

# **Alcohol hangover: underlying biochemical and neurochemical mechanisms**

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## **Abstract**

The aim of this review is to examine the current hangover research in both animals and humans and evaluate the key evidence for contributors to the alcohol hangover. Alcohol hangover occurs the day after a single episode of heavy drinking, possibly when the alcohol concentration in the blood approaches zero. The contribution of alcohol and its metabolites, neurotransmitter changes with particular reference to glutamate, neuroinflammation and congeners to hangover severity are presented. There are extensive variations in hangover research in humans with respect to the type of study, the definition of hangover, reliance on recall, uncontrollable variables, difficulty of blinding, and the different symptoms and methods for their measurement. Animal studies show variations with respect to the route of administration (intra-gastric (ig) or intra-peritoneal (ip)), the behavioural tests utilised and discrepancy in the timepoint used for hangover onset. Human studies of alcohol hangover have the significant advantage over animal models of easily being able to assess hangover severity and demonstrate that this correlates with specific behaviours or biochemical markers. However, animal models provide valuable insight into the neural mechanisms of hangover. Despite such limitations, several hangover models have identified pathological changes which correlate with the hangover state. Of these, alcohol metabolites, neurotransmitter alterations, inflammatory factors and mitochondrial dysfunction are the most likely to be involved in hangover pathology. Future research should aim to investigate the relationship between these factors and how they influence alcohol hangover.

## Introduction

Alcohol hangover is defined as the experience of various unpleasant physiological and psychological effects that follow the consumption of high quantities of alcohol. The symptoms occur several hours after hangover consumption, approximately 10h, (typically after a maximum blood alcohol content, BAC, is  $>0.8$ ) (Verster et al., 2014), which varies according to sex, weight and genetic factors. Hangovers can last for several hours or even more than 24 hours. Over 47 symptoms of hangover were identified (Penning et al., 2012), the most common symptoms being tiredness, headache, nausea and impaired attention (van Schrojenstein Lantman et al., 2017). Several of these symptoms also occur during chronic alcohol withdrawal, being more severe and of longer duration, in addition to other symptoms such as severe anxiety and tension, negative emotional state, sweating and seizures. Hangover also causes several neurocognitive impairments related to executive function (impairment of attention, memory and psychomotor skills) as well as everyday tasks such as driving. These cognitive impairments can reduce overall performance resulting in increased accidents, workplace absenteeism and reduced productivity. Reduced productivity has a huge overall impact on society; in 2010 the US Centre of Disease Control and Prevention estimated that alcohol hangovers cost approximately \$179 billion (Sacks et al., 2015).

Despite having such a large health and economic impact, there has been limited research into the biochemical and neurochemical changes that occur during this process. The symptoms of hangover begin several hours after the drinking session concludes, possibly when BAC falls to a very low level (Kim et al., 2003). or even approaches zero (van Schrojenstein Lantman et al., 2016). However, a range of biochemical and neurochemical parameters are likely to have altered prior to the occurrence of hangover symptoms, the intensity of such alterations could reflect the severity of the hangover

Common factors that have been identified as contributing to hangover severity, but not being the sole mechanism of action, are outlined in **Table 1A**. Symptoms of dehydration and sleep disturbances are commonly reported in subjects experiencing hangover, when changes in various hormones e.g. vasopressin and cortisol, as well as electrolytes and glucose content may be involved. Meanwhile genetic factors, e.g. individual difference in the propensity to experience hangover and of being resistant to hangover, are also important. Between 5% (Kruisselbrink et al., 2017) and 23% (Howland et al., 2008) of the population are reported to be hangover resistant and may therefore be worthy of further investigation to identify

biochemical and neurochemical differences between this population and those with a propensity to hangover (hangover sensitive) after alcohol ingestion.

Additives within alcoholic beverages, specifically congeners, are also known to contribute to hangover severity **Table 1B**. Congeners are substances that are produced during distillation and fermentation, and may contribute to the symptoms of hangover induced by ethanol. High concentrations of congeners are present in red wine and distilled spirit, e.g. brandy, and lowest in clear spirits such as vodka. A high content of congeners possibly results in a more severe hangover although the effect of ethanol itself will have a considerably stronger influence on hangover severity than congener content (Rohsenow and Howland, 2010). The list of congeners includes amines, amides, acetones, acetaldehyde, polyphenol, methanol, histamines, fusel oil, esters and tannins although the contribution of each compound to alcohol hangover is unknown. Methanol, a product of sugar fermentation is considered to be a major contributor to the symptoms of hangover. Alcohol dehydrogenase, ADH, will metabolise methanol at a slower rate than ethanol to form formaldehyde and formic acid both of which are highly toxic and may contribute to hangover. Young-Sup et al., (2005) assayed blood methanol in humans, 13h after ingestion of 1.5g/kg ethanol, and identified a positive correlation between methanol concentration and a subjective hangover scale. A highly significant correlation was also found between the presence of headache, nausea and vertigo and urinary methanol concentration in subjects 6-11h after ingestion of 50-80g ethanol (Bendtsen et al., 1998). Further studies on the role played by methanol on hangover severity are clearly warranted.

## **Biochemical and neurochemical factors involved in hangover**

### **a) Ethanol and its Metabolites**

It is unclear whether differences in ethanol elimination rates contribute to hangover. No correlation between breath alcohol content and hangover severity was identified in hangover sensitive or hangover insensitive individuals (Mackus et al., 2018). Interestingly urinary ethanol levels were significantly elevated in hangover sensitive subjects compared to hangover insensitive on the day after ethanol consumption, which correlated with a variety of hangover symptoms, including, nausea, concentration problems, sleepiness, weakness, apathy, sweating, stomach pain, thirst, heart racing, and anxiety as well as sleep problems in hangover sensitive individuals (Van de Loo et al., 2017). It therefore remains to be investigated whether changes in ethanol elimination rates may be responsible for reducing hangover severity.

Alcohol is initially metabolised by ADH to acetaldehyde which is rapidly metabolised to acetate by aldehyde dehydrogenase (ALDH). Acetate is a precursor of acetyl CoA which can be converted to carbon dioxide and water in the Krebs cycle. Although acetaldehyde is highly toxic, its rapid oxidation by mitochondrial ALDH may preclude any toxicity, although its rate of elimination in the brains unknown. Approximately 36% of East Asian subjects experience an alcohol-induced flush reaction after ingestion of a small amount of alcohol. It presents as flushing on the face, neck and shoulders, as well as nausea, tachycardia and headaches (symptoms often experienced in hangovers) which is caused by high levels of acetaldehyde due to a deficient gene variant of the mitochondrial ALDH. However, other populations, namely Native Americans, Australian, Irish and British also exhibit alcohol induced adverse effects (tachycardia, flushing and headache) after a small amount of alcohol, although this is not caused by an increase in circulating levels of acetaldehyde (Ward et al., 1994), but maybe caused by changes in the metabolism of vasoactive amines including histamine and catecholamines (Ward et al., 1994)

Ylikahri et al., (1974) reported that there was no correlation between blood acetaldehyde levels and hangover severity, thereby indicating that acetaldehyde played only a minor role in the adverse symptoms experienced during hangover. Another study showed that serum and urine acetaldehyde levels were relatively low and changed minimally following acute ethanol intake (Tsukamoto et al., 1989). Interestingly, in this study serum acetate levels were significantly elevated for at least six hours after ethanol ingestion which could indicate an increased acetate metabolism in the brain. Serum levels of acetate are reported to be in the mM range in contrast to acetaldehyde levels which are in the uM range. Since glucose metabolism may be altered after ethanol ingestion it has been suggested that acetate could be used as an alternative brain energy source. In dialysis patients it has been shown that increased blood acetate levels were associated with the presence of headache (Diamond and Henrich, 1987). In contrast, animal studies have suggested a role for acetaldehyde in hangover. Social interaction normalised after co-administration of 4g/kg ethanol with rosiglitazone (a peroxisome proliferator-activated receptor (PPAR)- $\gamma$  agonist) which increased ALDH2 activity (Jung et al., 2006), although acetaldehyde levels were not assayed. Inhibiting ALDH2 activity worsened hangover intensity in a chronic headache model in rats (Maxwell et al., 2010). Ethanol-induced effects on the central nervous system may be caused by acetate increasing adenosine content in many tissues, including the brain, (Campisi et al., 1997) and inducing reduced motor coordination and locomotor activity (Carmichael et al., 1991) and analgesic effects. These effects were reversed by administration of an adenosine receptor

blocker, suggesting that adenosine metabolically produced from ethanol may contribute to its central effects. Additional research using positron emission tomography-computed tomography, PET-CT, has recently shown colonic acetate can cross the blood brain barrier and be taken up by the brain (Frost et al., 2014). It has also been demonstrated to play a role in appetite suppression, a symptom which can occur during hangover (Penning et al., 2012). Further research is required to highlight how these metabolites contribute to alcohol hangover.

### **b) Neurotransmitters and receptors**

Acute ethanol consumption affects a variety of neurotransmitter systems which include: GABA, glutamate, dopamine, serotonin and endocannabinoid systems. The stimulating effects occurring in the initial alcohol intoxication relate to changes in dopamine and brain derived neurotrophic factor, BDNF, (Bosse et al., 2012), the latter promoting activation of the TrkB receptor and the downstream signalling pathways. The balance between inhibitory GABAergic and excitatory glutamatergic neurotransmission will also be altered, with reduced levels of GABA and GABA receptor insensitivity (Crews et al., 2005) and increased glutamate levels and glutamate receptor suppression. Meta-analysis data from published datasets of various regions of the rat brain, where the effect of acute ethanol administration on glutamate and GABA levels was determined, showed that extracellular levels of glutamate were decreased in the nucleus accumbens, while extracellular levels of GABA and glutamate were elevated in other regions (Fliegel et al., 2013). This was shown to correlate with intensity of withdrawal symptoms. The toxicity of such increased glutamate release plays an important role during the initial stages of alcohol withdrawal after chronic alcoholisation, in the striatum (Rossetti and Carboni, 1995; and the nucleus accumbens (Dahchour et al., 1996) as well as in 'binge drinking' (Ward et al., 2009). Future research should investigate the role of these neurotransmitter changes during hangover.

### **c) Inflammation**

Ethanol can profoundly impact inflammatory- related processes, both peripherally as well as in the brain in the absence and presence of an immune challenge. Furthermore, there is a complex relationship between ethanol exposure and the immune response, ethanol dampening cytokine expression in response to an antigen (e.g. Bhatti et al., 2011) yet exacerbating the cytokine response to bacterial challenge in other circumstances, (Qin et al., 2008). Toll like receptors (TLR) are activated by ethanol in both the periphery, e.g. monocytes, and the brain, e.g. microglia, (Alfonso-Loeches et al. 2010), (leading to

NFkappaB activation). In addition ethanol-induced dysbiosis in the gut (Moos et al., 2016), will induce the release of endotoxins from the 'leaky gut' to induce inflammation and oxidative stress in both glial and neuronal cells and the release of both anti-inflammatory and pro-inflammatory cytokines in the absence of an immunological challenge.

When ethanol is administered either chronically or voluntarily to rats, selective neuronal damage is induced as a result of increased oxidative-nitrosative stress and activation of the inflammatory response, elevating cytokine expression in the hippocampus and cortex ((Tiwari et al., 2009). However Marshall et al., (2013) reported that there was only partial microglia activation in a chronic ethanol model, suggesting that this was a consequence of alcohol induced damage rather than the source. An inflammatory response may lead to a variety of symptoms, e.g. nausea, vomiting, headache, confusion, tremor, as well as clinical depression (Harrison et al., 2009), (inducing mood changes and cognitive impairment), and learning and memory deficits (Dantzer, 2004), many of which are evident during hangover, although to a lesser degree than chronic alcoholism and withdrawal. Alteration in specific cytokine levels observed during hangover may contribute to such adverse effects (Dantzer, 2004). For example, in one study ethanol, 4g/kg, ip, was administered to adult Sprague-Dawley rats, and IL-1 and TNF $\alpha$  increased in the hypothalamus, IL-6 and TNF $\alpha$  were elevated in the hippocampus after 3h but there were no significant alterations of any of these cytokines in the cerebellum. Increases in each of these cytokines was detected in the liver and spleen (Doremus-Fitzwater et al., 2014) Following 4g/kg ethanol i.p., IL6 levels were elevated in the hippocampus, paraventricular nucleus (PVN) and amygdala after 3h (Doremus-Fitzwater et al., 2015). Administration of 6g/kg ethanol to C57BL/6J mice, induced an increase in IL4 as BAC approached 0 (Walter and Crews, 2017) although such studies were of brain homogenates such than subtle changes of cytokines in specific brain regions would have been lost. In an animal model of social anxiety, central injection of several cytokines (e.g., IL-1, TNF $\alpha$ ) augmented ethanol withdrawal-associated anxiety, possibly through corticotrophin releasing factor-related mechanisms (Knapp et al., 2011). Varying results are reported for changes in cytokines during hangover which may relate to the sampling timing, concentration of ethanol administered, route of administration and the different tissues used. Changes in cytokines should be evaluated prior to, during and after hangover to understand the role of each cytokine in hangover.

Increased levels of cytokine are evident in the blood, mononuclear cells and saliva during hangover. IL8 levels were significantly increased in healthy non-alcoholic volunteers 36h after an acute alcohol challenge (60g) (Gonzalez-Quintela et al., 2000) while elevated IL-1Ra and decreased MCP-1 were identified in 20 healthy males during intoxication following a peak BAC of 0.12%. However, in another study of healthy subjects, where vodka, 4.28ml/kg was

administered, MCP-1 was increased after 24h (Neupane et al., 2016). Mononuclear cells isolated from the blood of healthy subjects after Ingestion of 1.5g/kg ethanol, showed induced significant elevations of IL-10, IL-12, and IFN-gamma after stimulation with phytohemagglutinin during the hangover state, (after 13h hours), but no significant changes were evident for IL-1b, IL-4, IL-6 and TNF $\alpha$  by comparison to the pre-ethanol value (Kim et al., 2003). Furthermore, IFN $\gamma$  and IL-12 concentrations were positively correlated with total hangover scale values. Saliva and urine collected from 36 healthy participants after a night of heavy drinking approximately 11.6 alcoholic drinks (undefined in grams), showed an increase in saliva content of IL2, IL4, IL5, IL6, IL10, IFN $\gamma$ , and TNF $\alpha$  nine hours after ethanol consumption while increases in urinary levels of IL4 and IL6, IL8 were assayed (Raasveld et al., 2015). Interestingly urinary cytokine levels were less pronounced than saliva. BACs were not assayed, and it was assumed that hangover occurred at 9 hours. Dividing the subjects according to whether they were alcohol sensitive or alcohol insensitive did not identify any differences in the altered cytokine levels during the hangover period. Assessment of immune status by Immune Function Questionnaire (IFQ) which catalogues the occurrence of sore throat, headache, flu, runny nose, coughing, cold sores, mild fever, warts, pneumonia, bronchitis, sinusitis during the past year (van de Loo et al., 2018) identified that drinkers with hangovers had significantly higher self-reported overall immune function scores when compared to hangover-resistant drinkers (mean  $\pm$  SD = 10.5  $\pm$  3.6 versus 13.1  $\pm$  4.9,  $p$  = 0.0001), indicating a poorer immune status. The interpretation as to whether cytokines are altered in these subjects is unclear as it is unknown whether a good immune response is associated with higher or lower circulating cytokine levels.

Glial cells play an important role in alcohol induced neuroinflammation and may contribute to the alcohol hangover. Microglia are the primary mediators of the innate immune system within the brain (Lenz and Nelson, 2018). Increases in microglia numbers, as assayed by the presence of the microglia markers Iba and CD68 were quantitated 18h after ethanol administration when BAC approached 0. Depletion of microglia in male C57BL/6J mice using colony stimulating factor 1 receptor (CSF1R) inhibitor PLX5622 (Walter and Crews, 2017) resulted in a blunted response in pro-inflammatory cytokines (TNF $\alpha$  and Ccl2) after 6g/kg ethanol. However, depleting microglia did not reduce gene expression of all neuroinflammatory factors or change the behavioural response (motor impairment measured using a rotarod). The role of the microglia in ethanol induced cytokine changes was also investigated using minocycline (a microglia inhibitor) in Adult Sprague-Dawley rats (350-450g) given 4g/kg ethanol intraperitoneally (Doremus-Fitzwater et al., 2014). The results showed that the application of minocycline failed to reverse ethanol related changes in cytokine expression. These findings highlight that the microglia may not express cytokines in response

to an acute ethanol challenge and further investigation is required to identify the mechanism of this neural inflammation.

Indirect evidence for inflammation contributing to alcohol hangover was indicated by the fact that various anti-inflammatory agents, such as ibuprofen and other nonsteroidal anti-inflammatory drugs (NSAIDs) may help alleviate headache and other adverse symptoms. Tolfenamic acid, a NSAID, at a dose of 400mg, could reduce the severity of hangover symptoms (nausea, vomiting, thirst, dry mouth, tremor, and irritation) and hangover score when administered prior to and after ethanol consumption (Jayawardena et al., 2017) Plasma levels of prostaglandin E<sub>2</sub> and thromboxane B were also reduced.

#### **d) Mitochondrial dysfunction**

Ethanol-induced damage to mitochondrial DNA, particularly in the liver has been extensively studied. Since brain cells are reliant on mitochondria, as they operate at the limits of energy availability, even slight damage to mitochondrial may result in elevated free radical production to induce toxicity in a number of brain region e.g. cerebellum, prefrontal cortex and hippocampus. In various studies the onset of hangover was found to correlate with: mitochondrial dysfunction as exemplified by alterations in ROS and RNS in the cortex of mouse models (Bustamante et al., 2012) which also correlated with reduced motor performance. Mitochondrial dysfunction was also present in other brain regions (cerebellum) as a result of alcohol hangover (Karadayian et al., 2015). Administration of a free radical scavenger to male mice, e.g. melatonin, protected against mitochondrial dysregulation and the motor impairments that result from alcohol hangover (Karadayian et al., 2014). Studies of free radical production and antioxidant status in isolated mitochondria and synaptosomes from male Swiss mice concluded that the synaptic terminals were mainly affected, suggesting bioenergetic dysregulation may contribute to the pathology of alcohol hangover. (Karadayian et al., 2017). Such results indicate redox imbalance may contribute to the motor related symptoms of alcohol hangover in mice. Neuronal mitochondria play an important role in neuronal development and synaptic plasticity, such that their dysfunction can produce apoptosis and ROS both of which have been linked to cognitive impairment (Da Silva et al., 2018).

#### **e) MRI Spectroscopy**

One area of research that has not yet been applied to hangover is neuro-imaging. Techniques such as functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy (MRS), electroencephalogram (EEG), magnetoencephalography (MEG) and positron-



emission tomography (PET) offer unparalleled ability to explore regional changes in brain function and metabolism. This has revolutionised the study of many normal and abnormal brain functions and have recently been used to study the effects of alcohol. For example, fMRI has recently showed that when a steady state concentration of blood alcohol of 0.08% was achieved, there was increased brain blood flow in thalamus but no other brain regions (Quelch et al., 2017). This is an area of great potential and we plan to exploit this technique in our further studies.

## **Conclusions**

The hangover state is a multifactorial event involving a variety of biochemical, neurochemical events as well as genetic factors which lead to the appearance of hangover symptoms. However, one of the major drawbacks has been establishing the exact time that hangover commences. A variety of animal and human studies have been conducted with a wide variety of ethanol doses administered by a variety of different routes. Further limitations in this field include: the changing definition of hangover, reliance on recall, uncontrolled variables, difficulty blinding and the extent of symptoms encountered and their measurements. Human alcohol hangover studies have the significant advantage over animal models of easily being able to assess hangover severity and demonstrate that this correlates with specific behaviours or biochemical markers. However, animal models provide valuable insight into the neuronal mechanisms of hangover. Despite multiple limitations, several hangover models have identified pathological changes which correlate with the hangover state. Of these inflammatory factors, alterations in neurotransmitters and receptors, mitochondrial dysfunction and alcohol metabolites are the most likely to be involved in hangover pathology. Upcoming investigations should focus on the contribution of different factors to specific hangover symptoms or the hangover state in general. It is likely that multiple factors contribute to the production of the alcohol hangover, but individual factors may be attributable to a few of the symptoms. It is therefore important that ongoing research continues to assess alcohol hangover as an individual concept but also as a collection of symptoms. Future research should aim to investigate the relationship between these factors and how they influence alcohol hangover as well as considering confounding factors that may contribute to the severity of alcohol hangover, e.g. quantity of alcohol consumed, sleep, food intake, genetics, blood glucose levels, dehydration and congeners.

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Table 1A. **Factors influencing alcohol hangover.** factors that can contribute to hangover but are not solely responsible for hangover pathology.

<b>Factor</b>	<b>Factor effect</b>	<b>Reference</b>	<b>Evidence</b>	<b>Associated with hangover severity?</b>
<b>Dehydration</b> Dehydration can lead to thirst, dizziness, headaches and concentration and memory problems these are also common hangover symptoms (van Schrojenstein Lantman et al., 2017).	Changing hormone levels	(Linkola et al., 1978).	Vasopressin	YES
		(Penning et al., 2010)	Vasopressin, Aldosterone, Cortisol, Or Renin	NO
	Hypoglycaemia	(Ylikahri et al., 1974).	Blood Metabolites (Including Glucose)	NO
		(Kruisselbrink et al., 2006).	Blood Glucose Concentration	NO
		(Ylikahri et al., 1976)	Supplementation of Glucose to reduce hangover	NO
	Changing electrolyte levels	Ylikahri et al., 1974)	Electrolytes (Na <sup>+</sup> , K <sup>+</sup> , Cl <sup>-</sup> , Ca <sup>++</sup> , And Mg <sup>++</sup> )	NO
acid-base status			YES	
<b>Sleep disturbance</b> The most frequently reported symptom of alcohol hangover was drowsiness (Penning et al., 2012).	Alcohol disrupting circadian rhythms	Karadayian et al., 2014)	Resynchronisation of circadian rhythms	YES (improved recovery time)
	Alcohol consumption was shown to impact the quality and quantity of sleep (Rohsenow et al., 2010).	Rohsenow et al., 2010)	Sleep disruptions	YES (but, did not correlate with cognitive performance)
<b>Genetic influence</b> 8.1% of drinkers reported to be hangover resistant even at high levels of consumption (BAC >0.20%) (Verster et al., 2014). Another study found that above 0.8% the prevalence of hangover resistance was about 5% (Howland et al., 2008)	<i>Genetic factors</i>	(Slutske et al., 2014).	Twin study survey suggests that genetic factors account for hangover:  Frequency (45% and 40% (men and women)  Resistance (43%)  Susceptibility (24% (and 16% (men and women)	YES



**Table 1B. Alcohol Additives (Congeners)** that can contribute to hangover severity. Naturally occurring chemical compounds found in alcoholic beverages, often the products of the distilling or fermenting process used for alcohol production (Rohsenow et al., 2010)

<b>Congeners</b>			<b>Associated with hangover severity?</b>
General congener levels	Rohsenow et al., 2010)	Different levels of general congeners vodka compared with bourbon	YES (but not impaired cognitive performance)
<b>Examples of specific congeners influencing hangover severity:</b>			
Histamine	(Zimatkin and Anichtchik, 1999).	Individuals who suffer from the alcohol flushing reaction' have seen some relief if they take H-1 and H-2 receptor antagonists, which would reduce histamine function	N/A
Methanol	(Young-Sup et al., 2005).	Methanol Concentration in the blood	YES/NO (subjective hangover severity but not psychiatric evaluation)
	(Mackus et al., 2017).	Urine methanol concentration	NO