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# Cytisine modulates chronic voluntary ethanol consumption and ethanol-induced striatal up-regulation of ΔFosB in mice

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ABSTRACT

Chronic administration of ethanol induces persistent accumulation of ΔFosB, an important transcription factor, in the midbrain dopamine system. This process underlies the progression to addiction. Previously, we have shown that cytisine, a neuronal nicotinic acetylcholine receptor (nAChR) partial agonist, reduces various ethanol-drinking behaviors and ethanol-induced striatal dopamine function. However, the effects of cytisine on chronic ethanol drinking and ethanol-induced up-regulation of striatal ΔFosB are not known. Therefore, we examined the effects of cytisine on chronic voluntary ethanol consumption and associated striatal ΔFosB up-regulation in C57BL/6J mice using behavioral and biochemical methods. Following the chronic voluntary consumption of 15% (v/v) ethanol under a 24-h two-bottle choice intermittent access (IA; 3 sessions/week) or continuous access (CA; 24 h/d and 7 d/week) paradigm, mice received repeated intraperitoneal injections of saline or cytisine (0.5 or 3.0 mg/kg). Ethanol and water intake were monitored for 24 h post-treatment. Pretreatment with cytisine (0.5 or 1.5 mg/kg) significantly reduced ethanol consumption and preference in both paradigms at 2 h and 24 h post-treatment. The ΔFosB levels in the ventral and dorsal striatum were determined by Western blotting 18–24 h after the last point of ethanol access. In addition, cytisine (0.5 mg/kg) significantly attenuated up-regulation of ΔFosB in the ventral and dorsal striatum following chronic ethanol consumption in IA and CA paradigms. The results indicate that cytisine modulates chronic voluntary ethanol consumption and reduces ethanol-induced up-regulation of striatal ΔFosB. Further, the data suggest a critical role of nAChRs in chronic ethanol-induced neurochemical adaptations associated with ethanol addiction.



## Introduction

Chronic administration of drugs of abuse including ethanol produces long-lasting neuroadaptations, through altered gene expression in the midbrain dopamine system, that underlie the progression to addiction-related behaviors (Koob & Volkow, 2010; Robison & Nestler, 2011; Spanagel, 2009). Evidence indicates that repeated exposure to ethanol or other drugs of abuse induces up-regulation of various transcription factors that influence changes in gene expression in reward-relevant brain regions (McClung et al., 2004; Spanagel, 2009; Vilpoux, Warnault, Pierrefiche, Daoust, & Naassila, 2009). One important transcription factor that has been strongly associated with addiction-related plasticity is the stable isomer of ΔFosB (35–37 kDa protein), a truncated splice variant of the FosB gene that belongs to the Fos family of immediate early genes (McClung et al., 2004; Nestler, Barrot, & Self, 2001). Following its induction, ΔFosB forms a protein adduct with the Jun family of proteins that interacts with DNA at specific sites in the promoter regions of target genes and thus activates or represses the transcription of these genes (Robison & Nestler, 2011). Previously, chronic passive or voluntary ethanol administration in rats was shown to induce a region-specific ΔFosB accumulation, with significant induction in the ventral and dorsal striatum (Li et al., 2010; Perrotti et al., 2008).

Emerging evidence implicates the role of brain nicotinic acetylcholine receptors (nAChRs) in various behavioral and dopamine-activating effects of ethanol (Chatterjee & Bartlett, 2010; Larsson & Engel, 2004; Rahman, 2011; Rahman & Prendergast, 2012). For example, pharmacological or genetic manipulation of nAChRs such as α4β2*, α3β4*, α3/α6β2* or α7* subtypes (* indicates the possible inclusion of other subunits) was shown to modulate various ethanol-drinking behaviors and ethanol-induced dopamine release in the ventral striatum of rodents (Chatterjee et al., 2011; Hendrickson, Zhao-Shea, Pang, Gardner, & Tapper, 2010; Kuzmin, Jerlhag, Liljequist, & Engel, 2009).

Cytisine is a known nicotinic alkaloid and has been used for clinical management of smoking cessation (Tutka & Zatoński, 2006). The pharmacological profile of cytisine indicates that it is a high-affinity partial agonist ($K_i$ = 1.2 nM) at α4β2*-nAChRs

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(Coe et al., 2005; Papke & Heinemann, 1994; Slater et al., 2003). Cytisine has also been shown to be a partial agonist at other β2*- (α3/α6-associated) containing nAChRs and shown to be a full agonist at α3β4*- or α7*-nAChR subtypes (Carbonnelle et al., 2003; Papke, Wecker, & Stitzel, 2010). Previously, Coe et al. (2005) have demonstrated the ability of cytisine to reduce nicotine-induced dopamine activation *in vivo*. Recent studies suggest that cytisine reduces ethanol consumption and preference in mice (Hendrickson, Zhao-Shea, & Tapper, 2009) and in selectively bred high-alcohol drinking HAD-2 rats (Bell, Eiler, Cook, & Rahman, 2009). Similarly, varenicline (a derivative of cytisine and a high-affinity partial agonist at α4β2*-nAChRs; clinically approved for smoking cessation) was shown to suppress ethanol drinking in various rodent models (Hendrickson et al., 2010; Steensland, Simms, Holgate, Richards, & Bartlett, 2007) and to suppress ethanol-induced accumbal dopamine release in rats (Ericson, Löf, Stomberg, & Söderpalm, 2009). We have recently demonstrated the efficacy of cytisine in attenuating excess binge-like/free-choice ethanol drinking and ethanol-induced striatal dopamine function in mice (Sajja, Dwivedi, & Rahman, 2010; Sajja & Rahman, 2011). Further studies from our laboratory indicated the potency of cytisine to modulate chronic nicotine-induced free-choice ethanol drinking in mice (Sajja & Rahman, 2012).

Regarding the cellular mechanism of action of cytisine, we have reported that cytisine, a partial agonist at α4β2-nAChRs, significantly reduced ethanol-induced stimulation of the mesolimbic dopamine system (Sajja et al., 2010). This is consistent with previous studies in which varenicline, a cytisine derivative, significantly reduced ethanol-induced dopamine release in the nucleus accumbens in rodents (Coe et al., 2005; Rollema et al., 2010). It is likely that cytisine reduces ethanol-induced neurochemical actions by its partial agonist profile at α4β2-nAChRs, similar to varenicline, such that it provides minimum efficacy on dopamine stimulation in the absence of ethanol and inhibits the nAChR functional interaction in the presence of ethanol (Sajja et al., 2010). Alternatively, desensitization of nAChRs can also be involved in the pharmacological manipulation of the ethanol-induced dopamine function by cytisine (Rollema et al., 2010).

Recently, the intermittent access ethanol-drinking paradigm has attracted significant interest as a reliable model for human behaviors observed during a transition to ethanol addiction (Crabbe, Harkness, Spence, Huang, & Metten, 2012; Hwa et al., 2011; Melendez, 2011). Also known as the "every-other-day" drinking paradigm, this free-choice procedure resulted in escalated ethanol intake patterns at relatively high concentrations of ethanol in rodents over a shorter duration (Loi et al., 2010; Melendez., 2011), as opposed to binge-like or continuous free-choice ethanol drinking (Hwa et al., 2011), followed in our previous studies (Sajja & Rahman, 2011). Thus, the intermittent access model would provide a better means to understand important neurochemical and molecular adaptations during a transition to addiction-related phenotypes. Previously, Li et al. (2010) have demonstrated that chronic intermittent access to ethanol (20% v/v) led to excessive consumption and significant up-regulation of ΔFosB levels in a region-specific pattern, more specifically in rat striatal regions, in a manner similar to morphine (Muller & Unterwald, 2005). Importantly, repeated injections of naltrexone (a clinically approved medication for the treatment of ethanol addiction) significantly attenuated ΔFosB induction that had been up-regulated by chronic intermittent access to ethanol (Li et al., 2010). Similarly, sub-chronic treatment with varenicline also reduced escalated ethanol intake patterns during an intermittent access paradigm (Steensland et al., 2007). However, the effects of repeated injections of cytisine in this model are not known. Further, it is also important to assess the effects of nicotinic ligands on chronic ethanol-induced ΔFosB induction, given the evidence that passive or voluntary nicotine intake in rodents up-regulates striatal ΔFosB induction (Marttila, Raattamaa, & Ahtee, 2006) and nicotinic partial agonists reduce ethanol-induced dopamine release or function (Ericson et al., 2009; Sajja et al., 2010).

Therefore, the objectives of the present study were: i) to study the effects of repeated injections of cytisine on chronic voluntary ethanol consumption in C57BL/6J mice under 24-h free-choice intermittent or continuous ethanol access procedures; and ii) to determine whether cytisine modulates chronic ethanol-induced up-regulation of ΔFosB in the ventral and dorsal striatum during intermittent and continuous ethanol access paradigms.

## Materials and methods

### *Animals*

Male C57BL/6J mice (5–6 weeks old; The Jackson Laboratory, Bar Harbor, ME) were initially acclimated for one week in standard cages (4/cage) and were later individually housed in Plexiglas® cages. Throughout their stay, animals received food and tap water *ad libitum*, under a 12:12-h light/dark cycle (lights off at 1800 h) with controlled temperature and humidity. All procedures were in compliance with National Institutes of Health guidelines for care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at South Dakota State University.

### *Drugs and ethanol-drinking solutions*

Cytisine (Sigma–Aldrich, St. Louis, MO) was dissolved in saline and injected intraperitoneally (i.p., 10 mL/kg). Fresh drug solutions were made on alternate days and doses of cytisine including pre-injection time intervals were selected based on previous evidence (Hendrickson et al., 2009; Sajja & Rahman, 2011, 2012). Ethanol solutions (%, v/v) for drinking were prepared by diluting ethanol (190 proof, Fisher Scientific, PA) with tap water and its concentration was chosen based on earlier studies (Melendez, 2011).

### *Intermittent or continuous access free-choice 15% (v/v) ethanol-drinking procedure*

Following acclimation, mice ($n = 20$) received 24 h/d intermittent access (IA) on Monday, Wednesday and Friday (3 sessions/week), or continuous access (CA; 24 h/d and 7 d/week) to 15% ethanol and water using a modified two-bottle choice (ethanol vs. water) procedure (Melendez, 2011; Sajja & Rahman, 2011). Briefly, animals were exposed to ascending concentrations of ethanol (3% on Monday, 6% on Wednesday, and 10% on Friday) during the first week of the IA paradigm or 4% for 3 d, 8% for 4 d, and 12% for 5 d during the CA paradigm, followed by 15% ethanol access (vs. water) for the rest of the experiment (Hwa et al., 2011). For each ethanol-drinking session, fresh ethanol solution and water were provided at the beginning of the dark phase in two separate 15-mL centrifuge tubes containing ball-bearing steel sipper tubes. The positions of the tubes were rotated on alternate days of ethanol-drinking sessions to prevent the development of positional bias. In addition, mice under the IA paradigm received two tubes of water on the remaining sessions of the week when they had no ethanol access. To account for fluid loss due to experimenter handling or evaporation, control ethanol and water tubes were placed into empty cages and their average values were subtracted from the original readings (Sajja & Rahman, 2011). Fluid levels were measured to the nearest 0.1 mL and ethanol and water intake (g/kg or mL/kg, respectively) including ethanol preference (%) were