

Alcohol hangover: impairments in behavior and bioenergetics in central nervous system

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ABSTRACT: Alcohol hangover (AH) is defined as the temporary state after alcohol binge-like drinking, starting when EtOH is absent in plasma. Results from our laboratory have shown behavioral impairments and mitochondrial dysfunction in an experimental model of AH in mice. Our model consisted in a single i.p. injection of EtOH (3.8 g/kg BW) or saline solution in male and female mice, sacrificing the animals 6 hours after injection. Motor and affective behavior together with mitochondrial function and free radical production were evaluated in brain cortex and cerebellum during AH. Results showed that hangover animals exhibited a significant reduction in neuromuscular coordination, motor strength and locomotion together with a loss of gait stability and walking deficiencies. Moreover, an increment in anxiety-like behavior together with fear-related phenotype and depression signs were observed. In relation to bioenergetics metabolism, AH induced a reduction in oxygen uptake, inhibition of respiratory complexes, changes in mitochondrial membrane permeability, decrease in transmembrane potential, increase in O₂^{•-} and H₂O₂ production and impairment in nitric oxide metabolism. All together our data suggest that the pathophysiological state of AH involves behavioral impairments and mitochondrial dysfunction in mouse brain cortex and cerebellum showing the long lasting effects of acute EtOH exposure in CNS.

The harmful use of alcohol caused approximately 3.3 million deaths and 5.1% of the global burden of disease was attributable to alcohol consumption (World Health Organization, 2014). Alcohol intake could be sub-classified at least in three different types of consumption: moderate, chronic and acute. The first one is considered harmless to human health as less than 40 grams of ethanol is consumed daily. Chronic alcohol consumption presents a variety of serious consequences on health altering numerous physiological, endocrine and behavioral functions since moderate to huge quantities of alcohol are consumed in a dependent and compulsive way (Rao *et al.*, 2015). Acute alcohol consumption, particularly defined as *binge drinking pattern*, includes huge quantities of alcohol consumed in a short period of time not only by mixing different kind of alcoholic beverages but occasionally

also combining them with abuse drugs (Rehm *et al.*, 2003; Stephens *et al.*, 2008).

As a consequence of binge drinking, a particular state defined as alcohol hangover is manifested. Alcohol hangover is described in humans as a physiological state that involves unpleasant next-day effects following excessive alcohol consumption (Verster, 2009). Hangover begins when EtOH is absent in plasma and is characterized by a cluster of psychophysical symptoms which include headaches, nausea, diarrhea, fatigue and tremors combined with decreased occupational, cognitive and visuospatial skills (Kim *et al.*, 2003; Howland *et al.*, 2008). Even though hangover is related with serious implications for driving and job performance, it has received little attention from the scientific community in contrast to the study of other alcohol disorders (Verster and Penning, 2010).

The first studies carried out in animal models provided insights into the physiological changes that occur during

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hangover (Gauvin *et al.*, 1992; Gauvin *et al.*, 1997). Further investigations established that hangover provokes hypoactivity (Doremus and Spear, 2007), fluctuations in body temperature, anxiety-like behavior (Zhang *et al.*, 2007) and reduced wheel running activity (Brasser and Spear, 2002).

One of the most important aspects in the study of behavioral and physiological consequences of alcohol hangover is the duration of the ethanol after-effects. In order to clarify this point, we developed an experimental model of hangover in Swiss mice. For such purpose, animals received i.p. injections of 3.8 g/kg EtOH or saline (control animals). The dose of EtOH was chosen based on that previously applied in hangover models by other authors (Doremus and Spear, 2007). Considering that hangover begins when EtOH is absent in plasma, we measured blood alcohol concentration and found that hangover starts six hours after EtOH exposure (Bustamante *et al.*, 2012). Thus, we tested different behavioral parameters at a basal point and every 2 hours up to 20 hours after hangover onset.

In relation to behavior analysis, hangover animals exhibited a significant reduction in the neuromuscular coordination and locomotion during 20 h. Also, these impairments were followed by a significant loss of gait stability and walking deficiencies together with a 60-75% deficit in motor strength for 16-18 h after hangover onset (Karadayian and Cutrera, 2013). According to this, Ling *et al.* (2010) reported the impact of alcohol hangover on driving and flying where motor coordination is one of the most affected skills together with bad cognitive performance, poor sleep quality and concentration problems. These data are in accordance with other researchers who evidenced an increase in body temperature and vocalizations 24 hours after EtOH administration in the rat (Sinclair and Gustafsson, 1987).

In addition, humans suffering from hangover self-reported depression (Swift and Davidson, 1998) or a general "decreased mood" (Mc Kinney, 2010). However, no studies were conducted to determine the time-extension of these symptoms. Thus, together with the analysis of motor performance, we explored the possible changes in affective behavior due to hangover. In this case, we observed that animals exhibited an increment in anxiety-like behavior together with fear-related phenotype and depression signs for 14-16 hours after hangover onset (Karadayian *et al.*, 2013). Moreover, motor and affective signs were tested under different light-dark conditions. Thus, we found that a particular photoperiod condition such as constant darkness proved to be effective to significantly reduce the recovery time for the impairments due to EtOH, suggesting that the circadian clock may be involved in the improvement of alcohol hangover symptoms (Karadayian *et al.*, 2014a).

It is well known that EtOH exposure can induce an increased production of reactive oxygen species and lipid

peroxidation in brain that could be associated with the induction of mitochondrial permeability transition, increasing the sensitivity of cells to damage and proapoptotic signals (Hoek *et al.*, 2002; Comporti *et al.*, 2010). In this sense, we explored the effect of EtOH hangover on mitochondrial function and free radical production in mouse brain cortex and cerebellum. In both brain areas alcohol hangover induced mitochondrial dysfunction six hours after of acute ethanol exposure. As detailed in Table 1, impairment in mitochondrial energy metabolism included deprivation in oxygen uptake, inhibition in respiratory complexes, changes in mitochondrial membrane permeability, decrease in transmembrane potential, increase in $O_2^{\cdot-}$ and H_2O_2 production and impairment in nitric oxide metabolism.

Particularly, alcohol hangover induced a reduction in RC (respiratory control) being its effect different between brain areas; while in brain cortex the reduction in respiratory control was due to a significant decrease in state 3 respiration, in cerebellum the decrease in RC was related to an increase in state 4 respiration (Bustamante *et al.*, 2012). The activity of enzymatic complexes of the mitochondrial respiratory chain was significantly reduced in both brain cortex and cerebellum. Specifically, decreases of 19% in complex I-III and 42% in complex IV activity were found in brain cortex while decreases of 38% and 16% were observed in cerebellum respectively. Related to complex II-III activity, a 36% decrease was found in brain cortex while in cerebellum no differences were evidenced. Changes in mitochondrial respiratory complexes could induce the opening of mitochondrial permeability transition pore, which is one of the molecular mechanisms causing mitochondrial dysfunction (Petronilli *et al.*, 1994). According to our studies, alcohol hangover induced an increase in mitochondrial permeability, indicating the presence of swelling. Also, a significant mitochondrial depolarization was observed after acute ethanol exposure together with increments of 20% and 90% in $O_2^{\cdot-}$ and H_2O_2 production in both brain areas (Karadayian *et al.*, 2015). Specifically, it was suggested that the increase in free radical production in cerebellum could result in permanent loss of GABAergic Purkinje cells (Ramezani *et al.*, 2012).

In order to characterize the alterations in the redox state associated with the hangover condition, we also explored the activity of antioxidant enzymes in cerebellum. The results indicate that catalase and glutathione peroxidase activities were decreased during hangover. On the contrary, SOD-1 and SOD-2 activities were significantly increased in EtOH hangover. Also, the activity of monoamine oxidase was 79% increased in the alcohol hangover group. All together, it seems that the increase in H_2O_2 levels is due in part to decreased metabolism by the antioxidant enzymes catalase and glutathione peroxidase and the increment in SOD isoforms and monoamine oxidase activities.

TABLE 1
Mitochondrial alterations and free radical production in brain cortex and cerebellum at the onset of alcohol hangover

Parameter/Brain area	Brain cortex	Cerebellum
State 3 oxygen uptake	↓63%	=
State 4 oxygen uptake	=	↑56%
RC	↓40%	↓45%
Complex I-III	↓19%	↓38%
Complex II-III	↓36%	=
Complex IV	↓42%	↓16%
$\Delta\psi$	↓20%	↓50%
$O_2\cdot^-$	↑17%	↑28%
H_2O_2	↑90%	↑90%
nNOS expression	=	↓50%
iNOS expression	↓40%	↓20%
NO production	↓25%	=

Original data from Bustamante *et al.*, 2012; Karadayian *et al.*, 2014b, 2015.

RC: respiratory control; $\Delta\psi$: membrane potential; nNOS: neuronal nitric oxide synthase; iNOS: inducible nitric oxide synthase; NO: nitric oxide.

Previous researches indicate that alcohol dependence does not only alter oxidative status, but also induces nitrosative species imbalance in the rat brain (Reddy *et al.*, 2013). Moreover, changes in mitochondrial NO metabolism were associated to memory and learning disabilities due to acute EtOH consumption (Chandler *et al.*, 1994). In this sense, we evaluated NO metabolism at the onset of alcohol hangover and found a clear decrease in NOS expression and NO production in brain cortex and cerebellum. Decreases in NO were associated with an impaired mitochondrial metabolism as observed in other models of mitochondrial dysfunction (Lores-Arnaiz *et al.*, 2005). Our results could indicate that hangover induces alterations in mitochondrial bioenergetics and reactive oxygen species generation in brain cortex and cerebellum with the consequent opening of the permeability transition pore with significant consequences for neural cell surviving (Bustamante and Lores-Arnaiz, 2010).

Melatonin is considered an endogenous free-radical scavenger and broad-spectrum antioxidant (Carpentieri *et al.*, 2012). Particularly, melatonin reacts with hydroxyl radicals in a diffusion-limited way by a sequential electron proton transfer (Galano, 2011). Supporting the hypothesis that alcohol hangover compromises mitochondrial functionality by inducing free radicals production, we tested the ability of the free radical scavenger melatonin to prevent mitochondrial damage due to alcohol hangover. Results indicated that melatonin can reduce the increment in reactive oxygen species production due to hangover and

also maintain the mitochondrial transmembrane potential (Karadayian *et al.*, 2014b). Interestingly, melatonin resulted to be also effective in reducing motor impairment in male mice but failed to protect behavioral disturbances in females. Thus, we showed that estrogen can block the protective action afforded by melatonin on motor performance deficits induced by EtOH (Karadayian *et al.*, 2012).

Unmanaged neuronal sudden withdrawal of the excessive alcohol consumption adversely alters neuronal integrity in vulnerable brain regions such as cerebellum, hippocampus and cortex (Jung and Metzger, 2010). The vulnerability to oxidative damage in the central nervous system was associated with movement disorders in rats during ethanol-withdrawal (Jung, 2014). It has been suggested that there is no hypothetical model explaining the pathology of alcohol hangover or an effective animal representation to study this state (Penning *et al.* 2010). We consider that the results obtained in our studies contribute to the knowledge of this pathophysiological state and to the proposal for a 'standard' criterion for hangover impairments.

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References

- Brasser SM, Spear NE (2002). Physiological and behavioral effects of acute ethanol hangover in juvenile, adolescent, and adult rats. *Behavioral Neuroscience* **116**: 305-320.
- Bustamante J, Karadayian AG, Lores-Arnaiz S, Cutrera RA (2012). Alterations of motor performance and brain cortex mitochondrial function during ethanol hangover. *Alcohol* **56**: 473-479.
- Bustamante J, Lores-Arnaiz S (2010). Characteristics of the mitochondrial permeability transition in brain cortex mitochondria. In: *Mitochondria: Structure, Functions and Dysfunctions*. (Svensson Oliver L, ed.) p. 829-847. Nova Science Publishers Inc. New York.
- Carpentieri A, Diaz de Barboza G, Areco A, Peralta López M, Tolosa de Talamoni N (2012). New perspectives in melatonin uses. *Pharmacology Research* **65**: 437-444.
- Chandler LJ, Guzman NJ, Sumners C, Crews FT (1994). Magnesium and zinc potentiate ethanol inhibition of N-methyl-D-aspartate-stimulated nitric oxide synthase in cortical neurons. *Journal of Pharmacology and Experimental Therapeutics* **271**: 67-75.
- Comporti M, Signorini C, Leoncini S, Gardi C, Ciccoli L, Giardini A, Vecchio D, Arezzini B (2010). Ethanol-induced oxidative stress: basic knowledge. *Genes & Nutrition* **5**: 101-109.
- Doremus-Fitzwater TL, Spear LP (2007). Developmental differences in acute ethanol withdrawal in adolescent and adult rats. *Alcoholism, Clinical and Experimental Research* **31**: 1516-1527.
- Galano A (2011). On the direct scavenging activity of melatonin towards hydroxyl and a series of peroxy radicals. *Physical Chemistry Chemical Physics* **13**: 7178-7188.
- Gauvin D, Briscoe R, Baird T, Vallett M, Carl KL, Holloway FA (1997). Cross generalization of an EtOH hangover cue to endogenously and exogenously induced stimuli. *Pharmacology Biochemistry and Behavior* **57**: 199-206.
- Gauvin DV, Youngblood BD, Holloway FA (1992). The discriminative stimulus properties of acute ethanol withdrawal (hangover) in rats. *Alcohol Clinical and Experimental Research* **16**: 336-341.
- Hoek JB, Cahill A, Pastorino JG (2002). Alcohol and mitochondria: a dysfunctional relationship. *Gastroenterology* **122**: 2049-2063.
- Howland J, Rohsenow DJ, Allensworth-Davies D, Greece J, Almeida A, Minsky SJ, Arnedt JT, Hermos J (2008). The incidence and severity of hangover the morning after moderate alcohol intoxication. *Addiction* **103**: 758-765.
- Jung ME, Metzger DB (2010). Alcohol withdrawal and brain injuries: beyond classical mechanisms. *Molecules* **15**: 4984-5011.
- Jung ME. (2014). Alcohol Withdrawal and Cerebellar Mitochondria. *Cerebellum* **15**: 4984-5011.
- Karadayian AG, Busso MJ, Feleder C, Cutrera RA (2013). Alterations in affective behavior during the time course of alcohol hangover. *Behavioral Brain Research* **253**: 128-138.
- Karadayian AG, Bustamante J, Czerniczyniec A, Cutrera RA, Lores-Arnaiz S (2014b). Effect of melatonin on motor performance and brain cortex mitochondrial function during ethanol hangover. *Neuroscience* **269**: 281-289.
- Karadayian AG, Bustamante J, Czerniczyniec A, Lombardi P, Cutrera RA, Lores-Arnaiz S (2015). Alcohol hangover induces mitochondrial dysfunction and free radical production in mouse cerebellum. *Neuroscience* **304**: 47-59.
- Karadayian AG, Cutrera RA (2013). Alcohol hangover: type and time-extension of motor function impairments. *Behavioral Brain Research* **247**: 165-173.
- Karadayian AG, Lores-Arnaiz S, Cutrera RA (2014a). The effect of constant darkness and circadian resynchronization on the recovery of alcohol hangover. *Behavioral Brain Research* **268**: 94-103.
- Karadayian AG, Mac Laughlin MA, Cutrera RA (2012). Estrogens block the protective action of melatonin in a behavioral model of ethanol hangover in mice. *Physiology and Behavior* **107**: 181-186.
- Kim DJ, Yoon SJ, Lee HP, Choi BM, Go HJ (2003). The effects of alcohol hangover on cognitive functions in healthy subjects. *International Journal of Neuroscience* **113**: 581-594.
- Ling J, Stephens R, Heffernan TM (2010). Cognitive and psychomotor performance during alcohol hangover. *Current Drug Abuse Reviews* **3**: 80-87.
- Lores-Arnaiz S, Perazzo JC, Prestifilipo LN, Dámico G, Czerniczyniec A, Bustamante J, Boveris A, Lemberg A (2005). Hippocampal mitochondrial dysfunction with decreased mtNOS activity in prehepatic portal hypertensive rats. *Neurochemistry International* **47**: 362-8.
- Mc Kinney A (2010). A review of the next day effects of alcohol on subjective mood ratings. *Current Drug Abuse Reviews* **3**: 88-91.
- Penning R, van Nuland M, Fliervoet LAL, Olivier B, Verster JC (2010). The Pathology of Alcohol Hangover. *Current Drug Abuse Reviews* **3**: 68-75.
- Petronilli V, Constantini P, Scorrano L, Colonna R, Passamonti S, Bernardi P (1994). The voltage sensor of the mitochondrial permeability transition pore is tuned by the oxidation-reduction state of vicinal thiols. Increased of the gating potential by oxidants and its reversal by reducing agents. *Journal of Biological Chemistry* **269**: 16638-16642.
- Ramezani A, Goudarzi I, Lashkarboluki T, Ghorbanian MT, Abrari K, Elahdadi Salmani M (2012). Role of Oxidative Stress in Ethanol induced Neurotoxicity in the Developing Cerebellum. *Iranian Journal of Basic Medical Sciences* **15**: 965-974.
- Rao PS, Bell RL, Engleman EA, Sari Y (2015). Targeting glutamate uptake to treat alcohol use disorders. *Frontiers in Neuroscience* **9**: Article 144.
- Reddy VD, Padmavathi P, Kavitha G, Saradamma B, Varadacharyulu N (2013). Alcohol-induced oxidative/nitrosative stress alters brain mitochondrial membrane properties. *Molecular and Cellular Biochemistry* **375**: 39-47.
- Rehm J, Room R, Graham K, Monteiro M, Gmel G, Sempos CT (2003). The relationship of average volume of alcohol consumption and patterns of drinking to burden of disease - An overview. *Addiction* **98**: 1209-1228.
- Sinclair JD, Gustafsson K (1987). Behavioral changes in rats on the day after acute ethanol intoxication. *Alcohol* **4**: 503-507.
- Stephens R, Ling J, Heffernan TM, Heather N, Jones K (2008). A review of the literature on the cognitive effects of alcohol hangover. *Alcohol and Alcoholism* **43**: 163-170.
- Swift R, Davidson D (1998). Alcohol hangover: mechanisms and mediators. *Alcohol Health Research World* **22**: 54-60.
- Verster JC (2009). The "hair of the dog": a useful hangover remedy or a predictor of future problem drinking? *Current Drug Abuse Reviews* **2**: 1-4.
- Verster JC, Penning R (2010). Treatment and Prevention of Alcohol Hangover. *Current Drug Abuse Reviews* **3**: 103-109.
- World Health Organization (2014). Global status report on alcohol and health. Department of Mental Health and Substance Abuse, Switzerland. (http://www.who.int/substance_abuse)
- Zhang Z, Morse AC, Koob GF, Schulteis G (2007). 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. *Alcohol Clinical and Experimental Research* **31**: 1811-1819.