Progress in Neuropsychopharmacology & Biological Psychiatry 79 (2017) 220–233

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

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

Short-term selection for high and low ethanol intake yields differential sensitivity to ethanol's motivational effects and anxiety-like responses in adolescent Wistar rats

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ARTICLE INFO

Keywords:
Short-term selective breeding
Adolescents
Ethanol aversion
Ethanol intake

ABSTRACT

Alcohol use disorders are modulated by genetic factors, but the identification of specific genes and their concomitant biological changes that are associated with a higher risk for these disorders has proven difficult. Alterations in the sensitivity to the motivational effects of ethanol may be one way by which genes modulate the initiation and escalation of ethanol intake. Rats and mice have been selectively bred for high and low ethanol consumption during adulthood. However, selective breeding programs for ethanol intake have not focused on adolescence. This phase of development is associated with the initiation and escalation of ethanol intake and characterized by an increase in the sensitivity to ethanol's appetitive effects and a decrease in the sensitivity to ethanol's aversive effects compared with adulthood. The present study performed short-term behavioral selection to select rat lines that diverge in the expression of ethanol drinking during adolescence. A progenitor nucleus of Wistar rats (F_0) and filial generation 1 (F_1), F_2, and F_3 adolescent rats were derived from parents that were selected for high (STDRHI) and low (STDRLO) ethanol consumption during adolescence and were tested for ethanol intake and responsivity to ethanol's motivational effects. STDRHI rats exhibited significantly greater ethanol intake and preference than STDRLO rats. Compared with STDRLO rats, STDRHI F_2 and F_3 rats exhibited a blunted response to ethanol in the conditioned taste aversion test. F_2 and F_3 STDRHI rats but not STDRLO rats exhibited ethanol-induced motor stimulation. STDRHI rats exhibited avoidance of the white compartment of the light-dark box, a reduction of locomotion, and a reduction of saccharin consumption, suggesting an anxiety-prone phenotype. The results suggest that the genetic risk for enhanced ethanol intake during adolescence is associated with lower sensitivity to the aversive effects of ethanol, heightened reactivity to ethanol's stimulating effects, and enhanced innate anxiety.

1. Introduction

The literature suggests that 50% of the variability of alcohol use disorders (AUDs) is attributable to genetic factors (Dick and Agrawal, 2008; Ducci and Goldman, 2008, 2012). Seminal studies indicated that alcoholism runs in families. Children of alcoholics are 3- to 5-times more likely to be diagnosed with AUD than children of non-alcoholic parents (Cotton, 1979). Dozens of studies have indicated that a positive family history of AUD (FH +) is a risk factor for AUD.

Alcohol use disorder does not follow a simple dominant or recessive pattern of inheritance but instead appears to be polygenic (i.e., it is caused by the independent and interactive effects of several genes); (Rietschel and Treutlein, 2013) and impacted by environmental modulation. The identification of specific genes and their concomitant biological changes that are associated with a higher risk of AUD has been difficult. Genetic alterations in enzymes that metabolize alcohol (hereinafter referred to as ethanol) were shown to be associated with differential degrees of AUD, a finding that opened the door to promising therapies (Ocaranza et al., 2008; Rivera-Meza et al., 2012). Genome-wide association studies (Adkins et al., 2017) and copy number variation studies (Bae et al., 2012) have helped pinpoint promising target genes. One alternative to these technically demanding experimental approaches is to identify biobehavioral correlates of AUD that are linked to the genetic predisposition to AUD, even iB60n subjects that do not fully express the disease (Schuckit, 1994).

FH + subjects perceive the autonomic and subjective effects of

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http://dx.doi.org/10.1016/j.pnpbp.2017.06.027
Received 1 April 2017; Received in revised form 25 May 2017; Accepted 24 June 2017
Available online 27 June 2017
0278-5846/

moderate ethanol doses significantly differently from those who are not at risk. Several studies (Conrod et al., 1997a; Conrod et al., 2001) reported heightened psychomotor stimulation in FH + individuals than in their FH- counterparts during the rising limb of the blood ethanol curve. Other studies (Schuckit et al., 2004) suggest that FH + individuals exhibit a blunted response to the aversive and sedative effects of ethanol. Thus, alterations in the sensitivity to the motivational (e.g., appetitive, aversive, and anxiolytic) effects of ethanol may be one way by which genes modulate the initiation and escalation of ethanol intake. The latter is an intriguing hypothesis that has been investigated in preclinical studies using rats that are selectively bred for high and low ethanol consumption, such as alcohol-preferring (P) and alcohol-non-preferring (NP) rats (Bell et al., 2008), Universidad de Chile Abstinent and Bibulous (UChA and UChB, respectively) rats (Quintanilla and Tampier, 2011), and Marchigian Sardinian alcohol-preferring (msP) rats (Ciccocioppo et al., 2006). P rats exhibit lower sensitivity to the aversive effects of ethanol (Stewart et al., 1996) but heightened sensitivity to the motor-stimulating effects of ethanol (Waller et al., 1986), which are considered proxies of the positive rewarding effects of the drug. UChB but not UChA rats exhibit ethanol-induced conditioned place preference (CPP) after preexposure to free-choice ethanol drinking (Quintanilla and Tampier, 2011).

Strains that are selectively bred for high ethanol intake are valuable animal models. They are generated by crossing males and females with high preference for 10% alcohol during adulthood for 30, 40, or 70 generations. Still unknown, however, are the ways in which ethanol intake and ethanol-induced appetitive and aversive responses diverge or converge across the initial generations. Phillips et al. (2005) reported lower ethanol-induced conditioned taste aversion (CTA) in the second generation of female but not male mice that were short-term selected for high ethanol intake compared with their counterparts that were selected for high ethanol intake. Several other studies with selectively bred or heterogeneous rats and mice (Doremus et al., 2005) have yielded a negative correlation between ethanol-induced CTA and ethanol drinking [for review and references, see (Green and Grahame, 2008)]. For instance, adolescent rats usually drink significantly more ethanol than adult counterparts (Doremus et al., 2005; Vetter et al., 2007) and, unlike their mature counterparts, are relatively insensitive to ethanol-induced CTA (Vetter-O'Hagen et al., 2009). Overall, this supports the hypothesis that insensitivity to ethanol's aversive effects is a factor in the vulnerability to enhanced ethanol consumption. The intriguing study by Phillips et al. (2005) also found greater ethanol-induced CPP in a short-term line that was selected for high ethanol consumption compared with their low ethanol consumption counterparts (hereinafter referred to as STDRHI and STDRLO, respectively). Ethanol-induced CPP is notoriously difficult to observe in genetically heterogeneous rats, yet it has been found in Marchigian Sardinian alcohol-preferring (Ciccocioppo et al., 1999) and other, genetically selected, alcohol-preferring rats. These studies further suggest a genetic relationship between ethanol's motivational effects and ethanol intake. The association between ethanol-induced CPP and ethanol intake is, however, much more variable than that found between ethanol-induced CTA and ethanol intake (Green and Grahame, 2008).

Other short-term selection programs for ethanol intake have been used to map quantitative trait loci (Belknap et al., 1997) and analyze differences in behavioral traits other than ethanol responses. STDRHI mice exhibited deficits in response inhibition (Wilhelm et al., 2007), a component of the broader construct of impulsivity that is linked to the vulnerability to ethanol intake during adolescence (Pilatti et al., 2017). An exacerbated anxiety response is another innate trait that can promote ethanol drinking via negative reinforcement mechanisms. msP rats are less prone than non-selected rats to explore the open, potentially dangerous, arms of the elevated plus maze and the central area of the open field (OF; Roman et al., 2012). Roman high-avoidance rats exhibit greater anxiety and consume more ethanol than inbred Roman low-avoidance rats (Manzo et al., 2012). Moreover, our lab recently reported significantly higher intake of ethanol in female, adolescent, rats with high levels of inborn anxiety than in counterparts with standard levels of anxiety (Acevedo et al., 2016). Together, this evidence suggests that an “anxious” phenotype may facilitate the sustained engagement in ethanol intake in this line (Ciccocioppo et al., 2006).

To our knowledge, a selective breeding program has not been performed for low and high levels of ethanol drinking during adolescence in rats or mice. Early and highly influential typological accounts of alcoholism differentiated between type I alcoholism that emerges later in life (i.e., after years of heavy drinking) and type II alcoholism that emerges during adolescence, predominantly in males, and is driven by the appetitive, rewarding effects of ethanol (Cloninger et al., 1996; Sigvardsson et al., 1996). At the epidemiological level, the time course of ethanol intake is initiated, peaks, and is almost normative during adolescence in western youth (Pinsky et al., 2010a). By the end of high school, more than half of adolescents engage in heavy episodic drinking patterns every time they drink, and a similar percentage have engaged in at least one binge drinking episode within the past year (Pilatti et al., 2013). Epidemiological and preclinical studies have shown that the earlier initiation and escalation of ethanol drinking is associated with a higher probability of problematic ethanol intake later in life. Still unknown, however, is whether both events are casually linked or whether they are both symptoms of a third variable, namely genetic vulnerability. A previous study of college students found that the frequency of drunkenness and other ethanol-related consequences was related to the age of onset of ethanol use but only in FH + individuals (Pilatti et al., 2014). Preclinical studies have consistently indicated that adolescent rats exhibit patterns of ethanol responsiveness that may facilitate the initiation and escalation of ethanol use. Compared with adult counterparts, adolescent rats are more sensitive to the appetitive (Pautassi et al., 2008) and social-facilitating effects of ethanol (Varlinskaya and Spear, 2015) and the acute cognitive deficits that are induced by ethanol (Swartzwelder et al., 2014). Moreover, adolescent rats are less sensitive to the aversive and sedative effects of ethanol that serve as natural barriers to sustained engagement in ethanol drinking (Spear and Swartzwelder, 2014). These and other studies have changed the concept of AUDs, which are now considered developmental conditions that have etiological roots in adolescence (NIH, 2008).

The breeding of rats that are selected for high and low ethanol consumption during adolescence would facilitate analyses of the mechanisms by which genes increase the likelihood of AUD. Such selective breeding may reveal the putative relationship between motivational sensitivity to ethanol and ethanol drinking (Green and Grahame, 2008) or detect preexisting (i.e., before any contact with ethanol) differences in innate anxiety or other traits between adolescents that are derived from high- and low-ethanol progeny. Anxiety-related disorders usually begin during adolescence (Cunningham et al., 2002) and are significantly associated with the emergence of AUD (Hobbs et al., 2011). This breeding strategy could help uncover endophenotypes, stable heritability, and behavioral traits that are linked to the pathophysiology of AUD (Hines et al., 2005; Klee et al., 2012) during a key developmental stage for the initiation of ethanol use.

The present study produced rat lines that diverged in the expression of ethanol drinking during adolescence through short-term behavioral selection (Belknap et al., 1997; Linsenbardt and Boehm, 2013). A progenitor, F_0, nucleus of genetically heterogeneous Wistar rats and filial generation 1 (F_1), F_2, and F_3 STDRHI and STDRLO offspring that derived from the selective mating of animals with high and low ethanol intake were tested for ethanol intake throughout adolescence (postnatal days 32–57 [PD32–57], Exp. 1) or for responsiveness to ethanol's motivational effects. Our hypothesis was that selection pressure would yield significant differences between STDRHI and STDRLO rats in ethanol-induced motor stimulation (Exp. 2a), basal innate anxiety (Exp. 2b), and ethanol-induced motivational learning (measured by CTA and place conditioning; Exp. 3 and 4, respectively). The measurement of saccharin intake during CTA conditioning allowed us to evaluate the