Physiology & Behavior 139 (2015) 50–58

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Ethanol-induced tolerance and sex-dependent sensitization in preweanling rats

S. Castello, D.A. Revillo, J.C. Molina, C. Arias *

Instituto de Investigación Médica M. y M. Ferreyra, INIMEC-CONICET-Universidad Nacional de Córdoba, Friuli 2434, Córdoba, Argentina

HIGHLIGHTS

- The same ethanol dose induced locomotor sensitization and tolerance in infant rats.
- Only males displayed sensitization induced by ethanol.
- Tolerance was observed when the training and testing contexts coincided.
- Preexposure to the context attenuates the acute stimulating effect of ethanol.

ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 31 October 2014
Accepted 3 November 2014
Available online 8 November 2014

Keywords:
Ethanol
Locomotor activity
Infant rat
Tolerance
Sensitization
Context
Sex

ABSTRACT

According to genetic studies, the acute stimulating effect of ethanol seems to be associated with an increased predisposition to consume large quantities of ethanol. Ethanol-induced stimulation has been rarely reported in adult rats. However, infant rats, particularly during the second postnatal week of life, are highly sensitive to ethanol-induced behavioral activation. They also consume more ethanol than in later ontogenetic stages. In adult mice repeated ethanol experience usually results in sensitization to the stimulating effect of ethanol, while tolerance is the predominant result in rats. The present study was designed to explore in rats whether repeated exposure to ethanol during infancy modifies subjects' sensitivity to the stimulating effect of the drug, either increasing or decreasing its magnitude (i.e. sensitization or tolerance, respectively). Furthermore, we also explored the possible context-modulation of these effects. In two experiments, subjects were trained with water or ethanol (2.5 g/kg) between postnatal days (PDs) 8 and 12 (Experiment 1) or between PDs 14 and 18 (Experiment 2), and tested in response to water or ethanol two days later. In these experiments we identified three variables that critically modulate the effect of the repeated ethanol exposure: sex, context and age. Ethanol exclusively and consistently induced locomotor sensitization in males trained outside of the testing context (Experiments 1a and 1b), while tolerance to the stimulating effect of ethanol was observed in males and females trained in the testing context (Experiment 1a). In Experiment 2 tolerance was detected in females trained outside of the testing context. Finally, experience with the testing context during training strongly attenuated the stimulating effect of ethanol in the older subjects (Experiment 2). These results show that the same ethanol treatment can produce opposite effects (tolerance or sensitization) and demonstrate the involvement of Pavlovian conditioning in the development of tolerance. Furthermore, sex was revealed as an important factor to take into consideration in the analysis of chronic experience with ethanol during infancy. We can conclude that specific ontogenetic stages can be used to study the biological determinants underlying both ethanol-induced tolerance and sensitization, and the environmental modulators of these effects.

1. Introduction

Ethanol-induced locomotor stimulation in laboratory rats has been considered a valuable tool for studying the motivational effects of this drug [28,64,83]. In rodents, the expression of this ethanol effect depends on the complex interaction of a variety of environmental and genetic factors, including the rodent species, individual differences (sex, age, behavioral traits) and variables related to the prior experience with both the drug itself and the environment in which the drug effects are experienced, among others [46,59]. The specific biological correlates of the behavioral activation induced by ethanol have mainly been studied in mice, since this rodent species shows this effect under a wider range of experimental conditions than rats [59]. Usually, when adult rats are injected with ethanol using similar parameters to those used with mice, the effect produced tends to be one of sedation rather than stimulation [25,46]. However, the locomotor activating effect of ethanol has been consistently observed in genetically selected rats that consume

* Corresponding author.
E-mail address: carlosargr@gmail.com (C. Arias).

http://dx.doi.org/10.1016/j.physbeh.2014.11.008
0031-9384/

high amounts of ethanol [1,18,55,61], which may indicate a positive correlation between subjects' sensitivity to the stimulating effect of ethanol and their predisposition to ingest the drug.

Similarly to that observed with other drugs of abuse, repeated experience with ethanol can result in an increased (sensitization) or reduced (tolerance) sensitivity to some of its specific effects. From different theoretical perspectives, both tolerance and sensitization induced by ethanol (or by other drugs) have been linked to the development of dependence [39,66,72,75]. The way biological and environmental factors interact to modulate these opposite drug effects are still not fully understood, and also depend on the specific behavioral or physiological index analyzed. For example, even with the same ethanol treatment, tolerance to one ethanol effect (rearing or ataxia) and sensitization to another (locomotor activity) have been observed in the same animal [52]. While ethanol-induced locomotor sensitization is easily observed in mice, in rats this effect is infrequent [58], and even when behavioral activation is observed after ethanol administration, rats rapidly develop tolerance to this effect [9,34].

The importance of studying these effects of ethanol (i.e. tolerance and sensitization) during infancy is based on a considerable amount of converging evidence from human and laboratory studies, which have shown that early experience with ethanol is an important determinant of responsiveness to the drug in later stages of ontogeny [23,74]. This association highlights the importance of understanding those factors that modulate the outcome of early experience with the drug. In many studies, the infant rat has been characterized by an increased responsiveness to the acute effects of ethanol, showing particular sensitivity to this drug's motivational and motor stimulating effects, especially during the first two postnatal weeks [2,5,53,54]. This profile is accompanied by a clear predisposition to consume relatively large amounts of ethanol [68,78]. Beyond this, however, few studies have focused on sensitivity to ethanol during infancy after repeated experiences with the drug [7,9,24,35,71].

Another argument to justify the study of tolerance and sensitization during infancy is that these effects have been described in different animal models as being context-dependent, which means that when subjects are evaluated in a different context from the one in which they were trained, the effects are attenuated or eliminated [16,70,75]. Context effects are particularly important in studies with infant rats, because their capacity to retain context learning is a matter of current debate (see, for example, [37,60]). The involvement of classical conditioning in ethanol-induced sensitization or tolerance has been observed in adult rodents by measuring different behavioral indexes, the most common one being hyperlocomotion in sensitization studies with mice [21], and the hypothermic and depressant effects of ethanol in the study of tolerance [27,44,82]. Although few additional studies have reported tolerance to the locomotor stimulating effect of ethanol in rats [9,34], the context-dependence of this effect has not been analyzed. In infant rats, locomotor sensitization induced by psychostimulants has been described as context-independent [40,47,48], although other authors have found context-specific locomotor sensitization induced by cocaine during this ontogenetic period [77].

The present study was designed to explore whether the locomotor response to ethanol is increased (sensitization) or reduced (tolerance) in pups repeatedly exposed to the drug during the infantile period. The protocol used in the experiments described below also enabled us to explore the possible context-modulation of these ethanol effects during the second (Experiment 1) or third (Experiment 2) postnatal weeks of life.

2. Experiment 1a

In this first experiment we focused our attention on the second postnatal week of life. Our interest in this specific stage of development stems from studies which have observed heightened sensitivity to ethanol-induced behavioral activation during this period [5], along with a predisposition to consume large amounts of ethanol [68]. Interestingly, it has been shown that the development of locomotor sensitization induced by ethanol may be associated with an increased consumption of the drug in mice with high initial affinity of ethanol [42]. Therefore, it is likely that ethanol-induced sensitization can also be detected at this early stage of development. In a previous study we demonstrated that ethanol (2.5 g/kg) can produce biphasic locomotor effects, increasing activity soon after ethanol administration (5–10 min) and attenuating exploration 30 min after administration [4]. In Experiment 1a we explored the effect of a repeated ethanol treatment, between PDs 8 and 12, on the locomotor response induced by ethanol after two days of withdrawal (PD15). In order to capture the biphasic locomotor effects of the drug, independent samples of subjects were trained and tested at two post-administration intervals, 5–10 or 30–35 min. Finally, the influence of context learning on the acute and chronic effects of ethanol was also explored. We previously found that prior experience with the testing context attenuates the stimulating effect of ethanol, and that this ethanol effect critically depends on novelty of the testing environment [4]. Considering also that environmental novelty is an important factor that can influence the development and expression of sensitization, it can be expected that detection of ethanol-induced sensitization in our experimental model may be benefited if subjects have not experience with the testing context before testing.

2.1. Materials and methods

2.1.1. Subjects

For Experiment 1a, a total of 169 male and female Wistar pups were used, 108 animals representative of 18 litters corresponding to post-administration time 5–10 min, and 61 animals from 8 litters for post-administration time 30–35 min. In all the experiments, no more than one subject of each sex from a given litter was assigned to the same treatment condition, to avoid overrepresentation of a particular litter in any treatment. Animals were born and reared at the vivarium of the Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC–CONICET-UNC, under conditions of constant room temperature (22 ± 1.0 °C), on a 12 h light–12 h dark cycle. Births were examined daily and the day of parturition was termed postnatal day 0 (PD0). Subjects were 8 days old at the start of the experiment. All procedures were approved by the National Department of Animal Care and Health (SENASA — Argentina) and were in compliance with the National Institute of Health's general guidelines for the Care and Use of Laboratory Animals.

2.1.2. Apparatus

In Experiment 1a, all animals were tested in a circular open field (30 cm diameter), with a white plastic wall and floor. A piece of cotton impregnated with almond odor (almond scent, 1 ml of a 0.1% solution v/v, Esencias del Boticario, Córdoba, Argentina) was placed on the top of the open field. The odor was included as a feature of the context in order to favor contextual learning in the infant rat. This context was also used for training subjects from the Context condition (see Procedures section). In all experiments, locomotor activity was estimated through an index that was calculated by counting the number of quadrants that the subject crossed during the training or testing session. For this purpose, the floor of the open field was divided into four quadrants. Training and testing sessions were videotaped, and were later evaluated by a researcher blind to the treatments, who counted the number of quadrants crossed. Every time a pup passed its head and forepaws across one of the lines that divided the quadrant, a quadrant was considered to have been crossed.

2.1.3. Procedures

2.1.3.1. Training phase. This phase took place between PDs 8 and 12 (one session per day). On PD 8 subjects were assigned to one of the two context conditions (Context or No-Context). In each training session, pups from both conditions were separated from their mothers and placed in