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Peri-Adolescent Alcohol Consumption Enhances the Reinforcing and Stimulatory Properties of Ethanol Within the Adult Mesolimbic Dopamine System in Alcohol Preferring P Rats.

For the degree of Doctor of Philosophy

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PERI-ADOLESCENT ALCOHOL CONSUMPTION ENHANCES THE REINFORCING AND STIMULATORY PROPERTIES OF ETHANOL WITHIN THE ADULT MESOLIMBIC DOPAMINE SYSTEM IN ALCOHOL PREFERRING P RATS

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of

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by

Jamie E. Toalston

In Partial Fulfillment of the

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of

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For Zac and Char.

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LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine, serotonin
5-HIAA	5-hydroxyindoleacetic acid
AA	ALKO Alcohol Accepting rat
Acb	nucleus accumbens
AcbC	nucleus accumbens core
AcbSh	nucleus accumbens shell
aCSF	artificial cerebrospinal fluid
ADE	alcohol deprivation effect
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid
ANA	ALKO Alcohol Non-Accepting rat
aVTA	anterior ventral tegmental area
BAL	blood alcohol level
CRF	corticotropin releasing factor
DA	dopamine
D1	dopamine 1 receptor
D2	dopamine 2 receptor
DE	deprivation effect
DOPAC	3,4-dihydroxyphenylacetic acid, a dopamine metabolite
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Edition 4
EtOH	ethyl alcohol

FR	fixed ratio
g/kg	gram/kilogram (body weight)
GABA	gamma-aminobutyric acid
HAD	High Alcohol Drinking rat (HAD1 - replicate line 1, HAD2 - replicate line 2)
HPA	hypothalamic-pituitary-adrenal (axis)
HPG	hypothalamic-pituitary-gonadal (axis)
HPLC-EC	High Performance Liquid Chromatography-Electrochemical Detection
HVA	homovanillic acid, catecholamine metabolite
ICSA	intracranial self-administration experiment
LAD	Low Alcohol Drinking rat (LAD1 - replicate line 1, LAD2 - replicate line 2)
LMA	(spontaneous) locomotor activity
MicroMicro	microinjection/microdialysis experiment
NP	alcohol Non-Preferring rat
P	alcohol Preferring rat
PD	postnatal day
PE	polyethylene
PR	progressive ratio
PVN	paraventricular nucleus of the hypothalamus
pVTA	posterior ventral tegmental area
RN	red nucleus
SACC	saccharin
sP	Sardinian alcohol-preferring rat
v/v	volume/volume
VTA	ventral tegmental area

ABSTRACT

Toalston, Jamie E. Ph.D., Purdue University, August 2011. Peri-Adolescent Alcohol Consumption Enhances the Reinforcing and Stimulatory Properties of Ethanol Within the Adult Mesolimbic Dopamine System in Alcohol Preferring P Rats. Major Professor: James M. Murphy.

Research in the alcohol preferring (P) rat has indicated that peri-adolescent alcohol (EtOH) consumption enhances the acquisition of oral operant EtOH self-administration, inhibits the extinction of responding for EtOH, augments EtOH-seeking behaviors, and increases relative reward value of EtOH during adulthood. Experiment 1 was conducted to determine if these adult effects of peri-adolescent EtOH intake could be observed using an Intracranial Self-Administration (ICSA) model. It was hypothesized that an increased sensitivity to the rewarding actions of EtOH would be manifested in peri-adolescent-EtOH-exposed subjects compared to naive subjects when the opportunity to self-administer EtOH to the posterior ventral tegmental area (pVTA) is available in adulthood. The pVTA is a primary site for EtOH's reinforcing and rewarding properties in the mesolimbic dopamine (DA) system. Experiment 2 was a dose-response examination of the effects of EtOH administered to the pVTA on downstream DA efflux in the nucleus accumbens shell (AcbSh) via a joint Microinjection-Microdialysis (MicroMicro) procedure.

Male P rats were given 24-h free-choice exposure to 15% volume/volume EtOH from postnatal day (PD) 30 to PD 60, or remained experimentally naive, with ad lib food

and water. By the end of the periadolescent exposure period, average consumption was 7.3 g/kg/day of EtOH. After PD 75, periadolescent-EtOH-exposed and naïve rats were either implanted with an injector guide cannula aimed at the right pVTA for ICSA (Experiment 1), or two cannulae, one aimed at the right pVTA (injector) and one at the ipsilateral AcbSh (microdialysis) for MicroMicro (Experiment 2). Following one week of recovery from surgery, ICSA subjects were placed in standard two-lever (active and inactive) operant chambers. Test sessions were 60 min in duration and occurred every other day for a total of 7 sessions. Rats were randomly assigned to one of 5 groups (n=4-9/group) that self-infused (FR1 schedule) either aCSF (vehicle, 0 mg%), 50, 75, 100, or 150 mg% EtOH during 4 sessions, aCSF only for sessions 5 and 6 (extinction), and the initial concentration again for session 7 (reinstatement). MicroMicro subjects received six days of recovery from surgery, probe implantation the day before testing, and then continuous microdialysis for DA with 15 min microdialysis samples collected before, during, and then two hrs after 10-min pulse microinjection of either aCSF (vehicle, 0 mg%), 50, 75, 100, or 150 mg% EtOH.

Neither EtOH-exposed nor naive groups of P rats self-infused the aCSF or 50 mg% EtOH concentration. While the naive group did not self-infuse the 75 or 100 mg% EtOH concentrations, the peri-adolescent EtOH-exposed group of P rats did readily discriminate the active lever from the inactive lever at these concentrations. Both groups self-infused the 150 mg% EtOH concentration. Pulse microinjections of EtOH during the MicroMicro procedure revealed that 75 and 100 mg% concentrations of EtOH increased downstream DA in the AcbSh of EtOH-exposed, but not naïve, subjects. 150 mg% EtOH increased downstream DA in both adolescent treatment groups.

Overall, the results indicate that consumption of EtOH by P rats during peri-adolescence increases the reinforcing properties of EtOH in the pVTA in adulthood. The

results also indicate that there were differential effects of peri-adolescent EtOH exposure on DA efflux in the AcbSh. This provides evidence that peri-adolescent EtOH-exposure produces long-lasting alterations in neural circuitry involved in EtOH-reinforcement, during adulthood.

1. BACKGROUND

Alcohol and Adolescence: A Period of Vulnerability

Within the United States alcohol use by anyone under the age of 21 is illegal. Nonetheless, surveys have found that over 70 percent of high school seniors have consumed alcohol and 30 percent have reported binge drinking, which is defined as consumption of five or more drinks at a time (Johnston et al., 2007). Lifetime prevalence of diagnosed alcohol abuse or dependence disorders in the United States is between 5 and 13% of the population (Kessler et al., 2005). Worldwide, an estimated 76.3 million people have alcohol use disorders (Assanangkornchai and Srisurapanont, 2007). Given this large population of patients afflicted with continued maladaptive alcohol consumption, researchers have attempted to discover how, why, and particularly when alcohol consumption progresses from casual intake to a neuropsychological disorder.

What has been labeled as a "downward spiral" to EtOH addiction has several described phases (Koob and Le Moal, 1997). These include EtOH intake to the point of intoxication and pharmacologically rewarding effects, and at least one of the following: escalation of EtOH intake due to an increase in motivation to achieve greater reward, an increased clearance of EtOH from system (metabolic tolerance to its effects), requiring greater intake to create same rewarding effect, and systemic homeostatic adjustment requiring further EtOH to stave off withdrawal. This also involves changes that occur during withdrawal and/or long term EtOH abstinence. Forward and backward feedback

loops intertwine these effects in many ways, and are observable at several levels, behaviorally and cellularly.

It has been observed that drinking experience early in life is positively correlated with alcohol abuse in adulthood (Kandel et al., 1992, Clark et al., 1998). Long-term epidemiological studies have found that those who drink before the age of 14 have a four times greater chance of developing a dependence on alcohol during their lifetime, compared to those who do not drink until after age 20 (Grant and Dawson, 1997). Fifty percent of the alcoholic population first manifests DSM-IV-diagnosable alcoholism by 19 years of age (Moss et al., 2007), indicating that the biological basis for alcoholism has already completely manifested before legal adulthood (and presumed cognitive maturity) has even arrived. This suggests that adolescence may be a time when neural systems are particularly vulnerable to drugs of abuse, like alcohol. The full effects of alcohol administration during adolescence are not yet understood, although it is agreed that adolescent alcohol consumption disrupts proper scaffolding of some brain structures that develop during this time period, including the cerebral cortex and limbic system (Crews et al., 2007).

Binge drinking at these early ages has become a particular area of concern. Higher levels of EtOH in the blood putatively mean greater effects on neurochemistry. Greater effects on neurochemistry are likely to lead to long-lasting adaptive changes that disrupt normal functioning. Studies of human twins has found that excessive alcohol consumption in adolescence predicts later alcohol use problems better than lower levels of alcohol consumption (Poelen et al., 2009). Unfortunately, on average, underage alcohol drinkers have been found to consume more alcohol per bout than those surveyed over the age of 21 (4.9 vs. 2.8 drinks) (Substance Abuse and Mental Health Services Administration Office of Applied Studies, 2008).

Adolescence is a time of complexity. Puberty, or sexual maturity, is the hallmark of adolescence. Developmental transitions, including physical, social, and cognitive maturational factors, together contribute towards producing a time period sensitive to production of drug abuse (Spear, 2000). Hormones, adrenal and gonadal in nature, act on many systems of the body to change the composition and physical conformation of skeleton, body fat and muscle. Social and behavioral markers of adolescence include changes in stress, anxiety, and sensation-seeking thresholds. On a neural level, significant amounts of cortical and hippocampal synaptic pruning continues during late adolescence and early adulthood. Neurotransmitter receptors, including DA, 5-HT, and GABA, are reorganized systematically (Spear, 2000).

Temporally, human adolescence is generally considered to encompass much of the teenage years, covering a time period before, during, and after puberty (menarche/spermarche), while also encompassing the aforementioned intellectual and psychosocial maturational processes. Regarding maturation in the United States, a human is legally a minor under parental control until the age of 18. Biologically, the process of sexual maturation of human females as a result of hypothalamic-pituitary-gonadal (HPG) stimulation may start from as early as age 10 and continue for about 4 years, while maturation of males begins around age 12 and continues for about 6 years (Windle et al., 2008). Metabolic fuel availability appears to have an influence on the onset timing of these processes, linking childhood obesity to earlier onset of puberty (Ebling, 2005).

The modeling of adolescent EtOH consumption in animals is one strategy to elucidate a more precise understanding of the effects EtOH has on the developing brain. Skeletal maturation and body weight also have an influence on onset of sexual viability in rodents. Vaginal opening in the female rat occurs at about 100 grams of weight,

between days 33 and 42, and estrous begins approximately one week later. Sexual maturity occurs at body length of 148 to 150 mm in male rats. Testes descend to the scrotum at about day 15 of age, and sperm are produced around day 45, yet optimal production is not achieved until 75 days (Suckow et al., 2006). Adolescence in the rat has been described to begin as early as postnatal day 20 in the female, while most obvious signs of puberty in the male rat have occurred as late as day 55 (Odell, 1990, Spear, 2000). It is for these reasons that in the alcohol field, many studies use an approximate window from postnatal day 30 to 60 to encapsulate a time period for both male and female subjects that may be described as peri-adolescence (Spear, 2000, Bell et al., 2006). This captures maturational changes during and after sexual maturity in an attempt to mirror human adolescence, which extends to physical and psychosocial changes beyond mere menarche/spermarche.

Observation of Adolescent EtOH Intake

Changes following EtOH intake during adolescence have the potential to affect social interest, novelty-seeking behaviors, attentional properties, and emotional state. Given what is known about hormone changes during this time period (testosterone, estrogen, growth hormones, anxiety-related hormones), and brain changes due to hormone changes, a number of interactions may occur. Epidemiological evidence indicates that drug-taking behavior ultimately affects many of these changes increasing subsequent drug-seeking behavior and drug-reward incentive value.

As described with humans (Substance Abuse and Mental Health Services Administration Office of Applied Studies, 2008, Poelen et al., 2009), adolescent rats have been found to voluntarily consume more EtOH by body weight than adult rats given the same amount of access, in both free-choice and limited access experiments (Bell et

al., 2003, 2004, 2006, Doremus et al., 2005, Vetter et al., 2007, Vetter-O'Hagen et al., 2009). Adolescents are innately different from adults in regards to sensitivity to the hypnotic, anxiolytic, and motor-impairing effects of EtOH. The decreased sensitivity to these aversive effects of EtOH may fail to discourage further drinking in adolescence (Brunell and Spear, 2006). In addition, impulsive behavior in human adolescence has been found to predict heavy drinking in adulthood (Verdejo-Garcia et al., 2008), while heavy drinking in early adolescence has been shown to predict an increase in impulsive behavior in later adolescence (White et al., 2011).

EtOH consumption by the adolescent rat has been shown to have a significant impact on adult EtOH drinking behavior. For example, when juvenile (PD 22-28) and adult rats are given given similar access to EtOH, the juvenile-exposed rats show a greater willingness to drink 30% EtOH in adulthood (Truxell et al., 2007). In the alcohol preferring (P) rat, peri-adolescent EtOH consumption enhances EtOH intake during adulthood when the rats are tested under both free-choice and operant conditions (McKinzie et al., 1998b). Subjects given free-choice access to EtOH during adolescence acquire operant responding for EtOH faster than naïve subjects as well as those given the same amount of pre-exposure during adulthood (Rodd-Henricks et al., 2002a, b). These authors also reported differences that included increased time to extinguish responding when EtOH was no longer available in the operant setting, as well as enhanced relapse-like behaviors when EtOH availability was returned.

The alcohol deprivation effect (ADE), as modeled by relatively long-term EtOH abstinence followed by a return to EtOH access, simulates precipitated relapse in the human condition, and has been observed in multiple laboratory species (Sinclair, 1971, Kornet et al., 1990, Spanagel et al., 1996, Agabio et al., 2000, Le and Shaham, 2002). Overt withdrawal symptoms after prolonged EtOH exposure last no longer than 1 week

in the rat (Cicero et al., 1971), but the ADE deprivation/abstinence interval encompasses periods beyond one week. The ADE is observed with manifestation of a spontaneous increase in EtOH intake, over previously normal drinking behavior, once access is restored (Sinclair and Senter, 1968). Some studies have revealed the absence of an ADE between adult- and adolescent-exposed subjects, given the above conditions (Tambour et al., 2008), but this may be a function of decreased procedural sensitivity. The P rat shows an increased ADE response following adolescent access, compared to adult subjects receiving the same exposure/abstinence periods (Rodd-Henricks et al., 2002a, b). In Wistar rats, acamprosate injections reduce the expression of an ADE enhancement in adult-exposed subjects, but not in adolescent-exposed subjects, suggesting that adolescent-initiated EtOH drinkers incur some adaptive resistance to the neurochemical actions of acamprosate treatment (Fullgrabe et al., 2007).

All of these studies combined illustrate that EtOH consumption by the adolescent rat has significant impact on adult EtOH drinking behavior, above and beyond any effect of mere prior access (i.e., differences seen relative to adult exposure).

Adolescent EtOH in Selectively Bred Rats

Forced-access experimental designs are often necessary to examine effects of high levels of EtOH. Free-choice access, where the subject determines how much EtOH is administered, can shed light on changes in motivation to seek EtOH and related differences in adult neural makeup. For example, following free-choice access to EtOH during adolescence, Wistar rats show more pronounced adult stress-induced EtOH consumption, compared to those exposed to EtOH in adulthood (Fullgrabe et al., 2007). However, to observe the long-term effects of EtOH following high amounts of adolescent EtOH consumption it is very useful to employ subjects selectively bred for high EtOH

intake and/or preference. These subjects readily consume EtOH voluntarily and attain pharmacologically relevant elevation of blood ethanol concentrations.

Selective breeding for high EtOH consumption in laboratory subjects is meant to model humans with a positive family history of alcoholism, as there is evidence of a human genetic predisposition for high risk alcohol use. Rodents selectively bred for high EtOH consumption include the P rat, high alcohol drinking (HAD) replicate rat lines, Sardinian preferring (sP) rats, ALKO alcohol accepting (AA) rats, and high alcohol preferring (HAP) mice. Cicero (1980) created a set of criteria for producing a valid animal model of alcoholism. These include 1) subjects should orally self-administer EtOH, 2) the amount of EtOH consumed should relate to pharmacologically relevant blood alcohol levels (BAL), 3) subjects should consume EtOH for pharmacological effect, not just taste or caloric load, 4) subjects should find EtOH consumption positively reinforcing, 5) chronic EtOH should lead to metabolic/functional tolerance, and 6) chronic EtOH should lead to dependence/observable withdrawal. This list was expanded by McBride and Li to include evidence of relapse-enhanced drinking behavior (1998). The previously-mentioned P rat, which was selectively bred for preference of 2 to 1 for 10% v/v EtOH over water and at least 5 g/kg/day self-administered EtOH (Lumeng et al., 1977), is believed to meet these criteria (McBride and Li, 1998, Murphy et al., 2002, Bell et al., 2005, 2006). The P rat is the animal model used in this study.

In the P rat, peri-adolescent consumption has been demonstrated to enhance EtOH intake in adulthood in both free-choice and operant settings (McKinzie et al., 1998b., Rodd-Henricks et al., 2002a, b, McBride et al., 2005). Following peri-adolescent access to EtOH in the P rat, increased clearance of DA has been seen in the nucleus accumbens (Acb) as measured by no-net-flux microdialysis for DA (Sahr et al., 2004), where several concentrations of DA are perfused, then analyzed for DA change.

Adolescent EtOH exposure also increases breakpoint responding for operant oral EtOH access, indicating a change in the reward salience of EtOH (Toalston et al., In Preparation). Peri-adolescent EtOH consumption by the P rat therefore appears to cause significant impact on adult EtOH drinking behavior.

Change in Reward Value of EtOH Following Exposure

Operant techniques can examine alterations in the amount of work a subject will perform to obtain reinforcement, and are often used to examine subjective changes in the reinforcing properties of EtOH (Hodos, 1961, Ciccocioppo et al., 2001, Rodd et al., 2004a). It has previously been observed that while P rats will consume large volumes of sweetened liquids, they will still concurrently consume pharmacologically relevant volumes of EtOH (Lankford et al., 1991). But given access to sweetened liquids alone, P rats will work to consume sweetened liquids at high volume, higher even than that observed for EtOH (Nowak et al., 1999, Czachowski and Samson, 2006). This was also observed in our laboratory's previous study (Toalston et al., In Preparation), where peri-adolescent P rats that had free-choice access to SACC responded more as adults for SACC alone than for EtOH alone. When the operant breakpoint was tested, however, subjects with free-choice peri-adolescent access to EtOH were willing to work to a higher level for EtOH than subjects with free-choice peri-adolescent access to SACC were willing to work for SACC. This supports the idea that, in the P rat, EtOH is a salient reinforcer and that peri-adolescent access to EtOH has a greater effect on adult behavior than that observed following peri-adolescent SACC (Toalston et al., In Preparation). Repeated accesses and abstinences from EtOH also increases the oral operant breakpoint for EtOH in both P and HAD rats (Rodd et al., 2003, Oster et al., 2006).

Behavioral Changes Following EtOH Exposure

Various studies have observed long-lasting behavioral effects in adult animals following adolescent EtOH treatments, which point to possible detrimental cognitive consequences. EtOH administration impairs performance on a number of tasks in adolescence to a greater degree than observed in adult-exposed subjects. Chronic injections of EtOH during adolescence increase novelty-seeking behavior in adulthood compared to naïve subjects (Stansfield and Kirstein, 2007). Repeated EtOH injections impair performance of adolescent subjects to a greater degree on a Morris water maze task than subjects tested during adulthood (Sircar and Sircar, 2005). Intermittent vapor-chamber exposure to EtOH during adolescence affects spatial working memory in adulthood, as measured by performance on a Morris water maze task (Schulteis et al., 2008). Adolescent EtOH-vapor exposed subjects display enhanced prepulse inhibition response, suggesting a form of behavioral inflexibility has been induced (Slawecki and Ehlers, 2005). This form of EtOH inhalation administration results in constant high levels of EtOH in the blood stream (Gilpin et al., 2008).

Repeated administration of EtOH by injection during adolescence has resulted in adult subjects less sensitive to EtOH-induced loss of righting reflex, indicating long-term tolerance to some of the effects of EtOH, or alterations in the ontogeny of this effect (Matthews et al., 2008). Adolescent EtOH exposure has effects on sleep patterns that last long into adulthood (Ehlers and Criado, 2010), and responses to EtOH challenge in adolescence shows increased sensitivity to some EtOH effects (LMA, anxiolytic, ataxic) and decreased sensitivity to others (sedative/hypnotic) compared to adults (Hefner and Holms, 2007). These studies illustrate that EtOH treatment has measurable, specific effects on behavior and memory in adolescent laboratory subjects, both concurrent to EtOH administration as well as without EtOH on board later, in adulthood.

Mesolimbic Dopamine System and Effects of EtOH

Systemic EtOH application (like that seen via oral EtOH consumption) increases EtOH concentrations in the blood, which has the effect of *in vivo* bathing of all body cells and their communications systems in EtOH until it is metabolized and removed from the blood stream. Even acute, one-time EtOH administration has deleterious effects on the normal functioning of neural substrates involved with reward (Crews et al., 2006). Regarding these neural substrates, DA and related serotonin (5-HT) pathways have been repeatedly implicated in high EtOH consumption in rodent models (Murphy et al., 1985, McBride et al., 1991, Di Chiara, 1997).

Tsai's ventral tegmental area, the A10 seat of DA neurons cell bodies, is a heterogeneous structure, with five main nuclei (Tsai, 1925, Oades and Halliday, 1987). The VTA is strongly implicated in cognitive and behavioral processes including motivation and reward, as well as disorders arising from malfunction of these processes; e.g., addiction and schizophrenia (Laviolette, 2007, Murray et al., 2008). VTA dopaminergic projections run primarily to mesocortical and mesolimbic areas, but also mesostriatal, mesodiencephalic, and mesorhombencephalic areas (Ikemoto, 2007).

Mesolimbic VTA DA reward circuitry has been narrowed down further, into meso-ventromedial and meso-ventrolateral striatal systems (Ikemoto, 2007). The former projects from the posterior areas of the VTA to the AcbSh and has been implicated in processing rewarding stimuli, while the latter projects from the anterior/lateral areas of the VTA to the nucleus accumbens core (AcbC) and has been implicated in processing noxious stimuli (Faure et al., 2008, Brischoux et al., 2009). The rodent VTA has therefore commonly been separated into anterior (aVTA) and posterior (pVTA) dimensions, with this separation occurring at approximately 5.2/5.3 posterior to bregma as seen on the Paxinos and Watson rat atlas (1986). More generally speaking, posterior

VTA DA projections largely aim to the PFC and AcbSh, while anterior VTA DA cells project predominately to AcbSh and AcbC (Brog et al., 1993). A second main set of neurotransmitter-producing cell bodies within the VTA is GABAergic, which can interconnect with each other via gap junctions. Furthermore, terminating in the VTA are serotonergic, acetylcholinergic, GABAergic, and opioid projections (Ikemoto, 2007).

The mechanisms of action of rewarding drugs (including EtOH) involving the VTA is varied, but it has been observed that all drugs that are rewarding to humans are associated with DA release in the Acb, while drugs that are noxious decrease DA in this area (Di Chiara and Imperato, 1988). Thus, the reciprocal interaction that the VTA has with the Acb is a field of intense study.

Enhancement of neural activity in connection with EtOH within the VTA has been observed. In vitro, acutely administered EtOH increases the firing rate of DA neurons in the VTA (Brodie et al., 1999). VTA DA neurons of mice with higher sensitivity to EtOH, as evidenced by place preference conditioning (CPP) response, but lower propensity to consume EtOH, show stronger response to EtOH application than those with lower place preference conditioning and higher EtOH drinking behavior (Brodie and Appel, 2000). It has also been observed that chronic EtOH treatment increases the excitation of VTA DA neurons (Brodie, 2002).

Part of the effects EtOH has on DA in the VTA has been attributed to mediation by the serotonin system (Murphy et al., 1992). Serotonin neurons in the dorsal and median raphe nuclei terminate in both the VTA and Acb. 5-HT3 receptor antagonism reduces the stimulation of DA release by EtOH in the Acb (Carboni et al., 1989), suppresses EtOH-stimulated release of DA in the VTA (Campbell and McBride, 1995), and suppresses acquisition and maintenance of responding for ICSA of EtOH in the VTA (Rodd-Henricks et al., 2003). Increasing 5-HT enhances the excitability of DA neurons in

the VTA (Buck et al., 2004), however 5-HT3 antagonism does not suppress ICSA for the EtOH metabolite acetaldehyde (Rodd et al., 2005c).

Downstream from the VTA in the mesolimbic pathway, the Acb also shows evidence of enhancement of neural activity as a result of EtOH treatment or drinking. Systemically applied EtOH has been shown to increase DA release in the Acb (Di Chiara and Imperato, 1985). Chronic and intermittent EtOH exposure increases D2 receptor binding density in the AcbSh (Sari et al., 2006), showing specific evidence of compensatory changes in this area. Interestingly, however, self-administration of EtOH has also been observed to decrease DA response in the Acb following EtOH challenge, compared to yoked forced access subjects that do not show this effect (Nurmi et al., 1996). Overall, these findings are consistent with a strong link of the Acb to EtOH-seeking behavior; however, the relationship may be more complex than a simple unidimensional EtOH administration effect.

Enhanced EtOH intake in rodent subjects is related to lower endogenous basal 5-HT in regions of the mesocorticolimbic neural circuit (Murphy et al., 1987), as well as fewer 5-HT neurons in these areas (Zhou et al., 1994). EtOH challenge produces an increase in accumbal monoamine metabolites DOPAC, HVA, and 5-HIAA (Murphy et al., 1988), which suggests activation of these systems. However, in chronically exposed subjects this response is muted compared to naïve subjects (Murphy et al., 1988). Moreover, serotonin microinfused into the VTA increases DA efflux in the Acb (Guan and McBride, 1989).

The VTA projects GABA-releasing neurons to the Acb, with these VTA neurons receiving connections from the PFC (Carr and Sesack, 2000). GABA(A) antagonists cause a release of DA into the Acb when administered to the aVTA (Ikemoto et al.,

1997b). DA neurons of the VTA and medium spiny neurons of the Acb display evidence of increased excitability in synaptic areas, indicating a role from glutamate (Stuber et al., 2010).

Opioids are also a factor within the reinforcement neurocircuit (Di Chiara et al., 1996). A delta-opioid antagonist co-perfused into the Acb with EtOH prevented EtOH-induced DA increases, but not when co-perfused with cocaine, suggesting a role for delta-opioid receptors in the Acb specific to EtOH (Acquas et al., 1993). Genetic analyses of human families have shown that high risk for alcoholism is associated with carrying a variation in gene OPRK1, which reduces transcription activity of the kappa-opioid receptor (Edenberg et al., 2008).

Taken together, these findings illustrate how the effects of EtOH on the VTA-Acb system are obviously varied and complex, even without the added factor of adolescent development. As indicated above, animals genetically predisposed to excessive EtOH drinking or EtOH-seeking behavior (e.g., P Rats) are known to have endogenous alterations in neurochemical pathways compared with their alcohol-non-preferring (NP) or outbred (Wistar) counterparts. These neurochemical differences likely mediate some responses to EtOH. Thus the effects of adolescent EtOH on these pathways could further enhance neuroadaptations to enhance the already predisposed state for EtOH-seeking in the adult animal.

Neural Stunting Following EtOH Exposure

Binge EtOH results in greater brain damage in young adolescent rats compared to adult rats (Crews et al., 2000). EtOH exposure in adolescence dose-dependently stunts normal cell proliferation in the forebrain and dentate gyrus, with this effect occurring after a single peripheral injection of EtOH (Crews et al., 2006). In the

hippocampus, EtOH exposure during adolescence alters the track and survival of progenitor cells, possibly by reducing time in critical stages of the cell cycle (McClain et al., 2011a), as well as creating marked activation of select microglia, suggesting induction of immune-like responses (McClain et al., 2011b). These few recent studies show how on a level of cellular development, EtOH application during adolescence disrupts normal scaffolding of brain material, and that EtOH administration during this critical period of development can have long term harmful effects on neural functioning.

Changes in Neurotransmission Following EtOH Exposure

Adolescent subjects exhibit higher basal DA levels, as well as levels of D1 and D2 receptors, than adult subjects, emphasizing that DA tone differs overall in adolescents (Pascual et al., 2009). Elevated DA levels have been suggested by Melendez et al. to mediate ethanol-seeking behavior (2002) indicating that elevated basal DA may render ethanol-exposed adolescent rats more vulnerable to developing adult-onset ethanol-seeking behaviors than naïve subjects.

Repeated injections of EtOH during adolescence increases basal DA in the Acb during adulthood (Badanich et al., 2007). Repeated EtOH exposure in adolescence elevates accumbal DA levels of the adolescent Acb septi (Philpot and Kirstein, 1998). Similarly, this treatment elevates adult Acb DA levels, and decreases the DA response to an EtOH challenge within this brain structure (Philpot et al., 2009). Also, it has been suggested that repeated adolescent EtOH exposure decreases the time required to see the DA increase induced by EtOH administration into the Acb during adolescence (Philpot and Kirstein, 2004). Repeated injections of EtOH affects the accumbal ratio of DA to a primary metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), at several time points during adolescence (Philpot and Kirstein, 2004). This is in agreement with the

finding that free-choice adolescent EtOH exposure by P rats PD 30-60 results in greater DA clearance during no-net-flux microdialysis in the Acb, suggesting a general increase in DA release has occurred (Sahr et al., 2004). The authors also reported more protracted DA response to an EtOH challenge injection, compared to naïve rats. Repeated EtOH treatment decreases the amount of DRD2 in the Acb of adolescent but not adult subjects (Pascual et al., 2009)

Repeated injections of EtOH during adolescence results in subjects that have an increase in extracellular Acb glutamate in response to an EtOH challenge injection, as opposed to similarly-treated adult subjects which showed a decrease in extracellular glutamate (Carrarra-Nascimento et al., 2011) likely related to input from the PFC, a connection that may influence EtOH reward (Kalivas and Volkow, 2005). Low doses of naltrexone injections were more effective at slowing acquisition of EtOH drinking behavior in adolescent P rats compared to adult subjects, with less tolerance observed in the adolescent animals as well (Sable et al., 2006). This suggests that EtOH experience has a differential effect on adult and adolescent subjects, and that this can translate to an increase, or decrease, in the efficacy of traditional treatment options.

Interaction of Adolescence, Anxiety/Stress, and EtOH

Behavioral reaction to stress has been related to the development of alcoholism. In particular, EtOH abuse has been connected with dysfunction within the hypothalamic-pituitary-adrenal (HPA) axis, a large portion of neural system in place that processes physical and psychological stress (Koob, 2010). It is unknown whether ultimately this neuroendocrine disruption is causal in EtOH abuse, or vice versa, but evidence shows that EtOH dependence does indeed affect HPA functioning (Richardson et al., 2008). Furthermore, an abnormal stress response has been correlated with children of

alcoholics, who are identified as family history positive for alcoholism (Peterson and Pihl, 1990). Higher environmental stress exposure in both adolescent children of alcoholics and control subjects increased risk for developing substance use disorders (King and Chassin, 2008). Stress has been linked with higher relapse rates, making responses to stress a factor in the continuation of EtOH drinking (Sinha et al., 2011).

Within the HPA loop, corticotropin releasing factor (CRF) in the paraventricular nucleus (PVN) of the hypothalamus is strongly involved in stress response, and has been connected to hypotheses about creation of addictive states (Koob, 2010). For example, repeated stress has been shown to affect the VTA and Acb in ways similar to some drugs of abuse (Ortiz et al., 1996). Also within the HPA loop, increases in corticosteroid hormones have been shown to increase DA release in the Acb (Imperato et al., 1989). Adrenalectomy results in decreased basal DA in the AcbSh, as well as reduced DA reactivity during drug administration or stress (Barrot et al., 2000). Chronic corticosteroid administration results in decreased DA synthesis and DA turnover in the Acb (Pacak et al., 2002). Therefore, acute corticosteroid increases in turn result in increased basal DA, while chronic corticosteroid increases result in decreased basal DA in this area, resulting in sensitization that can enhance drug effects (Marinelli and Piazza, 2002).

Following repeated administration and withdrawals from EtOH, adolescents sensitized to decreased social interaction (anxiety-like behavior) longer than adult subjects given the same treatment (Wills et al., 2009), an effect that also requires higher doses of CRF to diminish (Wills et al., 2010). Combined with higher basal levels of CRF in the PVN and central nucleus of the amygdala, this has been interpreted as lower sensitivity to CRF compared to adults (Wills et al., 2010). Adolescents are less affected by the anxiogenic effects caused by both acute and chronic EtOH withdrawal, as

compared to adults, even when controlling for EtOH clearance concentrations (Doremus et al., 2003). This shows a form of insensitivity to EtOH effects (Spear, 2000).

Studies of macaque monkeys have shown that adolescent exposure to EtOH increases later drinking behavior compared to equivalent adult exposure in stressed peer-reared subjects compared to unstressed subjects, suggesting that stress has affected biological risk for high EtOH consumption (Barr et al., 2004). While chronic stress decreases adolescent EtOH intake, subjects still consume more EtOH g/kg than adults receiving the same treatment (Brunell and Spear, 2005). Male Wistar rats consuming EtOH during adolescence later increased EtOH consumption following stress to a greater degree than adult-exposed subjects (Siegmund et al., 2005). Following free-choice access to EtOH during adolescence, subjects show more pronounced adult stress-induced EtOH consumption, compared to those exposed in adulthood (Fullgrabe et al., 2007), and peri-adolescent EtOH vapor exposure affects later CRF responses in the PVN to an EtOH challenge, indicating that these subjects show a blunted response to this stressor (Allen et al., 2011b). P rats exposed to EtOH in adolescence show evidence of reduced novelty-induced anxiety compared to naïve subjects (Salimov et al., 1996).

As the stress-response system continues to develop during adolescence, there is therefore possibility for disruption of its natural course by EtOH administration at this time. This disruption may translate to differential response to EtOH, stress, and the interaction between the two, in adulthood.

Study Rationale and Hypotheses

Adolescent operant breakpoint studies (Rodd et al., 2003, Oster et al., 2006, Toalston et al., In Preparation), combined with ICSA (Rodd et al., 2000) and adolescent

work (Bell et al., 2003), have particular relevance in the development of the present study. As free-choice EtOH access during adolescence can be argued to have altered reward saliency of EtOH in our previous studies, interest in other parameters for examining the mesolimbic reward pathways after peri-adolescent EtOH drinking was peaked.

Past research has suggested that peri-adolescent EtOH consumption increases the subjective reinforcing properties of EtOH. The goal of this dissertation was to further examine the change in reinforcing properties of EtOH within the mesolimbic dopamine system induced by free-choice peri-adolescent EtOH consumption. Also, the intent was to examine whether peri-adolescent EtOH consumption enhances the stimulatory effect of EtOH on the mesolimbic dopamine system.

In the present study, two experiments evaluate the effects of free-choice EtOH intake by P rats during peri-adolescence on later adult EtOH-reward seeking behavior. Two questions were addressed: (1) Does EtOH experience during peri-adolescence affect responding for ICSA of EtOH into the pVTA in adulthood? (2) Does EtOH experience during peri-adolescence increase release of DA in the AcbSh of adult P rats following pulsed microinjection of EtOH into the VTA? Together, these studies will improve our knowledge of the effects of peri-adolescent EtOH exposure on the mesolimbic DA reward pathway. And, by extension, this knowledge will facilitate the development of treatments targeting alcohol abuse and dependence.

The overall hypothesis is that peri-adolescent self-administered EtOH results in long-term neuroadaptations within the mesolimbic DA system (VTA and AcbSh), increasing the reinforcing properties of EtOH in adulthood compared to EtOH-naïve subjects.

Experiment 1: ICSA

Experiment 1 will address whether EtOH experience in peri-adolescence affects later responding for EtOH ICSA into the pVTA. The ICSA method can determine whether a specific brain region is involved in drug reward, by measuring operant bar-press behavior that results in administration of drug discretely into the respective region. Comparison of number of responses between different concentrations of EtOH creates a dose-response curve for a particular set of treatment conditions. In this case, we chose to examine the pVTA, a nucleus of dopaminergic neurons previously shown to support intracranial EtOH self-administration (Gatto et al., 1994, Rodd-Henricks et al., 2000).

The research objective of the first experiment was to examine the extent to which EtOH consumption during peri-adolescence by P rats altered the neurocircuitry involved in the reinforcing properties of EtOH. The first working hypothesis was that EtOH-exposure-induced neuroadaptations during peri-adolescence result in an adult neuronal system projecting from the pVTA that is more sensitive to the reinforcing properties of EtOH compared to EtOH-naïve subjects. This increase in responsiveness to the stimulating effects of EtOH on pVTA DA neurons would be reflected by a shift of the dose-response curve to the left compared to EtOH-naive subjects. ICSA therefore will determine whether the dose-response curve for EtOH in adulthood differed between adolescent-EtOH-exposed and naïve subjects. The null hypothesis in this situation, where this neurocircuitry has no change in sensitivity following EtOH exposure in peri-adolescence, would be supported by no difference in responsiveness across EtOH concentrations to the stimulating effects of EtOH on pVTA DA neurons.

ICSA Background

Intracranial self-administration methods are useful for determining which discrete brain regions are involved in the initiation and maintenance of drug reinforcement (McBride et al., 1999). Comparison of the number of operant responses and infusions to the brain site between different concentrations of a drug creates a dose-response curve for a particular set of treatment conditions for a given microinfusion site in the brain. In this case, we chose to examine the pVTA, which is the seat of dopaminergic neuronal cell bodies that project axons to the AcbSh and other mesolimbic terminal sites.

The no-tangle form of microinjection of drug used in this experiment was pioneered by Criswell for use with chemotrodes (1977) to follow up previous chemical stimulation methods more difficult to administer (Myers, 1972). This was again refined by Bozarth and Wise (1980). In this procedure, a measured electric current passes between two electrodes submerged in a tank of infusate, producing hydrogen gas, and the increased pressure in the tank forces the desired small amount of infusate, which has been calibrated to the respective current, into the brain through a cannula attached to the tank. Monitored constant low current keeps infusate pressure stationary during inter-injection intervals (Goeders and Smith, 1987).

It is widely accepted that the VTA-Acb DA connection is involved in reward and reinforcement behavior (Wise and Rompre, 1989, Ikemoto and Panksepp, 1999). The pVTA has been shown to support EtOH self-administration, while the aVTA does not (Rodd-Henricks et al., 2000). Also self-administered in this area is acetaldehyde, a metabolite of EtOH (Rodd-Henricks et al., 2002c). D1/D2 agonists administered alone do not support ICSA in the AcbSh, while co-administration of the associated agonists does (Ikemoto et al., 1997a). Similar to EtOH, differences in opiate reward within the anterior and posterior VTA have also been observed (Carlezon et al., 2000). Regarding this,

GABA(A) antagonists are self-administered into the aVTA but not pVTA (Ikemoto et al., 1997c). GABA(A) receptors in the pVTA but not aVTA have been found to be involved in binge consumption of EtOH (Melon and Boehm, 2011). While VTA DA neurons are excited by AMPA administration, increasing DA in the Acb (Kretschmer, 1999), AMPA itself is not self-administered into the VTA (Ikemoto et al., 2004).

As described earlier, the research objective of the first experiment was to examine the extent to which EtOH consumption during peri-adolescence in P rats altered the development of neurocircuitry involved in the reinforcing properties of EtOH. The hypothesis was that EtOH-exposure-induced neuroadaptations result in an adult neurocircuitry system in the pVTA of adolescent-exposed animals that is more sensitive to the reinforcing properties of EtOH compared to EtOH-naive subjects. This increase in responsiveness to the stimulating effects of EtOH on pVTA DA neurons would be reflected by a shift of the dose-response curve to the left compared to EtOH-naive subjects. ICSA therefore determined whether the dose-response for EtOH in adulthood differed between adolescent-EtOH-exposed and -naïve subjects.

However, ICSA only examines effects on neurocircuitry at one site. To elucidate "downstream" effects of ICSA of EtOH in the pVTA, the current studies will also perform a concurrent analysis of a second site as pVTA EtOH is being infused. Because a concurrent analysis in the Acb during actual ICSA is technically very difficult and the findings may be confounded by variables such as motor effects, one way to accomplish this goal is an experimenter-administered (via a calibrated current generator) series of pulsed microinjections mimicking a classic ICSA bout with concurrent microdialysis in the downstream site. For the purpose of this paper, the method name is shortened to MicroMicro.

Experiment 2: MicroMicro

Experiment 2 will address whether EtOH experience during peri-adolescence enhances release of DA in the AcbSh of adults following EtOH administered into the VTA. This experiment used a microinjection-microdialysis methodology. This involves experimenter-administered EtOH directly into a cell body region (pVTA) at a concentration that has been shown to support self-administration in previous reports, coupled with a microdialysis probe implanted into a "downstream" DA cell terminal region (AcbSh), which provides a controlled examination of the EtOH effects on neurotransmitter release via the assay of the dialysate.

Specifically, the research objective of the second experiment was to examine the effects of peri-adolescent EtOH drinking on adult DA release in the AcbSh after microinjection of relevant concentrations of EtOH in the pVTA. The second working hypothesis was that peri-adolescent neuroadaptations result in an adult neurocircuitry system (DA neurotransmission between the "upstream" pVTA and "downstream" AcbSh projection area) that has greater sensitivity to EtOH stimulation. This increase in responsiveness to the stimulating effects of EtOH on pVTA DA neurons was expected to be reflected by an increase in DA release in the AcbSh in response to lower concentrations of EtOH, essentially creating a shift to the left in the dose-response curve. Therefore, the microinjection-microdialysis techniques compared DA *in vivo* efflux between peri-adolescent EtOH-exposed and EtOH-naïve subjects in the AcbSh following EtOH administration into the pVTA. The null hypothesis in this situation would be indicated by no change in responsiveness to the stimulating effects of EtOH on pVTA DA neurons, as measured by no difference in DA response, and would suggest no differences between the neurocircuitry of subjects exposed to EtOH during peri-adolescence compared with EtOH-naïve P rats.

MicroMicro Background

This pulsed MicroMicro methodology can be used to closely examine changes in downstream neurotransmitter release in response to ICSA-like administration in real time (Ding et al., 2011). Unlike ICSA, this involves experimenter-administered EtOH directly into a cell body region, in a concentration that was demonstrated to be self-administered as described in previous reports (Gatto et al., 1994, Rodd et al., 2004c), coupled with a microdialysis probe implanted into a downstream DA cell terminal region. This approach provides a controlled examination of the EtOH effects on neurotransmitter release via HPLC assay of the dialysate. In this study, EtOH is applied into the pVTA, while microdialysis for extracellular DA levels is performed in the AcbSh. The pulsing of EtOH mimics the pattern of EtOH administration during a bout of ICSA responding, complete with mock time-out intervals.

As described earlier, the research objective of the second experiment was to examine, in adulthood, the effects of peri-adolescent EtOH drinking on DA release in the AcbSh after microinjection of relevant amounts of EtOH into the pVTA. The hypothesis was that adolescent P rats exposed to EtOH would exhibit neuroadaptive changes in adult neurocircuitry (DA neurotransmission between the "upstream" pVTA and "downstream" AcbSh projection area) yielding an adult system that is more sensitive to EtOH reward, and reinforcement, compared to subjects not exposed to EtOH during peri-adolescence. This increase in responsiveness to the stimulating effects of EtOH on pVTA DA neurons was expected to be reflected by an increase in DA release in the AcbSh in response to lower doses of EtOH in the pVTA. This is presumed to mediate the behavioral outcome seen as a shift to the left in the dose-response curve for the ICSA of

EtOH. MicroMicro techniques, therefore, compared the AcbSh DA response between adolescent-EtOH-exposed and -naïve subjects following EtOH administration into the pVTA.

2. METHODS

Subjects

The adolescent EtOH access procedures used herein followed published procedures from our laboratory (Rodd-Henricks et al., 2002a, Bell et al., 2003, 2004, 2006, Sahr et al., 2004). Male P rats were chosen for use in the current study because their rapid growth post-adolescence increases skull strength for earlier cannula placement surgery. Previous work (McKinzie et al., 1998a, Bell et al., 2003) has described the differences between male and female P rats in EtOH drinking at these ages as minimal.

Animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All research protocols were approved by the Indiana University School of Medicine (Indianapolis, Indiana) Institutional Animal Care and Use Committee and were in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, the NIH, and the *Guide for the Care and Use of Laboratory Animals (2011)*.

Peri-Adolescent EtOH Exposure Procedure

Pups were single-housed in hanging stainless steel cages (Allentown Caging Equipment Co, Allentown, New Jersey) on PD 28. Subjects were initially maintained on a 12-hour light/dark cycle, lights on at 0900. On PD 30, subjects received either ad lib

water or continuous access to 15% v/v EtOH and water, until PD 60. Food was available ad lib. Bottle and body weights for all subjects were recorded every other day.

On PD 60, EtOH access ceased, and subjects were pair-housed in standard shoebox cages, within the same treatment condition. Subjects were also immediately transferred to a 12-hour reverse dark/light cycle, lights off at 1000, to optimize rats' nocturnal activity levels for later procedures. After PD 60, subjects received no further oral EtOH intake experience.

ICSA

ICSA Apparatus

Test chambers (Coulbourn Instruments, Allentown, Pennsylvania) were situated in sound-attenuating cubicles, as described previously (Rodd-Henricks et al., 2000, Rodd et al., 2004b). Briefly, chambers were illuminated by dim house lights during testing. Two identical levers were mounted on a single wall of the test chamber, 15 cm above a grid floor, separated by 12 cm. Directly above each lever were two cue lights, red and green. Red was illuminated over the active lever during resting conditions.

A desktop computer recorded the data (L2T2 system, Coulbourn Instruments, Allentown, Pennsylvania) and controlled the operant delivery of infusate following active lever response. An electrolytic microinfusion transducer (EMIT) system (Model 26, MNC, Shreveport, Louisiana) controlled the delivery of assigned infusate into the subject via calibrated pulses of current. To do this, a cylinder (28 mm length x 6 mm diameter; Plastics One, Roanoke, Virginia) with an attached 28 gauge injection cannula was filled with infusate. Two platinum electrodes extended into the cylinder from an airtight enclosure cap. This was connected by a spring-coated cable (Plastics One) and a swivel

(Mercotac, Inc.) to a constant current generator (MNC, Shreveport, Louisiana) that maintained low quiescent current between the electrodes. Depression of the active lever initiated a 5-sec infusion current of 200 μA, resulting in rapid generation of hydrogen gas, increasing the pressure inside the airtight cylinder, pressing a 100 nl bolus of infusate out through the injection tip. Between infusions, current returned to the low quiescent state.

During each infusion, there was a 5-sec time-out period where bar-press responses were recorded yet resulted in no further infusion. In this time-out, the house light and a red cue light were extinguished, while the green cue light over the active lever flashed in 0.5-sec intervals. The assignment of active and inactive lever with respect to the left or right position was counterbalanced among subjects, and remained the same throughout the experiment for each respective subject.

ICSA Procedure

Adolescent EtOH treatment occurred as described above. Food and water were available ad lib at all times, except during ICSA testing.

ICSA was performed as previously described (Rodd-Henricks et al., 2000, Rodd et al., 2004b). After postnatal day 75, the rats were implanted under isoflurane anesthesia with a guide cannula (22 gauge, Plastics One) stereotaxically aimed 1.0 mm above the pVTA. Coordinates were 5.8 to 6.1 mm posterior to bregma, 2.1 mm lateral, and 8.5 mm ventral from the surface of the skull at a 10 degree angle from the vertical (Paxinos and Watson, 1986). A place-holding stylet (28 gauge, Plastics One) extending 0.5 mm beyond the tip of the guide cannula was inserted at all times, except during test sessions. Subjects were single-housed post surgery, and allowed to recover for 7 days. Three days prior to testing, subjects were handled 5 min per day.

All infusates were prepared freshly on the day of the experiment. Artificial cerebrospinal fluid (aCSF) was used as the vehicle for ICSA infusions. This injection vehicle consisted of (in mM) 120.0 NaCl, 4.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 25.0 NaHCO3, 2.5 CaCl2, and 10.0 D-glucose, all filtered through a sterile filter (pore size 0.2 μM) as previously described (Rodd-Henricks et al., 2000, Rodd et al., 2004b). Ethyl EtOH (190 proof; McCormick Distilling Co., Weston, Missouri) was dissolved in the vehicle solution to the correct concentration. When necessary, 0.5 N HCl was added to adjust the pH to 7.4 (±0.1).

ICSA was conducted similar to procedures previously described (Rodd-Henricks et al., 2000, Rodd et al., 2004b). Briefly, subjects were brought to the testing room, the stylet was removed, and an injection cannula/infusate cylinder was affixed in place. The injection cannula extended 1.0 mm beyond the tip of the guide, into the pVTA. A single, noncontingent administration of infusate was given at the beginning of the session during this insertion procedure in order to prime the system. Test sessions occurred every other day. No operant shaping techniques were used. Active lever and inactive lever sides were counterbalanced between subjects, remaining the same for each individual rat. Within each 4 hr session, responses on the active lever resulted in 5 sec infusions on a fixed ratio 1 schedule of reinforcement. During infusion and time-out, responses on the active lever were recorded, but did not produce further infusions. Responses on the inactive lever were recorded but did not result in infusions at any time; these responses were used to index non-specific bar-pressing activity. During ICSA sessions 1 through 4 (acquisition), subjects received their respective dose of either the aCSF vehicle or EtOH. During ICSA sessions 5 and 6 (extinction), all subjects received aCSF vehicle only, and in session 7 (reinstatement), the original concentration was made available.

MicroMicro

MicroMicro Apparatus

Experimental housing was composed of Plexiglas chambers (40 x 28 x 40 cm). Polyethylene tubing connected to a dedicated Harvard pump (Harvart Apparatus, Holliston, Massachusetts) was used to administer aCSF continuously throughout the experiment for microdialysis in the AcbSh. The connection to the EMIT unit for microinjection administration to the pVTA was identical to that used in the ICSA experiment, although automated control of injections was programmed into the unit (Isolated Pulse Stimulator Model 2100, A-M Systems Inc, Sequim, Washington), instead of the separate computer controlling and recording operant self-administration.

Microdialysis probes were prepared as approximately 2-mm loop style probes, as previously described (Perry and Fuller, 1992, Engleman et al., 2000, Melendez et al., 2002). Probes were manufactured in the laboratory with regenerated cellulose Spectra/Por ® hollow fiber microdialysis tubing, molecular weight cut off of 13,000. In our laboratory, these have been previously found to have approximately 15% DA recovery, which is an average recovery rate for this design and probe length (Justice, 1993).

MicroMicro Procedure

Adolescent EtOH treatment occurred as in the first experiment. Food and water were available ad lib at all times, except during MicroMicro testing. MicroMicro was performed as previously described (Ding et al., 2011). After PD 75, and under isoflurane anesthesia, a microinjection guide cannula (22 gauge, Plastics One) was implanted in the right hemisphere of each subject, stereotaxically aimed 1.0 mm above the pVTA. Coordinates were 5.8 to 6.1 mm posterior to bregma, 2.1 mm lateral, and 8.5 mm ventral

from the surface of the skull at a 10 degree angle from the vertical (Paxinos and Watson, 1986). A place-holding stylet (28-gauge, Plastics One) extending 0.5 mm beyond the tip of the guide cannula was inserted at all times, except during final experimentation. Also in the right hemisphere, a guide cannula (18 gauge, Plastics One) was implanted, aimed 3.0 mm above the AcbSh. Coordinates were 1.7 mm anterior to bregma, 2.3 mm lateral, and 5.4 mm ventral to the surface of the skull at a 10 degree angle from the vertical (Paxinos and Watson, 1986). A place-holding stylet (Plastics One) extending 0.5 mm was inserted at all times, except during final experimentation.

Subjects were single-housed post surgery, and allowed to recover 6 days. Three days prior to testing, subjects were habituated to the testing chambers, 3 hours per day. One day before testing, microdialysis probes were inserted into the AcbSh under isoflurane anesthesia. When inserted, the dialyzing loop was oriented in an anterior-posterior direction to maximize exposure to the AcbSh. The probe extended 3 mm below the guide cannula, into the AcbSh.

The next day, the MicroMicro procedure was performed. Subjects were placed in the experimental chambers and the microdialysis probe tubing was connected to the pump for aCSF perfusion. The aCSF microdialysis perfusion medium was composed (in mM) as has been previously described (Melendez et al., 2002) of 145.0 NaCl, 2.7 KCl, 1.0 MgCl2, 2.5 CaCl2, and 2.0 Na2HPO4, filtered through a sterile filter (pore size 0.2 μM), always prepared fresh the day of the procedure. When necessary, pH was adjusted to 7.4 with 0.1 N acetic acid. Perfusate aCSF formula differed from injection aCSF to maximize diffusion across the probe dialysis membrane.

Flow speed of the perfusion medium was 1 μL/min. Microdialysis outflow was collected for 15 min per sample. After at least 90 min of washout flow, at least three

baseline dialysate samples were collected. This was followed by one microinjection dialysate sample.

During the microinjection sample, the pVTA-cannula stylet was removed and the injection cannula/infusate cylinder affixed in place. The injection cannula extended 1.0 mm beyond the tip of the guide, into the pVTA. Administration of infusate occurred automatically, as programmed into the EMIT machine. Each subject received microinjections of one solution only, prepared identically to that in the ICSA experiment; either infusate vehicle aCSF, or 50, 75, 100, or 200 mg% EtOH. All subjects received the same volume of microinjections. Microinjections were composed of a series of 100 nL boluses, each delivered over an interval of 5 sec. This was repeated every 20 sec for a total of 30 microinjections over a 10 min period into the pVTA.

Following the microinjection sample, 120 min of post-injection dialysate samples were collected. All samples were collected into tubes containing preservative of 5 µL of 0.1 N perchloric acid. After collection, samples were immediately frozen on dry ice and stored at -70 degrees C until HPLC analysis for DA content.

HPLC Apparatus

All microdialysis samples were analyzed with microbore HPLC-EC to determine extracellular levels of DA. Stored as described at -70 degrees C, there is no degradation of the DA samples (Campbell and McBride, 1995). Samples were loaded into a 10 µL sample loop and injected onto an analytical column. Detector output ran to a computer program for analysis (ChromPerfect, Justice Innovations, Inc., Palo Alto, California). DA levels were then determined by comparison with a standard curve. The lower sensitivity limit for DA was estimated to be 0.1 nM.

Histologies

Upon termination of ICSA experiments, a solution of 1% bromophenol blue dye was injected into the infusion site and the animals sacrificed. Brains were removed and immediately frozen at -70 degrees C, for slicing into 40-µm sections with a cryostat microtome. Slides were stained with cresyl violet and examined for infusion site verification using the atlas of Paxinos and Watson (1986).

Upon termination of MicroMicro experiments, a 1% bromophenol blue dye was injected into the infusion site and perfused through the microdialysis probe, and the animals were sacrificed. Brains were removed and immediately frozen at -70 degrees C, for slicing into 40-µm sections with a cryostat microtome. Slides were stained with cresyl violet and examined for infusion and microdialysis site verification using the atlas of Paxinos and Watson (1986).

Statistical Analysis

EtOH consumption was analyzed as pure EtOH intake (grams of EtOH per kilogram of body weight, g/kg). Change in preference ratio for EtOH intake vs. water was calculated by plotting ratios each day of EtOH exposure as a percentage of 15% EtOH solution consumed in relation to total fluids consumed ([g ethanol solution intake/g ethanol solution intake + g water intake] x 100). The first day of access was compared to the last day of access. Intake and Preference data were averaged in six blocks of 5 days, analyzed with a repeated measures ANOVA and linear regression between blocks.

ICSA data were analyzed with a Dose x Session mixed ANOVA, with repeated measures on Session performed on the number of active lever responses and the number of infusions separately. For each individual group, lever discrimination was

determined by "lever (active vs. inactive) x session" mixed ANOVA with repeated measures on session. Post hoc Tukey's b tests were employed when a significant main effect was found, $p < 0.05$. Extinction was determined by comparing the responses on the active lever during sessions 4, 5, and 6, while reinstatement was determined by comparing the responses on the active lever during sessions 5, 6, and 7.

MicroMicro data were expressed as a percentage of basal DA values, to correct for between-subject baseline variability, as previously described (Engleman et al., 2006). Basal dialysate values for each subject were calculated as the mean of three baseline samples prior to the injection sample. Also, absolute levels of baseline DA were calculated, and compared as group means between the two peri-adolescent Exposure Groups. The effects of peri-adolescent EtOH administration on basal extracellular DA levels and maximal drug effect with each Dose was calculated using Student's t-test. The effects of EtOH microinjection administration on extracellular DA levels as a function of time and adolescent treatment condition were analyzed using a two-way Adolescent Group x Time analysis of variance (ANOVA), with repeated measures on Time (15-min samples), followed by the Tukey's post hoc test. Alpha was set at a $P < 0.05$ significance level.

3. RESULTS

Cannula Placements

ICSA injector placements (Figure 1) located in the pVTA ranged from AP -5.5 to AP -6.1 mm relative to bregma as illustrated in Rat Brain in Stereotaxic Coordinates (Paxinos and Watson, 1986). Only subjects with the injector tip entirely within the pVTA (Rodd-Henricks et al., 2000) were used in the study. Prior to surgery, there were 104 peri-adolescent EtOH-exposed and 83 naïve subjects for the 10 groups of ICSA. After histologies, 69 total subjects were included in data analyses. Most misplaced guide cannulas aimed at the aVTA.

MicroMicro injector placements located in the pVTA (Figure 1) ranged from AP -5.5 to AP -6.1 mm relative to bregma. Only data from animals with the injector tip entirely within the pVTA (Rodd-Henricks et al., 2000) were used in the analyses. Microdialysis probe placements (Figure 2) ranged from AP 1.5 to

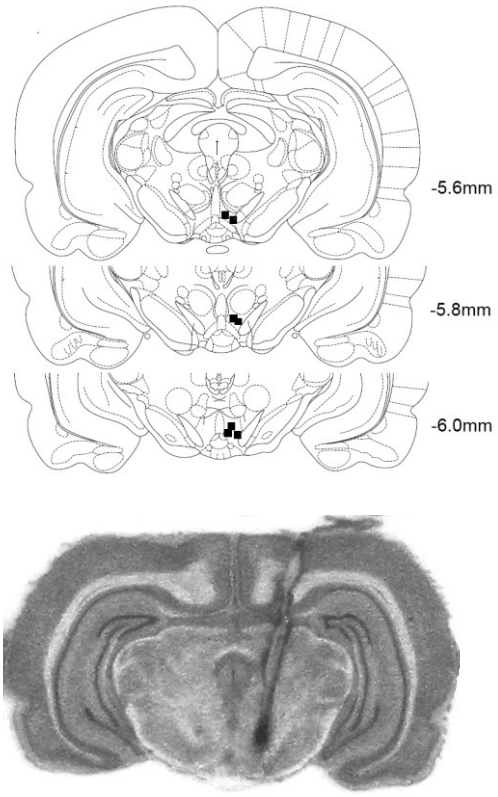

Figure 1. ICSA and MicroMicro Injector Placement. Top: Representation of placement sites within the pVTA. Black squares indicate representative locations of injection sites within the pVTA. Figure adapted from Paxinos and Watson (1986). Bottom: Depicts a photo of a representative brain slice, injection tip within the pVTA.

AP 1.7, and were located mainly in the AcbSh. Only animals with at least 75% of the microdialysis probe within the AcbSh were used in the analyses. Prior to surgery, there were 72 peri-adolescent EtOH-exposed and 56 naïve subjects for the 10 groups of MicroMicro. After histologies, 50 total subjects were included in data analyses. A large majority of AcbSh cannulas were correctly placed. Most missed pVTA cannulas were aimed either in the red nucleus or below the pVTA.

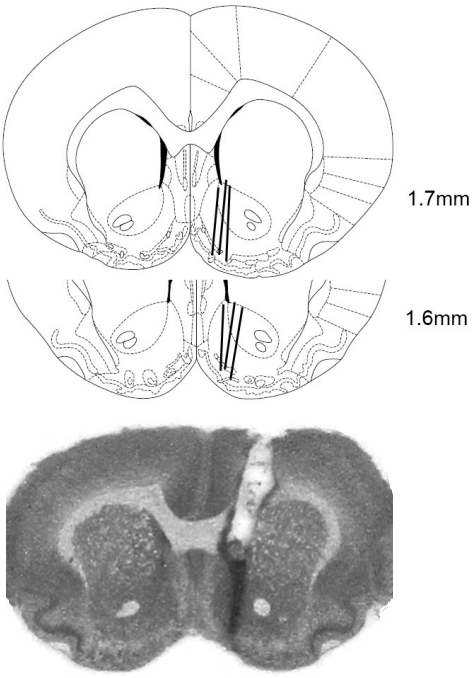

Figure 2. MicroMicro Dialysis Probe Placement.Top: Representation of placement sites within the AcbSh. Lines indicate representative locations of active probe in the AcbSh. Figure adapted from Paxinos and Watson (1986). Bottom: Depicts a photo of a representative brain slice, active probe membrane area within the AcbSh.

Body Weights

For body weight data illustration purposes, data were sampled every 4 days of the exposure period. There was a significant main effect of day ($F_{7,96} = 1370.7$, $p < 0.001$), but no significant main effect of exposure group ($F_{1,102} = 0.257$, $p=0.613$). There was no significant day x exposure group interaction ($F_{7,96} = 1.53$, $p=0.167$). This shows that while subjects gained body weight throughout the peri-adolescent time period, the EtOH-Exp

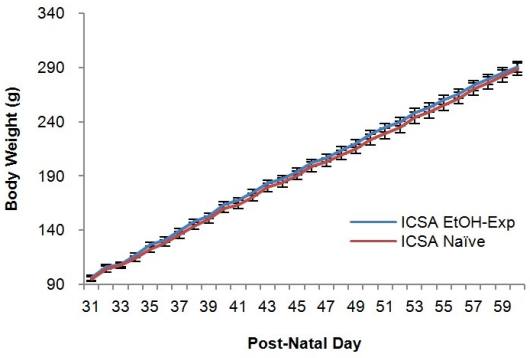

Figure 3. Body weights ICSA. Mean body weights (±SEM) over the 30 day peri-adolescent period for Naïve and free-choice EtOH-exposed (EtOH-Exp) subjects in the ICSA experiment. There was no significant difference between exposure groups.

and Naïve subjects did not differ from each other in bodyweight within the ICSA or MicoMicro experiments (Figures 3 and 4).

Peri-Adolescent EtOH Consumption and EtOH Preference

Initial consumption for all peri-adolescent EtOH-exposed subjects was 5.17 ±0.36 g/kg/day. At the end of the access period, consumption was 7.80 ±0.31 g/kg/day.

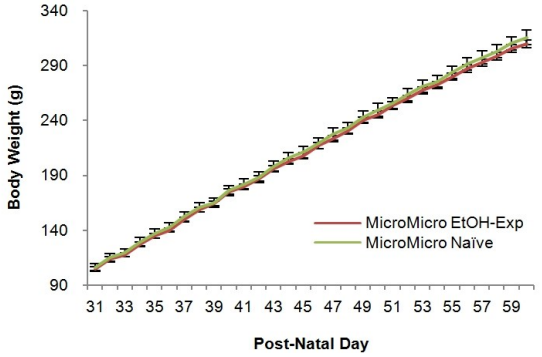

Figure 4. Body weights MicroMicro. Mean body weights (±SEM) over the 30 day peri-adolescent period for Naïve and free-choice EtOH-exposed (EtOH-Exp) subjects in the MicroMicro experiment. There was no significant difference between exposure groups.

For illustration of peri-adolescent EtOH consumption purposes, data (g/kg/day) were averaged in six blocks of 5 days. The repeated measures within subjects ANOVA revealed there was a significant main effect of Day ($F_{5,51} = 5.627$, $p < 0.001$), indicating that EtOH consumption changed over time. There was no significant effect of Study Group ($F_{1, 55} = 1.948$, $p = 0.168$).

There was no significant Day x Study Group interaction ($F_{5,51} = 0.582$, $p = 0.713$). Given this lack of difference between study groups, peri-adolescent EtOH consumption data were collapsed across all EtOH-drinking subjects, for a total N of 176 animals. With these data the EtOH

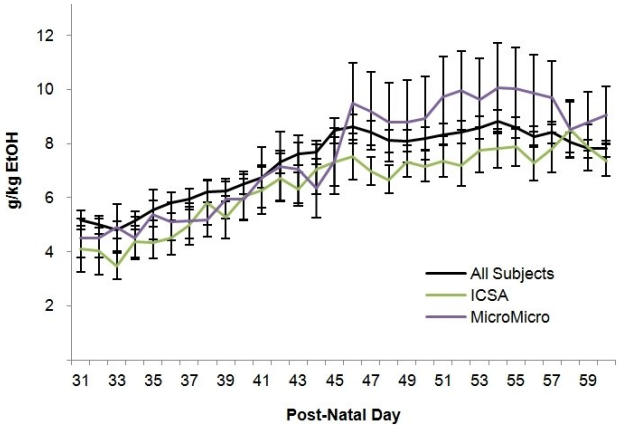

Figure 5. EtOH Intake. Represents the mean EtOH intake data for 15% v/v EtOH, g/kg. There was no significant difference between experimental groups. Differences over time are illustrated in the Appendix.

consumption average of each block of 5 days was compared to the other blocks of 5 days with paired samples T-tests. Every single block was significantly different from block 1 (M = 5.13, SEM = 0.29); block 2 (M = 6.13, SEM = 0.33, t(175)=-3.70, p < 0.001), block 3 (M = 7.56, SEM = 0.36, t(175)=-6.71, p < 0.001), block 4 (M = 8.27, SEM = 0.37, t(175)=-7.74, p < 0.001), block 5 (M = 8.54, SEM = 0.36, t(175)=-8.33, p < 0.001) and block 6 (M = 8.05, SEM = 0.31, t(175)=-7.21, p < 0.001). Block 2 was significantly different from block 3 (t(175)=-4.80, p < 0.001), block 4 (t(175)=-5.75, p < 0.001), block 5 (t(175)=-5.93, p < 0.001), and block 6 (t(175)=-4.85, p < 0.001). Block 3 was significantly different from block 4 (t(175)=-2.14, p = 0.03), but not block 5 (t(175)=-2.62, p = 0.09) or block 6 (t(175)=-1.27, p = 0.20). Block 4 was not significantly different from block 5 (t(175)=-1.29, p = 0.19) or block 6 (t(175)=0.632, p = 0.528), nor was block 5 significantly different from block 6 (t(175)=1.789, p = 0.07).

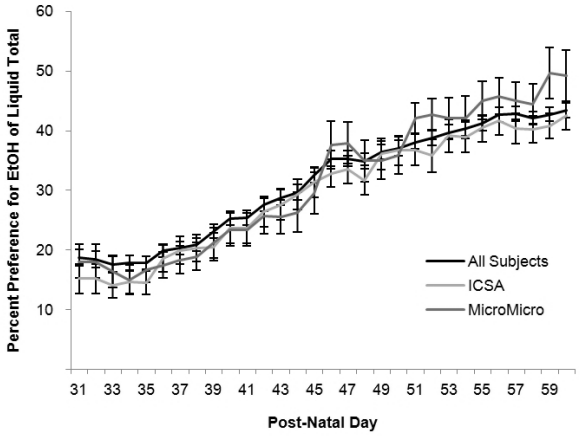

Figure 6. EtOH Preference. Represents the mean EtOH preference data for 15% v/v EtOH compared to water, as expressed in grams of fluid. There was no significant difference between experimental groups. Differences over time are illustrated in the Appendix.

These results indicate that EtOH intake increased over 5-day blocks, until block 4. This further suggests that EtOH consumption increase over time to a plateau between blocks 3 and 4, essentially the midpoint of EtOH access, whereupon amount of EtOH intake continued at prior levels and did not change.

For illustration of peri-adolescent EtOH Preference purposes, data (calculated by g 15% EtOH/g total fluids) were averaged in 6 blocks of 5 days. Following a within subjects repeated measures ANOVA for 5 day EtOH Preference blocks, there was a

significant main effect of Block ($F_{5,54}$ = 31.576), indicating that EtOH Preference changed over time. There was no significant effect of Study Group ($F_{1, 58}$ = 0.625, p = 0.433). There was no significant Block x Study Group interaction ($F_{5,54}$ = 0.822, p = 0.540).

Given this lack of difference between study groups, peri-adolescent EtOH Preference data were collapsed across all EtOH-drinking subjects, for a total N of 176 animals. This data was then used to compare the EtOH Preference average of each block of 5 days to the other blocks of 5 days with paired samples T-tests. Every single block was significantly different from block 1 (M = 18.09, SEM = 1.17); block 2 (M = 21.92, SEM = 1.04, t(175)=-3.91, p < 0.001), block 3 (M = 28.83, SEM = 1.17, t(175)=-9.31, p < 0.001), block 4 (M = 35.82, SEM = 1.18, t(175)=-13.48, p < 0.001), block 5 (M = 39.68, SEM = 1.16, t(175)=-15.44, p < 0.001) and block 6 (M = 42.77, SEM = 1.08, t(175)=-17.46, p < 0.001). Block 2 was significantly different from block 3 (t(175)=-10.16, p < 0.001), block 4 (t(175)=-13.90, p < 0.001), block 5 (t(175)=-16.50, p < 0.001), and block 6 (t(175)=-18.88, p < 0.001). Block 3 was significantly different from block 4 (t(175)=-8.55, p < 0.001), block 5 (t(175)=-10.99, p < 0.001), and block 6 (t(175)=-12.56, p < 0.001). Block 4 was significantly different from block 5 (t(175)=-5.87, p < 0.001) and block 6 (t(175)=7.28, < 0.001). Block 5 was also significantly different from block 6 (t(175)=4.26, p < 0.001). These results suggest that EtOH Preference versus water consistently increased over time.

Taken together, the results suggest that while EtOH total amount in g/kg plateaued at approximately 15 days of access, EtOH Preference continued to increase throughout the exposure period.

The Appendix shows visual expression of total EtOH intake data blocks and preference data blocks in predictive linear regression scatter plots of individual rats'

consumption at multiple paired time points, as well as proportion of explained variance (r^2) calculated from their associated Pearson's r correlation coefficients.

ICSA

Comparison of average acquisition responding during the first four days of ICSA with a between-subjects ANOVA revealed a significant main effect of Exposure Group ($F_{1,68} = 12.42$, $p = 0.001$), a significant main effect of Dose ($F_{4,68} = 8.43$, $p < 0.001$), and an Exposure Group by Dose interaction ($F_{5,51} = 3.70$, $p = 0.009$).

In peri-adolescent exposed subjects, a one-way ANOVA with Tukey's b post-hoc test was performed to compare acquisition responding by Dose to Infusion, Active Lever, and Inactive Lever data. There was a significant effect of Dose on Infusions ($F_{4,33} = 8.63$, $p < 0.001$) and a significant effect of Dose on Active Lever ($F_{4,33} = 7.43$, $p < 0.001$), but no significant effect of Dose on Inactive Lever ($F_{4,33} = 2.04$, $p = 0.114$).

Post-hoc comparison by Tukey's b tests (which corrects for unequal sample size) revealed that peri-adolescent exposed subjects bar-pressing for ICSA in adulthood of 75, 100, and 150 mg% EtOH, significantly ($p < 0.05$ level) infused these doses at a higher rate than both aCSF and 50 mg%. Also, similar analysis of bar-presses on the Active Lever by this group revealed significance at a 0.05 level for increased responding for 75, 100, and 150 mg% EtOH compared to aCSF and 50 mg%. There were no significant differences in response between doses on the Inactive Lever.

In naive subjects, a one-way ANOVA with Tukey's b post-hoc test was performed to compare acquisition responding by Dose to Infusion, Active Lever, and Inactive Lever data. There was a significant effect of Dose on Infusions ($F_{4,34} = 2.70$, $p = 0.049$) and a significant effect of Dose on Active Lever responses ($F_{4,34} = 2.91$, $p = 0.037$), but no significant effect of Dose on Inactive Lever responses ($F_{4,34} = 1.50$, $p = 0.226$).

Post-hoc comparison by Tukey's b tests revealed that naive subjects bar-pressing for ICSA of 150 mg% EtOH, significantly infused this dose at a higher rate than aCSF, 50, 75, and 100 mg% EtOH (Figure 6). Also, similar analysis of bar-presses on the Active Lever by this group revealed significance at a 0.05 level for increased responding for 150 mg% EtOH only compared to aCSF, 50, 75, and 100 mg% EtOH. There were no significant differences in response between doses on the Inactive Lever.

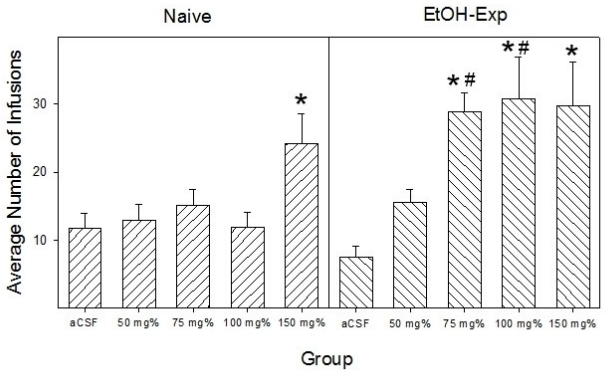

Figure 7. ICSA: Mean Acquisition Infusions. Average infusions (±SEM) by male P rats for self-infusion of aCSF, 50, 75, 100, or 150 mg% EtOH during the first four sessions, into the p-VTA. Asterisks indicate infusions were significantly different from aCSF, $p < 0.05$. Pound signs indicate infusions by peri-adolescent EtOH-exposed subjects (EtOH-Exp) were significantly different from naïve subjects, $p < 0.05$.

One-way ANOVAs were performed for each Dose. For aCSF, there was no significant difference between EtOH-exposed and naïve subjects for Infusion ($F_{1,11} = 2.42$, $p = 0.150$), Active Lever ($F_{1,11} = 0.96$, $p = 0.350$), or Inactive Lever ($F_{1,11} = 0.06$, $p = 0.806$). For 50 mg% EtOH, there was no significant difference between EtOH-exposed and naïve subjects for Infusion ($F_{1,19} = 0.79$, $p = 0.386$), Active Lever ($F_{1,19} = 0.50$, $p = 0.485$), or Inactive Lever ($F_{1,19} = 0.003$, $p = 0.954$). For 75 mg% EtOH, there was a significant difference between EtOH-exposed and naïve subjects for Infusion ($F_{1,16} = 14.23$, $p = 0.002$) and Active Lever ($F_{1,16} = 19.94$, $p < 0.001$), but no significant difference of Inactive Lever ($F_{1,16} = 0.15$, $p = 0.704$). For 100 mg% EtOH, there was a significant difference between EtOH-exposed and naïve subjects for Infusion ($F_{1,9} = 8.18$, $p =$

0.021) and Active Lever ($F_{1,9} = 6.89$, $p = 0.030$), but no significant difference of Inactive Lever ($F_{1,16} = 0.006$, $p = 0.940$). Finally, for 150 mg% EtOH, there was no significant difference between EtOH-exposed and naïve subjects for Infusion ($F_{1,9} = 0.49$, $p = 0.503$), Active Lever ($F_{1,9} = 1.397$, $p = 0.271$)., or Inactive Lever ($F_{1,9} = 0.05$, $p = 0.821$).

A repeated measures within-factor multivariate test was then performed for all groups. The repeated measure was Session, with a between subject comparison of Dose by Exposure Group. There was a main effect of Session ($F_{6,54} = 12.65$, $p < 0.001$), a Session by Exposure Group interaction ($F_{6,54} = 3.02$, $p = 0.013$), a Session by Dose interaction ($F_{24,228} = 2.11$, $p = 0.003$), and a Session by Exposure Group by Dose interaction ($F_{24,228} = 2.15$, $p = 0.002$).

Within peri-adolescent EtOH-exposed subjects, there was a significant effect of session ($F_{6,24} = 8.01$, $p < 0.001$) and a significant interaction of Session by Dose ($F_{24,108} = 1.95$, $p = 0.011$). Because there is a significant interaction, we break down one-way ANOVAs over the sessions. There is a significant difference for Session 1 ($F_{4,33} = 4.03$, $p = 0.010$), Session 2 ($F_{4,33} = 12.01$, $p < 0.001$), Session 3 ($F_{4,33} = 3.89$, $p = 0.012$), Session 4 ($F_{4,33} = 6.79$, $p = 0.001$), and Session 7 ($F_{4,33} = 12.86$, $p < 0.001$), but not Session 5 ($F_{4,33} = 2.46$, $p = 0.067$) or Session 6 ($F_{4,33} = 1.54$, $p = 0.216$). This is an indication of extinction, where significant difference does not appear between Doses on Sessions 5 and 6 (Figures 7 through 11).

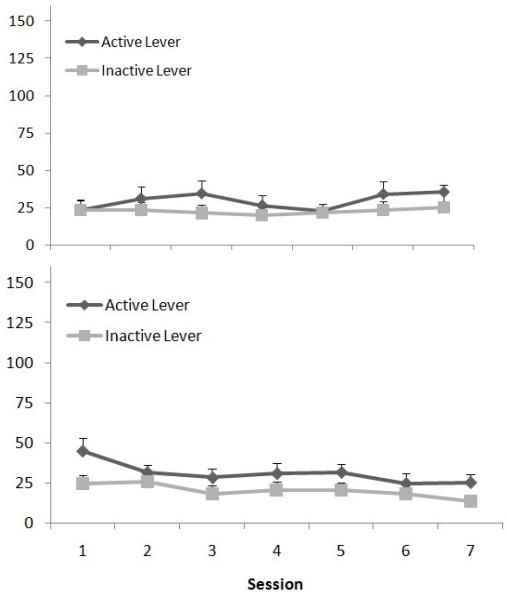

Figure 8. ICSA: aCSF Levers. Mean responses (±SEM) on the active and inactive lever by male P adolescent naïve (top) and E-Exposed (bottom) rats for self-infusion of aCSF. There was no significant difference between the two groups.

Within naive subjects, there was a significant effect of session ($F_{6,25} = 6.67$, $p < 0.001$) and a significant interaction of Session by Dose ($F_{24,112} = 2.46$, $p = 0.001$). Because there is a significant interaction, one-way ANOVAs are broken down over the sessions. There is a significant difference for Session 3 ($F_{4,34} = 3.32$, $p = 0.023$) and Session 4 ($F_{4,34} = 4.42$, $p = 0.006$). There was no significant difference for Session 1 ($F_{4,34} = 0.473$, $p = 0.755$), Session 2 ($F_{4,34} = 1.12$, $p = 0.364$), Session 5 ($F_{4,34} = 2.25$, $p = 0.087$), Session 6 ($F_{4,34} = 1.86$, $p = 0.143$), or Session 7 ($F_{4,34} = 2.16$, $p = 0.098$).

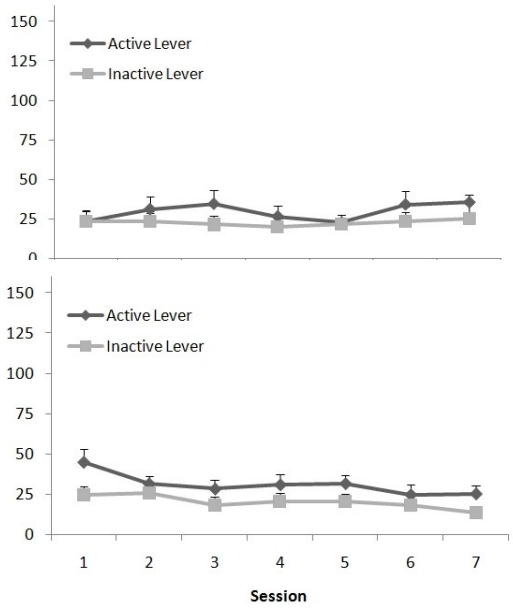

Figure 9. ICSA: 50 mg% Levers. Mean responses (±SEM) on the active and inactive lever by male P adolescent naïve (top) and E-Exposed (bottom) rats for self-infusion of 50 mg% EtOH. Sessions 5 and 6 were vehicle only. There was no significant difference between the two groups.

To determine presence (or lack) of lever discrimination in naïve subjects, a repeated measure analysis of Lever was examined, with a between subjects comparison of Dose and Exposure Group. There was no interaction of Session by Lever by Exposure Group ($F_{24,112} = 1.07$, $p = 0.384$). This was followed with paired-sample t-tests of both levers at each dose. Among naïve subjects at the 150 mg% dose, there was no observed lever discrimination ($p = 0.140$ and greater). Among peri-adolescent EtOH-Exposed subjects at the 75 mg% dose, there was lever discrimination on Session 1 ($p = 0.022$), Session 2 ($p = 0.007$), Session 3 ($p = 0.022$), Session 4 ($p = 0.004$) and Session 7 ($p = 0.011$). Among peri-adolescent EtOH-Exposed subjects at the 100 mg% dose, there was lever discrimination on Session 1 ($p = 0.002$), Session 2 ($p = 0.029$), Session 4 ($p = 0.017$) and Session 7 ($p = 0.001$). Among peri-adolescent EtOH-Exposed subjects at the 150 mg% dose, there

was lever discrimination on Session 2 ($p = 0.22$), Session 4 ($p = 0.043$) and Session 7 ($p = 0.019$).

When focusing on extinction data of the peri-adolescent EtOH-exposed subjects, repeated measures analysis with 3 sessions included (Sessions 4, 5, and 6) was examined for concentrations that supported infusions. There was a significant effect of Session ($F_{2,28} = 12.88$, $p < 0.001$) and a significant Session by Dose interaction ($F_{8,58} = 2.11$, $p = 0.048$). The interaction term was decomposed by examining Session for each Dose level, including follow-up t-tests. In EtOH-exposed subjects that were given 75 mg% EtOH to self-administer, the number of infusions during sessions 4 was significantly higher than session 5 ($p = 0.05$) and session 6 ($p = 0.05$). In EtOH-exposed subjects that were given 100 mg% EtOH to self-administer, the number of infusions

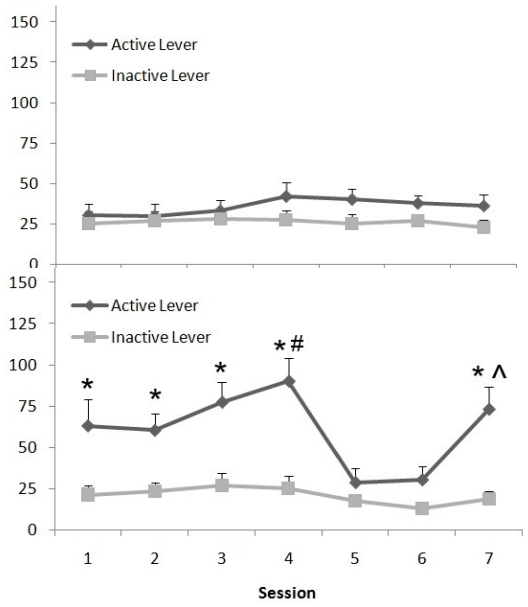

Figure 10. ICSA: 75 mg% Levers. Mean responses (±SEM) on the active and inactive lever by male P adolescent naïve (top) and E-Exposed (bottom) rats for self-infusion of 75 mg% EtOH. Sessions 5 and 6 were vehicle only. Asterisk (*) indicates lever discrimination and significant difference of E-Exp from naïve group, $p < 0.05$. Carat (^) indicates significant difference from extinction session 5, Pound sign (#) indicates significant difference from extinction sessions 5 and 6.

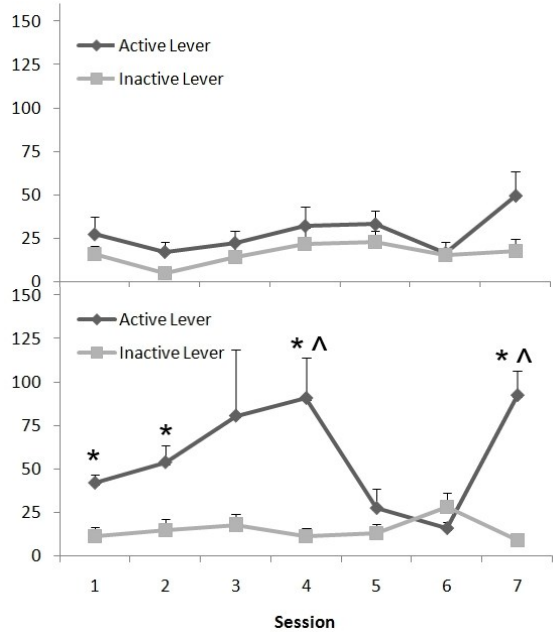

Figure 11. ICSA: 100 mg% Levers. Mean responses (±SEM) on the active and inactive lever by male P adolescent naïve (top) and E-Exposed (bottom) rats for self-infusion of 100 mg% EtOH. Sessions 5 and 6 were vehicle only. Asterisk (*) indicates lever discrimination, $p < 0.05$. Carat (^) indicates significant difference from extinction sessions and significant difference of E-Exp from naïve group.

during session 4 was significantly higher than session 6 (p = 0.015), while the difference between 4 and 5 approached significance (p = 0.67). In EtOH-exposed subjects that were given 150 mg% EtOH to self-administer, there was no significant difference between sessions 4 and 5 or 4 and 6 (p = 0.27 and 0.34).

Active lever data were also used for analysis of extinction data of peri-adolescent EtOH-exposed subjects. Repeated measures analysis revealed a significant main effect of Session ($F_{2,28}$ = 11.85 p < 0.001) and a significant Session by Dose interaction ($F_{8,58}$ = 2.95, p = 0.008). The interaction term was decomposed by examining Session for each Dose level, including follow-up t-tests. In EtOH-exposed subjects that were given 75 mg% EtOH to self-administer, the number of active lever responses during sessions 4 was significantly higher than session 5 (p = 0.004) and session 6 (p = 0.010). In EtOH-exposed subjects that were given 100 mg% EtOH to self-administer, the number of active lever responses during session 4 was significantly higher than session 5 (p = 0.018), as well as 6 (p = 0.14). In EtOH-exposed subjects that were given 150 mg% EtOH to self-administer, there was no significant difference between sessions 4 and 5 or 4 and 6 (p = 0.22 and 0.18).

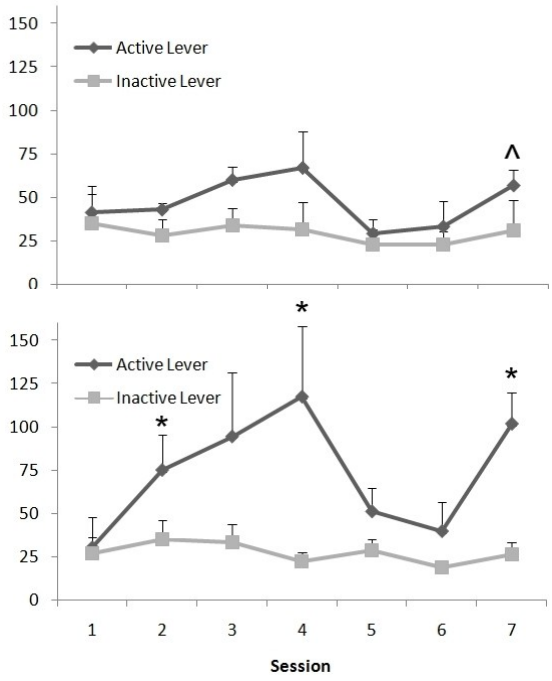

Figure 12. ICSA ICSA 150 mg% Levers. Mean responses (±SEM) on the active and inactive lever by male P adolescent naïve (top) and E-Exposed (bottom) rats for self-infusion of 150 mg% EtOH. Sessions 5 and 6 were vehicle only. Asterisk (*) indicates lever discrimination, p < 0.05. There was no significant difference of E-Exp from naïve group. Carat (^) indicates significant difference from extinction session 5.

When focusing on reinstatement data of the peri-adolescent EtOH-exposed subjects, repeated measures analysis with 3 sessions included (sessions 5, 6, and 7) was examined for concentrations that had previously supported infusions. There was a significant effect of Session ($F_{2,28} = 22.63$, $p < 0.001$) and a significant Session by Dose interaction ($F_{8,58} = 3.37$, $p = 0.003$). The interaction term was decomposed by examining Session for each Dose level, including follow-up t-tests. In EtOH-exposed subjects that were given 75 mg% EtOH to self-administer, the number of infusions during session 5 was significantly lower than session 7, ($p = 0.020$) as was 6 ($p = 0.024$). In EtOH-exposed subjects that were given 100 mg% EtOH to self-administer, the number of infusions during session 5 was significantly lower than session 7, ($p = 0.003$) as was 6 ($p < 0.001$). In EtOH-exposed subjects that were given 150 mg% EtOH to self-administer, there was no significant difference between session 5 and 7 or 6 and 7 ($p = 0.213$ and 0.142).

Active lever data were also used for analysis of reinstatement data of peri-adolescent EtOH-exposed subjects. Repeated measures analysis revealed a significant main effect of Session ($F_{2,28} = 16.91$, $p < 0.001$) and a significant Session by Dose interaction ($F_{8,58} = 3.88$, $p = 0.001$). The interaction term was decomposed by examining Session for each Dose level, including follow-up t-tests. In EtOH-exposed subjects that were given 75 mg% EtOH to self-administer, the number of active lever responses during sessions 5 was significantly lower than session 7 ($p = 0.008$) as was session 6 ($p = 0.034$). In EtOH-exposed subjects that were given 100 mg% EtOH to self-administer, the number of active lever responses during session 5 was significantly lower than session 7 ($p = 0.001$), as was 6 ($p = 0.001$). In EtOH-exposed subjects that were given 150 mg% EtOH to self-administer, there was no significant difference between active lever responses for sessions 5 and 7 or 6 and 7 ($p = 0.207$ and 0.154).

When focusing on extinction data of the naive subjects, repeated measures analysis with 3 sessions included (Sessions 4, 5, and 6) was examined for the one concentration that supported infusions. There was a no significant effect of Session ($F_{2,29}$ = 2.45, p = 0.103) but there was a significant Session by Dose interaction ($F_{8,60}$ = 4.73, p < 0.001). The interaction term was decomposed by examining Session for one Dose level, including follow-up t-tests. In naïve subjects that were given 150 mg% EtOH to self-administer, the number of infusions during session 4 was significantly higher than session 5 (p = 0.040) and 6 (p = 0.023).

Active lever data were also used for analysis of extinction data of naïve subjects. Repeated measures analysis revealed a significant main effect of Session ($F_{2,29}$ = 4.59 p = 0.019) and a significant Session by Dose interaction ($F_{8,60}$ = 3.05, p = 0.006).
The interaction term was decomposed by examining Session for each Dose level, including follow-up t-tests. In naive subjects that were given 150 mg% EtOH to self-administer, there was no significant difference between sessions 4 and 5 (p = 0.063) but there was a significant difference between 5 and 6 (p = 0.012).

When examining reinstatement data of the naïve subjects, repeated measures analysis with 3 sessions included (sessions 5, 6, and 7) was examined for the one concentration that had previously supported infusions. There was a significant effect of Session ($F_{2,29}$ = 9.214, p = 0.001) and a significant Session by Dose interaction ($F_{8,60}$ = 2.76, p = 0.012). The interaction term was decomposed by examining Session for each Dose level, including follow-up t-tests. In naïve subjects that were given 150 mg% EtOH to self-administer, the number of infusions during session 5 was significantly lower than session 7, (p = 0.001) but the same was not seen of 6 and 7 (p = 0.160).

Active lever data were also used for analysis of reinstatement data of naïve subjects. Repeated measures analysis revealed a significant main effect of Session

($F_{2,29}$ = 5.88, p = 0.007) and a significant Session by Dose interaction ($F_{8,60}$ = 2.10, p = 0.05). The interaction term was decomposed by examining Session for each Dose level, including follow-up t-tests. In naïve subjects that were given 150 mg% EtOH to self-administer, the number of active lever responses during session 5 was significantly lower than session 7, (p = 0.048) but the same was not seen of 6 and 7 (p = 0.196).

MicroMicro

Absolute baseline DA levels of adolescent experimental groups did not differ statistically between groups. Naïve subjects had an average baseline DA level of 1.10 (±0.12) nM, while EtOH-Exp subjects had an average baseline of 1.09 (±0.11) nM (Figure 13).

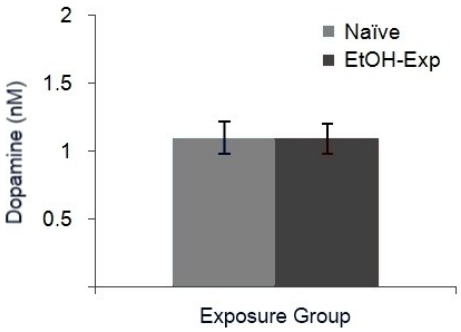

Figure 13. MicroMicro: Absolute DA Levels. Mean absolute DA levels between adolescent exposure groups. There was no significant difference between the two groups.

A between-subjects ANOVA revealed significant main effect of Time ($F_{11,30}$ = 3.895, p = 0.001) , a significant interaction of Time and Exposure Group ($F_{11,30}$ = 2.412, p = 0.027), a significant interaction of Time and Dose ($F_{44,116}$ = 1.69, p = 0.013), and a significant interaction of Time and Dose and Exposure group ($F_{11,33}$ = 3.31, p = 0.004).

To decompose the significant 3-way interaction, the initial variable held constant was adolescent exposure. Performing a similar repeated measures ANOVA in animals that were adolescent naïve, there was a significant Time x Dose interaction ($F_{11,14}$ = 6.84, p = 0.001). This significant 2-way interaction term was further reduced by examining the effect of dose at each time point. For naïve subjects, there was a significant difference at 15 min ($F_{4,25}$ = 4.57, p = 0.008), 30 min ($F_{4,25}$ = 7.64, p = 0.001), and 60 min ($F_{4,25}$ = 3.776, p = 0.018). Significance was approached at the 75 min time

point (p = 0.057). Post hoc tests performed for each individual time point (SNK and Tukey's b) indicated that in the 15 to 30 min post-injection block that the 150 mg% group was significantly different from the aCSF and 50 mg% group, with no other group differences. At the 30 min time point, the 150 mg% group was significantly different from all other dose levels. At the 60 min time point, the 150 mg% group was significantly different from the 50 mg% dose, but no other levels. Follow-up t-tests showed that in naïve subjects receiving the 150 mg% dose microinjection, DA levels at 15 min post injection were significantly different compared to baseline (p = 0.004), as was 30 min (p = 0.014). The 60 min time point approached significance (p = 0.057) (Figures 14 through 18).

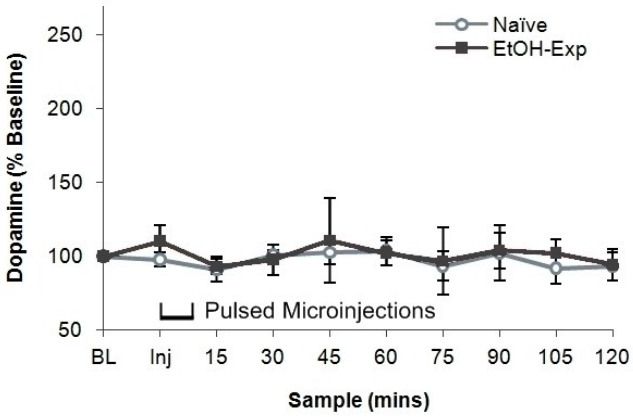

Figure 14. MicroMicro: aCSF. Microdialysis for DA (percent baseline, ±SEM) with concurrent pulsed microinjection of aCSF. There was no significant difference between the two peri-adolescent exposure groups, nor from baseline.

Performing a repeated measures ANOVA in animals that were peri-adolescent EtOH exposed, there was a significant main effect of Time ($F_{9,11} = 4.87$, p = 0.012) and significant Time x Dose interaction ($F_{11,12} = 428$, p = 0.009). This significant 2-way interaction term was further reduced by examining the

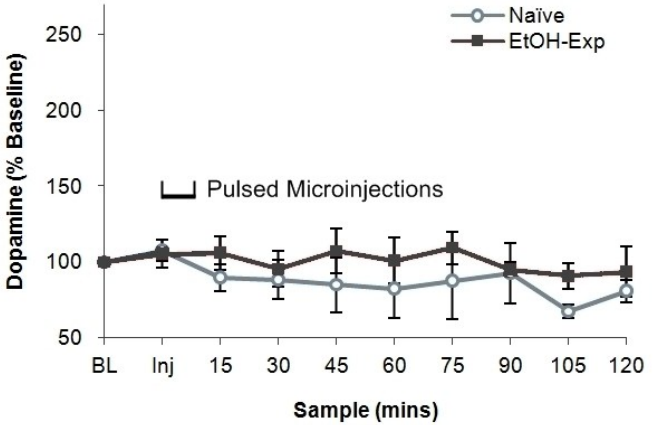

Figure 15. MicroMicro: 50 mg% Microdialysis for DA (percent baseline, ±SEM) with concurrent pulsed microinjection of 50 mg% EtOH. There was no significant difference between the two peri-adolescent exposure groups, nor from baseline.

effect of dose at each time point. For EtOH-exposed subjects, there was a significant difference at the Injection time point ($F_{4,23}$ = 4.25, p = 0.013), 30 min ($F_{4,23}$ = 5.23, p = 0.005), 60 min ($F_{4,23}$ = 3.88, p = 0.018), and 90 min ($F_{4,23}$ = 3.69, p = 0.022). Significance was approached at the 15 min time point (p = 0.078). Post hoc tests performed for each individual time point (SNK and Tukey's b) indicated that in the Injection time point, the 75 mg% group was significantly different from the aCSF and 50 mg% group, with no other group differences. At the 30 min time point, the 150 mg% group was significantly different from the aCSF and 50 mg% groups, with no other group differences. At the 90 min time point, the 150 mg% group was significantly different from the aCSF, 50, and 75 mg% groups, with no other group difference.

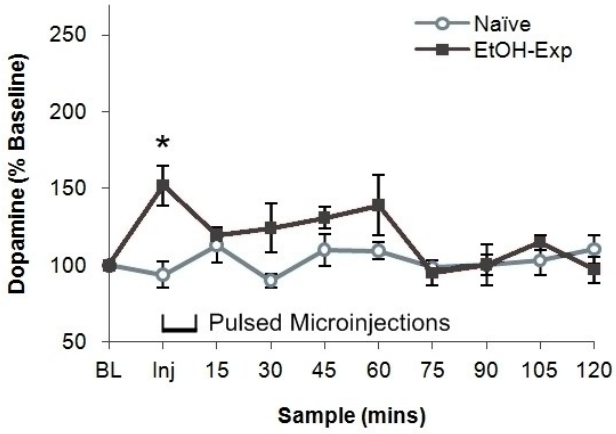

Figure 16. MicroMicro: 75 mg% Microdialysis for DA (percent baseline, ±SEM) with concurrent pulsed microinjection of 75 mg% EtOH. Asterisk (*) indicates a significant difference from baseline in the EtOH-Exp group (p < 0.05).

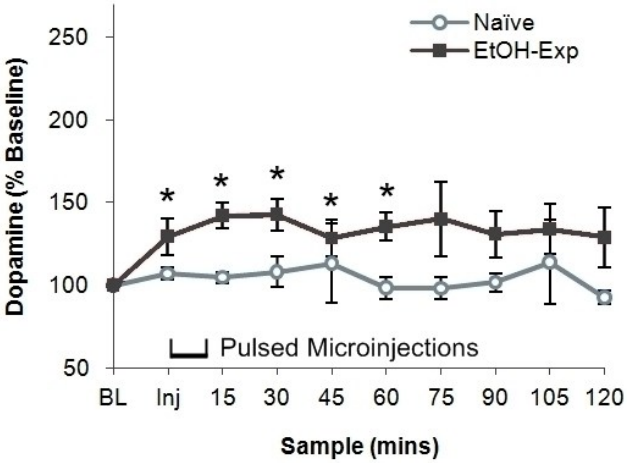

Figure 17. MicroMicro: 100 mg% Microdialysis for DA (percent baseline, ±SEM) with concurrent pulsed microinjection of 100 mg% EtOH. Asterisk (*) indicates a significant difference from baseline in the EtOH-Exp group (p < 0.05).

Follow-up t-tests showed that in peri-adolescent EtOH-exposed subjects receiving the 75 mg% dose microinjection, DA levels at the injection time point were significantly different compared to baseline (p = 0.029), while 15 min approached

significance (p = 0.065). In peri-adolescent subjects receiving the 100 mg% dose microinjection, DA levels at the injection time point (p = 0.005), 15 min (p = 0.027), 30 min (p = 0.011), 45 min (p = 0.042), and 60 min (p = 0.011) time points were significantly different compared to baseline. In peri-adolescent subjects receiving the 150 mg% dose microinjection, DA levels at the injection time point (p = 0.006), 30 min (p = 0.048), and 60 min (p = 0.007) time points were significantly different compared to baseline.

Performing a repeated measures ANOVA with a between subjects factor of Dose for the last baseline and first four post-baseline periods, at Dose levels of aCSF, 50, and 75 mg% EtOH, there are no significant differences between the adolescent exposure groups. There is a significant difference at 100 mg%, with a main effect of time ($F_{4,4}$ = 8.18, p = 0.033) and a Time by Adolescent Group ($F_{4,4}$ = 7.61, p = 0.037). A one-way ANOVA show that at this time point there is a significant difference at the Injection time point ($F_{1,8}$ = 6.19, p = 0.042), 15 min ($F_{1,8}$ = 29.69, p = 0.001), 30 min ($F_{1,8}$ = 8.35, p = 0.023), 60 min ($F_{1,8}$ = 15.56, p = 0.006), and 90 min ($F_{1,8}$ = 5.91, p = 0.045). The 75 min time point approached significance (p = 0.055). There was a significant difference at 150 mg%, with a main effect of Time ($F_{4,6}$ = 6.25, p = 0.025) but no interaction. This suggests that at 150 mg%, both Adolescent groups increased from baseline.

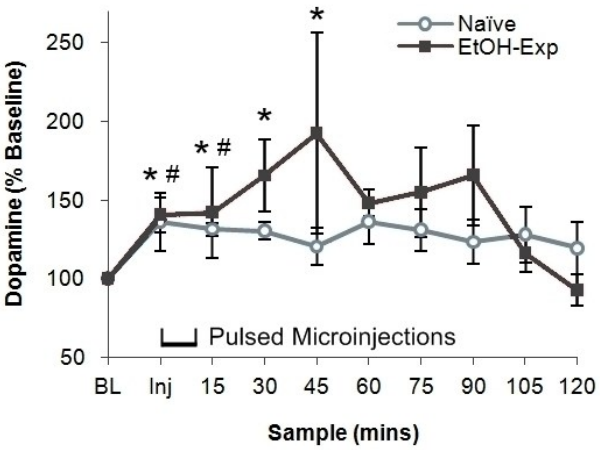

Figure 18. MicroMicro: 150 mg% Microdialysis for DA (percent baseline, ±SEM) with concurrent pulsed microinjection of 150 mg% EtOH. Asterisk (*) indicates a significant difference from baseline in the EtOH-Exp group (p < 0.05). Pound sign (#) indicates a significant difference from baseline in the Naïve group (p < 0.05).

4. DISCUSSION

The most salient results emerging from these experiments indicate that P rats given free-choice access to EtOH during peri-adolescence will voluntarily consume significant amounts of EtOH, and this early developmental drinking experience increases the reinforcing properties of EtOH in adulthood. The pVTA, a brain area known to be an important target area for EtOH's reinforcing effects, was found to manifest increased sensitivity to EtOH as a consequence of the adolescent drinking experience. This suggests that activation pVTA DA cell bodies of the mesolimbic DA system is a critical substrate that mediates the reinforcing properties of EtOH, and neuroadaptations in the pVTA apparently mediate the enhanced reinforcing actions of EtOH seen in adult animals exposed to EtOH during the adolescent period. Additionally, the findings of increased sensitivity in the mesolimbic system were confirmed with the observation that peri-adolescent EtOH exposure significantly increased DA efflux in the AcbSh, a primary terminal region for the pVTA axonal projections. Overall, the findings support the overarching hypothesis that peri-adolescent EtOH-exposure produces long-lasting alterations in neural circuitry involved in EtOH-reinforcement.

The findings of this study may have relevant translational impact for the importance of adolescent consumption in humans. Adolescent alcohol use appears to be experiencing a slight decline over the past two decades. In 1991, 88% of surveyed high school seniors reported alcohol consumption, whereas 2010 surveys reported this percentage to be slightly above 70% (Johnston et al., 2011). While this is good news,

particularly if the trend continues, there is still a large proportion (70%) of high school seniors who are drinking and experimenting with alcohol and have already chosen to undertake the risk of EtOH exposure.

Lifetime risk for alcohol use disorder declines with increasing age of first drink of alcohol (Prescott and Kendler, 1999), including subjects with family history positive for alcoholism, as examined in twin studies (Agrawal et al., 2009). Human adolescent binge drinking appears to be associated with alteration of white matter fiber tracts during adolescence, which has been related to cognitive performance deficits in adulthood (Jacobus et al., 2009, McQueeny et al., 2009). Also, fMRI data show that when anticipating reward, ventral striatal activation (an area analogous to the Acb examined in this study) is greater in adolescents compared to children and adults (Galvan et al., 2006), suggesting a greater range of plasticity for reward-associated behavior may be present during adolescence. There is something about the adolescent neural system that makes it fundamentally different from periods before and after adolescence (Spear, 2000).

Use of the P rat as a subject in the present studies is meant to model possible perturbations of the systems seen in human adolescents that are identified as family history positive for alcoholism, particularly since about 50% of the variance in alcohol drinking behavior is accounted for by heredity (Bell et al., 2005, 2006, McKinzie et al., 2005). Peri-adolescent EtOH consumption enhances EtOH intake in adulthood in both free-choice and operant settings (McKinzie et al., 1998b., Rodd-Henricks et al., 2002a, b, Bell et al., 2005). The present findings indicate that free-choice peri-adolescent EtOH consumption has long -lasting effects on EtOH reward pathways in adult P rats that are genetically predisposed to abuse EtOH. These effects increase the reward sensitivity of

lower concentrations of EtOH in the brain, as measured by a shift to the left of the dose-response curve for EtOH self-administered into the pVTA.

Adolescent EtOH exposure increases DA clearance in the Acb, suggesting a general increase in DA release has occurred (Sahr et al., 2004). Adolescent subjects exhibit higher basal DA levels, as well as levels of D1 and D2 receptors, than adult subjects, emphasizing that DA tone differs overall in adolescents (Pascual et al., 2009). EtOH during adolescence increases basal DA in the Acb during adolescence (Philpot and Kirstein, 1998) and adulthood (Badanich et al., 2007), and down-regulates the levels of DRD2 receptors in prefrontal cortex, striatum and Acb of adolescent animals, but not of adult rats (Pascual et al., 2009). This indicates that despite identical treatment in adults, the DA system can be uniquely altered in adolescence. Adolescent EtOH exposure causes faster DA increase induced by EtOH administration into the Acb during adolescence and affects the accumbal ratio of DA to DOPAC (Philpot and Kirstein, 2004, Sahr et al., 2004), yet decreases DA response to an EtOH challenge within this brain structure (Philpot et al., 2009). Thus, EtOH has both acute and long-lasting effects on the DA system terminating in the Acb, which may be causal or correlational to increased EtOH consumption in adulthood.

DA activation in the AcbSh in response to drug administration has been suggested to strengthen drug reward associations, a large part of addictive behavior (Koob and Le Moal, 1997, Di Chiara, 1998, Koob, 2010). Removal of EtOH access decreases DA and 5-HT in the Acb of EtOH-dependent subjects, an effect that is reversed when EtOH access is restored (Weiss et al., 1996). Suppression of EtOH operant reinforcement by naltrexone occurs simultaneously with a suppressed DA response in the Acb (Gonzalez and Weiss, 1998). During operant access for EtOH (as well as during an anticipatory pre-access period) DA levels are elevated in the AcbSh

(Weiss et al., 1993, Melendez et al., 2002). The literature evidencing DA change during reward is well established, but there is only a very limited focus when including the variable of adolescent EtOH exposure.

When it came to examining EtOH consumption during adolescence in the current study, it was observed that Intake volume of EtOH for each subject during the 30 day access period has reached its peak by the end of the second week. This lack in statistical significance between later time blocks suggests that some neurobehavioral change has occurred by, or occurs at, the 2 week peri-adolescent EtOH access, 45 day of age period. EtOH preference continued to increase throughout the exposure period, as evidenced by statistically significant differences between all blocks compared to each other. This corresponds to data reported previously in P rats (Bell et al., 2003, Bell et al., 2006). It appears that water volume consumption decreases by the end of a similar 30-day peri-adolescent EtOH exposure period, resulting in increased preference ratios. Bodyweight increases at a constant rate throughout the whole exposure period, indicating this is not due to an observable growth spurt. As seen in the Appendix, this increase in preference ratio is significantly correlated when subjects' drinking volumes are plotted in comparison of blocks, suggesting that while EtOH preference is significantly continuing to change over the full 30 day period, early EtOH preference predicts later EtOH preference. Levels of EtOH consumption by adolescent male P rats in this study was comparable to that of previous work with 15% v/v EtOH (Bell et al., 2006), in g/kg/day intake as well as EtOH percent preference observed throughout the 30 day access period. In the Bell et al., 2006 study, peri-adolescent subjects reached average BAC's of 56 mg% at two hours into the daily dark cycle, yet ranged as high as ~150 mg%. This average corresponds to a moderate amount of alcohol consumed in the

human condition, while the highest concentrations correspond to binge or greater levels of EtOH intake (Eckardt et al., 1998, Bell et al., 2006).

As in previous ICSA studies (Rodd-Henricks et al., 2000, Rodd et al., 2004, 2005a), P rats responded on the active lever to self-administer EtOH directly into the pVTA during ICSA sessions 1-4, and extinguished responding when only vehicle was available (sessions 5 and 6). Additionally, the operant responding was reinstated in session 7 when EtOH infusions were again available (see Figures 8 - 12). Experimentally naïve female P rats self-administer EtOH at concentrations that have varied by study (150 mg% EtOH, but not 100 mg%; Rodd-Henricks et al., 2000, 75 mg% and higher; Rodd et al., 2004, 2005a). In the current study, subjects were male instead of female and substantially larger as a result. These naïve male P rats failed to self-administer either vehicle or the three lowest EtOH concentrations (50, 75, and 100 mg%) to the pVTA, and did not discriminate the active lever from the inactive lever. At 150 mg% concentration, naïve rats self-infused EtOH at a statistically significant level, and approached significance for lever discrimination (possibly diminished due to high variability between subjects on both the inactive and active levers). Compared to extinction session 5, reinstatement responding on the active lever during session 7 was significantly different.

P rats that had free-choice access to EtOH during peri-adolescence also failed to self-administer 50 mg% EtOH and discriminate the active from the inactive lever. At the 75, 100, and 150 mg% concentrations, however, peri-adolescent EtOH-exposed subjects readily self-administered EtOH to the pVTA, responded more on the active than inactive lever during acquisition, extinguished responding on the active lever when only aCSF was given, and reinstated responding on the active lever when EtOH was

restored. Thus, free-choice peri-adolescent EtOH exposure increased the sensitivity of the pVTA to the rewarding effects of EtOH in adulthood.

Future work may find differences at higher concentrations of EtOH, as a previous study found that P rats exposed to EtOH constantly or intermittently in adulthood self-infused higher concentrations of EtOH (300 mg%) into the pVTA, while naïve subjects did not (Rodd et al., 2005b). This high concentration was not examined in the current study.

In the second study, the observed basal extracellular DA of the AcbSh (Figure 13; Naïve subjects 1.10 (±0.12) nM, EtOH-Exp subjects 1.09 (±0.11) nM) of P rats corresponded within ranges previously observed (Engleman et al., 2000; Melendez et al., 2002, Sahr et al., 2004, Ding et al., 2009, Franklin et al., 2009), indicating both probe recovery of DA and DA analysis by HPLC were comparable to previous studies. Furthermore, basal extracellular DA data compared between groups support a previous study which also failed to find differences in basal DA in the Acb of peri-adolescent EtOH-exposed P rats compared to -naïve subjects (Sahr et al., 2004).

Figures 14 - 18 illustrate the results of the MicroMicro experiments that measured DA extracellular concentrations (efflux) within the AcbSh in response to EtOH (50 - 150 mg%) administered into the pVTA. Naïve P rats failed to show an increase in DA in the AcbSh in response to microinjections of the three lower concentrations of EtOH (50, 75, and 100 mg%) into the pVTA. Subjects with free-choice access to EtOH during peri-adolescence did not show an increase at the 50 mg% level, but did display increases in extracellular DA within the AcbSh in response to microinjections of 75 and 100 mg% EtOH. The results from both the EtOH-exposed and -naïve subjects spanned a range of concentrations that were previously found to be below and above the threshold level that supported ICSA into the pVTA. Both groups showed an increase in AcbSh DA

response at the 150 mg% level, just as both groups displayed self-administration of 150 mg% EtOH. These results show that free-choice peri-adolescent EtOH consumption has changed the way the VTA responds to EtOH in adulthood. Thus, free-choice adolescent EtOH exposure enhances the release of DA downstream from the pVTA in the AcbSh, upon EtOH application to the pVTA.

Rodent subjects with a family history that predisposes them for high EtOH consumption have been shown to have lower levels of basal DA in the Acb compared to non-family-history-positive subjects, possibly connected with reduced DA release (Murphy et al., 1982, 1987). Long-term EtOH drinking has been shown to increase basal DA activity in the Acb of adult P rats (Thielen et al., 2004), which has been hypothesized to be related to increased sensitivity to the reinforcing effect of EtOH in the pVTA following EtOH access (Rodd et al., 2005b). While Acb DA transient events (fluctuations in DA under a second in duration) of adolescent subjects exist at baseline rates similar to adults, transient events in response to social and nonsocial stimuli differ, suggesting variations in novelty processing that may relate to reward salience (Robinson et al., 2011). Therefore, it appears that DA variations and responsiveness in the Acb is related to EtOH reward, reinforcement, and a predisposition for high EtOH intake.

The present study found that pulsed microinjections of EtOH administered to the pVTA, in a pattern similar to a bout of ICSA administration, are connected to increased extracellular DA concentrations in the AcbSh. This indicates further that stimulation of DA cell bodies in the pVTA projecting to the AcbSh is associated with the rewarding properties of EtOH. The present results also indicate that following peri-adolescent exposure to EtOH, lower concentrations of EtOH are required to produce observable DA in peri-adolescent exposed subjects than -naïve subjects. This indicates that the

subjective rewarding properties of EtOH within the pVTA can be been significantly altered by peri-adolescent EtOH exposure as well.

As observed in hippocampal cells following EtOH exposure in adolescence (Crews et al., 2007, McClain et al., 2011a, b), it is possible that EtOH administration is disrupting scaffolding of normal interconnections, or inducing pruning where pruning should not occur, in ways that affect the VTA-AcbSh neurocircuit. It is normal for neural connections and ratios of proteins and receptors to be changing at this time; neurogenesis in the hippocampus (He and Crews, 2007), as well as DA receptor binding in the cortex and striatum, have been shown to decline over time (Jucaite et al., 2010, Weickert et al., 2007), indicating that receptor density is higher in many brain regions of the adolescent relative to adults. For instance, DAR1 mRNA expression has been shown to peak in adolescence (Weickert et al., 2007). These basal differences may be involved in the vulnerability that the peri-adolescent time period presents; perturbations of systems already changing (neurogenesis, changes in receptor density and expression) may result in an altered mesolimbic DA system that is primed for increased EtOH reward in adulthood.

Conclusions

The mesolimbic DA system is a critical part of the neuro-circuit that is vulnerable during adolescence, and when perturbed, may promote future excessive EtOH drinking behavior. Overall, the results of this study indicate that the consumption of EtOH by P rats during peri-adolescence produces persistent neuroadaptations within this mesolimbic DA system. Specifically, following peri-adolescent free-choice EtOH-exposure in P rats, an increase in the sensitivity and reinforcing properties of EtOH in the pVTA was observed, as well as an increase in the sensitivity and efficacy of EtOH to

stimulate activation of the mesolimbic DA system. The focus on the mesolimbic DA system in the present study adds to a vast literature on the importance of this system in modulating alcohol abuse and alcoholism. It is, however, important to note that the mesolimbic DA pathway is potentially only one of many neurobiological substrates involved in the effects of alcohol and the mediation of drinking behavior.

An important aspect of the present study is that the EtOH was self-administered via free-choice drinking, compared with many other studies that used experimenter-administered EtOH via injections or other passive methods. Experimenter-administered EtOH is complicated by stress associated with the aversive effects of restraint and administration of EtOH amounts that are not necessarily reinforcing to the animal. An extensive literature indicates that developmental stressors can have multiple deleterious and long-lasting effects on the adult animal. The approach taken in this study is one of only a few studies that have used free-choice drinking as a method to administer EtOH to adolescent animals, and the results may be more comparable to human adolescent drinking than experimenter-administered approaches.

The translational implications of the present study are clear that adolescent alcohol drinking may increase the susceptibility to life-long alcohol abuse and alcoholism, particularly for individuals who have a high hereditary propensity to excessive alcohol use. According to a number of physical and neurobiological markers, the PD 30 - 60 used in the present study is reasonable approximation of the human adolescent period. However, one caveat of the present study is that adolescent children are likely to drink in binges when alcohol is available, unlike the continuous free-choice drinking approach used in this study. On the other hand, binge drinking has repeatedly been observed in many studies to cause even more severe deleterious effects than continuous drinking on behavior and neurobiological dependent measures. Thus, future

studies that investigate binge-like exposure in P rats would likely yield similar or even more pronounced effects as observed in the current results. Additionally, it is possible that similar findings would occur with EtOH exposure during other developmental periods before or after this PD 30 - 60 adolescent window, as well as free-choice drinking periods that span less than 30 days.

Future directions should explore other EtOH exposure parameters with regard to this adolescent EtOH exposure model to evaluate differences observed during EtOH drinking and/or post-EtOH binge-like access in adolescence. For example, studies might include examination of other neurotransmitters or brain areas, use of RT-PCR to determine changes in G-proteins or other intracellular signaling pathways, or receptor binding and receptor subunit analyses. Moreover, an important future direction is that these findings present an opportunity to develop pharmacological interventions that may prevent and/or ameliorate these peri-adolescent ETOH-drinking induced neuroadaptations.

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APPENDIX

APPENDIX

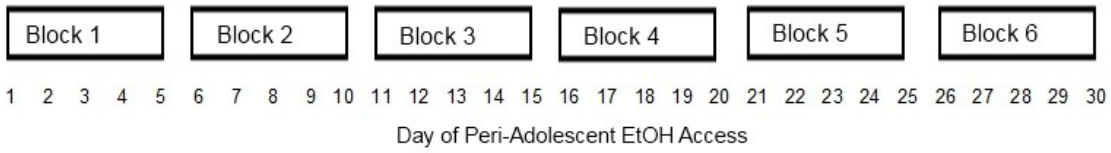

Figure A1. Blocks of EtOH Access. Illustration of breakdown of peri-adolescent EtOH access days into six blocks of five days each for comparison of means.

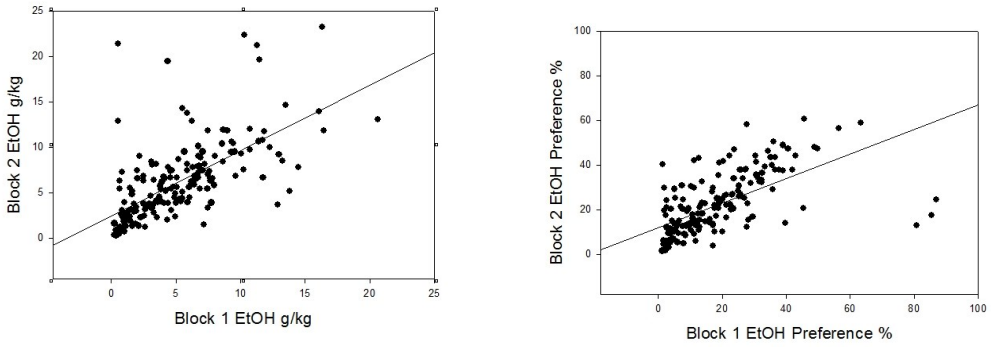

Figure A2. Block 1 Compared to Block 2. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.41 ($F_{1,174} = 121.23$, $p < 0.001$). The r^2 for Preference is 0.38 ($F_{1,174} = 105.95$, $p < 0.001$).

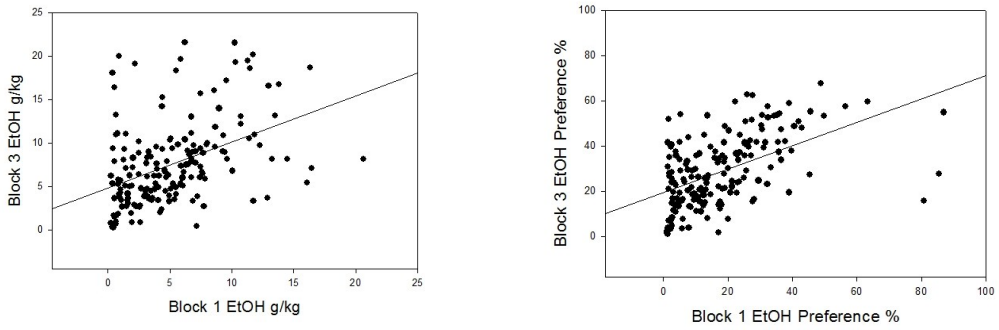

Figure A3. Block 1 Compared to Block 3. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.18 ($F_{1,174} = 38.38$, $p < 0.001$), while the r^2 for Preference is 0.26 ($F_{1,174} = 62.94$, $p < 0.001$).

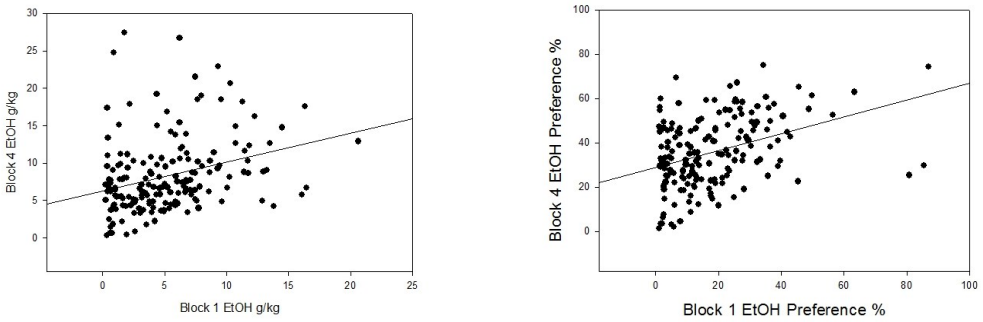

Figure A4. Block 1 Compared to Block 4. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.09 ($F_{1,174} = 17.5$, $p < 0.001$), while the r^2 for Preference is 0.14 ($F_{1,174} = 28.59$, $p < 0.001$).

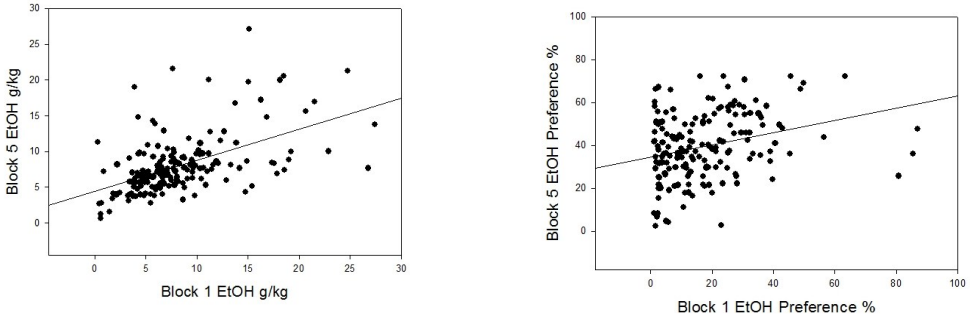

Figure A5. Block 1 Compared to Block 5. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.27 ($F_{1,174} = 11.81$, $p = 0.001$), while the r^2 for Preference is 0.08 ($F_{1,174} = 15.42$, $p < 0.001$).

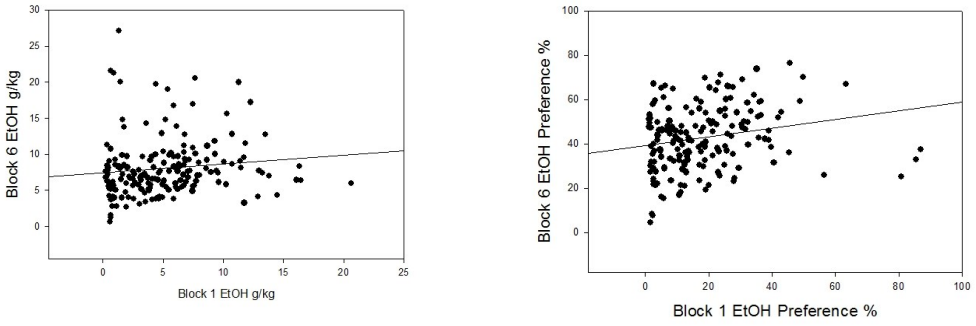

Figure A6. Block 1 Compared to Block 6. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.01 ($F_{1,174} = 2.52$, $p = 0.114$), while the r^2 for Preference is 0.04 ($F_{1,174} = 8.30$, $p = 0.004$).

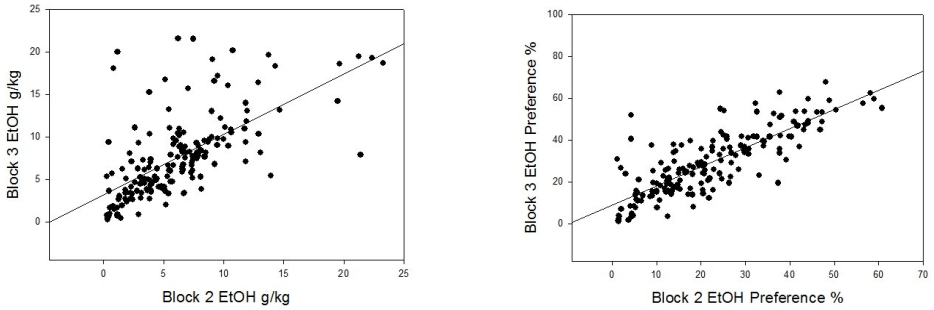

Figure A7. Block 2 Compared to Block 3. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.41 ($F_{1,174} = 124.85$, $p < 0.001$), while the r^2 for Preference is 0.67 ($F_{1,174} = 349.96$, $p < 0.001$).

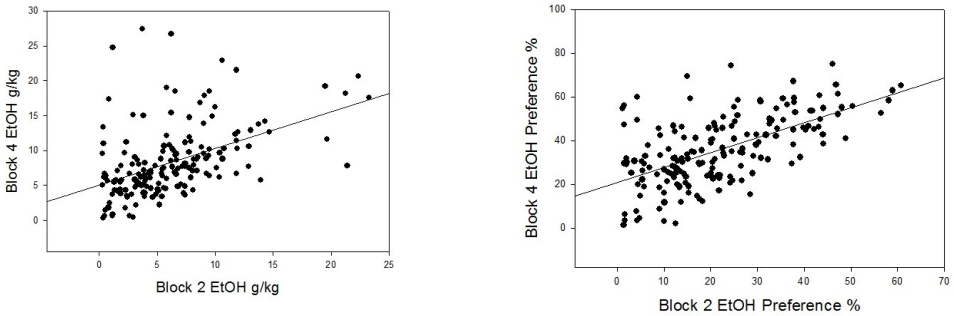

Figure A8. Block 2 Compared to Block 4. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.21 ($F_{1,174} = 47.88$, $p < 0.001$), while the r^2 for Preference is 0.36 ($F_{1,174} = 99.33$, $p < 0.001$).

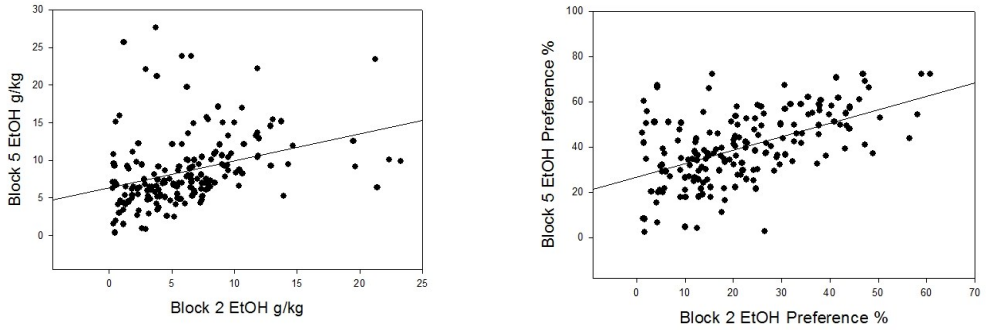

Figure A9. Block 2 Compared to Block 5. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.11 ($F_{1,174} = 21.23$, $p < 0.001$), while the r^2 for Preference is 0.28 ($F_{1,174} = 68.6$, $p < 0.001$).

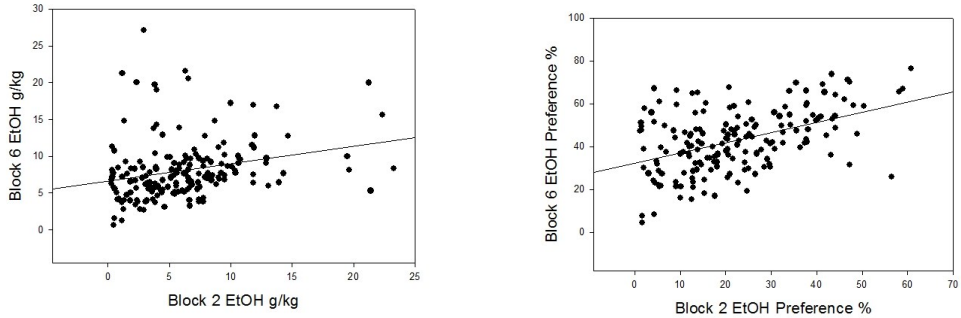

Figure A10. Block 2 Compared to Block 6. These blocks are significantly different for Intake ($p < 0.001$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.06 ($F_{1,174} = 12.00$, $p = 0.001$), while the r^2 for Preference is 0.21 ($F_{1,174} = 46.87$, $p < 0.001$).

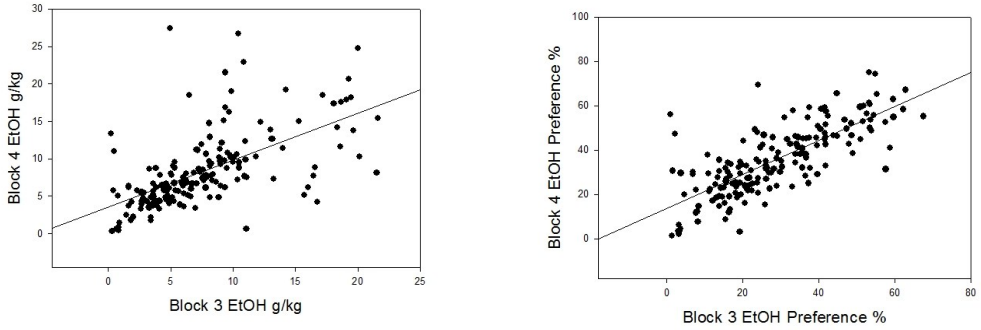

Figure A11. Block 3 Compared to Block 4. These blocks are significantly different for Intake ($p = 0.03$) and Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.37 ($F_{1,174} = 102.59$, $p < 0.001$), while the r^2 for Preference is 0.57 ($F_{1,174} = 235.62$, $p < 0.001$).

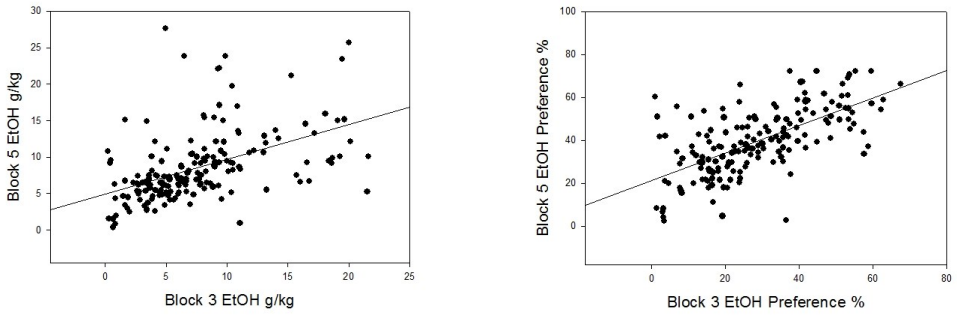

Figure A12. Block 3 Compared to Block 5. These blocks are not significantly different for Intake ($p = 0.09$) but are significantly different for Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.23 ($F_{1,174} = 51.71$, $p < 0.001$), while the r^2 for Preference is 0.41($F_{1,174} = 123.18$, $p < 0.001$).

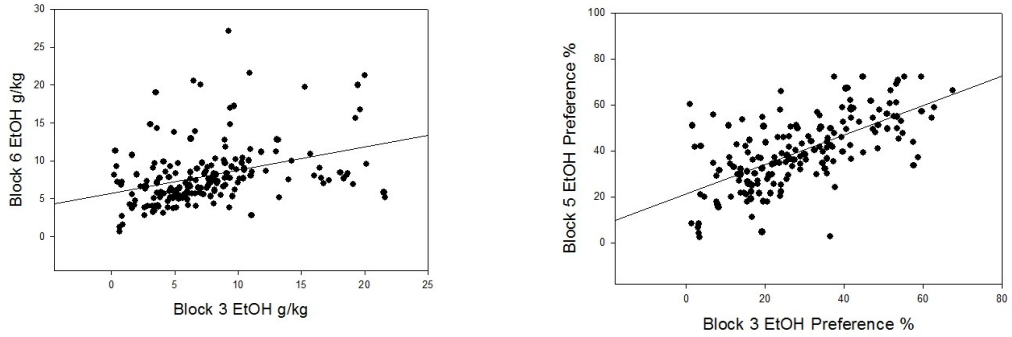

Figure A13. Block 3 Compared to Block 6. These blocks are not significantly different for Intake ($p = 0.20$) but are significantly different for Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.26 ($F_{1,174} = 26.44$, $p < 0.001$), while the r^2 for Preference is 0.13 ($F_{1,174} = 63.23$, $p < 0.001$).

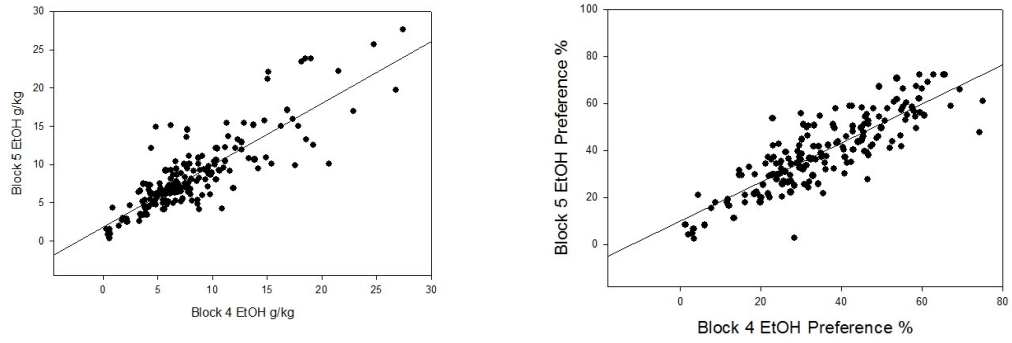

Figure A14. Block 4 Compared to Block 5. These blocks are not significantly different for Intake ($p = 0.19$) but are significantly different for Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.70 ($F_{1,174} = 417.32$, $p < 0.001$), while the r^2 for Preference is 0.71 ($F_{1,174} = 430.55$, $p < 0.001$).

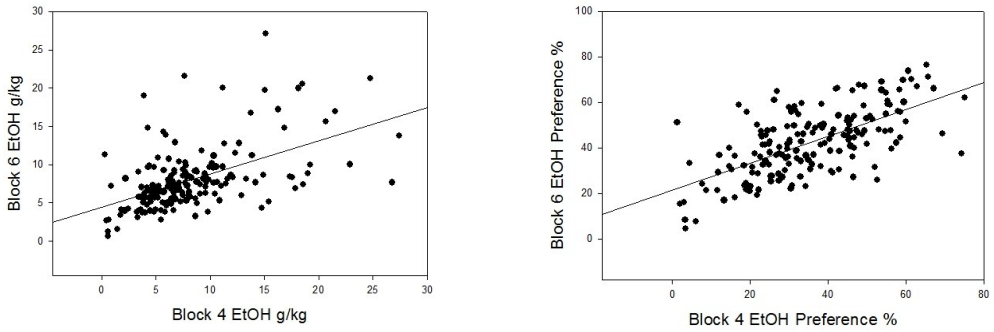

Figure A15. Block 4 Compared to Block 6. These blocks are not significantly different for Intake ($p = 0.53$) but are significantly different for Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.28 ($F_{1,174} = 66.73$, $p < 0.001$), while the r^2 for Preference is 0.42 ($F_{1,174} = 125.88$, $p < 0.001$).

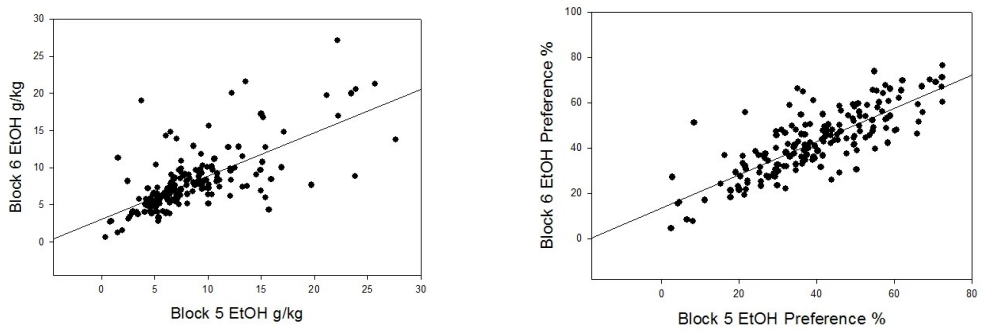

Figure A16. Block 5 Compared to Block 6. Blocks 5 and 6 are not significantly different for Intake ($p = 0.07$) but are significantly different for Preference ($p < 0.001$). Proportion of explained variance (r^2) for Intake is 0.47 ($F_{1,174} = 153.80$, $p < 0.001$), while the r^2 for Preference is 0.63 ($F_{1,174} = 299.56$, $p < 0.001$).

VITA

VITA

JAMIE E. TOALSTON

EDUCATION

Doctor of Philosophy. Psychobiology of Addictions, Purdue University. August 2011.
Dissertation: Peri-adolescent alcohol consumption enhances the reinforcing and stimulatory properties of ethanol within the adult mesolimbic dopamine system in alcohol preferring P rats.

Master of Science. Psychology, Purdue University. May 2008.
Thesis: Adolescent alcohol exposure in the alcohol preferring P rat: Operant response in the adult animal.

Bachelor of Arts with Departmental Honors. Biology and Psychology, Coe College. May 2002.
Thesis: The effects of chronic amphetamine exposure in domestic fowl cockerels: Tolerance or prevention of habituation?

RESEARCH SKILLS

Intracranial Self-Administration methods.
Microdialysis methods.
Microinjection methods.
Operant Self-Administration methods.
Conditioned Place Preference methods.
Rodent and fowl injection methods: intraperitoneal and subcutaneous.
Rodent surgery methods: intracranial cannula, intracardial catheter implantation.
High Performance Liquid Chromatography (HPLC): machine use and maintenance.
Water quality analysis methods: turbidity, temperature, pH, dissolved oxygen, and dissolved solids.

RESEARCH EXPERIENCE

Graduate Research Assistant, Indiana University Purdue University Indianapolis. May 2002 to August 2011.
Collect and analyze data for studies involving operant, microdialysis, microinjection, and intracranial self-administration methods.
Advisors: Dr. James M. Murphy, Department of Psychology. Dr. William J. McBride, Department of Psychiatry.

Research Assistant, Coe College. June 2001 to May 2002.
Collect and analyze data for behavioral amphetamine studies involving young domestic fowl.
Advisor: Dr. Michael R. Baker, Department of Psychology.

Research Assistant, Coe College. January 2001 to January 2002.
Collect data at eight creek sites for long-term water quality analysis.
Advisor: Dr. Martin St.Clair, Department of Chemistry.

ACADEMIC EXPERIENCE

Instructor, Department of Psychology, IUPUI. Spring and Fall 2008.
Introduction to Psychology as a Social Science.
Writing Consultant, Department of Rhetoric, Coe College. August 1998 to May 2002.
Consult students on idea clarification, organization, editing, and revision.

MEMBERSHIP IN PROFESSIONAL SOCIETIES

Student Member of the Society for Neuroscience (SFN).
Student Member of Research Society on Alcoholism (RSA).

PUBLICATIONS

Bell RL, Rodd ZA, Smith RJ, Toalston JE, Franklin KM, McBride WJ. (Revision submitted Pharmacology Biochemistry and Behavior, June 2011) Modeling binge-like ethanol drinking by peri-adolescent and adult P rats with a drinking-in-the-dark procedure.

Rodd ZA, Katner SN, Oster SM, Ding ZM, Deehan GA, Toalston JE, Hauser SR, McBride WJ. (In Press) Alcohol-preferring (P) rats are more sensitive than Wistar rats to the reinforcing effects of cocaine self-administered directly into the nucleus accumbens shell. Pharmacology Biochemistry and Behavior.

Hauser SR, Ding ZM, Getachew B, Toalston JE, Oster SM, McBride WJ, Rodd ZA. (2011) The posterior ventral tegmental area mediates alcohol-seeking behavior in alcohol-preferring rats. Journal of Pharmacology and Experimental Therapeutics 336:857-65.

Rodd ZA, Bell RL, Oster SM, Toalston JE, Pommer TJ, McBride WJ, Murphy JM. (2010) Serotonin-3 receptors in the posterior ventral tegmental area regulate ethanol self-administration of alcohol-preferring (P) rats. Alcohol 44:245-55.

Engleman EA, Ding ZM, Oster SM, Toalston JE, Bell RL, Murphy JM, McBride WJ, Rodd ZA. (2009) Ethanol is self-administered into the nucleus accumbens shell, but not the core: evidence of genetic sensitivity. Alcohol Clinical and Experimental Research 33:2162-71.

Ding ZM, Toalston JE, Oster SM, McBride WJ, Rodd ZA. (2009) Involvement of local serotonin-2A but not serotonin-1B receptors in the reinforcing effects of ethanol within the posterior ventral tegmental area of female Wistar rats. Psychopharmacology (Berl) 204:381-90.

Bell RL, Rodd ZA, Toalston JE, McKinzie DL, Lumeng L, Li TK, McBride WJ, Murphy JM. (2008) Autonomic activation associated with ethanol self-administration in adult female P rats. Pharmacology Biochemistry and Behavior 91:223-32.

Toalston JE, Oster SM, Kuc KA, Pommer TJ, Murphy JM, Lumeng L, Bell RL, McBride WJ, Rodd ZA. (2008) Effects of alcohol and saccharin deprivations on concurrent ethanol and saccharin operant self-administration by alcohol-preferring (P) rats. Alcohol 42:277-84.

Rodd ZA, Oster SM, Ding ZM, Toalston JE, Deehan G, Bell RL, Li TK, McBride WJ. (2008) The reinforcing properties of salsolinol in the ventral tegmental area: evidence for regional heterogeneity and the involvement of serotonin and dopamine. Alcohol Clinical and Experimental Research 32:230-9.

Rodd ZA, Gryszowka VE, Toalston JE, Oster SM, Ji D, Bell RL, McBride WJ. (2007) The reinforcing actions of a serotonin-3 receptor agonist within the ventral tegmental area: evidence for subregional and genetic differences, and involvement of dopamine neurons. Journal of Pharmacology and Experimental Therapeutics 321:1003-12.

Oster SM, Toalston JE, Kuc KA, Pommer TJ, Murphy JM, Lumeng L, Bell RL, McBride WJ, Rodd ZA. (2006) Effects of multiple alcohol deprivations on operant ethanol self-administration by high-alcohol-drinking replicate rat lines. Alcohol 38:155-164.

INVITED TALKS

Toalston JE, Rodd ZA, Czachowski CL, Bell RL, Melendez RI, Colombo G. Session: Modeling excessive alcohol intake and alcohol-seeking behaviors in rodents. Talk: The predictive validity of Pavlovian spontaneous recovery: An animal model of alcohol seeking. Slide presentation at the 2006 International Society for Biomedical Research on Alcoholism meeting. Sydney, Australia.

Toalston JE, Rodd ZA, Davids MR, Bell RL, Engleman EA, Murphy JM, McBride WJ. Microinjections of ethanol into the posterior, but not anterior, ventral tegmental area (VTA) increase dopamine release in the nucleus accumbens. Slide presentation and poster at the 2005 Research Society on Alcoholism meeting. Santa Barbara, CA.

SELECTED POSTERS AND PRESENTATIONS

Bell RL, Rodd ZA, Smith RJ, McConnell KK, Toalston JE, McBride WJ. The effects of ethanol intake by alcohol-preferring (P) rats using a drinking-in-the-dark multiple-scheduled-access (DID-MSA) procedure. Poster presentation at the 2010 Society for Neuroscience meeting. San Diego, CA.

Katner SN, Toalston JE, McClaren JA, Bard CD, Engleman EA, Rodd ZA, McBride WJ. Pharmacokinetics of nicotine and its metabolite cotinine in the brain of alcohol-preferring (P) rats following subcutaneous administration as measured by in vivo microdialysis coupled with HLPC-UV detection. Poster presentation at the 2010 Society for Neuroscience meeting. San Diego, CA.

Rodd ZA, Toalston JE, Bell RL, McBride WJ, Truitt WA. Adolescent oral consumption of ethanol, nicotine, or concurrent ethanol and nicotine alters gene expression of G coupled receptors in the anterior cingulate during adulthood. Poster presentation at the 2010 Society for Neuroscience meeting. San Diego, CA.

Toalston JE, Rodd ZA, Bell RL, McBride WJ, Truitt WA. Adolescent oral consumption of ethanol, nicotine, or concurrent ethanol and nicotine alters gene expression of G coupled receptors in the nucleus accumbens shell during adulthood. Poster presentation at the 2010 Society for Neuroscience meeting. San Diego, CA.

Oster SM, Pommer TJ, Toalston JE, Bell RL, McBride WJ, Rodd ZA. Operant oral EtOH self-administration with concurrent access to multiple EtOH concentrations in Alcohol-Preferring (P) rats: Evidence for drug escalation and excessive blood EtOH concentrations. Poster presentation at the 2010 Research Society on Alcoholism meeting. San Antonio, TX.

Bracken AL, Oster SM, Toalston JE, McQueen VK, McBride WJ, Rodd ZA. Positive contrast and sustained ethanol self-administration in alcohol-preferring (P) rats given concurrent operant access to ethanol and sucrose. Poster presentation at the 2009 Research Society on Alcoholism meeting. San Diego, CA.

Toalston JE, Rodd ZA, Oster SM, Bell RL, Murphy JM, McBride WJ. Effects of ethanol drinking by alcohol-preferring (P) rats during peri-adolescence on subsequent ethanol intracranial self-administration in the ventral tegmental area during adulthood. Poster presentation at the 2009 Research Society on Alcoholism meeting. San Diego, CA.

Cook JB, Bracken AL, Rodd ZA, Toalston JE, Lumeng L, Murphy JM, McBride WJ, Bell RL. The effects of ethanol consumption during peri-adolescence or adulthood on subsequent ethanol-induced motor impairment in alcohol-preferring (P) rats. Poster presentation at the 2008 Research Society on Alcoholism meeting, Washington, D.C.

Oster SM, Ding Z-M, Toalston JE, Rodd ZA, Bell RL, Pommer TJ, Lumeng L, Murphy JM, McBride WJ. Alcohol drinking increases the sensitivity of the nucleus accumbens shcell to the reinforcing effects of cocaine in alcohol-preferring (P) rats. Poster presentation at the 2008 Research Society on Alcoholism meeting, Washington, D.C.

Hauser SR, Ding Z-M, Getachew B, Dhaher R, Toalston JE, Oster SM, McQueen VK, McBride WJ, Rodd ZA. Involvement of the posterior ventral tegmental area (p-VTA) in mediating alcohol-seeking behavior in alcohol-preferring (P) rats. Poster presentation at the 2008 Research Society on Alcoholism meeting, Washington, D.C.

Toalston JE, Rodd ZA, Truit WA, Hong D, Wang M, Witzmann FA. Protein expression changes in the nucleus accumbens shell and central amygdala following ethanol self-administration into the posterior ventral tegmental area (VTA) in alcohol-preferring (P) rats. Poster presentation at the 2007 Society for Neuroscience meeting, San Diego, CA.

Oster SM, Toalston JE, Ding Z-M, Deehan GA, McBride WJ, Rodd ZA. Salsolinol self-infusion into the posterior ventral tegmental area (VTA) is dependent upon dopamine activity. Invited talk for the 2007 Society for Neuroscience meeting, San Diego, CA.

Rodd ZA, Toalston JE, Ding ZM, Oster SM, Davids MR, Bell RL, Engleman, EA, McBride WJ. Ethanol-activation of ventral tegmental area (VTA) dopamine neurons is mediated by local serotonin-3 receptors. Poster presentation at the 2007 Society for Neuroscience meeting, San Diego, CA.

Ding Z-M, Toalston JE, Oster SM, McBride WJ, Rodd ZA. Reinforcing effects of ethanol in the posterior ventral tegmental area (VTA) are not mediated by serotonin-1B receptors. Poster presentation at the 2007 Society for Neuroscience meeting, San Diego, CA.

Toalston JE, Rodd ZA, Oster SM, Murphy JM, Bell RL, McBride WJ. Effects of saccharin or ethanol drinking by adult alcohol-preferring (P) rats during peri-adolescence on subsequent ethanol self-administration during adulthood. Poster presentation at the 2007 Research Society on Alcoholism meeting, Chicago, IL.

Pommer TJ, Oster SM, Toalston JE, Murphy JM, Bell RL, McBride WJ, Rodd ZA. Introduction of a 'lock-out' during concurrent access to multiple ethanol concentrations: effects of repeated deprivations. Poster presentation at the 2007 Research Society on Alcoholism meeting, Chicago, IL.

Rodd ZA, Larson LA, Toalston JE, Cook JB, Oster, SM, Lumeng L, McBride WJ, Murphy JM. Peri-adolescent ethanol exposure reduces basal heart rate but not autonomic reactivity to self-administered ethanol in adult P rats. Poster presentation at the 2007 Research Society on Alcoholism meeting, Chicago, IL.

Bell RL, Rodd ZA, Schultz JA, Toalston JE, Oster SM, Murphy JM, Lumeng L, McBride WJ. An evaluation of the consummatory behavior of genetically selected alcohol-preferring rats during adolescence and adulthood. Poster presentation at the 2007 Research Society on Alcoholism meeting, Chicago, IL.

Toalston JE, Rodd ZA, Oster SM, Murphy JM, Bell RL, McBride WJ. Effects of peri-adolescent ethanol drinking on oral saccharin self-administration by adult Alcohol Preferring (P) rats. Poster presentation at the 2006 Indianapolis Chapter of the Society for Neuroscience meeting, Indianapolis, IN.

Bell RL, Rodd ZA, Toalston JE, Larson LA, Peper CL, Lumeng L, McBride WJ, Murphy JM. Heart rate activating effects of self-administered ethanol in alcohol-preferring (P) rats with or without prior home-cage access to ethanol. Poster presentation at the 2006 Society for Neuroscience meeting, Atlanta, GA.

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Pommer TJ, Rodd ZA, Bell RL, McQueen VK, Toalston JE, Engleman EA, Lumeng L, McBride WJ, Li T-K, Murphy JM, Effects of 5-HT3 antagonists within the nucleus accumbens shell on operant self-administration of ethanol by inbred alcohol-preferring (iP) rats. Poster presentation at the 2003 Indianapolis Chapter of the Society for Neuroscience meeting. Eli Lilly and Co, Indianapolis, IN.

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GRANTS

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HONORS AND AWARDS

Indianapolis Chapter of the Society for Neuroscience Poster Award, 2009.
Research Society on Alcoholism Student Merit Award Winner, 2005.
Research Society on Alcoholism Enoch Gordis Research Recognition Award Finalist, 2005.
IUPUI Research Incentive Fellowship, 2002-2003.