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Research paper

The effects of gonadectomy and binge-like ethanol exposure during adolescence on open field behaviour in adult male rats

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HIGHLIGHTS

- Gonadectomy in male rats result in impaired spontaneous locomotion in adulthood.
- Adolescent binge-like ethanol shows increased anxiety-like behaviors in adult rats.
- Binge-like ethanol and gonadectomy exert impact on depressive-like behaviors in rats.
- The testicular hormones are potent stimulators of ethanol-induced behaviors.

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ABSTRACT

Binge drinking ethanol exposure during adolescence can lead to long-term neurobehavioural damage. It is not known whether the pubertal surge in testosterone that occurs during adolescence might impact the neurobehavioural effects of early ethanol exposure in adult animals. We examined this hypothesis by performing sham or gonadectomy surgeries on Sprague–Dawley rats around postnatal day (P) 23. From P28-65,the rats were administered 3.0 g/kg ethanol using a binge-like model of exposure. Dependent measurements included tests of open field behaviour, blood ethanol concentrations, and testosterone levels. As adults, significant decreases in open field activity were observed in the GX rats. The open field behaviour of the GX rats was restored after testosterone administration. Binge-like ethanol exposure altered most of the parameters of the open field behaviour, suggestive of alcohol-induced anxiety, but rats treated with alcohol in combination with gonadectomy showed less motor behaviour and grooming behaviour and an increase in immobility, suggesting ethanol-induced depression. These results indicated that testosterone is required for ethanol-induced behavioural changes and that testicular hormones are potent stimulators of ethanol-induced behaviours.

1. Introduction

Adolescence is a time of gradual transition from childhood to adulthood. Activation of the hypothalamic-pituitary-gonadal axis leads to significant increases in circulating oestrogen and testosterone [1,2]. Pubertal increases in gonadal hormones can lead to some adolescent-typical neurobehavioural alterations,such as risk taking and reckless behaviour [3,4]. Animal studies have suggested that sex differences exist in fear and anxiety-related behaviors [5,6]. Pre-pubertal gonadectomy was found to have an impact on a variety of adult-typical behaviours, including reproductive behaviour, aggression, and anxiety-related behaviours [7,8]. Adolescence is also a developmental window; the brain is one of the major target organs for ethanol actions, and binge drinking during adolescence can lead to alterations of the brain structure, physiology and behaviour [9]. Several studies have found that binge drinking itself can result in anxious behaviour and mood disorders [10,11]. The role of gonadal hormones in the ethanol-induced anxiety behaviour remains relatively unexplored. The link between testosterone and alcohol use is of much interest, including whether

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the pubertal surge in testosterone that occurs during adolescence might impact the effects of early ethanol exposure on anxiety-related behaviours in adult animals.

The purpose of this study was to determine whether gonadectomy and binge-like ethanol exposure during adolescence altered open field behaviour in adulthood. Furthermore, we also wanted to examine the effects of ethanol-induced behaviours during adulthood after preventing the increase in testosterone during adolescence. Male Sprague–Dawley rats were gonadectomized or received sham surgeries before puberty, during the peri-adolescent period, and we then exposed them to saline or ethanol using a binge-like pattern of administration. Behavioural performances were measured by analysing open field behaviour after the animals entered the adult stage.

2. Materials and methods

2.1. Animals

Forty-two male Sprague–Dawley rats on postnatal day 21 (P21) were supplied by the Experimental Animal Center of Hebei Medical University. Food and water were available ad libitum. All of the procedures were approved by the Committee of Ethics on Animal Experiments at Hebei Medical University.

2.2. Surgery

The rats were randomly assigned to one of seven groups ($n = 6$/group): Intact, ethanol alone (Intact-eth); sham-operated (SH); gonadectomized (GX); GX and testosterone supplemented (GX-TP); GX plus alcohol (GX-eth); and GX-TP plus alcohol (GX-TP-eth). The animals (prepubertally on P23) were anaesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg). The rats received bilateral orchiectomies through a bilateral incision of the scrotum, or they were subjected to sham operations. All of the surgeries were performed under aseptic conditions.

2.3. Treatment

From P28 to P65, the rats were exposed intermittently to saline or 3.0 g/kg ethanol (25%v/v) via intraperitoneal injection (daily at 5:00 PM). The exposure schedule consisted of one injection per day for two consecutive days, followed by two days with no injections. This cycle was repeated ten times for a total of 20 injections over 38 days. The GX-TP rats received subcutaneous TP injections (1 mg/kg per day at 5:00–6:00 PM). This procedure was repeated daily until the end of the behavioural tests. The SH rats and GX rats were subjected to the same treatment using sesame oil as vehicle.

2.4. Apparatus

The open field apparatus was a box ($100 \times 100 \times 40$ cm) constructed from plastic board that consisted of four black walls and a white bottom without a cover. The bottom was divided into 25 squares (20×20 cm). Every square further consisted of 400 small grills (1×1 cm). The apparatus was located in a sound-attenuating chamber and was illuminated with luminance of 20 lux on the floor. A digital video camera (Canon HF100, Japan) was installed above the apparatus.

2.5. Procedure

The subjects were acclimated to the open field apparatus for 2 days before any experimental procedures began. Five days after the final ethanol administration, on P71and P72, each rat was individually placed in the centre of the open field apparatus and was allowed to explore the field for 15 min.

Table 1
Behavior patterns of rat in the open field test.

Behavior pattern	Unit	The action of rat
Immobile-sniffing	Number	Rat sniffs the environment standing on the ground [12,13]
Exploratory behavior		
Walking	Number	Rat walks around sniffing the environment [12,13]
Climbing	Number	Rat maintains an erect posture leaning against the wall [12,13]
Rearing	Number	Rat maintains an erect posture without leaning against the wall [12,13]
Thigmotaxic behavior		Rat prefers the periphery of the apparatus to activity in the central parts of the open field [14]
Time spent in the central area	Second	Total time spent in the central area in whole test period
Motor behavior		
Horizontal activity	Number	Total number of square crossings in whole test period (3 or more paws moved from the original square to an adjacent one) [14–16]
Total path length	Centimeter	Total length of crossings in whole test period [17]
Grooming behavior		Include rat paw licking, nose/face grooming, head washing, body grooming/scratching, leg licking and tail/genitals grooming [18,19]
Latency of grooming	Second	Time from the onset of the test until grooming behavior was first displayed
Number of grooming	Number	Number of all kind of grooming in whole test period
Duration of grooming	Second	Total time of grooming behavior in whole test period

2.6. Parameters

Five types of behavioural patterns were analysed in our study. Immobile-sniffing, exploratory behaviour, thigmotaxic behaviour, motor behaviour and grooming behaviour (Table 1). The rats' performances on P71 and P72 days recorded and analysed post hoc. Because there were no substantial differences in most of the behaviour parameters on P71 and P72 (ANOVA), the results are averaged for each rat. The averaged amount of individual behaviour parameters is presented for each rat in the results.

2.7. Blood Ethanol Concentration (BEC)

To measure blood ethanol concentrations, 60 min after the final ethanol administration (20 injections), blood samples were