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Combined exposure to tobacco smoke and ethanol during adolescence leads to short- and long-term modulation of anxiety-like behavior☆,☆☆

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ABSTRACT

Background: Tobacco smoking is associated with alcohol drinking and consumption of both drugs typically begins during adolescence. Since anxiety is considered a relevant factor for both smoking and drinking due to its motivating force for a continued consumption, anxiety alterations shared by these two drugs could explain their co-use and co-abuse.
Methods: Here, we investigated the short- and long-term effects of adolescent tobacco smoke and/or ethanol exposure on anxiety levels. From postnatal day 30–45, Swiss mice were exposed to tobacco smoke (SMK – whole body exposure, 8 h/day) and/or ethanol (ETOH – 25% solution, 2 g/kg i.p. injected every other day) as follows: (1) SMK + ETOH exposure; (2) SMK exposure; (3) ETOH exposure; (4) Control. Anxiety levels were assessed with the elevated plus maze and open field tests.
Results: By the end of exposure, SMK female mice presented an anxiolytic response in the elevated plus maze and this response was intensified by co-exposure to ethanol. A short-term deprivation from SMK elicited an anxiogenic state in females in this maze. Although neither smoke nor ethanol effects persisted one month post-exposure, SMK + ETOH male and female mice exhibited an anxiogenic response in the open field.
Conclusion: Adolescent female mice are more susceptible to the anxiolytic effects of SMK. The stronger effect in SMK + ETOH group suggests that, in females, the combined exposure leads to lower anxiety levels. Anxiety levels do not seem to be relevant during a short-term SMK + ETOH deprivation, however, increased anxiety during long-term smoking and drinking deprivation demonstrate late-emergent effects both in males and females.

1. Introduction

Both tobacco smoking and alcohol drinking are likely to initiate during adolescence (CDC, 2010; Doubeni et al., 2010) and epidemiological findings demonstrate a strong relationship between smoking and drinking (Dawson, 2000; Grant, 1998; Larsson and Engel, 2004; Meyerhoff et al., 2006). For instance, early drinking increases the likelihood of poly substance use (Ellickson et al., 2003) and alcoholism frequency increases when adolescents start smoking at a younger age (DiFranza and Guerrera, 1990; Grant, 1998). However, the biological bases of the co-use and co-abuse of tobacco and alcohol are poorly understood.

There is a close relation between smoking or alcohol drinking and mood. Tobacco smoking reduces anxiety while increased anxiety is a symptom of tobacco withdrawal (Hughes et al., 2000). These lead to the suggestion that smokers resort to smoking in order to modulate their anxiety levels (Gilbert et al., 1989; Picciotto et al., 2002). Regarding ethanol, its increased ingestion by anxious patients, a behavior possibly associated with its acute anxiolytic effects, and the elevated anxiety during early withdrawal that occur in most alcohol-dependent patients have led to the hypothesis that anxiety is involved in the etiology of alcohol consumption (for review: Heilig et al., 2010). The association between smoking and consumption of alcoholic beverages could be explained by cumulative mood-altering effects of tobacco and ethanol, particularly

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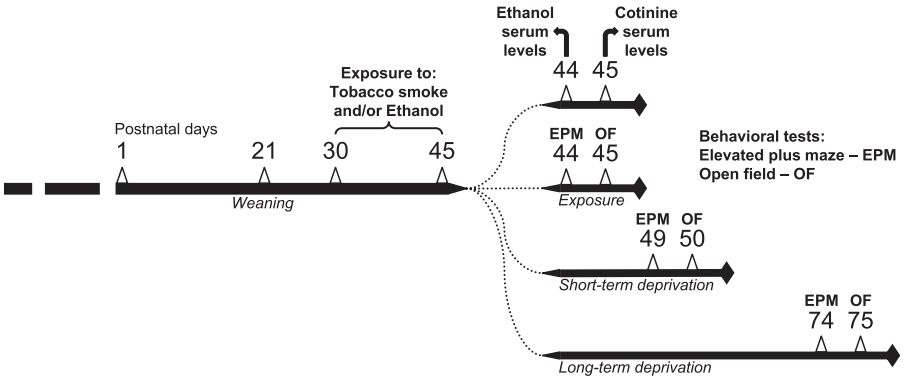

Fig. 1. Time-line of the experiment.

those related to anxiety. Moreover, epidemiological studies and experimental data indicate sex-dependent effects of several drugs of abuse (Dazzi et al., 2007; Lynch, 2006; Lynch and Sofuoglu, 2010; Tanapat et al., 1999). Despite that, there is scant information as to the consequences of tobacco and alcohol co-consumption on anxiety and on potentially sex-selective effects.

Nicotine has been described as the active component of cigarette smoke responsible for a wide variety of nervous system effects resulting from tobacco consumption (for review: Fowler et al., 2008; Slotkin, 2002). The majority of studies in experimental models of adolescent exposure have specifically assessed the consequences of nicotine exposure and, more recently, the consequences of the combined nicotine + ethanol exposure (Abreu-Villaça et al., 2007, 2008; Ribeiro-Carvalho et al., 2008, 2009, 2011; Trezza et al., 2009). This combined exposure was shown to result in biochemical and behavioral effects, including anxiety-related ones (Abreu-Villaça et al., 2008), that were distinct from those observed when the drugs were used separately, indicating that nicotine and ethanol interact, affecting the functioning of the central nervous system during this period of development.

Considering that more than 4500 substances have been identified in tobacco smoke and that there is evidence that nonnicotine components play an important role in tobacco effects in the central nervous system (Abreu-Villaça et al., 2010; Bruijnzeel, 2012; Rose, 2006; Villégier et al., 2010), an alternative approach to more closely investigate the effects of smoking would be to use animal models of tobacco smoke exposure. Despite that, there are scant experimental studies on the effects of tobacco smoke and ethanol co-exposure. This lack of information is particularly disconcerting when one considers that adolescents that both smoke cigarettes and drink alcoholic beverages expose themselves to a considerable number of substances that are present in the tobacco smoke and that may interact with nicotine and/or ethanol in affecting the central nervous system. Accordingly, the aim of the present study was to investigate the effects of tobacco smoke and ethanol when administered separately or in combination on anxiety levels. Based on evidence that distinct behavioral tests in animal models could be indexing different emotional aspects of anxiety-like behavior in rodent (for review: Ramos, 2008), we opted to use two tests in the present study: the elevated plus maze and the open field. Since adolescence is described as a key period for initiation of tobacco and ethanol consumption, exposure occurred during this period of development. The effects of exposure and deprivation on anxiety levels were investigated.

2. Methods

2.1. Animals and treatment (Fig. 1)

Experiments were carried out with the approval of the Animal Care and Use Committee of the Universidade do Estado do Rio de Janeiro (CEA/014/2011). Swiss mice were bred and maintained in our vivarium (21 ± 1 °C, lights: on 1:00 – off 13:00). Access to food and water was ad libitum. We used litters of 8–12 pups. At weaning (postnatal day 21 = PN21) animals were separated by sex in groups of 2–5.

During adolescence (PN30 to PN45) (Spear, 2000), mice were exposed to tobacco smoke and/or ethanol. Tobacco smoke was generated from reference research cigarettes (University of Kentucky, Lexington, KY, USA) type 2R1F (nicotine = 1.74 mg/cigt). Whole body exposure was for 8 h/day, from 9:00 to 17:00, 7days/week in a chamber that received the smoke generated in an automatic cigarette smoking machine (Teague Enterprises, Davis, CA, USA), as a surrogate for active smoking (Abreu-Villaça et al., 2010; Slotkin et al., 2001). Control mice were exposed to ambient air (detailed description in Supplementary Material S1[1]). As for ethanol exposure, 25% ethanol (2 g/kg) solution (v/v) in saline was injected (i.p.) every other day in order to mimic cyclical patterns of consumption (Pascual et al., 2007; White et al., 2000). Therefore, during the period of adolescent exposure, mice presented a period of ethanol intoxication followed by deprivation every 48 h. Control mice were exposed to saline.

Pups (44 litters: 106 females and 106 males) were distributed into four treatment groups (detailed description in Supplementary Material S1): VEH (air + saline), SMK (smoke + saline), ETOH (air + ethanol) and those receiving the combined treatment: SMK + ETOH (smoke + ethanol). Behavioral tests were conducted by the end of the drug administration period (PN44-45), during a short-term (PN49-50) or a long-term deprivation (PN74-75) and separate groups of mice were tested at each time-point. One male and one female from each litter were randomly assigned to each treatment group/age. All mice were submitted to both behavioral tests. Body weights were measured every day during the exposure period (PN30-PN45) and at two time points during deprivation (PN50 and PN75).

2.2. Behavioral testing

Elevated plus maze (detailed description in Supplementary Material S1[2]). The percentage of time spent in the open arms (%Time OA: the time spent in open arms divided by time spent in open + closed arms) and the percentage of open arms entries (%Entries OA: the number of entries in open arms divided by number of entries in open + closed arms) were used as anxiety measures. An entry was counted whenever the animal crossed with all four paws into an arm. Increased %Time OA and/or %Entries OA correspond to decreased anxiety-like behavior and *vice versa* (Rodgers and Dalvi, 1997). Ethological measures – protected head-dips and time spent in the center of the maze – were also quantified (detailed description in Supplementary Material S2[3]).

Open field test (detailed description in Supplementary Material S1[4]). The test was performed one day after the elevated plus maze. The ambulation was quantified on the basis of the number of rectangles crossed by the animals. Animals had to place all four legs on a given rectangle for a crossing to be counted. Total ambulation, which consists of the sum of rectangles crossed in the center and in the periphery (Cen + Pe), was evaluated (Filgueiras et al., 2009). The activity in the center, inversely related to levels of anxiety (Prut and Belzung, 2003), and assessed as the number of rectangles crossed in the center corrected by total ambulation Cen/(Cen + Pe), was used as a measure of anxiety-like behavior. Ethological measures – rearing, grooming and

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