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# Effects of binge-like ethanol exposure during adolescence on the hyperalgesia observed during sickness syndrome in rats

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ABSTRACT

Acute and chronic ethanol exposure increases the risk of infection by altering the innate host's defense system. Adolescence is a critical period for brain development. Insults during this period may have long-lasting consequences. The present study investigated the effects of binge-like ethanol exposure in adolescent rats on mechanical hyperalgesia during sickness syndrome that was induced by a systemic injection of lipopolysaccharide (LPS) or an intracerebroventricular (i.c.v.) injection of interleukin-1β (IL-1β) after the cessation of ethanol exposure. Male Wistar rats were exposed to ethanol from postnatal day (PND) 25 to PND 38 in a binge-like pattern. Hyperalgesia was assessed on the right hindpaw after an intraperitoneal injection of LPS (5 and 50 μg/kg, intraperitoneally) on PND 51 and PND 63 or an i.c.v. or intraplantar (i.pl.) injection of IL-β (3 and 1 ng, respectively) on PND 51. Ethanol exposure during adolescence did not alter mechanical thresholds which increased normally with age. The systemic injection of LPS (0.5–50 μg/kg) in adult rats induced dose-related mechanical hyperalgesia. Binge-like ethanol exposure significantly increased mechanical hyperalgesia that was induced by 50 μg/kg LPS on PND 51 and 63, which lasted until 24 h after the injection. This change was not observed at a lower dose of LPS (5 μg/kg). Acute oral treatment with ethanol 24 h prior to LPS administration did not alter mechanical hyperalgesia. The i.c.v. injection of IL-1β (1–10 ng) also induced dose-related mechanical hyperalgesia in the right hindpaw in non-exposed animals. In animals that were exposed to binge-like ethanol, the i.c.v. or i.pl. injection of IL-1β also increased hyperalgesia on PND 51. These results suggest that binge-like ethanol exposure during adolescence causes alterations in the central nervous system that can increase mechanical hyperalgesia that is observed during sickness syndrome, and this effect can be observed until adulthood after the cessation of ethanol exposure.

## 1. Introduction

Ethanol (EtOH) consumption usually begins in early adolescence (12–14 years old) and increases until 21 years of age (Masten et al., 2009). These individuals usually drink intermittently, frequently adopting a pattern of binge drinking (Masten et al., 2009). Binge drinking is a pattern of drinking that results in blood alcohol levels of 0.08 g/dl which is achieved approximately after five drinks for men and four drinks for women within a period of 2 h (NIAAA, 2016). The World Health Organization has a similar definition for heavy episodic drinking, which is the consumption of 60 g or more of pure EtOH (six or more standard drinks in most countries) on at least one single occasion at least monthly (World Health Organization, 2014). The consumption may reach 6.2 l of EtOH annually per person aged 15 years or older (World Health Organization, 2014).

Adolescence is a critical period of life when complex interactions between genetic factors and environmental experiences remodel certain brain areas that confer neural growth into adulthood (Fine and Sung, 2014). Excessive EtOH consumption during this period leads to changes in the prefrontal cortex, cerebellum, and hippocampus, followed by cognitive dysfunction and emotional and memory deficits (Fowler et al., 2014; Oliveira et al., 2015; Pascual et al., 2007; Pascual et al., 2014).

In addition to these changes in the central nervous system, EtOH consumption can alter innate and adaptive immune responses (Goral et al., 2008; Jimenez-Ortega et al., 2011). The pattern of EtOH ingestion is determining factor with regard to whether these changes are temporary or permanent. For example, Jimenez-Ortega et al. (2011) showed that intermittent EtOH consumption decreased lymph node and spleen lymphocyte cell populations more than acute or chronic EtOH

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consumption. The release of proinflammatory cytokines was shown to be suppressed after stimulation with lipopolysaccharide (LPS) and phytohemagglutinin in the blood after acute EtOH exposure in humans (Afshar et al., 2015). Conversely, microglial activation and an increase in the synthesis of proinflammatory cytokines, chemokines, and toll-like receptors (TLRs) through nuclear factor κB activation is seen in the human, rat, and mouse brain during EtOH addiction (Crews et al., 2011). Furthermore, intermittent EtOH exposure during adolescence induces inflammatory brain damage, characterized by higher levels of cyclooxygenase-2, nitric oxide synthase, and caspase 3 activity and apoptotic cell death, assessed by the determination of DNA fragments. This damage was associated with behavioral changes, such as worse performance on a conditional discrimination task, worse motor performance, lower discrimination of novel and familiar objects, and worse performance on a beam walking task (Pascual et al., 2007). The higher levels of inflammatory markers in the central nervous system that are associated with behavioral changes in animals suggest that EtOH consumption influences pain processing.

Despite the habitual use of EtOH by patients with chronic pain, recent studies showed that intermittent EtOH consumption did not alleviate thermal hyperalgesia that was associated with neuropathic pain (Gonzalez-Sepulveda et al., 2016). In fact, chronic EtOH administration induced tolerance to the antinociceptive effects of EtOH, and a hyperalgesic response was observed in the animals' paws 12 h after the last EtOH administration (Gatch, 2002). Other studies reported that binge EtOH drinking induced mechanical, thermal, and inflammatory hyperalgesia during the withdrawal period (Bergeson et al., 2016; Dina et al., 2006). The consumption of EtOH was shown to be a comorbid risk factor for peripheral neuropathy in patients who were treated for human immunodeficiency virus (Ferrari and Levine, 2010; Lopez et al., 2004; Moyle and Sadler, 1998; Nath et al., 2002)

We recently found that binge exposure to EtOH during adolescence reduced the febrile response that was induced by LPS and interleukin-1β (IL-1β), even 12 days after the cessation of EtOH consumption (Telles et al., 2017). The intraperitoneal (i.p.) administration of LPS caused a systemic inflammatory response syndrome that was characterized by fever, hyperalgesia, anorexia, and sleepiness. Altogether, these symptoms are referred to as sickness syndrome. The aim of the present study was to investigate whether binge-like EtOH administration during adolescence in rats affects LPS- and IL-1β-induced hyperalgesia during the sickness syndrome.

## 2. Materials and methods

### 2.1. Animals

The experiments were conducted in 21-day old ($n$ = 108) and 60-day-old ($n$ = 68) male Wistar rats that were obtained from the animal facility of the Federal University of Paraná. The animals were housed five per cage in a temperature-controlled room at 22°C ± 1°C under a 12 h/12 h light/dark cycle (lights on at 7:00 AM) with food and water available ad libitum. All of the procedures were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and Brazilian regulations on animal welfare. All of the procedures were previously approved by the institution's Ethical Committee for Animal Use and were in accordance with Brazilian and EU Directive 2010/63/EU Guidelines for Animal Care. All efforts were made to minimize the number of animals used and their suffering and the animals received only one injection of LPS or IL-1β.

### 2.2. Mechanical hyperalgesia

Mechanical hyperalgesia was assessed using an electronic Von Frey anaesthesiometer (Insight, Ribeirão Preto, SP, Brazil) as described previously (Redivo et al., 2016). The animals were individually placed in a Plexiglas compartment (20 cm length × 25 cm width × 15 cm height) on a wire mesh floor platform for 60 min for habituation. The mesh floor allowed the tip of the anaesthesiometer to stimulate the mid-plantar right region of the hindpaw using a disposable polypropylene tip (0.5 mm diameter) that was connected to a force transducer and digital display that allowed determination of the force (in grams) applied. A linear increase in force was applied to the paw until the occurrence of a paw withdrawal response. The mechanical threshold was calculated as the average value of three similar withdrawal responses. The occurrence of mechanical hyperalgesia was determined as the difference between the average mechanical threshold observed prior to and at different time points after the administration of LPS or IL-1β.

### 2.3. Intracerebral cannula implantation and microinjection

For intracerebroventricular (i.c.v.) administration, a 22-gauge stainless-steel guide cannula (0.8 mm outer diameter, 12 mm length) was stereotaxically implanted in the right lateral ventricle under ketamine/xylazine (90/10 mg/kg) anesthesia on postnatal day 45 (PND 45). The stereotaxic coordinates were the following: 0.8 mm lateral to midline, 1.5 mm posterior to bregma, and 2.5 mm below the brain surface, with the incisor bar lowered 3.3 mm below horizontal zero (Paxinos and Watson, 1998). The cannulas were fixed to the skull with jeweler's screws that were embedded in dental acrylic cement. The animals were allowed to recover for 5 days before the oral administration of EtOH on PND 50, followed by the injection of IL-1β on PND 51. After the end of the experiment, each rat was microinjected with Evans blue (2.5% in saline) in the lateral ventricle. The brains were removed, and animals that had cannula misplacements, cannula blockage during the injection, or abnormal body weight gain patterns after surgery were excluded from the study (2 animals in 56). IL-1β was administered over 1 min using polyethylene-10 PE-10 tubing.

### 2.4. Dose-response curve of LPS- and IL-1β-induced mechanical hyperalgesia

Basal mechanical thresholds in non-EtOH-exposed adult animals were measured as described above. After this measurement, the animals received 0.5, 5, and 50 μg/kg LPS, i.p. (*E. coli*, 0111:B4, Sigma-Aldrich, St. Louis, MO, USA). Hyperalgesia was evaluated every hour up to 6 h. Control animals received the same volume of vehicle (sterile saline). Basal mechanical thresholds were similarly measured in animals that received IL-1β (1, 3, and 10 ng/2 μl, i.c.v., R & D Systems, Minneapolis, MN, USA), and mechanical hyperalgesia was measured 3, 6, and 24 h after the injection.

### 2.5. Effect of EtOH exposure on mechanical hyperalgesia

#### 2.5.1. Binge-like EtOH exposure during adolescence

Ethanol (3 g/kg, 25% w/v in saline) was administered i.p. on PND 25 (EtOH-pretreated group). The control group received an equivalent volume of saline. On PND 26, 29, 30, 33, 34, 37, and 38, the animals received the same treatment. This binge-like pattern was chosen based on Lerma-Cabrera et al. (2013) and Forbes et al. (2013) to simulate a pattern of acute intoxication (i.e., binge) that is common during adolescence in humans. The i.p. route of administration was chosen to avoid variations in blood EtOH levels that can be caused by different patterns of consumption of drinking water or inconsistent gastrointestinal absorption. To evaluate hyperalgesia, the animals were divided into two groups. In one group, 12 days after binge-like EtOH exposure (PND 50), the animals received an additional dose of EtOH or distilled water orally (Fig. 1). In the other group, 24 days after binge-like EtOH exposure (PND 62), the animals received an additional dose of EtOH or distilled water orally (Fig. 1). The mechanical hyperalgesia experiments were performed the next day (PND 51 [Fig. 1A] or PND 63 [Fig. 1B]) when the animals were treated with saline, LPS (5 and 50 μg/