**Introduction**

Excessive alcohol consumption is common during human adolescence, which is a developmental period characterized by heightened synaptic plasticity. The adolescent brain, which develops well into a person’s 20s, undergoes various forms of synaptic plasticity including intense "reshaping" of neuronal circuits mediating alcohol and drug addiction (Toga et al., 2006).

Recent studies in our laboratory have shown that adolescent C57BL/6J mice have a greater capacity to drink 15% ethanol solutions compared to adults (Melendez, 2011). Nevertheless, the neurochemical mechanisms mediating adolescents’ vulnerability to heavy drinking are not well understood.

Notably, a growing number of studies indicate that the impairment of extracellular brain glutamate levels, particularly in the nucleus accumbens (NAC), plays a pivotal role in the development of addiction to nicotine, heroin, and cocaine (reviewed by Kalivas, 2009). However, little is known regarding alcohol addiction and much less is known in relation to alcohol use during the adolescent period.

For the present study, Experiment 1 determined the impact of early alcohol drinking on subsequent drinking during adulthood. Experiment 2 examined the extent to which adolescent and adult mice will drink 15% ethanol versus an alcoholic beer beverage (i.e., Medalla). Experiment 3 measured the brain levels of extracellular glutamate in the NAC of adolescent relative to adult C57BL/6J mice in the absence and presence of ethanol.

**Methods**

Male C57BL/6J mice used were obtained from Jackson Laboratory at 3 weeks (postnatal day [PND] 21) or 11 weeks (PND 84) of age. Upon arrival, mice were single housed and acclimated for 4-5 days in an IACUC-accredited animal housing facility prior to any behavioral or neurochemical manipulation. All mice were fed ad libitum and were given 4-5 days to habituate to the home cage before any testing began when mice were at 4-12 weeks of age, which approximates human adolescence and adulthood, respectively (Quinn, 2005).

**Ethanol Self-Administration Procedures**

For Experiment 1, adolescent and adult mice (n=7/group) were given access to 15% (v/v) ethanol in their home cages until they reached 17% (v/v) ethanol and were kept on a 12-hr light/dark cycle with lights on at 0700 hours. All mice were given 45 days to reach their respective ethanol consumption levels. The ethanol solution was delivered using a constant volume syringe pump (Columbus Instruments, Columbus, OH) that delivered 20 µl/min over 30 min per day. Mice were monitored daily for ethanol intake, and baseline ethanol consumption was determined for 7 consecutive days to ensure that mice were consuming ethanol at the desired levels. All mice were initially anesthetized and surgically implanted with a guide cannula aimed 1 mm above the NAC. Moreover, repeated ethanol exposure or ethanol drinking experience resulted in greater ethanol-induced increases in glutamate levels in the NAC of adolescent relative to adult mice. This suggests that adolescent mice are more responsive to the effects of ethanol on glutamate release, which may be a potential mechanism mediating heavy drinking.

**Image and Localization of Microdialysis Probes in the NAC of C57BL/6J Mice.**

**Elevated Glutamate Transporter Function in the Prefrontal Cortex (PFC) of Adolescent Mice.**

**Conclusions**

Experiment 1 demonstrated that adolescent mice consume greater amounts of ethanol on a daily basis during adolescence, which persisted well into adulthood. These findings are consistent with clinical studies showing that early alcohol use enhances the risk of heavy drinking in adulthood.

Experiment 2 indicated that intermittent (every-other-day) ethanol exposure produces greater escalation of beer and 15% ethanol consumption in adult mice. These findings suggest that adolescent mice have a greater capacity for ethanol intake regardless of its concentration or type of solution (e.g., beer). They also support the notion that adolescents are more vulnerable to the addictive properties of ethanol such as high-ethanol preference.

Experiment 3 revealed that adolescent (ethanol-naive) mice have greater basal levels of extracellular glutamate in the NAC. Moreover, repeated ethanol exposure or ethanol drinking experience resulted in greater ethanol-induced increases in glutamate levels in the NAC of adolescent relative to adult mice. This suggests that adolescent mice are more responsive to the effects of ethanol on glutamate release, which may be a potential mechanism mediating heavy drinking.

In summary, these findings show a clear relationship between enhanced ethanol drinking during adolescence and elevated glutamatergic neurotransmission within the PFC-NAC circuit.