Introduction

Alcohol is the most commonly abused drug among adolescents and shows the highest liability of all abused substances. In humans, adolescent alcohol use can predict later drug abuse potential, and can lead to negative alterations in decision-making functions. Adolescence is characterized by maturation and remodeling of key brain regions implicated in reward and decision making and this malleable nature of the adolescent brain renders it uniquely vulnerable to environmental insults such as alcohol exposure. We have previously demonstrated that voluntary consumption of alcohol by adolescent rats results in increased maladaptive risk-taking behavior on a probability discounting task when later tested as adults. Measurement of pharmacological (DA) release via fast scan cyclic voltammetry (FSCV) has demonstrated that rats exposed to alcohol in adolescence show increased DA transmission within the nucleus accumbens (NAc) core in response to risky, but not to safe, options in adulthood. This finding suggests that changes in striatal DA release, as a consequence of alcohol exposure, could bias choice by assigning greater value to the risky option, but the mechanism of action remains unknown. GABA<sub>A</sub> receptors located in the ventral tegmental area (VTA) are a potential candidate for mediating these effects, as these receptors are a main target of alcohol, undergo extensive development during adolescence, and can modulate DAergic neuron activity and subsequent dopamine release in the NAc. Thus, we hypothesize that adolescent alcohol exposure confers persistent changes to risk-taking behavior through alcohol-mediated GABA<sub>A</sub> receptor modulation of DA release in the VTA. In order to investigate this possibility, here we use electrical stimulation of the pedunculopontine tegmental nucleus (PPT) to elicit GABA<sub>A</sub> receptor dependent currents in VTA DA neurons. GABA<sub>A</sub> receptor subunits, specifically the α2, α3, and δ subunits, are preferentially expressed in the VTA DA neurons, while VTA GABA neurons preferentially express the α1 subunit. Thus, in order to specifically target GABA<sub>A</sub> receptors on DA neurons, we utilize L-838,417, an α1 allosteric agonist at the α2, α3, and δ subunits. The results of these experiments provide unique insight into the potential role that GABA<sub>A</sub>ergic modulation of DA release plays in maladaptive risk-taking behavior following adolescent alcohol consumption.

Methods

Ethanol presentation: Alcohol was presented to male Sprague Dawley adolescent rats (PND 50–60) in a 1% gel matrix consisting of distilled water, gelatin, fructose (10%), and 10% ethanol. Ethanol was replaced with distilled water for control gel matrix. Alcohol availability was 24 h/day for 20 days in addition to ad lib water and chow, and fresh gel containing jars were presented every day. Upon completion of the 20 day exposure, gel access was discontinued and animals were monitored daily for withdrawal symptoms for the following 20 days. No overt signs of withdrawal were recorded.

Fast scan cyclic voltammetry and PPT stimulation-induced NA<sub>C</sub> dopamine release

Probability discounting task: As previously described, following the 20 days of withdrawal from ethanol, rats began food restriction to ~90% of their free-feeding body weight. Following lever-pressing training for single sucrose pellets and autoshaping, animals were tested on a concurrent instrumental response task involving the presentation of two levers. The certain lever was associated with the delivery (100%) of two sucrose pellets and the uncertain lever was associated with the probabilistic delivery (either 75%, 50%, 25%, or 0%) of four sucrose pellets. Daily sessions consisted of 24 forced trials followed by 24 free choice trials. During forced trials, administration of a single lever was always correct, while during free choice trials the same probability of reinforcement for the two levers was presented. Probability of reinforcement for the uncertain lever was varied pseudorandomly.

Fast scan cyclic voltammetry and PPT stimulation-induced NA<sub>C</sub> dopamine release

In vivo phasic dopamine release is modulated by adolescent alcohol intake

The ImageStream® combines flow cytometry and quantitative fluorescent microcopy for single cell analysis

Adolescent alcohol intake promotes suboptimal risk preference in adulthood

Adolescent alcohol intake results in increased NA<sub>C</sub> DA release in response to PPT stimulation in adulthood

Imaging flow cytometry for isolation and analysis of midbrain dopamine neurons

Conclusions

Voluntary alcohol intake during adolescence produces enduring maladaptive risk-taking behavior later in adulthood.

Adolescent alcohol intake results in enhanced NA<sub>C</sub> DA release via PPT stimulation, suggesting disinhibition of VTA DA neurons.

L-838,417, a GABA<sub>A</sub> α2, α3, and δ allosteric agonist, inhibits NA<sub>C</sub> DA release to a greater extent in adult rats previously exposed to alcohol during adolescence.

Imaging flow cytometry is a novel technique for isolation and subsequent analysis of midbrain dopamine neurons. Future directions with this technique will investigate the effects of adolescent alcohol intake on midbrain GABA<sub>A</sub> subunit expression levels in dopamine neurons.