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Research report

Exposure to nicotine and ethanol in adolescent mice: Effects on depressive-like behavior during exposure and withdrawal

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ABSTRACT

Depression and use of addictive substances are two of the most frequent public health problems of adolescents. However, little is known about the association between depression and drug use. Considering that ethanol and nicotine are the most widely used and abused drugs by adolescents, here, we evaluated the depressive-like behavior of C57BL/6 male and female mice exposed to nicotine (NIC) and/or ethanol (ETOH) from the 30th to the 45th (PN30–45) postnatal day. Four groups were analyzed: 1) concomitant NIC (50 μg/ml in 2% saccharin to drink) and ETOH (25%, 2 g/kg i.p. injected every other day) exposure; 2) NIC exposure; 3) ETOH exposure; 4) vehicle. Immobile behavior, an animal model of depressive behavior, was assessed in the forced swimming test (FST) while the anhedonic state was assessed in the sucrose preference test (SPT) by the end of exposure (PN45–47) as well as during short- (PN50–52) and long-term (PN75–77) withdrawal. In the FST, ETOH female mice showed a reduction in immobility time by the end of exposure while, during long-term withdrawal, immobility time was increased. Short-term withdrawal elicited an increase in immobility time only in female NIC mice. In the SPT, males from both NIC and NIC + ETOH groups showed increased sucrose consumption, suggesting a reward-craving effect during short-term withdrawal. During long-term withdrawal, NIC male mice showed an anhedonic effect. Adolescent nicotine, ethanol and nicotine + ethanol combined exposures during adolescence thus elicit gender-selective effects both during exposure and withdrawal that may contribute to the increased prevalence of depression among drug users.

1. Introduction

Adolescence is characterized by neuroendocrine alterations [59] as well as by maturational changes in the nervous system [22]. It is well-documented that adolescence is a period in which many behavioral and psychiatric problems begin [59], characterizing adolescence as a period of vulnerability. Particularly, depressive disorders and substance use disorders are considered two of the most frequent public health problems of adolescents [55].

Alcohol and nicotine are the most widely used and abused drugs by adolescents. Most cigarette smokers begin their habit as adolescents [45]; epidemiological data have shown a great number of high school students smoking cigarettes – approximately one-fifth in the US [13]. Exploratory alcohol use also typically initiates during adolescence [58]. In addition, there is a high rate of co-occurrence of smoking and alcohol use [18] and, perhaps most importantly, there is a strong correlation between onset of tobacco consumption at an early age and alcohol addiction [25]. By using animal models of nicotine and ethanol exposures during adolescence, we recently found that these drugs interact at behavioral and neurochemical levels [1,2,50,51]. Nicotine and ethanol co-exposure elicited cumulative memory/learning deficits during exposure [1] and long-term withdrawal promoted an anxiogenic effect [2]. In addition, we showed that the cholinergic system is a target for nicotine and ethanol interactions during exposure and withdrawal periods [50,51], sug-

Abbreviations: ANOVA, analysis of variance; ChAT, choline acetyltransferase; ETOH, ethanol-exposed group; FPLSD, Fisher's Protected Least Significant Difference; FST, forced swimming test; HC-3, hemicholinium-3; nAChR, nicotinic acetylcholine receptor; NIC, nicotine-exposed group; NIC + ETOH, nicotine and ethanol-exposed group; PN, postnatal day; rANOVA, repeated-measures analysis of variance; uANOVA, univariate analysis of variance; VEH, vehicle group.

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gesting that these interactions might underlie the association between tobacco and alcohol consumption during adolescence. We demonstrated that the co-exposure elicited a more pronounced cholinergic nicotinic receptor upregulation than nicotine [50] and promoted a long lasting cholinergic synaptic impairment [51].

Adolescence is the highest-risk period for the development of depressive disorders [31]. Interestingly, prospective studies indicate that there is a high prevalence of depressive disorders among adolescents with substance use disorders in both clinical and non-clinical populations [30]. This association could be explained in two different ways. First, depressive and substance use disorders could be caused by the same underlying genetic and/or environmental factors. On the other hand, these disorders could have a causal relationship, so that depression would increase the risk of substance use disorders and vice versa. Lending support to this last possibility, some studies have reported early drug use as a predictor of depression [9,27]. It was described that smoking cessation frequently precipitates depressive symptoms that can be reversed with the reintroduction of smoking [60]. Moreover, transdermal nicotine patches exert an antidepressant-like activity in non-smokers [53]. Animal model studies corroborate these effects of nicotine: An acute administration of nicotine elicits an antidepressive-like behavior [63] while long-term nicotine withdrawal promotes increased depressive-like behavior in adult mice [28,41]. Regarding alcoholic beverage consumption, epidemiologic studies have shown that depressive symptoms often co-occur with heavy alcohol drinking [40,52]. In fact, there is evidence that heavy drinking, and in particular a binge pattern of consumption, produces depressive symptoms [48]. Animal models also provide some evidence regarding the association of alcohol consumption and depressive disorders. It has been demonstrated that subchronic ethanol exposure increases depressive-like behavior of rats [24].

Considering: 1. the association between depression and drug use during adolescence, 2. that the use and co-use of tobacco and alcoholic beverages begin during this period and, 3. that the association between smoking and consumption of alcoholic beverages during adolescence could be explained by the cumulative behavioral effects of these drugs of abuse, the aim of the current study was to investigate the effects of adolescent nicotine (the main psychoactive component of tobacco smoke) and/or ethanol administration on depressive-like behavior during drug administration as well as during short- and long-term withdrawal. Therefore, in keeping with earlier experimental designs [1–3,50,51], we chose to give mice free access to a nicotine solution in the drinking water, which allows for consumption during their active time. This pattern of exposure was shown to elicit moderate levels of cotinine (nicotine metabolite) in mice [51]. For ethanol, we chose a moderate dose to be injected (i.p.) every other day, mimicking adolescent binge drinking. The depressive-like behavior was evaluated in the forced swimming test (FST) and sucrose preference test (SPT). In the FST we assessed the immobile behavior of mice. This test is based on the observation that rodents, after initial escape-oriented movements, develop an immobile posture when placed in an inescapable stressful situation (for review: [15]). Since several antidepressant drugs reduce immobility time in this paradigm [15,32,49], it has been suggested that an increased immobility time reflects a state of lowered mood [49], indicative of depressive-like symptomatology [19,39]. In order to rule out the possibility that deficits in motor performance could confound the analysis of immobility time, mice were also submitted to the endurance swimming test. The SPT is based on a two-bottle-choice drinking paradigm in which reduction in sucrose consumption reflects a state of anhedonia [67]. This symptom is a useful endophenotype to investigate depressive-like behavior in mice [7,16]. In addition, sucrose consumption has been used as a measure of reward-craving [44].

2. Material and methods

2.1. Animal treatment

This study was conducted under the institutional approval of the Universidade do Estado do Rio de Janeiro (protocols: CEA/144/2006 and CEA/030/2009). All experiments were carried out in accordance with the declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. C57BL/6 mice were chosen because prior reports demonstrate that adult and periadolescent mice from this strain consume nicotine in the concentration used in the present study [1–3,33,34,50,51]. All mice were bred and maintained in our laboratory. Animals were derived from a C57BL/6 colony maintained at the Universidade Estadual de Campinas (São Paulo, Brazil) for over 70 generations. Animals were kept in a temperature-controlled room on a 12 h light/dark cycle (lights on at 1:00 am). Access to food and water was *ad lib*. On the first postnatal day (PN1), litters were culled to a maximum of 8 mice to ensure standard nutrition. At weaning (PN25) animals were separated by sex and allowed free access to food and water.

On PN29, pups from 46 litters (140 females and 129 males) were housed in groups of two or three animals to begin treatment. Animals were exposed to nicotine and/or ethanol from PN30 to PN45, the approximate age range during which animals of both genders and most breeding stock exhibit adolescent-typical behavioral characteristics and particular neurochemical and endocrine patterns when compared to adulthood and pre-pubertal periods (Spear, 2000). During this period, 25% ethanol (2 g/kg) solution (v/v) in saline or saline only were injected (i.p.) every other day in order to mimic cyclical patterns of alcohol consumption while (−)-nicotine free base (50 μg/ml) in 2% saccharin or 2% saccharin only were administered in the drinking water (the sole source of fluid) in order to mimic intermittent nicotine consumption. Accordingly, a complete, self-contained study of the impact of adolescent nicotine and ethanol exposures required four treatment groups: VEH (oral saccharin + injected saline), ETOH (oral saccharin + injected ethanol), NIC (oral nicotine/saccharin + injected saline) and those receiving the combined treatment: NIC + ETOH (oral nicotine/saccharin + injected ethanol). Bottles were cleaned and refilled daily. Loss due to leakage was measured from a bottle placed in an empty cage ("blank"), and subtracted from fluid consumption data. Body weights and fluid consumption were also measured every day. Since body weight increases significantly during adolescence, daily fluid intake data were obtained by dividing the values of fluid intake of each cage by the combined body weight of the animals of that cage.

Studies were conducted by the end of the drug administration period (PN45–47), during a short-term withdrawal (PN50–52) and during a long-term withdrawal (PN75–77). For each treatment group/age, 19–25 animals were examined, equally divided into males and females.

2.2. Behavioral tests

In an effort to avoid possible behavioral biases, at each testing day, mice from all treatment groups at a given time-point (during exposure, short-term or long-term withdrawal) were tested. All animals were submitted to the three behavioral tests described below. The depressive-like behavior was initially investigated through the analysis of immobility time in the FST. The FST was performed between 2:00 pm and 7:00 pm. The endurance swimming test was performed the day after the FST. This test, which is commonly used to study muscular resistance [28], was performed between 9:00 am and 12:00 am. The SPT initiated at 2:00 pm in the same day of the motor test.

2.2.1. Forced swimming test

Each mouse was submitted to a 6 min FST session. The test procedure is described in detail elsewhere [23,38]. Briefly, each mouse was placed in a plastic container (diameter = 21 cm, height = 23 cm) filled with 16 cm of water at about 25 °C. The animal's behavior was continuously recorded throughout the testing session with an overhead video camera. Animals were considered to be immobile when they remained floating with all limbs and tail motionless. The time the animals spent in this condition was considered to be the measure of immobility, which was assessed using the video images of the tests. The total testing time was subdivided into 6 consecutive intervals of 1 min each in order to analyze immobility as a function of time. Immobility was assessed for the total testing time and for each one of the six time intervals.

2.2.2. Endurance swimming test

Each mouse was submitted to a swimming session bearing a constant load, corresponding to 12% of its body weight, attached to the tail. The animal was considered to be fatigued when it failed to rise to the surface of the water to breathe within 5 s [28]. The test was carried out in a rectangular container (length = 48 cm, height = 29.5 cm, breadth = 24 cm) containing water (at about 25 °C) to a depth of 25.5 cm.