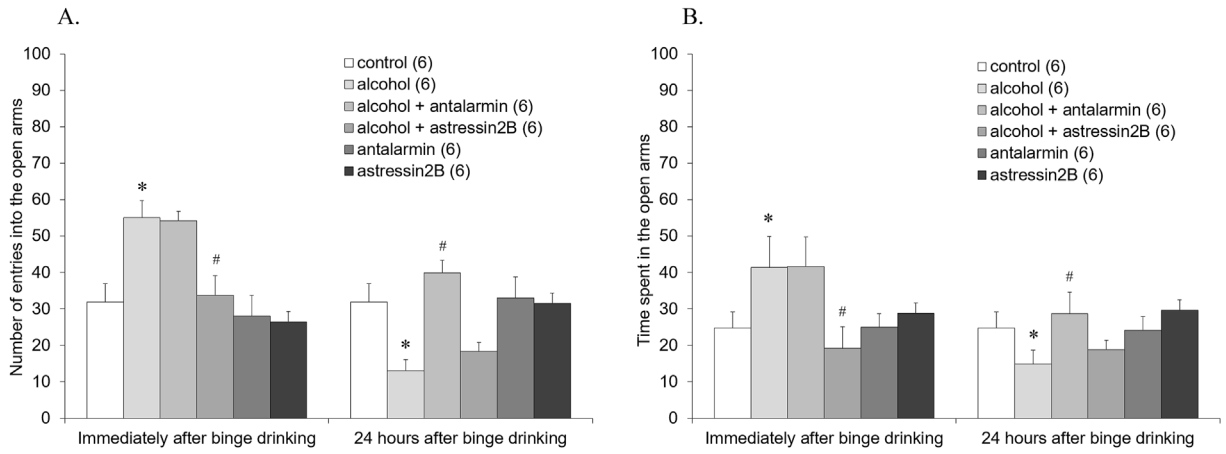

Fig. 1. The effects of binge drinking on the number of entries into (**A**), and the time spent in the open arms (**B**) in mice investigated in an elevated plus-maze test for signs of anxiety. Values are presented as means ± SEM; a statistically significant difference was accepted for $p < 0.05$ and indicated with * for alcohol vs. control, and # for alcohol + CRF antagonist vs. alcohol alone.

Discussion

The present study demonstrates that alcohol exerts different effects on anxiety-like, depression-like, and social behavior immediately and a day after binge drinking. Binge drinking produces anxiolytic and antidepressant effects when mice are tested immediately after drinking in the dark. Previous studies have already suggested that a single cycle of binge drinking is not necessarily associated with anxiety and depression (Evans, Rodríguez-Borillo, Font, Currie, & Pastor, 2020; Olney et al., 2018). In concordance, a recent study using a slightly modified version of the drinking in the dark paradigm showed that binge drinking has no short-term effect on the behavior of adolescent C57BL/6 mice but evokes anxiety- and depressive-like behavior during adulthood (Van Hees et al., 2022). Based on the present experiments, a single session of binge drinking in adolescent C57BL/6 mice seems to have rather anxiolytic and antidepressant effects. In addition, binge drinking enhances the sociability and the preference for social novelty of male mice when they are tested immediately after drinking in the dark, a finding that can be related to the anxiolytic and antidepressant effects observed. In general, alcohol is known to have a biphasic effect on social behavior, as low doses increase and high doses decrease the number of social contacts (López-Cruz et al., 2016; López-Cruz, Salamone, & Correa, 2013). Furthermore, mice exposed to alcohol spend more time in interaction with a conspecific than the object, regardless of the dose of alcohol (López-Cruz et al., 2013, 2016).

In contrast, mice exposed to alcohol presented anxiety-like and depression-like signs 24 h after binge drinking, which may correspond for hangover in humans. Hangover is a state that occurs after a single episode of heavy drinking when BAC approaches zero and is associated with a combination of physical signs and affective symptoms, including anxiety and depression. The affective symptoms of hangover – a term used by some authors interchangeably with acute alcohol withdrawal (Marsland et al., 2021; Palmer et al., 2019) – usually emerge at 10 h and may persist even after 24 h following alcohol administration (Karadayian, Busso, Feleder, & Cutrera, 2013; Karadayian & Cutrera, 2013). In accordance, a previous study has already reported that a history of 30 days of binge drinking elicits negative affect in mice, most notably anxiety-like signs, which emerge after 24 h of withdrawal and persist for at least 21 days following the last episode of binge drinking (Lee et al., 2015, 2017). However, in another study previously published, only a weak negative affect, including a few signs of anxiety-like and depression-like behavior, and no elevation of the circulating corticosterone levels, as a biochemical index of stress, were

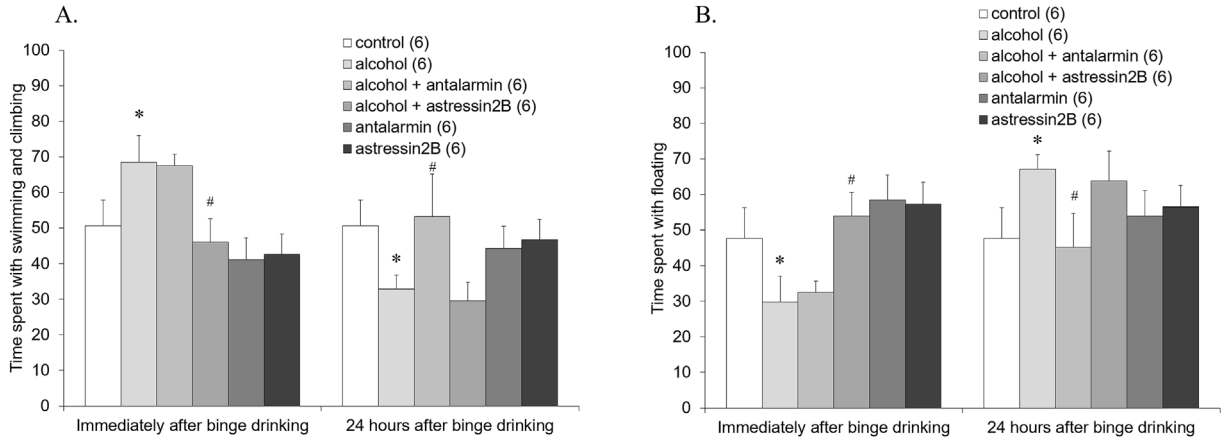

Fig. 2. The effects of binge drinking on the time spent with swimming and climbing (**A**), and floating (**B**) in mice investigated in a forced swim test for signs of depression. Values are presented as means ± SEM; a statistically significant difference was accepted for $p < 0.05$ and indicated with * for alcohol versus control, and # for alcohol + CRF antagonist vs. alcohol alone.

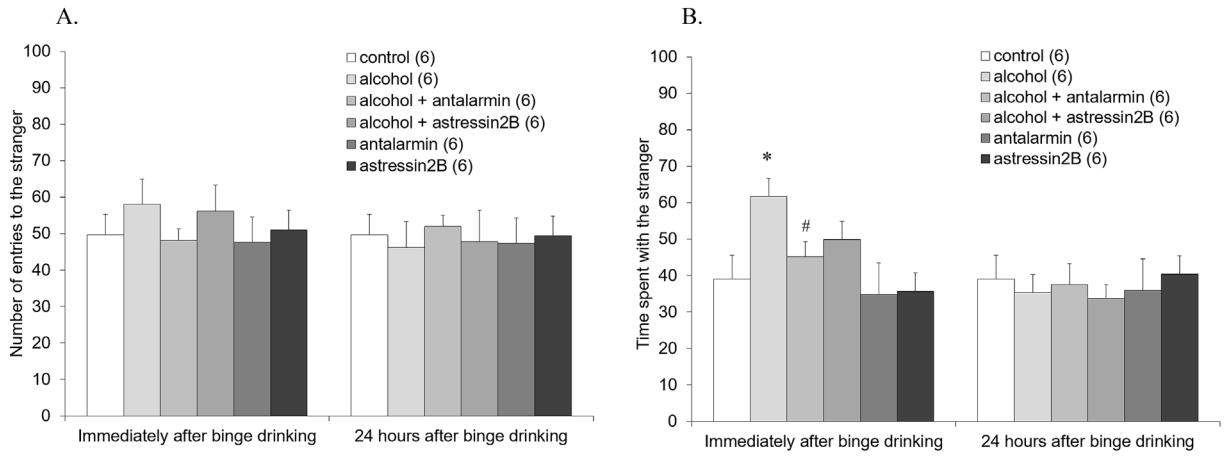

Fig. 3. The effects of binge drinking on the number of entries to (**A**), and the time spent with the stranger (**B**) in mice investigated in a three-chamber social interaction test for their sociability. Values are presented as means ± SEM; a statistically significant difference was accepted for $p < 0.05$ and indicated with * for alcohol vs. control, and # for alcohol + CRF antagonist vs. alcohol alone.

detected after 24 h of binge drinking in mice (Jimenez Chavez et al., 2020). In this study, male and female adolescent and adult mice were subjected to 14 consecutive days of binge drinking using a multi-bottle choice drinking in the dark procedure (Jimenez Chavez et al., 2020). The authors of this study concluded that incubation of negative affect during alcohol withdrawal is age-dependent, and not sex-selective, but also admitted that procedural differences might have accounted for the relatively weak effect of binge drinking on anxiety-like and depressive-like behavior, when compared to other studies (Jimenez Chavez et al., 2020). In addition, binge drinking does not affect the social interaction of male mice, when they are tested 24 h after drinking in the dark. A recent study has already suggested that binge drinking has no impact on the sociability and the preference for social novelty of mice, at least when they are tested 24 h after drinking in the dark (Van Hees et al., 2022). Another study recently published underlined the anxiogenic and cognitive impairing effects of binge drinking (Jimenez Chavez et al., 2022). In this study C57BL/6 mice were exposed to drinking in the dark for a 1-month period and investigated in a battery of behavioral tests, including elevated plus-maze, forced swim, and Morris water-maze tests (Jimenez Chavez et al., 2022). The authors reached the following conclusions: 1) both biological sex and the age of drinking onset are subjective factors that impact voluntary alcohol consumption by mice into old age; 2) binge drinking during later life elicits a negative affective state that is relatively sex-independent; 3) binge drinking during both mature adulthood and old age impairs spatial learning and memory; 4) binge drinking during mature adulthood accelerates deficits in working memory; and 5) mature adult females tend to exhibit more alcohol-induced cognitive impairments than males (Jimenez Chavez et al., 2022). We find these studies very inspiring for our future investigations regarding immediate and persistent effects of binge drinking on male and female mice, at adolescence and adulthood.

The present study also demonstrates that the anxiolytic and antidepressant effects produced by binge drinking are mediated by CRF2, whereas the anxiety-like and depression-like signs observed the next day are promoted by CRF1. This is consistent with the original hypothesis, which proposed that CRF1 and CRF2 play dualistic roles in the brain (Bale, 2014; Bale & Vale, 2004), with CRF1 promoting activation of the hypothalamic-pituitary-adrenal (HPA) axis, anxiety, and depression, and with CRF2 mediating anxiolytic and antidepressant actions. However, a recent hypothesis states that the role of CRF1 and CRF2 in anxiety and depression is not a matter of simple dualism but depends on the brain regions

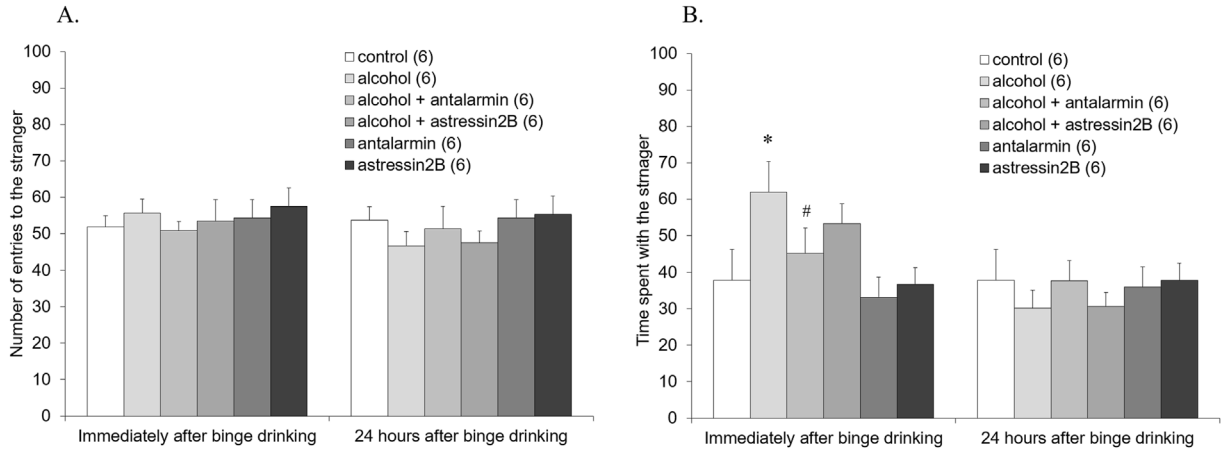

Fig. 4. The effects of binge drinking on the number of entries to (**A**), and the time spent with the stranger (**B**) in mice investigated in a three-chamber social interaction test for their preference for social novelty. Values are presented as means ± SEM; a statistically significant difference was accepted for $p < 0.05$ and indicated with * for alcohol vs. control, and # for alcohol + CRF antagonist vs. alcohol alone.

Table 1
Results of the statistical analysis for elevated plus-maze test.

Number of entries into the open arms		
Groups	Binge drinking	Hangover
Alcohol vs. Control	**$F(5,30) = 9.826$ $p = 0.0063$**	**$F(5,30) = 6.419$ $p = 0.0249$**
Alcohol vs. Alcohol + Antalarmin	$F(5,30) = 9.826$ $p > 0.999$	**$F(5,30) = 6.419$ $p = 0.0006$**
Alcohol vs. Alcohol + Astressin2B	**$F(5,30) = 9.826$ $p = 0.0147$**	$F(5,30) = 6.419$ $p = 0.9311$
Time spent in the open arms		
Groups	**Binge drinking**	**Hangover**
Alcohol vs. Control	**$F(5,30) = 2.263$ $p = 0.0423$**	**$F(5,30) = 2.006$ $p = 0.0440$**
Alcohol vs. Alcohol + Antalarmin	$F(5,30) = 2.263$ $p > 0.999$	**$F(5,30) = 2.006$ $p = 0.0180$**
Alcohol vs. Alcohol + Astressin2B	**$F(5,30) = 2.2630$ $p = 0.0149$**	$F(5,30) = 2.006$ $p = 0.9804$

Table 2
Results of the statistical analysis for forced swim test.

Time spent with swimming and climbing		
Groups	Binge drinking	Hangover
Alcohol vs. Control	**$F(5,30) = 4.295$ $p = 0.0298$**	**$F(5,30) = 1.798$ $p = 0.0508$**
Alcohol vs. Alcohol + Antalarmin	$F(5,30) = 4.295$ $P > 0.999$	**$F(5,30) = 1.798$ $p = 0.0365$**
Alcohol vs. Alcohol + Astressin2B	**$F(5,30) = 4.295$ $p = 0.0107$**	$F(5,30) = 1.798$ $p = 0.994$
Time spent with floating		
Groups	**Binge drinking**	**Hangover**
Alcohol vs. Control	**$F(5,30) = 4.223$ $p = 0.0335$**	**$F(5,30) = 1.429$ $p = 0.0486$**
Alcohol vs. Alcohol + Antalarmin	$F(5,30) = 4.223$ $p = 0.9996$	**$F(5,30) = 1.429$ $p = 0.3547$**
Alcohol vs. Alcohol + Astressin2B	**$F(5,30) = 4.223$ $p = 0.0870$**	$F(5,30) = 1.429$ $p = 0.9996$

and neuron populations being activated (Henckens, Deussing, & Chen, 2016; Janssen & Kozicz, 2013). Therefore, future experiments using modern techniques of CRF overexpression and global or local CRF1 and CRF2 knockout animal models should determine the intimate brain regions and mechanisms involved in binge drinking. Our pre-clinical study may have clinical implications. A previous study demonstrated that pre-treatment with a CRF1 antagonist or CRF2 agonist prior to alcohol self-administration could reduce the amount of alcohol administered (Lowery et al., 2010). The present study using the same animal model suggests that pre-treatment with a selective CRF1 antagonist and a selective CRF2 antagonist could attenuate both the positive, rewarding effects, and the negative, aversive effects of alcohol and alcohol withdrawal, respectively. In this order of thought, co-administration of these drugs might prevent spiraling of repeated cycles of binge drinking into alcohol addiction. In addition, selective CRF2 agonists, such as urocortin 2 and urocortin 3, may also prove useful in the therapy of alcohol addiction, since our previous study revealed that these neuropeptides ameliorate the anxiety- and depression-like state developed during nicotine addiction, as well (Bagosi et al., 2016).

Many other investigators have examined the role of CRF1 and CRF2 in binge or heavy drinking of alcohol (Albrechet-Souza et al., 2015; Kaczmarek, 2017; Kaur, Li, Stenzel-Poore, & Ryabinin, 2012; Sparta et al., 2013). We believe that any inconsistencies found between our study and others investigating the anxiety-like, depression-like, and social behavior using the same animal model of binge drinking could be due to the changes in the drinking in the dark paradigm. In our experiments, C57BL/6 mice were exposed to alcohol for 4 days, according to the classical drinking in the dark paradigm, even if the animals did not always reach the BAC of 0.08 g/dL within 2 h (Thiele et al., 2014; Thiele & Navarro, 2014). In comparison, in other experiments the mice were exposed repeatedly to alcohol, at different times, and for longer periods in order to reach the alcohol level that is characteristic for binge drinking (Lee et al., 2015, 2017; Van Hees et al., 2022). As regards the robust negative affect that was observed 24 h after a single session of binge drinking in our case, and that was described after several cycles of binge drinking and withdrawal in other cases, we presume that these may also arise from the different methodology. In our experiments, mice with BACs lower than 0.08 g/dL were excluded from the statistical analysis

Table 3
Results of the statistical analysis for social interaction test (sociability).

Number of entries to the stranger		
Groups	Binge drinking	Hangover
Alcohol vs. Control	$F(5,30) = 0.5843$ $p = 0.8988$	$F(5,30) = 0.1044$ $p = 0.9987$
Alcohol vs. Alcohol + Antalarmin	$F(5,30) = 0.5843$ $p = 0.8175$	$F(5,30) = 0.1044$ $p = 0.9863$
Alcohol vs. Alcohol + Astressin2B	$F(5,30) = 0.5843$ $p > 0.999$	$F(5,30) = 0.1044$ $p > 0.999$
Time spent with the stranger		
Groups	**Binge drinking**	**Hangover**
Alcohol vs. Control	**$F(5,30) = 3.782$ $p = 0.049$**	$F(5,30) = 0.1693$ $p > 0.999$
Alcohol vs. Alcohol + Antalarmin	**$F(5,30) = 3.782$ $p = 0.0261$**	$F(5,30) = 0.1693$ $p = 0.998$
Alcohol vs. Alcohol + Astressin2B	$F(5,30) = 3.782$ $p = 0.6125$	$F(5,30) = 0.1693$ $p > 0.999$

Table 4
Results of the statistical analysis for social interaction test (preference for social novelty).

Number of entries to the stranger		
Groups	Binge drinking	Hangover
Alcohol vs. Control	$F(5,30) = 0.2391$ $p = 0.9940$	$F(5,30) = 0.3691$ $p = 0.9255$
Alcohol vs. Alcohol + Antalarmin	$F(5,30) = 0.2391$ $p = 0.9827$	$F(5,30) = 0.3691$ $p = 0.9866$
Alcohol vs. Alcohol + Astressin2B	$F(5,30) = 0.2391$ $p = 0.9996$	$F(5,30) = 0.3691$ $p = 0.9866$
Time spent with the stranger		
Groups	**Binge drinking**	**Hangover**
Alcohol vs. Control	$\mathbf{F(5,30) = 2.779}$ $\mathbf{p = 0.0138}$	$F(5,30) = 0.4130$ $p > 0.999$
Alcohol vs. Alcohol + Antalarmin	$\mathbf{F(5,30) = 2.779}$ $\mathbf{p = 0.0490}$	$F(5,30) = 0.4130$ $p = 0.9399$
Alcohol vs. Alcohol + Astressin2B	$F(5,30) = 2.779$ $p = 0.9387$	$F(5,30) = 0.4130$ $p > 0.999$

Table 5
Results of the blood alcohol concentration (BAC) measurements.

Blood alcohol concentration (BAC) g/dL		
Animals	Binge drinking	Hangover
Control #1	0.00	0.00
Control #2	0.00	0.00
Control #3	0.00	0.00
Control #4	0.00	0.00
Control #5	0.00	0.00
Control #6	0.00	0.00
Alcohol #1	**0.08**	0.00
Alcohol #2	**0.10**	0.00
Alcohol #3	**0.12**	0.00
Alcohol #4	**0.08**	0.00
Alcohol #5	**0.08**	0.00
Alcohol #6	**0.09**	0.00
Alcohol + antalarmin #1	**0.09**	0.00
Alcohol + antalarmin #2	**0.10**	0.00
Alcohol + antalarmin #3	**0.09**	0.00
Alcohol + antalarmin #4	**0.11**	0.00
Alcohol + antalarmin #5	**0.08**	0.00
Alcohol + antalarmin #6	**0.08**	0.00
Alcohol + astressin2B #1	**0.10**	0.00
Alcohol + astressin2B #2	**0.10**	0.00
Alcohol + astressin2B #3	**0.08**	0.00
Alcohol + astressin2B #4	**0.09**	0.00
Alcohol + astressin2B #5	**0.11**	0.00
Alcohol + astressin2B #6	**0.12**	0.00
Antalarmin #1	0.00	0.00
Antalarmin #2	0.00	0.00
Antalarmin #3	0.00	0.00
Antalarmin #4	0.00	0.00
Antalarmin #5	0.00	0.00
Antalarmin #6	0.00	0.00
Astressin2B #1	0.00	0.00
Astressin2B #2	0.00	0.00
Astressin2B #3	0.00	0.00
Astressin2B #4	0.00	0.00
Astressin2B #5	0.00	0.00
Astressin2B #6	0.00	0.00

that led to a relatively small sample size for each group. It is also important to mention that in other experiments no surgical procedures were used before the behavioral tests and mice were not selected based on their alcohol level; therefore, a larger sample size and consequently a more complex statistical approach were used that may lead to statistically different outcomes (Lee et al., 2015, 2017; Van Hees et al., 2022).

Nevertheless, we would like to emphasize that the present study does not simply replicate but expands upon existing results based on the following observations. First, our study suggested for the first time that a single session of binge drinking produces anxiolytic and antidepressant effects immediately after binge drinking, rather than inducing anxiety-like and depression-like behavior, which mimics more closely how alcohol acts on humans. Second, our study was the first to investigate the role of CRF receptors in the affective component of binge drinking, reaching the conclusion that the anxiolytic and antidepressant effects produced by binge drinking are mediated by CRF2, whereas the anxiety-like and depression-like signs observed the next day are promoted by CRF1, an observation that may have therapeutic implications in humans.

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