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Effects of adolescent onset voluntary drinking followed by ethanol vapor exposure on subsequent ethanol consumption during protracted withdrawal in adult Wistar rats

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ABSTRACT

Epidemiological studies have demonstrated that heavy drinking and alcohol abuse and dependence peak during the transition between late adolescence and early adulthood. The objective of the present study was to determine whether a model of early onset adolescent ethanol drinking exposure that is followed by an ethanol vapor regimen during late adolescence and young adulthood leads to an increase in drinking in adulthood. In this model, initiation of voluntary ethanol drinking in adolescence, using a sweetened solution, was followed by an 8-wk intermittent ethanol vapor regimen in Wistar rats. A limited-access two-bottle choice paradigm was then used to measure intake of a 10% (w/v) ethanol solution. No differences in water intake (g/kg), total fluid intake (ml/kg) and body weight (g) were observed between air-exposed and ethanol-vapor exposed groups during the pre-vapor and post-vapor phases. The 8 weeks of ethanol vapor exposure was found to produce only a modest, but statistically significant, elevation of ethanol intake during the protracted withdrawal period, compared to air-exposed rats. A significant increase in ethanol preference ratio was also observed in ethanol-vapor exposed rats during the sucrose-fading phase, but not during the protracted withdrawal period. The findings from the present study suggest that in addition to alcohol exposure, environmental variables that impact appetitive as well as consumptive behaviors may be important in developing robust drinking effects that model, in animals, the increased risk for alcohol dependence seen in some human adolescents who begin drinking at an early age.

1. Introduction

Adolescence is a critical time period for brain development when cognitive, emotional and social maturation occur (see Dahl and Spear, 2004). The 2007 National Survey on Drug Abuse and Health has reported that approximately 16% of teens between the age of 12 and 17 were current users of ethanol, with 10% of these individuals classified as binge drinkers (U.S. Department of Health and Human Services, 2008). Additionally, underage college students have been shown to be more likely to drink to excess, when they drank, than their older peers (Wechsler et al., 2002). Evidence from the Monitoring the Future (MTF) study showed that 30-day prevalence and heavy drinking in men peaks at ages 21–22 and then declines linearly into adulthood (Bachman et al., 1997). Consistent with these findings, Grant et al. (2004) reported that individuals within the ages 18–29 exhibit the highest rates of past-year ethanol abuse and dependence.

For some individuals ethanol use during early adolescence is clearly a risk factor for the later development of alcohol dependence (Ehlers et al., 2006; Grant, 1998; Grant and Dawson, 1997; Hicks et al., 2010; Hingson et al., 2008). How early adolescent drinking causes an increased risk for alcohol dependence in some individuals is not known. One hypothesis posits that early heavy drinking can disrupt the normal course of social and intellectual development leading to an increased risk for a number of social and psychological pathologies including drug addictions (De Wit et al., 2000; York, 1999). An alternate hypothesis is that some individuals who initiate drinking during early adolescence may be more likely to have an underlying predisposition to disinhibitory behavior and psychopathology that drives their early drinking (Iacono et al., 2002; Jessor and Jessor, 1977). These hypotheses are difficult to disentangle in human studies; however, the development of an animal model in order to study the effects of adolescent ethanol exposure on drinking behaviors in adulthood could be useful in the understanding of the brain mechanisms underlying the effects of early adolescent drinking.

The adolescent period in rodents has many similarities to the human condition making it a good model to study the short- and long-term consequences of adolescent ethanol exposure (Spear, 2000b, 2000c; Spear and Varlinskaya, 2005). Variables that have been used to investigate adolescent drinking patterns in animal

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models include sex, age, ethanol fluid concentration, isolate-housing and use of different sipper-tube types in paradigms with 24-h access to ethanol solutions (Bell et al., 2006; Brunell and Spear, 2005; Doremus et al., 2005; Ehlers et al., 2007; Fullgrabe et al., 2007; Lancaster et al., 1996; Siciliano and Smith, 2001). Studies characterizing developmental differences in drinking patterns indicate that adolescent rats show greater levels of ethanol intake than adult rats (Brunell and Spear, 2005; Doremus et al., 2005; Spear, 2004, 2007; Vetter et al., 2007; Vetter-O'Hagen et al., 2009). In studies of alcohol preferring rats (AA) male AA rats were shown to decrease their ethanol consumption with age (Sarviharju et al., 2001).

Studies using animal models have also indicated that voluntary ethanol drinking during adolescence can, in some models, be shown to facilitate the acquisition of alcohol self-administration, increase craving behavior, and increase the probability of relapse in adults (see McBride et al., 2005; Spear, 2000a; Gilpin et al., 2012). However, other studies have shown that ethanol exposure during adolescence has no effect on subsequent ethanol consumption in adulthood. For instance, Vetter et al. (2007) found that adult rats trained to drink ethanol during adolescence showed no differences in ethanol drinking when compared to a control group not exposed to ethanol during adolescence. Additionally, Siegmund et al. (2005) have shown that Wistar rats that initiated alcohol consumption during adolescence, when not exposed to stress, actually consumed less alcohol and showed lower preference than rats who were initiated into drinking as adults. The reason that disparate findings have been obtained between studies is at this point is not clear. Potentially important factors include: the strain of the rats, whether the alcohol exposure during adolescence was voluntary, the length of alcohol exposure, the effects of stress, and the dose of alcohol.

Since in humans, drinking typically begins during adolescence but then continues during young adulthood and then declines into later adulthood, studying a model that assesses the consequences of ethanol dependence during a period that includes late adolescence and young adulthood could provide insight into the importance of this transition period on the maintenance of ethanol drinking patterns in adulthood. One potential approach to developing such an animal model is based on experiments in adult animals that assess whether alcohol exposure, that is high enough to induce symptoms associated with withdrawal, is important in the development and maintenance of subsequent ethanol addiction (see Koob and Le Moal, 2008). Typically such a model uses intermittent exposure to ethanol vapor to study the increase in voluntary consumption and self-administration of ethanol following periods of abstinence in animals with a history of prolonged vapor exposure (see Heilig et al., 2010). Findings from studies in adult rats have shown that chronic intermittent ethanol vapor exposure produces a robust increase in voluntary ethanol consumption (Rimondini et al., 2002a,b, 2003; Sommer et al., 2008; Thorsell et al., 2005a) and operant ethanol self-administration (O'Dell et al., 2004; Roberts et al., 1996, 2000; Thorsell et al., 2005b; Walker and Koob, 2007). However, studies evaluating the effects of exposure to 2 weeks of alcohol vapor in male Sprague–Dawley rats during adolescence on subsequent drinking behavior revealed that the alcohol exposed animals did not show increases in drinking in adulthood, even after noise stress (Slawecki and Betancourt, 2002). It was suggested by the authors that forced exposure to ethanol vapor during adolescence does not seem to be sufficient to alter the initiation or maintenance of ethanol self-administration (Slawecki and Betancourt, 2002).

There is evidence to suggest that intermittent ethanol vapor requires a minimum duration of ethanol exposure in order to produce a lasting upregulation of ethanol preference in adult rats. For instance, studies have shown that a minimum duration of intermittent ethanol vapor exposure is needed to produce a lasting increase of ethanol preference in adult rats (e.g., O'Dell et al., 2004; Rimondini et al., 2002a; Slawecki and Betancourt, 2002; Sommer et al., 2008). Taken together, these findings suggest that (1) a longer duration of ethanol vapor exposure may be required in adolescent rats to produce long-lasting effects in ethanol drinking and (2) that it may be necessary to initiate voluntary drinking in rats prior to vapor exposure during adolescence in order to see subsequence increases in drinking in adulthood. Therefore, the present study evaluated a model of early onset ethanol drinking that is followed by an ethanol vapor exposure regimen during late adolescent/young adulthood designed to resemble the pattern of heavy drinking and alcohol-related problems and dependence shown to peak during late adolescence and early adulthood and to decline linearly into adulthood (Bachman et al., 1997; Baer, 1993; Grant et al., 2004; Johnston et al., 2001a,b). Rats in the present study were exposed to an 8-wk intermittent ethanol vapor regimen shown to produce behavioral, neurophysiological and neurochemical changes that persist into the late protracted withdrawal period in adult Sprague–Dawley and Wistar rats (Criado and Ehlers, 2010; Criado et al., 2008a,b, 2011; Slawecki, 2002; Slawecki et al., 2001).

The present report is part of a larger study characterizing the risks and consequences of adolescent ethanol drinking in animal models and humans (Criado et al., 2008b; Ehlers et al., 2006; Pian et al., 2008a,b, 2010; Slawecki et al., 2001; Walker et al., 2008). These experiments were designed to investigate whether initiation of voluntary drinking followed by prolonged exposure to ethanol vapor during late adolescence/young adulthood increases or decreases adult ethanol intake during protracted withdrawal relative to: (1) pre-exposure ethanol drinking levels; and (2) to an air-exposed control group. The working hypothesis of this study is that initiation of voluntary ethanol drinking in adolescence followed by an 8-wk intermittent ethanol vapor regimen during late adolescence and young adulthood would produced a significant increase in ethanol intake in adult Wistar rats during protracted withdrawal.

2. Materials and methods

2.1. Subjects

Thirty-six male Wistar rats (Charles River, Wilmington, MA) were used in the present study and were 23 days of age (P23) on arrival. The animals were pair housed in standard home cages measuring 25 cm wide × 20 cm high × 45 cm long in a temperature-controlled room maintaining a 12 h light/dark cycle (lights on at 6 am). Upon arrival, animals were weighed and handled daily. Ad libitum food and water were provided for the duration of the experiment, except during the 30-min limited-access sessions. The work described herein adheres to the guidelines stipulated in the NIH *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80–23, revised 1996) and was reviewed and approved by The Scripps Research Institute's Institutional Animal Care and Use Committee.

2.2. General procedure

All limited-access drinking tests occurred 2 h after the onset of the light phase of the light/dark cycle, which has previously been shown to promote enhanced ethanol consumption in adolescent animals (Walker et al., 2008). Animals were weighed and transported to the testing room 30 min prior to the initiation of the two-bottle choice sessions and 10 min prior to the start of the session were transferred to plastic cages [25 (w) × 20 (h) × 45 cm (l)] with wire bar cage tops that were separated into two equal-sized compartments using a Plexiglas divider. Solutions were presented using 100 ml graduated cylinders (Nalgene Labware, Rochester, NY) fitted with curved ball-point sipper tubes (Ancare, Bellmore, NY). Animals were given 30 min of access to the solutions without food availability, after which the cages were cleaned with 70% ethanol. Following