THE EFFECT OF DIFFERENTIAL REARING CONDITIONS ON THE CONSUMPTION OF AND OPERANT RESPONDING FOR ETHANOL IN THE INDIANA UNIVERSITY SELECTIVELY BRED ALCOHOL-PREFERRING (P) AND -NON-PREFERRING (NP) RAT LINES

by

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B.A., The Richard Stockton College of New Jersey, 2001

M.S., Kansas State University, 2006

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Psychology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2009

Abstract

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I would like to thank the members of my supervisory committee, Drs. Stephen Kiefer, Mary Cain, Jerome Frieman, Mark Weiss, and Tonatiuh Melgarejo for their assistance during all phases of this dissertation. I offer a special level of gratitude to Dr. Mathew Palmatier for his assistance throughout all of the operant experiments conducted for this dissertation. I would also like to thank my immediate family and friends for their unending support.

Differential rearing conditions and the consumption of and responding for ethanol in alcohol preferring and non-preferring rats.

In 2006, 50.9 percent (125 million people) of the American population aged 12 or above considered themselves “drinkers” of alcohol (Office of Applied Studies, 2006). Of the 125 million people identified, 57 million aged 12 or older reported binge drinking while 17 million Americans (6.9%) reported heavy alcohol use within the 30 days preceding the survey (Office of Applied Studies, 2006). The operational definitions of current use or “drinker” is one drink in the past 30 days which includes both binge drinking (5 or more drinks on a single occasion within a few hours of each other) and heavy drinking (5 or more drinks on a single occasion for at least 5 consecutive days) (Office of Applied Studies, 2006). In 2006 alone there were over 34,000 alcohol related deaths not including alcohol related accidents or homicides (National Center for Health Statistics, 2006). Thus, alcohol abuse and alcoholism are prevalent disorders in the United States that result in a large number of fatalities each year.

According to the DSM-IV-TR (2000; text revision), alcoholism in humans falls under the category of substance abuse and can be diagnosed if a person exhibits a level of substance use that leads to a “clinically significant impairment or distress” and the person meets one or more of 4 criteria. Criterion 1 addresses the ability of a person using a substance to fulfill their life obligations, specifically those related to work, school, or home life (e.g., poor work performance or a large number of absences from work or school related to the use of the substance and/or child or household neglect). The second criterion involves a person continually using a substance even in the face of negative consequences and danger oneself or others (e.g., operating an automobile or

heavy equipment while under the influence of the substance). Criterion 3 states that the person continues to take the substance even though they have experience repeated legal problems associated with the substance (e.g., substance related arrests). And finally, criterion 4 addresses the continued use of the substance when the person experiences recurring social/interpersonal problems associated with the use of the substance (e.g., verbal and physical fights with others when intoxicated) (*DSM-IV-TR,* 2000).

To date several lines of both human and animal research have been developed to better characterize alcohol abuse and alcoholism as a disorder as well as to elucidate successful methods of treatment. In pursuing such goals researchers have made use of a number of methods ranging from pharmacological manipulations (e.g., naltrexone) to environmental manipulations (e.g., rearing environment). Additionally, researchers have probed the genetic aspects of alcoholism through the use of selectively bred and inbred animals which offer a close approximation of the disorder of alcoholism. By utilizing such animal models, researchers are provided a method by which to assess genetic contribution to the development of alcoholism (nature). On the other hand the use of environmental manipulations (e.g., rearing paradigms) provide researchers with a venue to explore the contribution of external influences on the development of alcoholism (nurture).

Neuroscience researchers have made use of a number of elegant techniques to develop genetically altered lines of animals (animal models) to probe the relationship between various genes and several disorders and diseases (for example: Eriksson, 1968; Grahame et al., 1999; Li et al., 1987; Li et al., 1993; Mardones & Segovia-Riquelme, 1983 McClearn & Rodgers, 1959). Alternatively, researchers have also

made use of differential rearing environments, maternal separation, and stress paradigms to examine the effect of environment/experience on adult traits (Brown et al., 2003; Bruel-Jungerman et al., 2005; Escorihuela et al., 1995; Huang et al., 2006; Kempermann et al., 1997; Murtha et al., 1990; Rampon et al., 2000; Renner & Rosenzweig, 1987; Segovia et al., 2006; Silva-Gomez et al., 2003 ; van Praag et al., 2000). Through such techniques (genetic alteration, maternal separation, etc.) a number of animal models, that display symptoms that closely resemble human disorders and diseases, have been developed which afford neuroscience researchers the ability to develop novel treatments for various disorders and diseases. One such area of neuroscience that has benefited from the use of animal models (genetic alteration) and environmental manipulation is the study of alcohol use, abuse, and alcoholism.

Rodent Models of Alcohol Use, Abuse, and Alcoholism

Simply put, animal models cannot completely account for the complex nature of a disorder such as alcoholism. However, animal models of alcoholism have proven to be useful tools that allow researchers to investigate alcoholism in a controlled setting utilizing techniques and paradigms that would otherwise be impossible using human participants because of ethical constraints. Currently there are several selectively bred and/or inbred lines of mice and rats that are considered useful as animal models of alcoholism. Selectively bred lines consist of divergent groups of rodents that exhibit either a strong preference for and high consumption level of alcohol (preferring line) or do not prefer and consume very little alcohol (non-preferring line). Inbred lines, on the other hand, represent populations of homozygous animals that share the same alcohol preference/consumption due to their identical genetic makeup. Because the current

experiments used rats, the majority of the discussion to follow will focus on rat models of alcoholism. However, it is important to note that there are several lines of mice that have been studied for their alcohol preference and that are currently being used in alcohol research.

To identify alcohol preferring rodents as an animal model of alcoholism, Lester and Freed (1973), Cicero (1979), and more recently McBride and Li (1998) have established a set of seven criteria. Specifically, to qualify as an animal model for alcoholism, rats should 1) self-administer alcohol orally (e.g., drink from a sipper), 2) consume enough alcohol to attain a pharmacologically high blood alcohol level (BAC), 3) consume alcohol for its pharmacological effects and not for reasons such as taste, smell, or caloric value 4) be willing to work for alcohol (e.g., operant responding), 5) express both metabolic and functional tolerance after chronic alcohol access, 6) show an alcohol dependence as characterized by withdrawal symptoms (e.g., seizure thresholds and anxiety) when no longer provided access to alcohol, and 7) exhibit a "loss of control" (an increase in consumption levels over baseline) when alcohol is reinstated after a period of imposed abstinence (the alcohol deprivation effect; ADE) (Lester & Freed, 1973; Cicero, 1979; McBride & Li, 1998). Several alcohol preferring rat lines exist and each have been evaluated (to some extent) using the 7 criteria listed above.

Alcohol Preferring Rat Lines

Over the past half-century, several selectively bred alcohol preferring and non-preferring rat lines have been developed in multiple countries around the globe. Two of the earliest lines to be developed were the University of Chile B (UChB; alcohol preferring) and A (UChA; alcohol non-preferring) rat lines which date back to the early

1950's (Mardones & Segovia-Riquelme, 1983). Approximately 15 years after the development of the UChA and UChB lines, researchers in Helsinki, Finland began breeding the Alko-Alkaline (AA; alcohol preferring) and Alko-non-Alkaline (ANA; alcohol non-preferring) rat lines (Eriksson, 1968). The alcohol preferring (P) and non-preferring (NP) rat lines followed in the next decade, bred originally at the Walter Reed Army Institute of Research in Washington, DC and then continued at the Indiana University school of Medicine (Li et al., 1977). In 1981, researchers at the University of Cagliari (Italy) began breeding the Sardinian alcohol-preferring (sP) and non-preferring (sNP) rats (Mardones & Segovia-Riquelme, 1983). Finally, in the mid 1980's, the high-alcohol drinking (HAD) and low-alcohol drinking (LAD) selectively bred replicate rat lines were developed at the Indiana University School of Medicine (Li et al., 1993).

The UChA/UChB, P/NP, and sP/sNP represent rat lines that were selectively bred from Wistar foundation stock rats (Colombo et al., 2006; Eriksson, 1968; Li et al., 1993; Quintanilla et al., 2006). The AA and ANA rat lines were also originally bred from a Wistar foundation stock. However, due to inbreeding (loss of heterozygosity), it was necessary for both lines to be revitalized using Brown Norwegian and Lewis rat strains in the 37^{th} generation (Hilakivi et al., 1984). The HAD and LAD rats, on the other hand, were originally bred from a stock of N/NIH rats (Li et al., 1993). The N/HIH rat line was selected to be the breeding stock for the HAD and LAD rat lines due to the fact that they possess a greater degree of heterozygosity compared to the Wistar line (Hansen & Spuhler, 1984; Li et al., 1993). Furthermore, replicate lines were bred (HAD_1/LAD_1 and HAD_2/LAD_2) to ensure a greater number of breeding families as well as to maintain heterozygosity for generation after generation to more accurately reflect the inherent variation in the population of human alcoholics (Li et al., 1993).

All of the alcohol-preferring selectively bred rat lines mentioned above have been evaluated to some extent using the criteria for an animal model of alcoholism. For instance, the UChB, AA, P, sP, and $HAD_{1\&2}$ rat lines were bred using a selection criterion which focused on the consumption of 10% alcohol. As such, all lines meet criteria 1 and 2 as they will readily consume alcohol from a sipper tube in amounts that range from 4 to 8 g/kg/day (achieving significant BAC's) while their non-preferring counterparts avoid alcohol (Colombo et al., 1995; McBride & Li, 1998; Quintanilla et al., 2006; Ritz et al., 1986; Ritz et al., 1994b; Sinclair et al., 1989). Only the P and sP rat lines have been shown to consume alcohol for its pharmacological effects and not for taste, smell, or caloric value (*Criteria 3*; Colombo et al., 2006; Lankford et al., 1991; Li et al., 1987). The AA, P, sP, and $HAD_{1\&2}$ rat lines will readily work (operantly respond) for alcohol (*Criteria 4*; Files et al., 1998; Murphy et al., 1989; Penn et al., 1978; Ritz et al., 1994a; Ritz et al., 1994b; Samson et al., 1998; Vacca et al., 2002) with the AA, P and $HAD_{1\&2}$ lines learning to respond for alcohol without requiring a sucrose fading procedure (Hyytiä & Sinclair 1989; Rodd-Henricks et al., 2002 a,b). The AA and P lines also express functional and metabolic tolerance to alcohol (*Criteria 5*; Forsander & Sinclair, 1992; Gatto et al., 1987a; Gatto et al., 1987b; Lumeng & Li, 1986; Waller et al., 1983) while the UChB and $HAD_{1\&2}$ lines have only been shown to exhibit functional tolerance to the motor impairing effects of alcohol (Quintanilla et al., 2006; Suwaki et al., 2001) and the sP line has yet to be investigated fully in this respect. The P rat line has been the only line shown to express dependence and withdrawal effects following chronic alcohol consumption (Kampov-Polevoy et al., 2000; Rodd et al., 2004b; Waller et al., 1982) as well as an ADE following various lengths of imposed abstinence (McKinzie et al., 1998; Rodd et al., 2003; Rodd-Henricks et al., 2000b; Sinclair & Li,

1989). The AA and sP rat lines will display an increase in alcohol consumption over baseline following short deprivations (AA = 12-24 hrs; sP = 3 hrs) but do not display an ADE following longer deprivation periods (Serra et al., 2003; Sinclair & Li, 1989). The $HAD_{1\&2}$ lines will also display an ADE however it is contingent upon exposing the rats to repeated cycles of alcohol access and abstinence (Rodd-Henricks et al., 2000a). A thorough literature search yielded no results indicating whether the UChB rat line has been evaluated to establish if the line shows an ADE.

The current research will utilized the Indiana University alcohol-preferring (P) and non-preferring (NP) rat lines to investigate the interaction between rearing environment and the genetic proclivity to consume alcohol. The P rat line was chosen as it is the only preferring rat line that has been found to meet all 7 criteria for an animal model of alcoholism. Specifically, when provided 24 hour free access to alcohol P rats will consume over of 5 g/kg/day and NP rats will consume less than 1 g/kg/day of alcohol (*Criteria 1*; Li et al., 1986; Li et al., 1987). Additionally, when provided either limited-access or 24-hour free-access to alcohol, P rats will consume enough alcohol to establish blood alcohol level's (BAC's) in the 50-70 mg% range with some rats reported to establish BAC's up to 200 mg% (*Criteria 2*; Bell et al., 2006; Murphy et al., 1986; Rodd-Henricks et al., 2001; Li et al., 1987). The P rat line clearly consumes alcohol for its pharmacological effects and not taste, smell, or caloric value as dietary changes or the addition of flavored tastants do not affect alcohol preference (*Criteria 3*; Lankford et al., 1991; Li et al., 1987). Furthermore, P rats will self-administer alcohol both intragastrically and intracranially (*Criteria 3*; Gatto et al., 1994; Rodd et al., 2005; Rodd et al., 2004a; Waller et al., 1984).

Several researchers have also shown that the P rat line will readily learn operant self-administration of alcohol and work to high break points (> FR-30) to obtain a single 0.1 ml of alcohol solution (*Criteria 4*; Files et al., 1998; Murphy et al., 1989; Penn et al., 1978; Ritz et al., 1994b; Samson et al., 1998). The P rats exhibit both functional and metabolic tolerance to alcohol characterized by an increase in alcohol elimination (metabolic) and a decrease in the aversive, ataxic, and motor impairing effects (functional) of alcohol compared to NP rats (*Criteria 5*; Gatto et al., 1987; Lumeng & Li, 1986; Stewart et al., 1991) The P line exhibits alcohol dependence characterized by physical withdrawal symptoms following chronic 24-hour free-access to alcohol (*Criteria 6*; Kampov-Polevoy et al., 2000; Waller et al., 1982). Unlike other selectively bred alcohol-preferring lines, the P rats will display an ADE following deprivation periods ranging from 1 to 8 weeks in length (McKinzie et al., 1998; Rodd-Henricks et al., 2000a; Sinclair & Li, 1989) as well as show an in increase in their ADE magnitude and length following successive access/deprivation periods (Rodd-Henricks et al., 2001; Rodd-Henricks et al., 2000b).

Several underlying neurological/neurochemical differences between the P and NP lines exist and are believed to contribute to the differential levels of alcohol consumption and/or responding between the two rat lines. Overall, P rats exhibit a decreased number of serotonin neurons (Zhou et al., 1991; Zhou et al., 1995a) and subsequently, decreased levels of serotonin (5-HT) in a number of brain areas, including the limbic system, compared to NP rats (Murphy et al., 1982; Murphy et al., 1987; Strother et al., 2005). The P rat line has also been found to have a lower number of dopamine (DA) neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAC) (Zhou et al., 1995b), fewer D2 receptors (DA receptor) in the

VTA and NAC (McBride et al., 1990), and decreased levels of DA and DA metabolites (3,4-Dihydroxy-Phenylacetic Acid: DOPAC and Homo-Vanilic Acid: HVA) in the NAC compared to NP rats (Murphy et al., 1982; Murphy et al., 1987; Strother et al., 2005). Hwang et al. (1990) found a higher number of GABA terminals within the NAC in P rats. Additionally, greater densities of the μ opioid receptor have been found in the limbic system of the P rat compared to the NP rat (McBride & Li, 1998).

These findings are of interest as the differences observed between the P and NP rat lines in various alcohol related behaviors are believed to be a function of differences in the underlying neurochemical systems mentioned above. For instance, abnormalities found in the 5-HT system of P rats have been linked to the increases in alcohol seeking (Stewart & Li, 1997). Low levels of DA in the NAC of P rats has been directly correlated with high alcohol preference and increased levels of alcohol consumption/responding (Bell et al., 2006; Stewart & Li, 1997). The increased number of GABA terminals is believed to contribute to the increased alcohol tolerance and withdrawal symptoms observed (Davis & Wu, 2001) as well as a decrease in the sensitivity to the motor impairing effects of alcohol witnessed in the P line (Murphy et al., 2002). While greater densities of the μ opioid receptor are believed to increase DA transmission in the reward pathway, working in an indirect manner to increase the reinforcing properties of alcohol (Herz, 1997).

In summary, several selectively bred alcohol preferring and non-preferring rat lines exist and have been examined to various extents. Selectively bred rodent lines offer an advantage over the inbred rodent lines as selective breeding for alcohol consumption allows for the normal distribution of non-selected traits (those traits other than alcohol consumption/preference) which produces an animal model that more

closely represents the genetic variation in the overall population of human alcoholics (Yoneyama et al., 2008). Of all the selectively bred alcohol-preferring rat lines, the P rats make up the only selectively bred rat line that meets all 7 of the criteria put forth for an animal model of alcoholism. Moreover, the P and NP rat lines display differences in their neurochemcial properties, which are believed to underlie their divergent levels of alcohol consumption/preference, that are similar to human alcoholics which makes them the closest approximation to human alcoholism in a selectively bred animal model. Therefore, the P and NP rat lines represent the most viable candidates for probing the interaction between the genetic predisposition to prefer or not prefer alcohol and differential rearing environments.

Differential Rearing Conditions (General)

The idea that the brain's physiology, once fully developed according to a pre-determined genetic outline, could no longer change was a common view shared by many neuroscientists during the early to mid 20th century (Renner & Rosenzweig, 1987). Hebb (1947) was the earliest researcher to develop an alternative theory to this view as he reported a clear difference in learning between rats that had been enriched (taken home and treated as pets) compared to their standard laboratory counterparts. Later, Rosenzweig, et al. (1962a; 1962b) were able to show that rats exhibited changes in brain chemistry as a result of being reared in a complex and novel environment. Since this landmark study, several researchers have made progress toward more fully characterizing the effects of raising animals in differential rearing environments (complex, novel, and/or enriched environments; EC, social/group housing environments; SC, or impoverished/isolated rearing environments; IC). Much of the research has been focused toward elucidating the effect of rearing on specific brain

areas, pathways, and neurochemical systems and how such rearing-evoked changes affect visible/quantifiable behaviors ranging from learning to drug taking.

Rats that are reared in an enriched environment (EC) are typically housed in a large communal cage with several cohorts (up to 12) as well as several novel objects (plastic toys and objects) with which the rats interact. Rats reared in a social condition are housed in standard shoebox cages or comparable caging with cohorts (usually 2-4 rats per cage). Animals reared in an impoverished condition are housed singly in hanging metal cages. Additionally, rats in the EC are handled daily, rats in the SC are handled once per week during scheduled bedding changes, and rats in the IC are not handled for the length of the rearing period (usually between 30 – 60 days in length). It is important to note that not all the paradigms that investigate rearing effects on brain changes and behavior are identical and that the description above is a general description of the paradigm currently being used in our laboratory (for specific paradigm see Methods).

It has been observed that a number of brain structures and neuronal processes are affected by differential rearing environments. For instance, rats reared in an EC have been found to display a significantly greater thickness in their cerebral cortex (occipital, motor, and somatosensory) compared to rats reared in an IC. The greater cortical thickness is due to an increased density of the neurons (Murtha et al., 1990; Renner & Rosenzweig, 1987) as well as a greater number of glial cells (greater metabolic support) within their cerebral cortex compared to IC rats (Renner & Rosenzweig, 1987). This increase in the number of support cells allows for increased neuronal activity which in turn is believed to facilitate an increase in neuronal connections and an overall increase in dendritic density for EC rats (Renner &

Rosenzweig, 1987) whereas IC rats display decreased dendritic spine density in the prefrontal cortex (PFC) and hippocampus (Silva-Gomez et al., 2003). Rearing rodents in an EC increases neurogranin levels subsequently increasing hippocampal long-term-potentiation (an increase in synaptic sensitivity) as well as hippocampal neurogenesis, improving learning and memory via enhanced synaptic efficiency compared to their controls (Brown et al., 2003; Bruel-Jungerman et al., 2005; Escorihuela et al., 1995; Huang et al., 2006; Kempermann et al., 1997; Rampon et al., 2000; Segovia et al., 2006; van Praag et al., 2000).

It has been reported that differential rearing conditions affect, either directly or indirectly, several neurotransmitters/neurotransmitter systems as well. For instance, social isolation has been found to decrease benzodiazepine (BZ) receptor (a γ-aminobutyric-acid (GABA) receptor) binding in the cortex, hippocampus, tectum, and cerebellum in both pre- and post-weanling rats (Insel, 1989; Miachon et al., 1990). Isolate reared rats also exhibit decreased levels of allopregnanolone, a hormone that has been found to modulate GABA. Theilen et al. (1993) housed adult P and NP rats in either an isolate or pair housing condition (2 per cage) and reported an increase in $GABA_A$/BZ receptor function in isolate housed animals over pair-housed animals, independent of rat line.

Andin et al. (2007) also categorized EC-induced changes to the glutamatergic system in the hippocampus as they reported an upregulation of N-methyl-D-aspartic acid (NMDA) receptor messenger ribonucleic acid (mRNA) in enriched rats in comparison to their controls. Andin et al. (2007) did not report an upregulation in Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid (AMPA) receptor mRNA yet Lee et al. (2003) observed enhanced spatial learning in EC rats, reflected by increases in

both NMDA and AMPA receptor activation. As a corollary, Mlynarik et al. (2004) witnessed an increase in AMPA glutamate receptor 1 (GLUR1) gene expression in the hippocampus. Looking at metabotropic rather than ionotropic receptors, Melendez et al. (2004) found that rats reared in an IC show a "blunting" of metabotropic GLUR's extracellular GLU regulation. Additionally, rearing rats in an EC has been observed to reverse NMDA NR1 subunit and associated spatial learning deficits induced by pre-natal/pre-weaning lead exposure (Guilarte et al., 2003). Thus, it would appear that rearing in an EC exerts a prolific effect on both AMPA and NMDA GLU receptors.

Differential rearing conditions also affect the serotonergic system. For example, when injected with amphetamine, it has been noted that IC rats exhibit a decrease in 5-HT release (Dalley et al., 2002). This has been hypothesized to be a result of rearing-induced changes in the sensitivity of the 5-HT_{1A} receptor (pre-synaptic autoreceptor) in certain regions of the rat brain. Rearing animals in an IC increases 5-HT_{1A} receptor functioning (marked by an increase in G-protein interaction) in the dorsal raphe nucleus of C57/B6 mice (Advani et al., 2007) as well as decreases 5-HT_{1A} binding in the frontal pole of the cortex, dentate gyrus, and the ventral hippocampus in male Long-Evans rats (Hellemans et al., 2005). An increase in 5-HT_{1A} receptor functioning has also been observed by Wright et al. (1991) as they reported that IC rearing produced a "supersensitivity" of the 5-HT_{1A} receptor in that both forepaw treading and flat body posture were significantly increased following injection of a 5-HT_{1A} agonist. Rasmuson et al. (1998) documented an increase in 5-HT_{1A} mRNA expression in the dorsal hippocampus of EC reared rats. Muchimapura et al. (2003) observed rearing in an IC to increase the sensitivity of presynaptic 5-HT_{1B} but not affect postsynaptic 5-HT_{1A} receptors in the hippocampus of Lister-Hooded rats. Further, rats reared in an IC have

a decreased turnover of 5-HT in the NAC suggesting that rearing animals in an IC has a rather profound effect on the serotonergic system (Heidbreder et al., 2000).

Differential rearing environments have been implicated in changes to the opioid system as well. An early study reported that rats raised in an IC for 44 days consumed significantly more morphine solution than those raised in a group housing condition (Alexander et al., 1981). Vanderschuren et al. (1995) investigated the effect of 7 days of social isolation on opioid receptor binding in several areas of the rat brain. Interestingly, rats housed in the IC exhibited an increase in opioid binding in the mPFC and the parafasicular area (Vanderschuren et al., 1995). More recently, Smith and colleagues (2003; 2005; 2008) have further characterized the effect of rearing condition, specifically rearing in an EC, on the mu (μ) and kappa (κ) opioid receptors. Rearing rats in an EC for 49 days increased the sensitivity of both the μ receptor (Smith et al., 2005) as well as the κ receptor (Smith et al., 2003) in male but not female rats (Smith et al., 2008). Delta (δ) opioid receptors do not appear to be affected by differential rearing conditions (Van den Berg et al., 1999).

Exposure to novelty has been shown to enhance cholinergic signaling in the hippocampus (Degroot et al., 2005) and choline acetyltransferase activity in the caudate increasing acetylcholine (ACh) synthesis compared to IC reared rats (Park et al., 1992). Additionally, Del Arco et al. (2008b) report that rearing rats in an EC decreases ACh efflux in the prefrontal cortex (PFC) in response to handling stress and introduction to an open field test compared to their IC reared counterparts. Along with these findings, Del Arco et al. (2008a) also found a significant decrease in D1 (dopamine) receptors in the PFC of EC rats. Other research on dopamine (DA) in the PFC has shown that dopamine transporter function (DAT) as well as DA metabolism is decreased in rats

reared in an EC in relation to those reared in an IC (Zhu et al., 2004). Similarly, Jones et al. (1992) reported that rats reared in an IC showed decreased dihydroxyphenylacetic acid (DOPAC) levels and subsequently increased levels of DA within the NAC and caudate and putamen, compared to SC reared rats, following an injection of *d*-amphetamine (2 mg/kg, s.c.). Post mortem analysis also revealed higher levels of *d*-amphetamine stimulated DA in the PFC of IC rats in relation to SC rats (Jones et al., 1992). Bowling et al. (1993) reported that rats reared in an EC for 39 days and injected with *d*-amphetamine (0.5 and 2 mg/kg s.c.) exhibited an increase in DA synthesis in the striatum following only the 2 mg/kg s.c. dose. Additionally, EC rats showed a decrease of DA metabolism in NAC (at both 0.5 mg/kg and 2 mg/kg s.c.). Further, Engleman et al. (2004) reported that Wistar rats housed singly in hanging wire cages exhibited greater sulpiride (D_2 agonist)-induced DA release in the NAC compared to rats housed two per cage in standard shoebox cages.

The behavioral correlates to neurochemical changes in EC rats have been documented in a number of ways, including decreased time to solve a Hebb-Williams maze (Murtha et al., 1990; Wainwright et al., 1993), enhanced performance in a Morris water maze (Tees, 1999), and an increase in novelty seeking, measured by head dipping behavior, in a hole board test (Fernandez-Teruel et al., 2002). Additionally, rearing rodents in an enriched environment increased the time to onset of Huntington's, Parkinson's, and Alzheimer's disease, epileptic seizures, and Fragile X syndrome (Faherty et al., 2005; Lazarov et al., 2005; Nithianantharajah & Hannan, 2006; Spires & Hannan, 2005). Further, rearing in an EC can reverse/eliminate some of the behavioral effects of fetal alcohol spectrum disorder (FASD) and autism in rodent models (Hannigan and Berman, 2000; Schneider et al., 2006).

Perhaps even more interesting are the findings that have occurred in relation to the effects of differential rearing environments on drug use and/or abuse in animals. Rats reared in an EC are more sensitive to rewarding and stimulating effects of amphetamine (Bardo et al., 1995; Bowling & Bardo, 1994), will self-administer less low dose amphetamine (Bardo, et al., 2001; Bowling, et al., 1993; Green et al., 2002), and exhibit a decrease in extinction time and an increase in reinstatement threshold for amphetamine maintained responding (Stairs et al., 2006) compared to IC reared rats. When provided access to cocaine, differentially reared rats exhibit disparate behaviors relative to the testing paradigm used. For example, when cocaine is provided in a two-bottle free-access situation in which the rats can consume the cocaine fluid (cocaine + water), EC rats consume more cocaine fluid than IC rats (Hill and Powell, 1976). However, when differentially reared rats are provided access to cocaine via operant responding and intravenous infusions, IC rats respond significantly more for cocaine than do their EC counterparts (Ding et al., 2005; LeSage et al., 1999; Schenk et al., 1987; Yajie et al., 2005) possibly due to a greater sensitivity to the stimulant effects of cocaine (Howes et al., 2000; Smith et al., 1997). Similar findings have been reported for heroin and/or morphine. Rats reared in a colonial housing condition (group housing) consumed less morphine than IC rats when it was available in solution (Alexander et al., 1981) which may be a result of the finding that IC rats are less sensitive to the reinforcing/pharmacological effects of morphine (Wongwitdecha & Marsden, 1996). When tested for operant self-administration acquisition, IC reared rats acquire responding for intravenous heroin faster than EC reared rats (Bozarth et al., 1989).

Differentially reared rats display neurochemical and behavioral differences in response to nicotine as well. Neugebauer et al. (2004) prenatally treated rats with

cocaine then, following weaning, reared them in either an EC or and IC to establish if there would be an interaction between prenatal cocaine, behavior, and mPFC DAT function. Interestingly, the only observable difference in DAT due to the interaction between differential rearing and prenatal cocaine treatment was found following a nicotine challenge which resulted in a decrease of DA clearance in the mPFC in EC relative to IC rats (Neugebauer et al., 2004). In non-prenatally cocaine exposed rats, Zhu et al. (2007) found the opposite in that EC rats exhibited an increase in DA clearance or an increase in the function of DAT whereas IC rats did not. Further, Green et al. (2003) showed that rearing in an EC reduced nicotine-induced hyperactivity (associated with a higher dose of nicotine: 0.8 mg/kg) compared to rats reared in an IC.

Thus, differential rearing environments affect every major neurotransmitter system and the effects of such rearing can be observed via numerous behavioral and neurochemical testing paradigms. Furthermore all of the major neurotransmitter systems have, in one way or another, been implicated in drug use, abuse, and/or addiction. Therefore, it is plausible, and has been shown to some extent, that differential rearing conditions affect drug consumption and/or the behavioral/motivational aspects of drug taking in rats to via neurochemical mechanisms. However, there are a limited number of studies focusing on the interaction between rearing environment and its affect on animal models of drug use/abuse (specifically alcohol use/abuse and alcoholism). The current research will contribute more knowledge to this area.

Differential Rearing Conditions (Ethanol)

Researchers have intermittently investigated the effects of differential housing/rearing environments on the consumption of alcohol for more than 3 decades.

The majority of studies over this time period have focused on housing density (e.g., social vs. isolate housing and crowding) and the effects of such environmental manipulations on alcohol consumption. The effect of post-weaning rearing conditions (as compared to studies focused on housing density) on alcohol consumption has received much less attention. The primary differences between housing density/crowding paradigms and differential rearing paradigms involve the time at which animals begin their housing/rearing and the time at which testing commences. For housing/crowding studies, animals usually arrive at the laboratory as adult rats and are housed in their respective condition for a short amount of time before beginning the experiment. On the other hand, for studies using differential rearing conditions, animals arrive at the laboratory immediately following weaning (21-30 days of age) and are reared in their respective condition (usually between 30 to 90 days) that involves no experimental testing. Thus, research using various housing/crowding conditions focuses more on the effects of such housing conditions on behavior as the animal lives in them (e.g., stress of overcrowding) while research using differential rearing conditions focuses more on the effect that the rearing conditions have on the development of the rat (e.g., brain development) and how such development affects adulthood behavior. The current research will focus on the effect of rearing conditions on the responding for and consumption of alcohol in alcohol preferring and non-preferring rats. However, both paradigms are important as the rats will be reared and housed (in adulthood) in their respective conditions.

An early study by Deatherage (1972) found that Long-Evans rats housed in an isolate condition for 30 days consumed significantly more 20% alcohol than rats housed in a social environment (6 per cage). However, in the same experiment an additional

group of isolate housed Long-Evans rats (30 day rearing period) did not differ in their consumption of 10% alcohol compared to socially housed animals (Deatherage, 1972). A total of 6 groups were utilized with each having access to only one of the three solutions (20% alcohol, 10% alcohol, or water) as their sole source of fluid for 24 hrs a day during testing (Deatherage, 1972). Rats in the social conditions were not separated during consumption testing and had equal access to 3 bottles (per cage) of their respective solution (20% alcohol, 10% alcohol, or water) making it impossible to control for individual variation in body weight and fluid consumption between rats in each social condition.

Heminway and Furumoto (1972) sought to identify whether crowding affected the consumption of alcohol in rats. Using three housing conditions (un-crowded: UC, moderately crowded: MC, and over-crowded: OC) researchers wanted to identify the effects of low, medium, and high population densities on alcohol consumption. At 15 weeks of age 72 random-bred albino rats were randomly assigned 12 per cage to one of the three conditions which corresponded to different circular cage dimensions (UC = 11.3 ft^2, MC = 6.1 ft^2, and OC = 2.5 ft^2). There were a total of 6 groups, 1 experimental (received alcohol) and 1 control (received water only) group for each cage size. Rats in each condition had access to a total of 4 bottles per cage positioned exactly 90° apart around the circumference of the cages. Two bottles in each experimental group contained alcohol solution (5% and 10% alcohol v/v respectively) while all other bottles in both groups contained water (Heminway & Furumoto, 1972). Bottle locations in the experimental groups were rotated daily so that both concentrations of alcohol were presented equally at every location. The rats in the MC housing condition consumed a significantly greater amount of alcohol than either of the other two groups. Furthermore,

there were no significant differences in alcohol consumption between the UC and OC groups. However, as with the previous experiment by Deatherage (1972), the Heminway and Furumoto (1972) study did not account for group drinking confounds. All rats in each group had access to the same 4 bottles and there was no possibility of teasing apart the individual variability in alcohol consumption between rats in each condition.

A similar set of studies was completed by Hannon and Donlon-Bantz (1975; 1976) in which they looked at housing conditions (crowding) on the consumption of alcohol. In the first study female Sragues-Dawley rats were housed either 8 per cage in a crowded condition or individually. Rats were obtained in adulthood (average weight: 266 g's) and placed into the two conditions and provided access to water or alcohol (10% v/v) flavored with milk. Rats housed in the crowded condition were observed to drink significantly more milk flavored alcohol than those housed in the isolated condition. A subsequent study expanded on the number of solutions available as well as the number of housing conditions. Hannon and Donlon-Bantz (1976) housed rats in either an isolated (I), medium population density (MG), or a large density condition (LG) and provided them access to three solutions: water, .01 M saccharin, and 10% (v/v) alcohol flavored with .01 M saccharin. Animals were placed individually into drinking cages for 3 drinking sessions (10 min in length) per day. Bottle positions were randomized daily to control for position bias and no food was available during the drinking sessions. Rats in the LG group consumed significantly more alcohol-saccharin solution than the I and MG groups which did not differ significantly from one another. Groups did not significantly differ in water or saccharin solution consumption. The findings of both studies suggest that animals housed in cages with higher population

densities tend to consume more alcohol than those housed in less population dense cages.

Kazmaier et al. (1973) performed one of the earliest studies investigating the effects of differential rearing environments on alcohol consumption. Six male Sprague-Dawley rats were equally divided amongst 3 rearing conditions (enriched: handled daily, normal: metal hanging cage with no handling, and deprived: suspended cage with opaque sides and no handling). Rats were weaned and then placed in their respective condition for a 25 day rearing period. At the conclusion of the rearing period all rats were housed in individual cages and alcohol consumption and water consumption was measured for 10 days. Drinking tube position was alternated daily to control for position bias. Kazmaier et al. (1973) reported that the groups did not differ significantly in the consumption of 12% alcohol.

Another study that concentrated more on adult housing conditions was completed by Parker and Radow (1974) and used a daily 8 hr housing period in which animals were either housed individually or in pairs. The researchers found that isolate housed Wistar rats consumed significantly more 25% alcohol than those housed in the social condition. In this experiment 12 rats were reared in a large colony cage for approximately 100 days (until 120 days of age). Rats were then equally divided into isolate and social groups. Both groups underwent food and fluid deprivation while being housed (1 per cage: isolate; 2 per cage: social) in 26 x 10 ¼ x 7 ¼ inch cages from 1200 hrs to 2000 hrs daily (Parker & Radow, 1974). To control for group drinking confounds, following the 8 hr housing/deprivation period all rats were placed (1 per cage) in smaller cages (8 x 10 ¼ x 7 ¼ in.) with access to water, 25% alcohol, and food from 2000 hrs to 1200 hrs the following day. This regimen of housing/testing continued

for 60 days with rats differing in 25% alcohol consumption only during the second half of the experiment. Upon examination of the adrenal glands of all 12 animals, Parker and Radow (1974) reported that isolation housing (for 8 hrs a day) produced hyperadrenalcorticism which may contribute to the increased alcohol preference and consumption in isolate reared rats.

Animals prenatally exposed to alcohol that were isolated following weaning (28 days of age) have also been observed to consume significantly more alcohol (5% v/v concentration) than their socially (2 per cage) reared, prenatally exposed counterparts (Buckalew, 1979). In the cited study, 3 pregnant dams (hooded rats) were given access only to 5% alcohol during gestation and lactation. The 15 weanling rats were reared in different environments and then tested for alcohol preference and consumption. During the 30 day rearing period, following weaning, all animals were given access to both water and 5% alcohol and consumption measures were taken every 3 days. All rats showed a preference for the alcohol solution compared to water, however, rats reared in the isolated condition consumed significantly more alcohol than rats reared in the social condition (Buckalew, 1979).

Using a slightly different paradigm, Ellison et al. (1979) and Kulkosky et al. (1980) looked at the differences in alcohol consumption between colonial housed versus isolate housed rats. Ellison et al. (1979) had the more elaborate colony cage of the two studies which housed 36 male Long-Evans rats (170 g's at the start of rearing) in an environment with 36 individual burrows, a behavior arena, and a feeding arena. The isolate condition consisted of 36 rats that were housed in stainless steel cages. These rats underwent all aspects of the experiment in the same manner as the colonial housed animals. Alcohol, 1% v/v (flavored with .05% anise), was available from rearing

day 1 and was gradually increased in concentration every 5 days until a 10% v/v concentration of alcohol was reached (Ellison et al., 1979). Feeding occurred in both environments for 1 hr per day and water was available ad libitum. Data collected over a 30 day period found that the isolate rats consumed significantly more 10% alcohol than those in the colonial housing condition (Ellison et al., 1979).

Kulkosky et al. (1980) randomly assigned 18 Long-Evans rats (9 male and 9 female; 60 days of age) to one of three rearing conditions: isolate (1 per cage), group housed (6 rats per cage), and “colonial” (6 rats per cage with several natural objects and a dirt floor; simulation of natural environment). Following a 25 day adaptation phase, all rats had access to water, alcohol (10% v/v), and flavored alcohol (.125% sodium saccharin, 3.0% glucose, 1.0% sodium chloride and 10% alcohol) solutions for 16 days (phase II) at the end of which the flavored alcohol solution was removed. The rats then had access to water and 10% alcohol for an additional 5 days (phase III). Over the course of both phases II and III, rats in the colonial housing condition consumed significantly less alcohol (g’s/kg) than rats in both the isolate and group housing conditions which were not significantly different from one another (Kulkosky et al., 1980). Thus, colonial housed rats consumed significantly less alcohol than rats in the other rearing conditions in both the Ellison et al. (1979) and Kulkosky et al. (1980) experiments.

However, in an additional study by Ellison (1981) using the same colonial and isolate housing conditions, rats in both groups did not significantly differ in alcohol consumption but did show differences in alcohol preference. Again, alcohol (1% v/v with .05% anise) was available from day 1 with the concentration of alcohol gradually increasing over the course of 30 days until it reached a final 10% concentration where it

remained for the duration of the experiment. Unlike the previous experiment, Ellison (1981) transferred colonial housed animals to isolation following 6 months of rearing. Following a 5 day habituation period for the colonial housed rats, fluid consumption was compared for an additional 14 days between the groups. Both groups consumed similar amounts of 10% alcohol but on average, the isolation reared animals showed a significantly greater preference for the alcohol solution compared to the colony reared animals. The authors suggest that this was due, in part, to the distribution of alcohol consumption in the colony housed animals as a few rats consumed a large quantity of alcohol and relatively little water while the majority of colony animals consumed primarily water and little to no alcohol (Ellison 1981).

Between the mid 1980's and the early 1990's Rockman and colleagues performed a series of experiments that investigated the effects of environmental enrichment on rats' proclivity to consume alcohol. In their studies, rats in an environmental enrichment condition (EC) were housed in a large cage with a number of novel objects (i.e., toys, pipes, running wheel, etc.). The objects were changed daily to promote a novel environment during the entire rearing period. The EC condition in these experiments represents a different rearing environment from the previously mentioned studies in that rats were constantly exposed to a novel environment whereas previous endeavors focused primarily on housing conditions (i.e., isolate, social, and/or colonial) where the environments remained static.

In their first study addressing the effects of rearing conditions on alcohol consumption, Rockman et al. (1986) reared male Wistar rats in either an EC or an IC (1 per cage) from weaning (21 days) for a period of 90 days. At the conclusion of the rearing period rats were placed individually in standard cages. Food was available for 2

hrs daily and the rats had access to two calibrated drinking tubes, one with water and the other with 3% v/v alcohol which was gradually increased to a 9% v/v alcohol concentration. Solution position was changed for each presentation to control for position bias. Alcohol intake for each rat was calculated in grams/kilograms of alcohol consumed as well as a ratio of mean percentage of total fluid intake for 24 days (Rockman et al., 1986). Contrary to what one might expect given the past literature on rearing conditions and alcohol consumption, it was reported that rats reared in the EC condition consumed significantly more 9% alcohol than those reared in the isolate condition (Rockman et al., 1986). These results were replicated in a later study as well (Rockman et al., 1988).

In their third study Rockman et al. (1989) made use of an additional 2 housing conditions at the conclusion of the 90 day rearing period. At the conclusion of the rearing period half of the rats from the EC and half from the isolate condition switched housing conditions and formed an additional two groups (4 groups total: enriched/enriched, isolated/isolated, enriched/isolated, and isolated/enriched). Interestingly, the EC/EC (enriched/enriched) consumed significantly more 9% alcohol than rats in the other three groups. Therefore, given these findings, Rockman and colleagues suggested that both post-weaning rearing conditions as well as adult housing conditions affect alcohol consumption in rats. Furthermore, an additional study by Rockman and Gibson (1992) added that the length or rearing condition also played a role in alcohol consumption as rats reared in an EC for 60 days did not significantly differ from those reared in an isolate, isolate/enriched, or enriched/isolate condition.

Using a slightly different paradigm than those already mentioned in this section, Wolffgramm (1990) examined the effects of social deprivation on alcohol consumption

in adult male Wistar rats. Rats (140 – 160 g's) were housed in either an individual cage (1 per cage), a "contact" cage (1 per cage with partial contact with 4 other rats), or a social cage (4 per cage); for the latter, rats were separated and placed into an isolated cage for one 24 hr period per week. All animals received water (4 bottles available) for the initial 4 weeks of the experiment. At the start of 5th week rats received access to water and alcohol solutions of 5, 10 and 20% v/v (4 bottles available). Overall, rats housed in the isolate condition consumed a significantly greater amount of alcohol (g's/kg) than the other groups eventually preferring the 20% solution over all other alcohol solutions available. Interestingly, during weeks 5-8, rats in the social caging/24 hr isolation condition were observed to consume significantly more alcohol during the 24 hr isolation period compared to any other group (during the 24 hr period only). However, this effect of social housing/24 hr isolate housing was no longer evident after the eighth week of testing. Rats in the isolated condition never showed this same attenuation of alcohol intake therefore, the IC group only showed increased ethanol intake compared to contact and SC groups following extended exposure to ethanol (Wolffgramm, 1990).

Schenk et al. (1990) examined the effect of both rearing and housing conditions on alcohol consumption in rats. Long-Evans rats at either 21 days of age (weaning) or 65 days of age were assigned to a social (4 per cage) or isolated condition (1 per cage). All rats were reared or housed in their assigned condition for a period of 84 days at the conclusion of which they were transferred to individual cages for acquisition and testing. During every other day of acquisition (17 days), rats were provided access to two bottles: one with water and another with alcohol starting at 2% v/v and gradually increasing to 10% v/v on day 17. After acquisition rats began a 20 day testing period

and were provided daily access to both water and 10% alcohol with consumption measures being taken every 24 hrs. Shenk et al. (1990) reported that rats reared in an isolated condition exhibited a significant preference for and consumption of 10% alcohol compared to rats in the social condition. Rats housed beginning at 65 days of age did not differ in alcohol consumption (Shenk et al., 1990).

Stress due to housing condition has also been examined as a potential influence toward elevated alcohol consumption. Using 56 day old male Wistar rats, Roske et al. (1994) housed animals in isolation (stress) or a "group" (non-stress) cage. Rats remained in their housing condition for 119 days and were then tested over the course of 3 weeks for alcohol preference and consumption using a two-bottle free-access paradigm (water available in one bottle, 10% alcohol v/v available in the other). According to the authors, rats housed in the isolated condition showed an increase in the "alcohol-preferring coefficient" and an overall increase in total volume consumed of both solutions compared to rats housed socially. Thus, Roske et al. (1994) conclude that housing rats in social isolation increases their preference for alcohol compared to their group housed counterparts.

Using the Maudsley inbred rat strain, Adams and Oldham (1996) exposed animals with an anxiogenic phenotype to a semi-natural, social, or isolated housing condition to explore the interaction between the genetic predisposition toward anxiety and differential housing conditions on alcohol consumption. Rats were 42 days of age at the start of the experiment and were either housed singly in a Wahmann cage (isolate; n = 11), 3 per cage in expanded stainless steel cages with nestbox (social; n = 12), or 8 per cage in a large semi-natural cage that contained plastic burrows and tunnels (semi-natural; n = 8) for a period of 16 weeks. Following the housing period all

animals were transferred into individual Wahmann cages for the remainder of the experiment. After a 2 week habituation period in the Wahmann cages rats underwent a free-access, two-bottle choice test (water and 10% alcohol v/v) for an additional 8 weeks. Interestingly, the rats originally housed in the semi-natural housing consumed significantly more alcohol than rats in the other two settings.

A rather interesting experiment by Hall et al. (1998a) investigated the effect of social (2 per cage) and isolated (1 per cage) rearing conditions on the consumption of alcohol in two rat lines (Fawn-Hooded and Wistar) simultaneously. Rats from both lines were obtained at 21 days of age and were randomly assigned to the two rearing conditions. The rearing period lasted for 60 days and was followed by a two-bottle free-access voluntary alcohol consumption test. Similar to many of the previously mentioned studies, rats had access to one bottle containing water and another containing alcohol with bottle positions switched daily to control for position bias. The initial concentration of the alcohol solution began at 2%, shifted to 4%, then to 8% and eventually 16% (all v/v). Each time the alcohol solution concentration was increased the authors waited until consumption at that concentration was stable before proceeding to the next concentration. Overall, the Fawn-Hooded rats consumed significantly more low concentration alcohol than Wistar rats. When the animals were presented with higher concentrations of alcohol there were no significant differences between the rat strains. However, rats reared in the isolate condition, regardless of strain, consumed significantly more high concentration alcohol than rats in the socially reared group (Hall et al., 1998a). These findings would suggest that rearing environment affects two different rat strains in a similar manner.

Research completed by Fernandez-Teruel et al. (2002) used the Roman high- and low avoidance rat lines (RHA/verh and RLA/verh) to explore the effects of enriched versus social rearing on the consumption of alcohol in rats that possess divergent predispositions for sensation/novelty seeking. A total of 60 male rats (15 RHA/verh and 15 RLA/verh in each housing condition) were reared from 30 days of age in either an EC (7-8 rats per cage) or an SC (2 per cage) for a 120 day rearing period. Following the conclusion of the 6 month rearing period the EC rats were removed from the enriched environment and housed in social cages for the remainder of the experiment. All rats remained in the social housing cages until they reached 20 months of age at which time testing commenced with a two-bottle saccharin/water test followed by a hole-board test, and finally an alcohol/water choice test. Animals were not provided the opportunity to consume alcohol until approximately 3 months following the start of testing (23 mo's of age). Alcohol testing involved a two-bottle choice between water or 10% (v/v) alcohol for 4 days. The data showed that rats reared in the EC consumed significantly more alcohol than those reared in the SC. This finding coincides with the results reported by Rockman and colleagues (1986; 1988; 1989) (where EC rats consumed more alcohol in general) and adds that rearing weanling rats in differential rearing conditions can produce enduring effects on alcohol consumption.

Another study using the Fawn-Hooded rat strain reared rats (21 days of age) in either a social (3-4 per cage) or isolate condition for 63 days (Lodge and Lawrence, 2003). All rats were then individually housed and subjected to an acquisition phase using a free-access, two-bottle choice paradigm with water in one bottle and 5% alcohol (v/v) in the other. At the conclusion of acquisition, if a rat had not exhibited a preference for the 5% alcohol solution, the rat was provided with two bottles of 5% alcohol as the

sole source of fluid on their cage for a period of 2 days to make sure all rats experienced the alcohol as well as to discern if they were non-preferring or avoiding the alcohol for other reasons (e.g., taste). Once animals had completed the acquisition phase the two-bottle paradigm was reinstated for all rats and measurements were recorded daily for 4 weeks. Lodge and Lawrence (2003) did not observe significant differences in alcohol consumption between socially or isolate housed Fawn-Hooded rats. The authors did, however, report that the rats reared in the isolate condition acquired a preference for 5% alcohol significantly faster than those reared in the social condition. The fact that isolate and socially reared Fawn-Hooded rats did not differ in consumption of a lower concentration of alcohol may be a function of the rat strain as the Hall et al. (1998a) paper also did not observe differences between socially and isolate reared Fawn-Hooded rats at lower concentrations of alcohol.

A study completed by Juarez and Vazquez-Cortes (2003) examined the effects of rearing rats in either a social or isolate condition as well as the effects of exposing rats to the opposite condition during rearing and adulthood. For this experiment male Wistar rats were reared in their respective condition for 10 days (25-35 days of age). There were 8 rats per group in 4 groups: isolate group (reared 1 per cage), social (reared 8 per cage), isolate/social (isolate rearing but switched to social every other day for 12 hr), and social/isolate (social housing but switched to isolate every other day for 12 hr). Additionally, during this rearing period, all rats were exposed to 8% alcohol (v/v) every other day for 12 hr. For rats that switched housing every other day, the drinking period coincided with the time spent in the opposite rearing condition. Following the 10 day rearing period with alcohol exposure, all rats were placed into a social condition (8 rats per condition; 4 groups) for 20 days. Following 14 days of exposure to the SC for

all rats, 24 hour two-bottle (8% alcohol and water) consumption testing occurred for 6 days. All rats were then housed in an isolate condition for the remainder of the experiment and once again alcohol consumption was measured using a 24 hr two-bottle consumption test for an additional 10 days (8% alcohol and water).

Rats maintained in the isolate condition for the entire rearing period were found to consume significantly more alcohol than those in the other three groups. However, during the adulthood social housing phase, the group reared in the social condition but switched every other day to the isolate condition consumed significantly more alcohol than any other group. Finally, there were no significant differences in alcohol consumption between groups during the isolate housing phase for the final 10 days of the experiment (Juarez & Vazquez-Cortes, 2003). The authors explain their data as a response to separation stress however, given that all rats were separated from their social counterparts at the conclusion of the experiment, this is unlikely. Furthermore, periadolescent alcohol exposure on the average of 8 g's/kg or more per testing session further confounds the findings of this experiment as several unexplored developmental changes could have occurred in the rats due to alcohol exposure during this period in turn affecting alcohol consumption (Sahr et al., 2004; Siciliano & Smith, 2001; Slawecki et al., 2001).

Another experiment completed in 2003 investigated the effects of differential rearing/housing on alcohol consumption in rats at a number of different starting ages at the beginning of the study. Yoshimoto et al. (2003) acquired male Kyoto-Wistar rats at 1, 4, 10, and 16 months of age and reared/housed them in aggregated (3-4 rats per group) or isolated (1 per group) conditions for 6 months. For 15 days following the rearing/housing phase all rats received water and 10% alcohol (v/v) in a two-bottle free-

access consumption test. Isolate and social rats that were 7 and 10 months old at the start of testing did not significantly differ in the amount of alcohol consumed between groups. However, older rats (16 months and 22 months old at the start of testing) in the social condition consumed significantly less alcohol than their isolate-housed counterparts. The authors reported a decrease in alcohol consumption among the aggregated housed rats as the isolate reared/housed rats consumed approximately the same amount of alcohol (g/kg) across all four age conditions.

Thorsell et al. (2005) used male Wistar rats at 46 days of age to investigate the effects of social versus isolate housing on alcohol consumption. Rats were housed either in pairs or singly and remained in their housing condition for a period of 7 weeks prior to testing. Following the 49 day housing period rats were provided with 10% alcohol (v/v) as their sole source of fluid for 3 days to facilitate later drinking behavior. After the 3 day introduction period all rats underwent a two-bottle free-access paradigm with water in one bottle and an alcohol solution that was gradually increased in concentration, in the other: 2% alcohol for 3 days, 4% alcohol for 3 days, 6% alcohol for 10 days, and 8% alcohol for 5 days (Thorsell et al., 2005). During the entire fade saccharin was present in the alcohol drinking solution at a concentration of 0.1% (w/v). Results showed that the only significant difference between social and isolate housed rats in this experiment was during Day 1 of forced 10% alcohol intake where the socially housed rats consumed significantly more alcohol than the isolate housed animals.

Recent research in our lab has found that differential rearing conditions affect both operant responding for and the consumption of 10% alcohol (Deehan et al., unpublished data; Deehan et al., 2007). In both of these studies, rats were reared in an enriched condition (EC), social condition (SC), or an impoverished condition (IC) for a

period of 90 days. The EC is comparable to that described in the summary of the experiments by Rockman and colleagues in that animals housed in this condition resided in a large cage with 14 novel objects (toys) which were changed and rearranged daily. Rats in the EC were also handled daily during the scheduled toy change. The SC is a pair housed condition in standard shoebox cages and the rats are handled once per week during bedding changes. Unlike all of the previous studies discussed in this section, the IC represented an impoverished environment in which rats are housed 1 per cage in hanging metal cages. It is termed an impoverished environment as rats in this condition were not only isolated from other rats but lived on a wire mesh floor for the length of the experiment. Additionally, rats in this condition were not handled during the rearing period.

Using the EC, SC, and IC rearing conditions, it was found that male Long-Evans rats reared for 90 days in an IC responded significantly more for 10% alcohol solution (v/v) than those reared in an EC during 30 min sessions and significantly more than both the EC and SC groups during 60 min sessions (Deehan et al., 2007). Furthermore, rats reared in the IC were the only subjects to show a clear preference for alcohol during a two lever preference test in which water was present on one lever and alcohol on the other. Once the 90 day rearing period was complete all rats began operant responding acquisition for 20% sucrose. Once the rats had acquired responding for the sucrose solution they were faded using a modified fading procedure similar to that reported by Samson (1986). This fading procedure involved gradually fading the alcohol concentration (0%-10%) up while simultaneously fading the sucrose concentration down (10%-0%). The conclusion of the fading procedure marked the start of access to 10% alcohol solution with no sucrose present. Following alcohol

testing rats were faded in reverse order ending on a 10% sucrose solution (w/v). Interestingly, the difference observed between the EC, SC, and IC groups in operant responding for alcohol was specific to only the 10% alcohol solution as the groups did not significantly differ in responding while sucrose was present in the solution.

In a subsequent study using male Long-Evans rats, identical rearing conditions, the same 90-day rearing period, and the same modified sucrose fading procedure our lab has found that IC animals consume significantly more 10% alcohol (v/v) during a limited access consumption test than their EC reared counterparts (Deehan et al., unpublished data). Upon the completion of the 90 day rearing period all rats were individually caged for a 15 min limited access consumption test each morning (approximately 1 hr out of rearing condition) and returned to their home condition for the remainder of the day. During the beginning of the experiment all rats had access to 10% sucrose during the limited access testing period. Following 5 days of 10% sucrose access, sucrose was faded out and the alcohol faded in to reach a final concentration of 10% alcohol. The testing continued for 10 days with 10% alcohol as the only solution available during limited access testing. Alcohol was then faded back out of the solution and sucrose back in until a 10% sucrose solution was available for the final 5 days of the experiment. Much like the first study, the groups did not differ in amount consumed while sucrose was present in the solution. However, IC rats consumed significantly more 10% alcohol solution than rats in the EC group. The SC group did not significantly differ from the EC or IC groups in limited access consumption.

The conclusion of the limited access testing marked the beginning of 24 hour access testing. During 24 hr access rats were maintained individually in standard shoebox cages. All rats had access to 2 bottles (water and 10% alcohol) for 24 hours a

day for 10 days. Consumption levels were measured daily. Groups did not significantly differ during a 24 hr free-access two-bottle choice test. This finding does not agree with the reports from Schenk (1990) and Hall et al. (1998a) both reported isolate reared animals consuming more alcohol than those reared in a social condition when provided 24 hour access. While Hall et al. (1998a) used two different rat strains than the strain used in our laboratory, Schenk (1990) also used male Long-Evans rats and reared them for a comparable rearing period of 84 days. However, given that spillage was a common occurrence and consumption measures were not recorded at intervals less than 24 hrs further research will be needed.

To date, few investigators have examined the effect of differential housing environments on alcohol consumption in rats genetically bred for alcohol consumption. Ehlers et al. (2007) represents the only article that approximates this line of research in that they investigated the effects of differential housing conditions (social and isolate) on alcohol consumption in adult P and NP rats (average of 47 days old upon arrival). The results of the Ehlers et al. (2007) study seem promising as adult, isolate housed P rats (one per standard laboratory cage) consumed significantly more alcohol than group housed P rats (2 per standard laboratory cage). Given the direction of this finding it would appear that group housing exerts a type of protective factor against increased alcohol consumption in rats that consume a lot of alcohol.

Rather than housing mature rats in differential conditions, the current work focused on rearing weanling P and NP rats in differential rearing conditions during the critical period of brain development (days 21 – 45). Additionally, the current research investigated both alcohol consumption as well as operant responding for alcohol as the use of these two paradigms has been shown previously to support disparate levels of

consummatory versus motivated behavior for cocaine (Hill & Powell, 1976; LeSage et al., 1999; Schenk et al., 1987; Yajie et al., 2005) and alcohol (Rockman et al., 1986; Rockman et al., 1988; Rockman et al., 1989; Deehan et al., 2007).

Ethanol Consumption (Liking/Consummatory) vs. Ethanol Responding (Wanting/Appetitive)

It has been argued that there is a dissociation between the subjective hedonic experience of a substance and the amount of motivated behavior that an animal will exhibit to obtain said substance. Such differences in hedonic experience have been observed with substances ranging from sucrose to drugs of abuse (cocaine, alcohol, etc.). Robinson and Berridge (1993) termed the subjective hedonic experience of a substance (drug, sucrose, etc.) "liking" and the motivational properties of a substance experienced by the animal "wanting" or "incentive salience." That is, for an animal, the greater the hedonic experience of a particular substance the more the animal can be said to like the substance. Similarly, the more the animal wants (craves) the substance, the more the animal will be motivated to obtain the substance (e.g., through operant responding). Liking and wanting are usually positively correlated in that if an animal likes a substance, they will be motivated to obtain that substance but it is important to note that this is not always the case (i.e., an animal may consume more of a substance or "like" it but may not respond/work to obtain it) as they rely on different neurological components and both can occur without conscious experience (Berridge, 1996).

Liking

Recent research has shown that liking can be directly linked to opioid transmission in the nucleus accumbens (NAC) and the ventral pallidum (VP) and γ-amino-butyric-acid (GABA) signaling in the parabrachial nucleus of the brain stem

(Higgs & Cooper, 1996; Kelley & Berridge, 2002; Pecina & Berridge, 1996; Pecina & Berridge, 2000; Pecina & Berridge, 2005; Pecina et al., 2006; Smith & Berridge, 2005; Söderpalm & Berridge, 2000a; Söderpalm & Berridge, 2000b). Using microinjections of DAMGO (a selective μ agonist), naloxone (a general opioid antagonist) and the immunohistochemical technique of Fos plumes, Pecina and Berridge (2000; 2005) located a 1 mm^3 "hedonic hot spot" as well as a small "hedonic cold spot" in the medial shell of the nucleus accumbens (NACms). Using the same technique, a slightly smaller (.84 mm^3) "hedonic hot spot" was also discovered in the ventral pallidum (Kelley & Berridge, 2002; Smith & Berridge, 2005). Microinfusions of DAMGO into the NACms hot spot increased "liking reactions" to sucrose 4 fold while also decreasing "disliking reactions" to quinine (an aversive bitter taste) by 75% compared to vehicle microinfusions (Pecina et al., 2006). In a similar manner, microinfusions of DAMGO into the VP "hot spot" produce double the "liking reactions" to sucrose compared to vehicle controls (Smith and Berridge, 2005). However, when DAMGO was infused into the "cold spot" exactly the opposite occurred as "liking reactions" were suppressed to levels below that of the vehicle controls (Pecina & Berridge, 2005). It is important to note that the NACms and the VP are not autonomous structures in the mediation of liking. The VP serves as the primary output of the NAC and DAMGO stimulated increases in "liking reactions" in both structures can be blocked via simultaneous microinfusions of naloxone into the opposing structure (Pecina et al., 2006; Smith & Berridge, 2005).

Hedonic "liking reactions" and food consumption are altered via GABA manipulation in the brain stem as well. An early study by Berridge (1988) reported that chlordiazepoxide (a benzodiazepine receptor agonist) increased "positive ingestive reactions" in mesencephalic decerebrate rats. Microinjections of benzodiazepine into

the 4th ventricle, but not lateral ventricle, increased hedonic reactions and feeding (Pecina & Berridge, 1996). More specifically, microinjections of midazolam (a benzodiazepine receptor agonist) directly into the parabrachial nucleus of the pons increased feeding behavior in a similar manner as reported by Pecina and Berridge (1996) (Higgs and Cooper, 1996; Söderpalm & Berridge, 2000b). This effect was blocked when flumazenil (a benzodiazepine receptor antagonist) was microinjected prior to the midazolam microinjection (Higgs & Cooper, 1996).

Recent research has now also implicated the cannabinoid receptors in the NAC as influential in taste processing. Mahler et al. (2007) observed an amplification of positive hedonic responses to sucrose when they microinjected Anandamide (a cannabinoid receptor agonist) into the shell of the NAC. Through the use of Fos plumes, Mahler et al. (2007) were able to localize the effects of Anandamide to a "hot spot" in the dorsal medial shell of the NAC overlapping with the already established opioid hot spot mentioned above. Speculation has implicated a number of other structures throughout the brain to be involved in the processing of hedonics for taste stimuli and these are currently being explored further.

Wanting

The physiological components of "wanting" are controlled by primarily the same structures and neurochemicals as "liking" (i.e., the NAC and the VP; opioids and GABA) with a few additions. For example, microinjections of DAMGO (a μ opioid agonist) into the NAC shell region served to increase food consumption (Pecina & Berridge, 2005). This effect was more global in nature than that seen with "liking" as food consumption was increased by microinjections throughout the shell including the "hedonic cold spot" (Pecina & Berridge, 2005). Further, bilateral microinjections of morphine (an opioid

agonist) and muscimol (a $GABA_A$ agonist) into the NAC shell both increased feeding behavior (Reynolds & Berridge, 2001; Reynolds & Berridge, 2002; Söderpalm & Berridge, 2000a). However, wanting has also been found to be affected by alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor manipulation in the NAC suggesting a role for glutamate (Glu) in the process as well. Reynolds and Berridge (2003) microinjected DNQX (a specific AMPA/Kainate receptor antagonist) in the shell of the NAC and witnessed an increase in feeding behavior similar to that seen with the $GABA_A$ agonist (Söderpalm & Berridge, 2000a). Others have reported similar findings using AMPA/Kainate antagonists in the NAC shell (Kelley & Swanson, 1997; Maldonado-Irizarry et al., 1995; Stratford et al., 1998).

An additional glutamaterigic mechanism has been discovered in the lateral hypothalamus (LH). Microinjections of the N-Methyl-D-aspartic acid (NMDA) receptor antagonist D(-)-AP-5 has been shown to attenuate NAC shell GABA evoked feeding behavior (Stratford & Kelley, 1999). However, focus on the LH as a mediator of feeding/motivated behavior is by no means a new concept. Berridge and Valenstein (1991) electrically stimulated the LH and observed an increase in feeding behavior (wanting) without a corresponding increase in the hedonic responses (liking) as rats exhibited more aversive than ingestive reactions (Berridge & Valenstein, 1991). Follow up studies have since found a “wanting” projection directly from the NAC to the LH whereby DAMGO and naloxone infusions into the NAC increase or decrease Fos expression in the LH respectively (Smith & Berridge, 2007).

Microinjections of DAMGO into the posterior and central but not anterior VP produced an increase in food consumption over vehicle controls (Smith & Berridge, 2005). Additionally, microinjections of Bicuculline ($GABA_A$ antagonist) increased food

intake throughout the VP yet failed to increase “liking reactions” to sucrose (Smith & Berridge, 2005). The VP, being the main output for the NAC, does receive “wanting” information from the NAC but the relationship is not reciprocal as with “liking.” Microinjections of DAMGO into the VP and NAC both increase “wanting” and microinjections of naloxone into the NAC suppresses DAMGO mediated increases of “wanting” in the VP (Smith & Berridge, 2007). However, naloxone does not suppress DAMGO stimulated “wanting” in the NAC suggesting that the NAC bypasses the VP when it comes to motivated behavior (Smith & Berridge, 2007).

And finally, the neurotransmitter dopamine (DA) has been implicated in “wanting” in the NAC as well. Wyvell and Berridge (2000; 2001) found that microinjections of amphetamine (a potent DA agonist) selectively increased sucrose “wanting” but not sucrose “liking.” Two elegant studies have illuminated the DA/wanting relationship further. Pecina et al. (2003) made use of hyperdopaminergic mutant mice (mice with 70% more synaptic DA due to a decrease in DA transporter functioning) to show an increase in synaptic DA was accompanied by an increase in sucrose “wanting” but not sucrose “liking.” Conversely, Robinson et al. (2005) used DA-deficient mice (tyrosine hydroxylase knockout mice) to show that DA is necessary for mice to want a sucrose reward but does not affect hedonic reactions to sucrose. Thus DA represents a neurotransmitter that is necessary for an animal to exhibit “wanting.”

Up to this point the discussion has been focused on “liking” and “wanting” in relation to the hedonic reactions to and consumption of food and sucrose. Because the mesocorticolimbic reward pathway is involved both with the processing of food and drug reward these constructs can also be applied to drug taking (consumption) and drug responding (motivation) as well. With this, the term “incentive sensitization” needs to be

introduced whereby an animal (human, rat, etc.) experiences a “sensitization” (increase in the sensitivity) of their reward system (the reward pathway) (Berridge & Robinson, 2003). This is thought to eventually cause an increase in the incentive salience or “wanting” of the drug (Robinson & Berridge, 2008; Berridge & Robinson, 2003). The increase in incentive salience following prolonged or repeated exposure is believed to be a direct result of changes occurring in the underlying neural circuitry responsible for reward processing and as such, can remain intact over long periods of time (Berridge & Robinson, 2003; Robinson & Berridge, 2008)

As alcohol is normally consumed orally, the consumption of alcohol is affected by how the solution tastes (hedonic impact). However, incentive salience also comes into play as prolonged and/or repeated experience with alcohol can cause one to become addicted to or “want” alcohol more. Thus, both “liking” and “wanting” can have a role in whether an animal takes that next drink. Furthermore, alcohol has been shown to affect GABA, opioid, DA, endocannabinoid, and Glu systems within the mesocorticolimbic reward pathway affecting, in one way or another, every structure implicated in “liking” and/or “wanting” (Koob, 2004; for reviews see Maldonado et al., 2006; Oswald & Wand, 2004; Vengeliene et al., 2008).

To examine fully the relationship between the positive and negative experiences of the hedonic and motivational aspects of alcohol, researchers have developed a number of useful models. For instance, home-cage drinking and preference paradigms measure the amount of alcohol solution consumed over a given period of time (usually between 15 min to 24 hrs) either alone or in comparison to another readily available fluid (usually water; preference test) (Richter & Campbell, 1940). Consumption tests, whereby an animal need only consume fluid from a sipper tube/bottle, are believed to

activate a consummatory system which is involved only with the maintenance and termination of drinking (Cunningham et al., 2000). On the other hand, an operant paradigm in which an animal is required to work (e.g., press a lever) for access to a substance is believed to enact an appetitive system that serves to motivate and direct behavior to obtain the substance, at which time the consummatory processes take over (Cunningham et al., 2000).

As mentioned previously, the results from past research investigating the effects of differential rearing conditions on alcohol consumption are inconsistent. Some investigators have reported an increase in alcohol consumption in EC rats compared to IC rats. Other researchers have found that IC rats consume more alcohol than EC or socially housed rats while other researchers have reported no differences between differentially housed/reared animals (for review see Rearing/Alcohol section). Furthermore, as has been reviewed in preceding sections, differential rearing environments have been found to affect opioids, dopamine, and the structures intricately linked to both liking (consumatory) and wanting (appetitive) in animals. Therefore, the current research made use of a consumption paradigm as well as an operant paradigm to fully characterize the effect of differential rearing environments on both the consumatory (liking) and appetitive (wanting) processes in selectively bred P and NP rats.

General Summary, Research Aims, and Hypotheses

While researchers in the early to mid 20th century were divided as to whether nature or nurture played the primary role in how an animal develops, the current view is that of a synergistic relationship between the two. This view can be carried over to virtually every field in neuroscience, including addiction research, and, when utilized,

has led to the discovery of several aspects of a wide range of addictive disorders. One such disorder is alcohol abuse/alcoholism which affects millions of people in the US. Within the field of alcohol research, specifically rodent research, several selectively bred and inbred rodent lines have been developed to model the disorder of alcoholism. These lines have been thoroughly evaluated, granted some more thoroughly than others, and utilized to probe the genetic components of alcoholism. A number of researchers have also explored the effect of environmental manipulations, both in adolescent and adult rats, on alcohol abuse and alcoholism and observed changes to drinking and responding behavior for alcohol. The current research made use of the selectively bred P and NP rat lines as well as differential rearing conditions to more fully characterize the interactions between nature and nurture and the effect such interactions have on an animals' proclivity to consume and/or work for alcohol.

The reasoning behind using the selectively bred P and NP rat lines for the current research is that they are the only rodent model of alcoholism that fully meets all 7 criteria that have been put forth for an animal model of alcoholism. Further, a thorough literature review resulted in only one article that focused on the effect of housing environment on alcohol consumption in P and NP rats (Ehlers et al., 2007). However, Ehlers et al. (2007) did not utilize an environmental enrichment paradigm that includes an impoverished condition (1 per cage; hanging metal cage) which created the question: would there be more pronounced differences in alcohol consumption in rats reared during development in conditions that represent more of an extreme (e.g., IC vs. EC)? Additionally, would rearing in such conditions affect operant responding for alcohol in P and NP rats in the same manner it affected operant responding for alcohol in Long-Evans rats as previously reported by our laboratory (Deehan et al., 2007)?

These questions were pertinent as several lines of research have described variable alcohol consumption when rats are either reared or housed in differential environments (for review see Differential Rearing/Housing Section).

The research questions for the current experiments are founded on a wide body of research implicating differential rearing conditions in the changes of several brain structures and virtually every major neurotransmitter system as well as several observable behaviors. As reviewed above, differential rearing conditions affect several neurotransmitter systems and have been linked to the consumption of and operant responding for alcohol. Furthermore, a number of these neurochemical systems have been documented as being part of the causal factors for the disparate drinking and responding behaviors of the P and NP rat lines. Therefore, it is possible that differential rearing conditions may affect the consumption of and responding for alcohol in selectively bred P and NP rats.

The current research made use of both consumption and operant paradigms to analyze whether differential rearing conditions affect consumatory behavior (or liking) and/or operant behavior (motivation to obtain alcohol; wanting) in P and NP rats. Past research focused on differential rearing conditions and alcohol consumption or responding illuminated a number of disparate findings between these two paradigms (e.g., Hill & Powell, 1976; compared to: LeSage et al., 1999; Schenk et al., 1987; Yajie et al., 2005; and: Rockman and colleagues, 1986; 1988; 1989 compared to: Deehan et al., 2007). Additionally, differential rearing conditions affect the same neurotransmitter systems and brain structures that underlie both “liking” and “wanting.” The majority of “liking” research presented above deals primarily with oral-facial reaction (i.e., taste reactivity test) and “wanting” is discussed in terms of consumption as well as motivated

behavior. However, for the current research we will not utilize a taste reactivity paradigm. It is believed, however, that by first depriving the rats of fluid prior to testing each day (for limited access consumption) and then starting them all with a 10% sucrose solution and gradually fading to a 10% ethanol solution (Experiment 1) or by starting them with 6% ethanol and gradually fading them up to 10% ethanol (Experiment 2), all rats will have adequate experience with each solution. Therefore, rats that did or did not "like" the 10% ethanol solution should have shown increased or decreased consumption respectively (Experiment 1). Similarly, rats that did or did not "want" the 10% alcohol solution should have displayed increased or decreased operant responding respectively (Experiment 2). Provided past research, it was a possibility that differential rearing conditions might have affected alcohol consumption and operant responding for alcohol differently. Thus, by using both paradigms the current research was able to more thoroughly examine the effect of differential rearing conditions on the consumatory and motivated behaviors toward ethanol.

The current research reared both P and NP rats in an IC, EC or SC for a period of 60 days. Following this rearing period, for Experiment 1, rats were first tested for limited-access consumption of 10% alcohol. Immediately following limited-access testing, rats were evaluated for 24-hr consumption and preference for ethanol as well. For Experiment 2, rats were reared in the same manner but at the conclusion of their rearing period rats were tested for operant responding for ethanol. Rats also underwent an operant preference test during concurrent access to two levers (ethanol lever vs. water lever) that was used to assess the extent to which rearing conditions affect the genetically predisposed motivation to obtain alcohol as a reinforcer in P and NP rats.

During Experiment 3, rats were assessed for their motivation to operantly respond for 10% sucrose.

Research Aims and Hypotheses (Experiment 1; Ethanol Consumption)

The first experiment focused on illuminating any interactions between differential rearing environments and the genetic predisposition to consume/prefer or not consume/not prefer alcohol in P and NP rats. It was hypothesized that P and NP rats raised in differential rearing conditions would exhibit differences in both limited- and free-access alcohol consumption and preference. Overall, it was hypothesized that rearing rats in the EC condition would act to significantly decrease limited- and free-access consumption as well as free-access preference of alcohol in both P and NP rats compared to IC reared P and NP rats while SC reared animals would not significantly differ from either of these groups respective to genetic predisposition. Given that the current research utilized both P and NP rats as well as three rearing conditions, several interactions were also predicted. In stepwise fashion, the hypotheses were as follows: 1) EC P rats would consume significantly less alcohol than IC P rats while SC P rats would not significantly differ from the EC P or IC P groups. 2) Rearing in an EC would act to attenuate while rearing in an IC would act to potentiate alcohol consumption such that EC P and IC NP rats will not significantly differ in alcohol consumption or alcohol preference. 3) EC NP rats would consume significantly less alcohol than the IC P, IC NP, SC P, EC P groups as well as exhibit significantly less of a preference for alcohol compared to these groups. The EC NP group would not differ significantly from the SC NP group.

Research Aims and Hypotheses (Experiment 2; Operant Responding: Ethanol)

The second experiment attempted to uncover any differences in motivation to obtain alcohol in differentially reared P and NP rats. Again, given the nature of the compound effect of both genetic predisposition and rearing environment, an interaction similar to that hypothesized in Experiment 1 was hypothesized for Experiment 2. Further, provided that past research in our laboratory has identified a similar trend between alcohol consumption and responding for alcohol in outbred rats, hypotheses for Experiment 2 will be identical in their predictions to those made for Experiment 1.

Research Aims and Hypotheses (Experiment 3; Operant Responding: 10% Sucrose)

The third experiment sought to determine if differentially reared P and NP rats would exhibit differences in their motivation to respond for 10% sucrose. Previous research has observed differences in the preference for sweet solutions in P versus NP rats (Stewart et al., 1994). Therefore, the current experiment hypothesizes that the P line will respond significantly more for sucrose than the NP line. However, it is hypothesized that differential rearing conditions will not significantly affect sucrose responding in P and NP rats.

Methods

General

Subjects

57 Male Alcohol Preferring (P) and 60 Alcohol Non-Preferring (NP) rats (Indiana University, Indianapolis, IN, USA) were obtained at 21 days of age for two experiments. All rats were provided with ad libitum access to food and water throughout the

experiment except during limited access testing (Experiment 1) and operant testing (Experiments 2 and 3). The animal colony room will be on a 12h light/12h dark cycle (lights on at 7:00 am). All protocols and procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) before the start of the experiments.

Environmental Conditions

Following arrival, each animal was randomly assigned to one of three rearing conditions for the entire study: environmental enrichment condition (EC), social condition (SC), or the impoverished condition (IC). Rats reared in the EC condition were housed in a large metal cage (60 x 120 x 45 cm) with cohorts (12 per cage). The EC also contained 14 plastic objects (children's toys, large plastic bowls, etc.). Each day all rats were handled and removed from the EC cage so that 7 of the 14 objects could be replaced with new objects while the remaining 7 objects were arranged in a novel configuration. SC rats were housed 2 per cage in a standard shoebox cage with wire rack top. SC rats were handled once a week during their scheduled bedding change. The SC condition was used as it conforms to the guidelines for typical housing conditions set in the NIH *Guide for the Care and Use of Laboratory Animals* (1996). Rats in the IC group were housed 1 per cage in hanging metal cages (17 x 24 x 20 cm) with a wire mesh floor and front panel, and solid metal sides, back, and top. The hanging metal cages were chosen for the current study as food, water, and bedding changes can be completed without handling the rats for the entire rearing period (21-81 days of age).

Apparatus

Operant responding for ethanol (Experiment 2) and sucrose (Experiment 3) were conducted in standard two-lever operant chambers (Colbourn Instruments, Allentown, PA, USA) that are contained within sound attenuated, ventilated (by fan), environmental boxes. Each chamber contains a house light (2.8 watts) that remained illuminated for the duration of the trial. The inside of the chamber has a dimension of 28 x 21 x 21 cm with plexiglass walls on the front and back and aluminum ceiling and side walls. Two levers, 12 cm apart, are located on the same aluminum side wall directly above their corresponding dipper trough where a dipper can present 0.1 ml of response contingent fluid. Upon dipper presentation a light (1 watt) was illuminated within the dipper trough for the duration of the dipper presentation (4 seconds). A desktop computer with the MedPC program installed controlled all operant chamber functions as well as recording all lever presses and dipper presentations. For all sessions during ethanol and sucrose testing the response requirement was set at a Fixed Ratio 1 (FR-1) schedule except during ethanol FR increase testing and progressive ratio testing. All operant sessions were 60 minutes in length.

Solutions (Sucrose and Ethanol)

Throughout all experiments solutions were mixed fresh daily. Ethanol concentrations were calculated as volume/volume (ethanol/deionized water) and sucrose concentrations were calculated as weight/volume (sucrose/deionized water).

Experiment 1 (10% ethanol consumption)

Subjects

A total of 19 male P and 19 male NP rats from the 65th generation were used for Experiment 1.

Procedure

Following the 60 day rearing period rats were tested in standard shoebox cages using a modified sucrose fading procedure comparable to that outlined by Samson (1986). Sucrose fading and alcohol testing consisted of: 10% sucrose for 5 testing sessions, 10% sucrose/2% alcohol for 3 sessions, 5% sucrose/5% alcohol for 3 sessions, 2% sucrose/10% alcohol for 3 sessions, and finally 10% alcohol for 10 sessions. At the conclusion of 10% alcohol testing all rats were faded in reverse order back to 10% sucrose to observe any changes to baseline sucrose consumption. The current research made use of a limited access paradigm whereby rats received a 15 minute limited-access test session each morning. Animals were provided 1 hr of water access in the afternoon to ensure proper hydration.

At 0900 each day all rats were weighed and placed, 1 per cage, in standard shoebox cages. At 0930, after each animal had been weighed and placed in their cage, 50 ml centrifuge tubes fitted with rubber stoppers and stainless steel drinking spouts were filled with the appropriate solution and attached to the cages. After 15 minutes of drinking time, the tubes were removed and the amount of solution consumed by each rat was recorded. After the final centrifuge tube was removed and consumption level recorded, rats were placed back in their home environment. At 1430 each day during limited-access testing, water bottles were placed on the cages for a 1 hr drinking period.

Bottles were made available in the same order every day and the EC cage had 4 bottles fixed across the front of the cage so that all 12 rats were provided adequate opportunity to drink during this period. Following the 1 hr water access, bottles were removed and all rats were deprived until the start of limited-access testing the following day. This procedure was continued for the duration of limited-access sucrose fading and 10% alcohol testing as described above.

After limited-access testing all animals were then removed from their home environment and placed, 1 per cage, into standard shoebox cages for the remainder of the experiment (10 days). In this phase rats were tested using a free-access paradigm where both a bottle of water and a bottle of 10% alcohol were available 24 hr a day. Bottles were placed on the cages at 0900 the day following the termination of limited-access testing. Every 24 hr, following the initial bottle placement, bottles were removed from the cages and the rats and bottles were weighed and the data were recorded. After being weighed, each rat was placed back into their cage and the bottles were replaced with bottle positions alternating each day to control for place preference. Additional bottle weighing occurred at the start of and 2 hours into the rats' dark cycle (7:00 p.m. and 9:00 p.m.) to assess, as much as possible, drinking associated with the onset of the rats' active period. The room was illuminated via low-level red lighting to maintain the circadian rhythm of the rats. Free-access testing continued for a total of 10 days to ascertain if differentially reared P and NP rats exhibit differences in 24 hr consumption of and preference for 10% alcohol.

Data Analysis

Data from the limited-access fading procedure, limited-access 10% alcohol testing, and 24 hr free-access choice testing was analyzed using separate mixed

factorial Analyses of variance (ANOVA) for each fading/testing concentration. For all mixed factorial ANOVA's performed, Rearing Condition (EC, IC, and SC) and Line (P and NP) represented the between subjects factors and Session represented the within subjects factor. Data from 24 hr free-access testing with 10% alcohol was analyzed utilizing a 3 x 2 x 10 (Line x Rearing x Session) mixed factorial design ANOVA for alcohol consumption. Further, alcohol preference was calculated (via preference scores: amount of ethanol consumed divided by total fluid consumed) and analyzed by a separate 3 x 2 x 10 (Line x Rearing x Session) mixed factorial ANOVA. Tukey's post hoc tests were used to examine significant treatment effects observed. All analyses (for Experiment 1 and succeeding experiments)were conducted using SPSS 12.0 for Windows®.

Experiment 2 (Operant responding for 10% ethanol)

Subjects

Experiment 2 used 20 male P rats from the 66th generation and 22 male NP rats from the 65th generation as subjects.

Procedure

After the 60 day rearing period all rats were deprived of water by removing the water bottles from their cages for 16 hrs prior to the first operant session. This marked the start of magazine training during which animals were placed in the operant chambers without levers present and received non-contingent presentations of 6% ethanol randomly from both dippers. After 5 days of magazine training the levers were replaced and all animals underwent acquisition of operant responding for 6% ethanol on both levers. An animal was considered to have successfully acquired operant

responding for 6% ethanol when they made at least 50 lever responses in one session. Following acquisition all animals remained fluid deprived while undergoing a fading procedure whereby the concentration of ethanol was gradually increased from 6% to 10%. Briefly, the maintenance phase consisted of 3 days of 6% ethanol, 3 days of 8% ethanol, and finally 10 days of 10% ethanol. After the final day of the maintenance phase with 10% ethanol all rats were provided home cage water ad libitum for the remainder of the experiment.

All rats were then provided concurrent access to 10% ethanol on one lever and water on the other for a total of 38 days (days 21 – 59). During days 21 – 45 10% ethanol was available on the right lever while water was available on the left lever. On day 46, ethanol was switched to the left lever and water to the right lever. The lever switch was completed to ascertain if animals would track the ethanol solution and to make sure that their responding was not due to a lever bias. On day 60 all animals underwent extinction on the water (right) lever for 3 days in which the right lever became inactive and a response on the lever would result in no fluid delivery. For the next 4 days (days 64 – 66) the FR schedule on the ethanol lever was increased from an FR 1 to an FR 2. The FR schedule was then increased to an FR 5 for days 67 – 73. After the final day of FR 5 testing all animals underwent progressive ratio (PR) schedule testing. The animals were first subjected to a shallow PR for 10% ethanol by which the response requirement increased by 2 following every third ethanol delivery as incrementing the PR in this way has been shown to be effective at measuring breakpoint in the P and NP lines (Rodd et al., 2003; Oster et al., 2006). The rats started on an FR 2 schedule and following three ethanol deliveries (6 operant responses: 2 per delivery) the schedule was increased to an FR 4. Following 3 more ethanol deliveries

the FR schedule was increased to an FR 6 and so on. For the next 5 days, all rats were required to respond on a steeper logarithmic PR for 10% ethanol. For the logarithmic PR, rats started at an FR 2 and following each subsequent ethanol reinforcer the schedule increased logarithmically according the formula published by Richardson and Roberts (1996): 5*(exp(R*.012)) – 5. Following 5 days of testing on the logarithmic PR for 10% ethanol all rats were then tested on a logarithmic PR with15% ethanol for 5 days. At the conclusion of PR testing with 15% ethanol there were an additional 5 days of logarithmic PR testing with 10% sucrose. For all PR testing, the session length was 60-minutes and the last response requirement that the animal successfully completed was considered their break point. After PR testing with 10% sucrose, the experiment concluded and data was analyzed.

Data Analysis

Data for the acquisition of 6% ethanol responding was analyzed using a univariate ANOVA with Rearing and Line as the between groups variables. Data collected during fluid deprived responding where ethanol concentration was increased from 6% - 10% was analyzed using a mixed factorial analysis of variance (ANOVA). Data collected during non-deprived responding where the rats had concurrent access to water on one lever and 10% ethanol on the other was analyzed using 2 mixed factorial ANOVA's (one for each lever). For all mixed factorial ANOVA's, Rearing (EC, IC, and SC) and Line (P and NP) represented the between subjects factors and Session represented the within subjects factor. The concurrent access data was analyzed using 3 x 2 x 38 (Rearing x Line x Session) mixed factorial design ANOVA for each lever. Preference testing data was analyzed with six 2 x 38 (Lever by Session) mixed ANOVAs (one analysis per line per rearing condition). The FR increase data was

analyzed using a series of 3 separate mixed ANOVA's, one for each FR value. Progressive ratio data was analyzed using a series of four 3 x 2 x 5 (Rearing x Line x Session) mixed ANOVA's, one for each concentration/solution. Tukey's post hoc tests were used to analyze significant main effects.

Experiment 3 (Operant responding for 10% sucrose)

Subjects

Experiment 3 used 18 male P rats from the 65th generation and 19 male NP rats from the 64th generation as subjects.

Procedure

Following the 60 day rearing period all rats underwent an identical procedure for magazine training and operant responding acquisition as described for Experiment 2. The only difference was that magazine training and acquisition sessions occurred with 10% sucrose. Like Experiment 2, an animal was considered to have successfully reached criterion of acquisition of operant responding for 10% sucrose when they completed at least 50 lever responses in one session. Following successful acquisition of responding fluid deprivation was no longer continued. After the final animal had acquired, all rats underwent testing for maintenance of 10% sucrose responding where 10% sucrose was available on both levers and total number of responses were measured for 10 sessions.

Data Analysis

The 10% sucrose acquisition data was analyzed using a univariate ANOVA with Rearing and Line as the between groups variables. Data collected during the

maintenance of responding for 10% sucrose was analyzed using a 3 x 2 x 10 (Rearing x Line x Session) mixed factorial analysis of variance (ANOVA). For the analysis on 10% sucrose responding Rearing and Line represented the between groups variables while session represented the within groups variable. Tukey's post hoc tests were used to analyze significant main effects.

Results

Experiment 1

Sucrose and Sucrose/Ethanol Fading Consumption

After the 60-day rearing period all rats were first tested for limited-access consumption of 10% sucrose and then gradually faded from 10% sucrose to 10% ethanol. Differential rearing conditions did not significantly affect limited-access consumption of 10% sucrose (see Figure 1A.) or any of the other fading concentrations (10% sucrose/2% ethanol, 5% sucrose/5% ethanol, and 2% sucrose/10%ethanol; see Figure 1B.) presented during the fading procedure that preceded access to 10% ethanol. There was, however, a significant Line difference throughout 10% sucrose testing and sucrose/ethanol fading as P rats consistently consumed more of the solutions than NP rats [Fs>= 10.13, ps<.05].

As shown in Figure 1C, after the 10 days of access to 10% ethanol, a significant difference was evident among the groups in the consumption of the 2% sucrose/10% ethanol. A mixed analysis of variance (ANOVA) performed on g/kg ethanol consumed during the 3 days of access to 2% sucrose/10% ethanol revealed a significant effect of Rearing [$F(2,32) = 3.99$, $p<.05$, $\eta_p^2 = .44$] and a significant effect of Line [$F(1,32) = 9.91$, $p<.05$, $\eta_p^2 = .49$] but no Rearing by Line interaction [$F(2,32) = .07$, $p=.93$], no effect of

Day [$F(2,64) = .08$, $p=.93$], no Rearing x Day interaction [$F(4,64) = .60$, $p=.66$], and no Rearing x Line x Day interaction [$F(4,64) = .41$, $p=.80$]. A Tukey's post hoc analysis found that the IC P rats consumed significantly more ethanol than the EC P rats ($p<.05$) and the IC NP group consumed significantly more ethanol than the EC NP group ($p<.05$). The SC P and SC NP groups did not differ significantly from the other P and NP groups respectively. During the remainder of the fade out procedure rearing was not a significant factor in the consumption of the final 2 fading solutions (5% sucrose/5% ethanol and 10% sucrose/2% ethanol; see Figure 1C) or the 10% sucrose solution presented at the end of limited access testing (right panel, Figure 1A). There was an effect of line present throughout the ethanol fade out [$F(1,32) = 41.39$, $p<.05$, $\eta_p^2 = .56$] and the final sucrose testing [$F(1,32) = 55.97$, $p<.05$, $\eta_p^2 = .64$]. Again, P rats consistently consumed more solution than the NP rats.

Limited-Access Consumption of 10% Ethanol

Differential rearing conditions had a significant effect on limited-access ethanol consumption (see Figure 2). A 3 x 2 x 10 (Rearing x Line x Day) mixed ANOVA revealed a significant effect of Rearing [$F(2,32) = 10.39$, $p<.05$, $\eta_p^2 = .40$], a significant effect of Line [$F(1,32) = 48.04$, $p<.05$, $\eta_p^2 = .60$], but no significant Rearing x Line interaction [$F(2,32) = .22$, $p=.44$]. There was a significant effect of Day [$F(14,488) = 14.82$, $p<.05$, $\eta_p^2 = .32$] as virtually all groups increased their consumption of ethanol over the course of limited access testing. There was also a significant Rearing x Day interaction [$F(28,488) = 2.05$, $p<.05$, $\eta_p^2 = .11$], however, there was no significant interaction of Line x Day [$F(14,488) = 1.37$, $p=.18$] nor Rearing x Line x Day [$F(28,488) = .65$, $p=.92$]. A Tukey's post hoc analysis indicated that P rats reared in the IC consumed significantly more 10% ethanol solution than P rats in the EC group ($p<.05$).

The SC P group did not differ significantly from the IC P or EC P groups. There was a similar trend of limited-access ethanol consumption across the NP groups; however, it did not reach statistical significance. Throughout limited-access ethanol testing there was a significant line difference as P rats consumed significantly more ethanol than NP rats.

Free Access Consumption and Ethanol Preference

For 24-hr free-access, a 3 x 2 x 10 (Rearing x Line x Day) mixed ANOVA performed on g/kg ethanol consumed (see Figure 3A) did not reveal a significant effect of Rearing [$F(2,32) = 2.26$, $p=.12$] nor a significant Rearing x Line interaction [$F(2,32) = .84$, $p=.44$] but did show a significant effect of Line [$F(1,32) = 34.15$, $p<.05$, $\eta_p^2 = .52$] and a significant effect of Day [$F(9,288) = 8.20$, $p<.05$, $\eta_p^2 = .20$]. There was no significant Rearing x Line interaction Rearing x Day interaction [$F(18,288) = .77$, $p=.72$], Line x Day interaction [$F(9,288) = .99$, $p=.45$] nor Rearing x Line x Day interaction [$F(18,288) = .57$, $p=.92$]. Planned comparisons using a Tukey's post hoc test showed that the IC P group consumed significantly more ethanol than the EC P group ($p<.05$) while the SC P group did not significantly differ from either of these groups. Differential rearing environments did not exert a significant effect on the 24-hr free access consumption of ethanol in the NP rat line. It appears that over the course of the 10 testing days all rats slightly decreased EtOH consumption with the NP line consuming less than 1 g EtOH/kg BW, something that has been previously observed in this rat line. As in the limited-access testing, P rats consumed more ethanol overall than NP rats.

Differential rearing conditions also significantly affected 24-hour ethanol preference (see Figure 3B), the data of which look quite similar to the absolute amount of ethanol ingested. A 3 x 2 x 10 (Rearing x Line x Day) mixed ANOVA, conducted on

ethanol preference scores revealed a significant effect of Line [$F(1,32) = 33.13$, $p<.05$, $\eta_p^2 = .51$], but no effect of Rearing [$F(1,32) = 2.58$, $p=.09$] or Rearing x Line interaction [$F(2,32) = .93$, $p=.40$]. There was a significant effect of Day [$F(9,288) = 5.10$, $p<.05$, $\eta_p^2 = .14$] and a significant Day x Line interaction [$F(9,288) = 4.15$, $p<.05$, $\eta_p^2 = .12$] but no Rearing x Day interaction [$F(18,288) = .72$, $p=.79$] nor a Rearing x Day x Line interaction [$F(18,288) = .99$, $p=.47$]. For planned comparisons, a Tukey's post hoc test found that the IC P group preferred ethanol over water to a significantly higher degree than EC P rats ($p<.05$). The SC P group did not differ in their preference for ethanol from the IC P or EC P groups. There were no significant differences in ethanol preference among the NP groups.

Experiment 2

Fluid deprived acquisition and maintenance of operant responding for EtOH

Figure 4 presents the mean number of sessions required by each group to acquire the operant responding criterion. There was no significant effect of Rearing [$F(2,39) = .28$, $p=.76$] or Line [$F(5,36) = .32$, $p=.90$] on the acquisition of operant responding for 6% ethanol. Following successful acquisition of operant responding for 6% ethanol all rats were gradually presented with increasing concentrations of ethanol from 6% ethanol to 10% ethanol on both levers (3 days of 6% ethanol, 3 days of 8% ethanol, and 14 days of 10% ethanol). A mixed ANOVA did not reveal a significant effect of Rearing [$F(2,36) = 1.75$, $p=.19$], Line [$F(1,36) = 1.39$, $p=.25$], or a Rearing by Line interaction [$F(2,36) = 1.05$, $p=.36$] for operant responding across the fading procedure (see Figure 5). For the final day of fluid deprivation (Day 20), the mean ($\pm$ SEM) number of responses for 10% ethanol on both levers for each group were: IC P =

117.29 ± 7.96, IC NP = 115.88 ± 6.15, EC P = 114.71 ± 10.72, EC NP = 98.38 ± 7.01, SC P = 129.17 ± 25.81, SC NP = 102.67 ± 7.62.

Operant responding for 10% ethanol and ethanol lever preference

After the final day of responding for 10% ethanol in a mildly fluid deprived state, all rats were provided with water on their home cages 24-hours a day for the remainder of operant testing. Over the course of the next 39 days ethanol was available on one lever while water was available on the other to assess non-fluid deprived operant responding. As shown in Figure 6A, a mixed ANOVA performed on ethanol lever responding found a significant effect of Rearing [$F(2,36) = 14.40$, $p<.05$, $\eta_p^2 = .44$], Line [$F(1,36) = 47.50$, $p<.05$, $\eta_p^2 = .57$], as well as a significant Rearing x Line interaction [$F(2,36) = 10.10$, $p<.05$, $\eta_p^2 = .36$]. The analysis also revealed a significant effect of Session [$F(38,1368) = 18.03$, $p<.05$, $\eta_p^2 = .33$], Session x Rearing interaction [$F(76,1368) = 3.18$, $p<.05$, $\eta_p^2 = .15$], Session x Line interaction [$F(38,1368) = 10.54$, $p<.05$, $\eta_p^2 = .23$], and Session x Rearing x Line interaction [$F(76,1368) = 2.81$, $p<.05$, $\eta_p^2 = .14$]. A Tukey's post hoc analysis showed that the IC P rats responded significantly more on the ethanol lever than either the EC P or SC P rats ($p<.05$). Also, the SC P rats responded significantly more on the ethanol lever than the EC P rats ($p<.05$). Differential rearing conditions did not significantly affect ethanol lever responding in NP rats.

The mixed ANOVA performed on water lever responding (Figure 6B) did not find a significant effect of Rearing [$F(2,36) = 1.43$, $p=.25$], Line [$F(1,36) = .74$, $p=.40$] nor a significant Rearing x Line interaction [$F(2,36) = 2.20$, $p=.13$]. There was, however, a significant effect of Session [$F(38,1368) = 2.35$, $p<.05$, $\eta_p^2 = .06$] and a significant Session x Rearing interaction [$F(76,1368) = 1.76$, $p<.05$, $\eta_p^2 = .09$]. Statistical analyses

revealed that there was not a significant Session x Line interaction [$F(38,1368) = .85$, $p=.72$] nor a Session x Rearing x Line interaction [$F(76,1368) = 1.03$, $p=.42$]. Upon closer examination of the data it appears that the several minimal increases and decreases in responding for water across the sessions were responsible for the significant effect of Session and that the groups look essentially the same.

For ethanol lever preference, a series of 6 mixed ANOVAs were run using Lever as the between subjects factor and Session as the within subjects factor. Rats in the IC P group displayed a significant preference for the ethanol lever (Figure 7A) compared to the water lever [$F(1,12) = 34.54$, $p<.05$, $\eta_p^2 = .74$]. There was also a significant effect of Session [$F(38,456) = 6.20$, $p<.05$, $\eta_p^2 = .34$] and a significant Session x Lever interaction [$F(38,456) = 7.26$, $p<.05$, $\eta_p^2 = .38$]. Alcohol-preferring rats reared in the SC also displayed a significant preference for the ethanol (Figure 7B) lever over the water lever [$F(1,10) = 14.56$, $p<.05$, $\eta_p^2 = .59$] along with a significant effect of Session [$F(38,380) = 5.14$, $p<.05$, $\eta_p^2 = .34$] and a significant Lever x Session interaction [$F(38,380) = 5.62$, $p<.05$, $\eta_p^2 = .36$]. As shown in Figure 7C, animals in the EC P group did not exhibit a significant preference for the ethanol lever [$F(1,12) = 1.71$, $p=.22$], yet , there was a significant effect of Session [$F(38,456) = 2.53$, $p<.05$, $\eta_p^2 = .17$] and a significant Lever x Session interaction [$F(38,456) = 1.86$, $p<.05$, $\eta_p^2 = .13$]. As can be seen in Figures 8a through 8c differential rearing conditions did not significantly affect ethanol lever preference in NP rats [$Fs <= 5.1$, $ps >= .05$].

Active (ethanol) versus inactive lever responding (FR increasing)

After the 39th day of concurrent access to ethanol and water, the water lever was put on extinction and the rats could then only receive reinforcement (ethanol) for responding on the left lever. Subsequently the number of responses required to obtain

one ethanol reinforcer was increased gradually from an FR 1 (Days 60 – 62) to an FR 2 (Days 63 – 66) and then an FR 5 (Days 67 – 73). A series of 3 separate mixed ANOVAs were used to analyze ethanol responding data for each FR schedule (see Figure 9). During FR 1 responding (water lever extinction), a 3 x 2 x 3 (Rearing x Line x Session) mixed ANOVA found a significant effect of Rearing [$F(2,36) = 6.38$, $p<.05$, $\eta_p^2 = .26$], Line [$F(1,36) = 29.02$, $p<.05$, $\eta_p^2 = .45$], and a Rearing x Line interaction [$F(2,36) = 7.63$, $p<.05$, $\eta_p^2 = .30$]. However, the analysis did not reveal a significant effect of Session [$F(2,72) = .16$, $p=.85$] nor any significant within subjects interactions [$Fs<= 2.07$, $ps>=.09$]. A Tukey's post hoc test revealed that the IC P group responded significantly more on the active lever than the EC P group ($p<.05$). The SC P group did not significantly differ from either the IC P or the EC P groups. Interestingly, the Rearing x Line was due to the fact that only the IC P group significantly differed from the NP groups. The NP groups did not significantly differ in active lever responding.

For data collected during the FR 2 schedule a 3 x 2 x 4 (Rearing x Line x Day) mixed ANOVA showed a significant effect of Rearing [$F(2,36) = 14.66$, $p<.05$, $\eta_p^2 = .45$], Line [$F(1,36) = 56.63$, $p<.05$, $\eta_p^2 = .61$], and a significant Rearing x Line interaction [$F(2,36) = 15.34$, $p<.05$, $\eta_p^2 = .46$]. The analysis failed to reveal a significant effect of Day [$F(2,36) = .78$, $p=.51$] or any within subject interactions [$Fs<= 1.65$, $ps>=.14$]. However, a Tukey's post hoc analysis found that the IC P group responded significantly more on the active lever than the SC P and EC P groups ($p<.05$). Additionally, the SC P group responded significantly more on the active lever than the EC P group ($p<.05$). There were no significant differences in responding on the active lever among the NP groups.

A final 3 x 2 x 7 (Rearing x Line x Day) mixed ANOVA analyzing the 7 days of responding during the FR 5 schedule found a significant effect of Rearing [$F(2,36) = 12.98$, $p<.05$, $\eta_p^2 = .42$], Line [$F(1,36) = 61.99$, $p<.05$, $\eta_p^2 = .63$], as well as a significant Rearing x Line interaction [$F(2,36) = 11.59$, $p<.05$, $\eta_p^2 = .39$]. For FR 5 responding the analysis also found a significant effect of Day [$F(6,216) = 2.15$, $p<.05$, $\eta_p^2 = .06$], a significant Day x Rearing interaction [$F(12,216) = 2.68$, $p<.05$, $\eta_p^2 = .13$], a significant Day x Line interaction [$F(6,216) = 3.82$, $p<.05$, $\eta_p^2 = .10$], as well as a significant Day x Rearing x Line interaction [$F(12,216) = 2.91$, $p<.05$, $\eta_p^2 = .14$]. A Tukey's post hoc test revealed that the IC P group responded significantly more on the active lever than either the SC P or the EC P rats ($p<.05$). Further, the SC P rats responded significantly more on the active lever than the EC P group ($p<.05$). Differentially reared NP rats did not significantly differ in active lever responding.

For inactive lever responding during FR testing, a 3 x 2 x 14 (Rearing x Line x Day) mixed ANOVA was performed. The analysis revealed non-significant effects of Rearing [$F(2,36) = 2.39$, $p=.06$], Line [$F(1,36) = .96$, $p=.33$], and the Rearing x Line interaction [$F(2,36) = .35$, $p=.71$]. There was however, a significant effect of Day [$F(13,468) = 3.96$, $p<.05$, $\eta_p^2 = .10$] and a significant Day x Line [$F(13,468) = 2.35$, $p<.05$, $\eta_p^2 = .10$] interaction. Yet, there was no significant Day x Rearing interaction [$F(26,468) = 1.44$, $p=.07$] nor a Day x Rearing x Line interaction [$F(26,468) = .61$, $p=.94$]. After further examining the data, it appears that the significant effect of Day was primarily driven by an increase in the variability of the behavior of all groups during FR 5 responding. Further, the significant Day x Line interaction looks to be a result of a large increase of inactive lever responding on the first day of FR 5 testing by all three P rat groups.

Progressive ratio responding for ethanol and sucrose

After the FR schedule increase tests, rats were subjected to a shallow progressive ratio (PR) schedule in which the response requirement started at 2 and increased by 2 after every three reinforcers earned. Figure 10 (Left Panel) shows the 5 days of shallow PR testing where a 3 x 2 x 5 (Rearing x Line x Day) mixed ANOVA resulted in a significant effects of Rearing [$F(2,36) = 11.57$, $p<.05$, $\eta_p^2 = .39$], Line [$F(1,36) = 60.30$, $p<.05$, $\eta_p^2 = .63$] and Rearing x Line interaction [$F(2,36) = 7.14$, $p<.05$, $\eta_p^2 = .28$]. A Tukey's post hoc test revealed that the IC P rats had a significantly higher break point on the shallow PR schedule than the EC P rats ($p<.05$). The SC P group, while it did not differ significantly from the IC P group, did exhibit a significantly higher break point than the EC P group ($p<.05$). Interestingly, the EC P group did not exhibit a significantly different break point than rats in all three NP groups. There was also a significant effect of Day [$F(4,144) = 4.87$, $p<.05$, $\eta_p^2 = .12$] but no significant within groups interactions [$Fs <= 1.75$, $ps >= .09$]. The NP groups did not significantly differ in break point on the shallow PR schedule.

Immediately after the shallow PR all animals were placed on a steeper logarithmic PR with access to 10% ethanol (Figure 10; Middle Panel) for an additional 5 days. A 3 x 2 x 5 (Rearing x Line x Day) mixed ANOVA on the data collected during logarithmic PR testing revealed a significant effect of Rearing [$F(2,36) = 9.43$, $p<.05$, $\eta_p^2 = .34$], a significant effect of Line [$F(1,36) = 47.89$, $p<.05$, $\eta_p^2 = .57$] and a significant Rearing x Line interaction [$F(2,36) = 9.68$, $p<.05$, $\eta_p^2 = .35$]. However, there were no significant effects of day or within group interactions [$Fs <= 2.10$, $ps >= .08$]. A Tukey's post hoc test showed that the trend observed in the shallow PR continued throughout the steeper PR as the IC P and SC P groups exhibited significantly higher break points

than the EC P group (ps<.05). The IC P and SC P groups did not significantly differ from one another. Also, the break point for the EC P group did not significantly differ from any of the NP groups. The NP groups did not significantly differ in their break point for 10% ethanol.

After the logarithmic PR for 10% ethanol all animals were kept on the same PR schedule for an additional 5 days with access to 15% ethanol (Figure 10; Right Panel). Another 3 x 2 x 5 (Rearing x Line x Day) mixed ANOVA revealed significant effects of Rearing [$F(2,36) = 8.10$, $p<.05$, $\eta_p^2 = .31$], Line [$F(1,36) = 37.67$, $p<.05$, $\eta_p^2 = .51$] and the Rearing x Line interaction [$F(2,36) = 6.92$, $p<.05$, $\eta_p^2 = .28$]. Analyses did not reveal any significant within group effects [Fs<=1.59, ps>=.13]. A Tukey's post hoc analysis found that the IC P group exhibited a significantly higher break point for 15% ethanol than rats in the EC P group ($p<.05$). The SC P group did not significantly differ in break point for 15% ethanol from either the IC P group or the EC P group. The EC P group did not significantly differ from any of the NP groups. Furthermore, there were no significant differences in break point for 15% ethanol between the three NP groups.

A final phase of logarithmic PR testing was carried out with 10% sucrose following 15% ethanol PR testing. Similar trends to what was observed during ethanol PR testing were present as a 3 x 2 x 5 (Rearing x Line x Day) mixed ANOVA revealed a significant effects of Rearing [$F(2,36) = 9.01$, $p<.05$, $\eta_p^2 = .33$] and Line [$F(1,36) = 18.66$, $p<.05$, $\eta_p^2 = .34$] as well as a significant Rearing x Line interaction [$F(2,36) = 5.53$, $p<.05$, $\eta_p^2 = .24$]. A Tukey's post hoc analysis showed that the IC P group had a significantly higher break point than the EC P group ($p<.05$). The SC P and EC P groups did not significantly differ from one another nor all of the NP groups. The three NP groups did not significantly differ in break point. Upon a closer examination of the

data it appears that the Rearing x Line interaction is being driven by an increase in average break point for both the EC NP and IC NP groups over the course of the 5 days of testing. By the final day both the EC NP and IC NP group means are slightly above that of the EC P group.

Experiment 3

Acquisition and maintenance of operant responding for 10% sucrose

Experiment 3 was completed to observe whether differential rearing conditions affected the acquisition of and operant responding for sucrose in P and NP rats. Figure 12 shows the average number of sessions it took the groups to reach criterion (50 responses for sucrose). A one-way ANOVA performed on the average number of days to criterion revealed a significant effect of Line [$F(1,31) = 14.50$, $p<.05$, $\eta_p^2 = .21$] but did not find a significant effect of Rearing [F(2,31)=1.95, p=.13], nor a Rearing x Line interaction [F(2,31)=.81, p=.45]. In general, NP rats took longer to acheive criterion than P rats in acquiring responding for sucrose. For the maintenance of operant responding for 10% sucrose (Figure 13), a 3 x 2 x 10 (Rearing x Line x Session) mixed ANOVA did not find a significant effect of Rearing [F(2,31)=1.71, p=.20] nor a significant Rearing x Line interaction [F(2,31)=.41, p=.67]. However, the analysis did reveal a significant effect of Line [$F(1,31) = 10.22$, $p<.05$, $\eta_p^2 = .25$] as P rats generally responded at a higher rate than the NP rats.

Discussion (Experiment 1)

Data from Experiment 1 indicate that differential rearing conditions affect limited- and free-access ethanol consumption as well as ethanol preference in the Indiana University alcohol-preferring (P) rat line but not the non-preferring (NP) line. Alcohol-

preferring rats reared in an IC for 60-days consumed significantly more ethanol during limited- and free-access testing and exhibited a significantly higher preference for ethanol over water compared to P rats reared in an EC. The SC P rats did not significantly differ from either the EC P or the IC P rats. There was a similar pattern observed for the NP line during limited-access testing and early on in free-access, however, the differences did not achieve statistical significance.

The results are of interest as the rats selected for both the P and NP lines are weaned and reared/housed one per cage up to and during the ethanol consumption/preference selection process which occurs about 3 weeks after weaning (Lumeng et al., 1977). As mentioned previously, the critical development period in which the rat brain undergoes changes due to differential rearing experiences extends to post-natal day 45 (Ennon & Morgan, 1977; Renner & Rosenzweig, 1987; Robbins et al., 1996). Thus, it is possible that the individual rearing of rats during this time may have played a part in the increased ethanol consumption exhibited by animals selected as preferring rats. However, the NP line was also reared individually and selected due to their lack of consumption. It may be the case that during the early selection process of the NP line, the rats classified as non-preferring possessed an innate resistance to the effects of rearing in an IC. The underlying mechanism(s) that contribute to the low levels of ethanol consumption in the NP line do not appear to be as affected by differential rearing conditions as those in the P line. This is supported by the findings of Experiment 1 where rearing in an IC slightly increased ethanol consumption among the NP groups. However, it would appear that differential rearing conditions are not able to significantly affect ethanol consumption in the NP animals.

Overall, it would appear that the consumption levels of the IC P rats are consistent with levels thoroughly documented in standard housed P rats (Li et al., 1987). Given this finding, it would seem that rearing P rats in an EC is offering protection, via neurological changes during rearing, against increased ethanol consumption. Many studies have reported that IC rearing increases ethanol consumption (Deatherage, 1972; Hall et al., 1998a; Parker & Radow, 1974; Roske et al., 1994; Schenk et al., 1990). However, in the current study, not only did the IC P rats show comparable ethanol consumption to standard housed P rats but rearing the NP line in an IC failed to significantly increase ethanol consumption above that of the other two housing conditions. It is possible that the rats in the IC P group reached a ceiling effect in the amount of ethanol they could consume but this is unlikely as previous research has shown that with 24-hour access, P rats have been found to consume over 5 g/kg BW per day (Li et al., 1987). In the current study, during free-access testing, the average consumption of the IC P group was just below 5 g/kg BW per day. Furthermore, while there was an overall effect of line throughout testing, the EC P group did not significantly differ from the IC NP and SC NP groups during limited-access testing. For free-access and preference testing the EC P group did not differ from any of the NP groups. This would suggest that rearing in an EC is acting to decrease ethanol consumption and preference to the extent that P rats reared in an EC exhibit comparable consummatory behavior to the NP line. Therefore, it seems that rearing P rats in an EC is acting to attenuate ethanol consumption as compared to the idea that rearing P rats in an IC is increasing ethanol consumption. It is important to note that throughout testing (including both fading periods) the P line exhibited a significantly

higher consumption of, and preference for, ethanol compared to the NP line regardless of rearing condition.

While EC rearing significantly decreased ethanol consumption in P rats in the current study, differential rearing conditions did not affect the consumption of 10% sucrose or the majority of the fading solutions for any of the groups. There was a significant effect of rearing for the first solution available following 10% ethanol testing as IC P rats consumed significantly more 10% ethanol/2%sucrose solution than EC P rats; also, IC NP rats consumed significantly more of the solution than EC NP rats. With the addition of 2% sucrose in the solution, all rats slightly increased their consumption. Overall though, this effect is most likely a carry over from 10% ethanol testing as the concentration of ethanol remained the same and the concentration of sucrose in the solution was low. It is possible that the differences between the IC NP and EC NP rats became more pronounced as the solution became a bit more palatable with the addition of 2% sucrose. Yet, once the concentration of sucrose is increased to 5% and the concentration of ethanol is decreased to 5% the effect of rearing condition in both lines disappeared. An effect of line was present during both sucrose testing periods and throughout both fading periods as P rats consumed significantly more solution than NP rats regardless of rearing condition.

Discussion (Experiment 2)

Differential rearing conditions significantly affected operant responding for 10% ethanol in the P rat line. Rats from the P line reared in an IC for a period of 60-days responded significantly more for 10% ethanol than P rats reared in either the EC or SC groups. Further, P rats in the SC group responded significantly more for ethanol than P rats reared in the EC. Interestingly, the effect of rearing was selective to non-deprived

responding only. Rearing rats in an IC, EC, or SC did not significantly affect the acquisition of 6% ethanol responding or operant responding for any of the ethanol concentrations (6% - 10%) during the maintenance phase of the experiment where the animals were in a mildly fluid deprived state. Differential rearing conditions did not affect responding for 10% ethanol in the NP groups. Additionally, there was no significant effect of rearing condition on water responding in the P or NP lines.

When comparing the overall responding levels exhibited by each of the groups during the 39 days of concurrent water and ethanol access, rearing P rats in an EC protected these animals from increased levels of ethanol self-administration (as observed in the IC P and SC P groups). It is believed that the data from Experiment 2 represent an effect of EC rearing rather than IC rearing for a number of reasons. For instance, responding levels exhibited by the IC P group were comparable to operant responding levels that had been previously observed in hanging cage, individually housed P rats (Samson et al., 1998). Also, rearing NP rats in an IC failed to increase operant responding above that of the other two rearing conditions. Granted, rearing NP rats in an EC did not significantly decrease ethanol responding to levels that were lower than the other two groups. Thus, due to the low levels of responding for ethanol exhibited by the NP group, it is difficult to draw any hard conclusions. Even though statistical analyses did not show a significant effect of rearing for the NP groups, a closer look at the preference testing data shows an ethanol lever preference for all of the NP groups toward the end of testing (days 52 – 59). As can be seen in Figure 8, this preference looks to be slightly larger in the IC NP and the SC NP groups compared to the EC NP group. This is an observation that would add further support for the protective nature of rearing in an EC.

Another argument for why rearing in an EC is protective is that the EC P rats did not show a significant preference for the ethanol lever compared to the water lever during concurrent access. Past research has found that alcohol-preferring rats will readily choose to respond for ethanol over water in a two lever choice paradigm (McBride & Li, 1998). Furthermore, during the FR increase, following concurrent access testing, the EC P group did not significantly increase their response rates as the requirement increased, yet, both the IC P and SC P groups did. Finally, EC P rats in Experiment 2 did not significantly differ in response rate compared to all three NP groups throughout concurrent access, FR increase testing, and PR testing. Here rats that have been selectively bred to consume/prefer ethanol and reared in an EC are exhibiting similar levels of appetitive behaviors toward 10% ethanol as a rat line bred to not consume/prefer ethanol. That is, by exposing animals to an ever changing and novel environment (the EC) it altered, to some extent, the underlying mechanisms that are responsible for the increased motivation to respond for ethanol.

After the three phases of ethanol PR testing all rats were tested on a logarithmic PR with access to 10% sucrose. Unlike previous research that has shown that differentially reared rats do not differ in the consumption of, and/or responding for 10% Sucrose, the current study observed a significant effect of rearing on break point for 10% sucrose. However, it warrants mentioning that the animals in the differential rearing conditions had different learning histories during the ethanol PR. For instance, EC P rats and the three NP groups were less motivated to obtain ethanol reinforcement than the IC P and SC P groups. The IC P and SC P groups had more experience with responding deeper into the progressive ratio. When 10% sucrose was substituted for 15% ethanol, it is not surprising that the IC P and SC P groups continued to achieve

significantly higher break points. Thus, provided observations of sucrose responding in past research in our lab as well as Experiment 3 in the current study, it is evident that this rearing difference was mainly a result of learning history and not entirely an effect of rearing.

Discussion (Experiment 3)

Due to the observation that differential rearing conditions appeared to affect responding for sucrose on a PR in Experiment 2, Experiment 3 sought to determine if sucrose and ethanol naïve P and NP rats would differ in responding for 10% sucrose. Unlike the findings of Experiment 2, differential rearing conditions did not significantly affect the acquisition or maintenance of operant responding for 10% sucrose in either rat line. While there was an effect of rat line, this difference in sucrose consumption/responding is most likely due to a greater preference for sucrose in P compared to NP rats that has been reported previously (Stewart et al., 1994). This finding further suggests that while differential rearing conditions affect the motivation of P rats toward obtaining ethanol reinforcement, the same is not true for 10% sucrose. Additionally, this finding further supports the idea that differences observed during sucrose PR testing in Experiment 2 are indicative of a learning history and not true differences in the motivation of the animals to obtain sucrose.

General Discussion

The findings from the current research show that differential rearing conditions affect both the consumption of and the operant responding for ethanol in the selectively bred P rat. Alcohol-preferring rats, reared in an EC, consumed less and exhibited less of a motivation to respond for ethanol compared to P rats reared in an IC. The direction

of the effect of rearing can be inferred, in this case, due to the well documented data on average levels of ethanol consumption and ethanol responding in P rats (Files et al., 1998; Li et al., 1987, for review see McBride & Li, 1998; Murphy et al., 1989; Samson et al., 1998; Rodd et al., 2003). Additionally, the observation, in the current study, that EC P rats did not significantly differ from the NP groups in any measure of ethanol consumption or responding, further supports this notion. From the current data it would seem that rearing P rats in an EC acts as a type of "protective factor" against increased consumption of and/or increased motivation to obtain ethanol.

Previous studies in this laboratory have found that rearing Long-Evans rats in an IC, EC, or SC for 90-days affected operant responding for and the consumption of 10% ethanol (Deehan et al., 2007; Deehan & Kiefer, unpublished findings). These findings along with the current data suggest that differential rearing conditions affect operant responding for ethanol in P rats and outbred Long-Evans rats similarly. A thorough literature search resulted in one other study (that of Ehlers et al., 2007) that had examined the effects of differential housing conditions on the consumption of ethanol in P and NP rats. Additionally, to date there has been only one other study (besides Deehan et al., 2007) focusing on the effect of differential rearing conditions on operant responding for ethanol (McCool and Chappell, 2009). The latter study used Long-Evans outbred rats. Thus, the current research represents the beginning of a new line of research investigating the effect of rearing environment (nurture) on the consummatory and appetitive behavior exhibited toward ethanol by rodents selectively bred (nature) to prefer or not prefer ethanol.

Ehlers et al. (2007) report that adult rats housed in an isolate condition show an "increase" in ethanol consumption compared to those housed in a social condition.

However, upon further examination of their data it appears that housing P rats in a SC acted to attenuate ethanol consumption rather than the IC increasing ethanol intake. For instance, P rats housed in the IC for the Ehlers study consumed approximately the same amount of ethanol during limited-access testing as the IC P rats in the current study. Additionally, the levels of ethanol consumption by the IC P and SC P groups during 24-hour preference testing in the Ehlers et al. (2007) study were approximately the same as that observed in the current study (4 g EtOH/kg BW), which as previously stated, is below what has been documented as the average consumption levels for standard housed P rats (5 – 8 g EtOH/kg BW per day; McBride & Li, 1998). Therefore it would appear that Ehlers et al. (2007) observed an effect of SC rather than an effect of IC. In the current study, however, IC P and SC P rats did not consume significantly different amounts of ethanol during limited-access or 24-hour preference testing. There are several methodological differences between the current study and that by Ehlers et al. (2007). The most evident difference is the age at which the animals were placed into their rearing/housing conditions. For the current study rats arrived at approximately 21 days of age and were reared in an IC, EC, or SC for a period of 60 days. For the Ehlers et al. (2007) study, rats arrived at their lab at approximately 47 days of age and were housed in an IC or SC for a period of 63 days.

Early reports by Ennon and Morgan (1977) and Renner and Rosenzweig (1987) and more recently Robbins et al. (1996) clearly implicate the early rearing period (from 21 to 45 days of age) to be the critical period in which rearing environment causes neurological changes in the rat. Such changes are believed to be the mechanism by which differential rearing conditions affect a host of adulthood behaviors (including drug intake). Yet, for ethanol consumption, several investigators, including Ehlers et al.

(2007) have shown that housing rats in isolate versus social conditions following this critical period can be effective at altering ethanol intake as well. For example, a study by Yoshimoto et al. (2003) examined the effect of housing animals of different ages (1, 4, 10, and 16 months of age) in either an isolate or social condition for 6 months on ethanol consumption. The authors reported that the most significant difference in ethanol consumption between isolate and socially housed rats occurred in the oldest group, which was tested at 22 months of age (Yoshimoto et al., 2003). A number of other studies have also reported that adult rats housed in social isolation consume significantly more ethanol than those housed in a social condition (Adams & Oldham, 1996; Lodge & Lawrence, 2003; Roske et al., 1994; Thorsell et al., 2005; Wolfgramm, 1990).Therefore, it would seem that additional influences (occurring after 45 days of age) are at work affecting ethanol consumption in differentially housed adult rats.

Studies have found housing rats (between 45 – 90 days of age) individually in adulthood results in isolation stress which is believed to contribute to a number of physiological and behavioral changes (Heinrichs & Koob, 2006). Further, it has been documented that a rat's ability to maintain homeostasis following stress decreases with age (Gil et al., 1999). Thus, a possible factor influencing ethanol intake in differentially housed adult rats could be housing stress (e.g., isolation stress). However, for the current study, rats were reared in their respective conditions during postweaning and remained in the conditions into adulthood. While there was a trend for IC rats to consume more ethanol than SC rats it did not reach statistical significance. The evidence presented above would lead one to think that differential rearing conditions would exacerbate any effect between IC and SC reared animals. The rats are in the rearing conditions during the critical period of 21 – 45 days of age and remain there for

an additional 36 days during adulthood. Additional research will be necessary to examine further the effects of differential rearing compared to the effects of differential housing conditions on ethanol consumption in the rat.

Concurrent with previous findings by Deehan et al. (2007), McCool and Chappell (2009) reported that IC reared Long-Evans rats responded to a greater extent for ethanol than SC reared rats. The McCool and Chappell (2009) study represents the only other study, than those performed in the current laboratory, exploring the effect of differential rearing conditions on operant responding for ethanol. Although the paradigms of Deehan et al., (2007) and McCool and Chappell (2009) differ, the overall picture is the same as IC rats exhibit greater motivation to obtain ethanol than SC rats. However, McCool and Chappell (2009) failed to utilize an EC condition in their study. Because the current research observed significant decreases in ethanol responding in P rats reared both the EC and SC conditions compared to rats rearing in an IC, it is possible that McCool and Chappell (2009) may be observing an effect of IC rather than SC rearing. This suggestion should be taken with caution as a well documented level of operant responding for ethanol in standard housed Long-Evans rats has not been established as it has for the P line. Also, Long-Evans rats are outbred and as such do not possess many of the neurological changes that have occurred due to the selective breeding of P line.

It has been previously suggested that rearing in an EC acts to protect animals from an increased proclivity to consume and/or respond for drugs of abuse (Bardo & Dwoskin, 2004; Bardo, et al., 2001; Green et al., 2002). The data from the current experiments would support this as rearing P rats in an EC decreased both their consumption of and operant responding for ethanol relative to IC P rats (and in some

cases SC P rats) to the extent that they did not significantly differ from NP rats (regardless of rearing condition). This would suggest that rearing animals in a novel environment produces neurological changes that counteract some of the neurological changes that have occurred due to several generations of selective breeding in the P line. The current experiments did not utilize any specific pharmacological agents or in vivo techniques to further probe the changes occurring due to rearing P rats in an EC. This will be an important direction for future research to classify fully the underlying neurological changes due to rearing an animal model of alcoholism in an enriched environment. Probing the exact interactions between rearing and selective breeding will focus on a number of neurological systems.

There are several neurological systems in the brain that are different between the P and NP lines that are believed to contribute to the high and low levels of ethanol intake and ethanol responding, respectively. Further, differential rearing conditions have been found to affect a number of these systems which in turn could be affecting ethanol consumption and ethanol responding. Selective breeding has been shown to produce differences in the DA, 5-HT, GABA, as well as opioid systems between the P and NP lines (for review see McBride & Li, 1998). All of these neurological systems have all been shown to have a role in ethanol consumption and/or responding (Davis & Wu, 2001; Heinz, 2002; Herz, 1997; Lovinger, 1999) and have been found to be affected by differential rearing condtions (Advani et al., 2007; Del Arco & Mora, 2008a; Del Arco et al., 2008b; Miachon et al., 1990; Smith et al., 2008).

Overall, P rats show decreased levels of DA in the reward pathway relative to NP rats (McBride & Li, 1998). Operant responding for ethanol has been shown to increase DA levels in the NAC of P rats to a greater extent than in Wistar rats (Weiss et al.,

1993). Interestingly, rearing rats in an EC has been found to decrease the metabolism of DA in the NAC, subsequently increasing DA levels following an acute amphetamine challenge (Bowling et al., 1993). Additionally, when given an injection of amphetamine (2 g/kg s.c.) IC reared rats showed greater increases of DA in the NAC than SC reared rats (Hall et al., 1998b; Jones et al., 1992). Thus, it would seem that rearing in an EC may act to increase levels of DA in the NAC subsequently decreasing ethanol intake and/or responding.

Using Wistar rats, Engleman et al. (2004) observed a greater sulpiride (D_2 agonist)-induced DA release in the NAC of those housed in an IC compared to an SC. This may be due to an upregulation of D_2 receptors in the NAC as Djouma et al. (2006) reported increased levels of the receptor in both the core and shell of the NAC as well as the basolateral amygdala (bAMG) and central nucleus of the amygdala (cnAMG) of Fawn Hooded rats reared in an IC compared to those reared in an SC. Rearing rats in an IC may also change the sensitivity of the D_2 receptor. A recent experiment by King et al. (2009) reported that in the striatum, IC reared rats show a significantly greater proportion of D_2 receptors in the high affinity state (D_2^{High}) compared to SC rats. However, there have also been a number of studies showing no effect of rearing in an EC on the D_2 receptor within the striatum (Del Arco et al., 2004; Djouma et al., 2006; Bardo & Hammer, 1991; Por et al., 1982) while others have reported rearing in an EC to down regulate the D_2 receptor in both the striatum and the NAC (Bean & Lee, 1991; Rilke et al., 1995). This remains one area that will require more research to thoroughly investigate the contribution of the D_2 receptor in the differences observed in ethanol intake among differentially reared P rats.

The serotonergic system is another possible candidate for where differential rearing conditions may be affecting the ethanol intake of alcohol preferring rats. Through generations of selective breeding the P rat expresses lower levels of serotonin (5-HT) due to a decreased number of 5-HT neurons and subsequently less sertonergic innervations in several brain areas (Murphy et al., 1982; Murphy et al., 1987). These differences in the 5-HT system are believed to contribute to the high ethanol intake of the P line (Murphy et al., 1982; Murphy et al., 1987). For instance P rats have been found to have a lower amount of extracellular 5-HT in their medial prefrontal cortex (McBride & Li, 1998). It should be noted that Brenes et al. (2008) found that rearing rats in an EC acts to increase 5-HT levels in the prefrontal cortex. Alcohol-preferring rats have also been shown to exhibit 30% lower 5-HT content in the NAC which has been described as a contributing factor to their higher preference for ethanol (McBride et al., 1995). Whereas the IC P rats in the current study may not have increased their consumption of and responding for ethanol above that of the average P rat, it is interesting to note that rearing rats in an IC decreased basal levels of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, in the NAC (Jones et al., 1992).

There have also been a number of differences observed in the GABAergic system of P rats compared to the NP line. The P line possesses a greater amount of GABAergic innervation in the NAC as well as a greater sensitivity to benzodiazepines overall (McBride & Li, 1998). To date, there have been two studies that have examined the effect of social isolation during adulthood in the P rat line. Engleman et al. (2004) found that the social isolation of adult P rats for 84 days increased sulpiride-induced dopamine release in the nucleus accumbens relative to SC P rats. Theilen et al. (1993)

housed adult P rats in social isolation for a period of 1-2 days and found that isolate housed P rats exhibited greater $GABA_A$ receptor sensitivity than rats continually reared in pair housing. While IC P rats in the current study did not show an increase in ethanol consumption and/or responding over that of what has been observed in P rats in general, is important to mention that both NAC dopamine activity (Doyon et al., 2003; Kaczmarek & Kiefer, 2000; Kiianmaa et al., 1995; Löf et al., 2007; Pelligrino & Druse, 1992) as well as $GABA_A$ receptor functioning (Boyle et al., 1993; Follesa et al., 2006; Kralic et al., 2003; Mody et al., 2007; Samson et al., 1987; Sanna et al., 2003; Santhakumar et al., 2007; Smith et al., 1992; Wegelius et al., 1994) have been positively correlated with ethanol consumption. Additionally, these systems are affected both by selective breeding and rearing so further studies are warranted to probe any interactions between the two.

Several studies have observed a relationship between the reinforcing properties of ethanol and the function of the opioid system. In P rats, a higher density of mu opioid receptors have been noted in several brain areas relative to NP rats: olfactory tubercle, NAC (shell and core), basolateral and lateral amygdaloid nuclei, lateral septal intermediate nucleus, caudate and putamen patches, lower in layers of CA1 hippocamus and posterior medial cortical amygdaloid nucleus compared to NP rats (McBride & Li, 1998). The higher mu densities are believed to contribute, to some extent, to their elevated intake of ethanol. Interestingly, rearing rats in an IC has been observed to increase their consumption of morphine as well as their responding for heroin compared to SC housed animals (Alexander et al., 1981; Bozarth et al., 1989). On the other hand, rearing in an EC for 49 days has been shown to increase the sensitivity of the mu receptor (Smith et al., 2005). Therefore, the mu opioid receptors of

P rats that have been reared in an EC may have greater sensitivity and as such may require much less ethanol to be stimulated than rats reared in an IC.

It also appears that the underlying neurological mechanisms of “liking” and “wanting” are affected by differential rearing conditions in a similar fashion. Previous research has reported that differential rearing conditions affect the consumption of (liking; consummatory behavior) and the operant responding for (wanting; appetitive behavior) of certain drugs of abuse in an opposing manner. However, recent research completed in the current laboratory found that outbred rats reared in an IC consumed significantly more ethanol than rats reared in an EC (Deehan & Kiefer, unpublished finding). Similarly, another study found that rats reared in an IC responded significantly more for ethanol than EC rats (Deehan et al., 2007). The current research observed similar findings as P rats reared in an IC exhibited significantly higher levels of ethanol consumption, ethanol responding, and ethanol preference than P rats reared in an EC. Thus, it would seem that, for alcohol, if differential rearing conditions are affecting “liking” and “wanting,” the changes to the neurological correlates underlying “liking” and “wanting” are in the same direction.

Various rearing period lengths have been used when investigating the effects of differential rearing/housing conditions on ethanol consumption and may be a contributing variable to the disparate results observed among studies. Given the current findings, however, it would seem that rearing period length is not a contributing variable. The current research used a 60-day rearing period and obtained comparable results to past research in our lab that used a 90-day rearing period (Deehan et al., 2007; Deehan & Kiefer, unpublished data). Yet, past experiments in the laboratory utilizing a 90-day rearing period used Long-Evans rats as subjects whereas the current

research used P and NP rats. Therefore, rat strain and rearing period may be interacting somehow.

A study completed by Hall et al. (1998a) reared both Fawn-Hooded and Wistar rats in either an IC or an SC for 60-days and reported that IC reared animals, independent of strain, consumed significantly more ethanol (at 16% v/v concentration) than did SC reared rats. With the same rearing length, the two strains of rats exhibited comparable consummatory behavior specific to ethanol. A more recent study completed by McCool and Chappell (2009) found that a 42-day rearing period altered ethanol consumption (lick rate) as well as the number of operant responses per second for ethanol in Long-Evans rats. Furthermore, ongoing research in the current laboratory is showing that a 30-day rearing period is as effective at altering ethanol responding as the 60-day rearing period in P rats (Deehan & Kiefer, unpublished data). Thus, it seems that ethanol consumption and ethanol responding are affected similarly by a number of different rearing lengths and that the results observed in the current experiments were primarily due to the early post-weaning effects (up to post-natal day 45) of differential rearing environments. However, future studies that house adult rats in the same conditions will be needed to confirm this.

The only studies, other than those completed in the current laboratory, looking at the effect of the environmental enrichment paradigm (EC, SC, and IC conditions) on the consumption of ethanol were those done by Rockman and colleagues (1986; 1988; 1989; 1991). All other rearing/housing studies have looked specifically at differences in ethanol consumption and responding when animals were reared/housed in an IC or an SC. While the IC always involves singly housing animals the SC varies widely in the number of animals housed together across studies. Studies have reared/housed as few

as 2 animals (e.g. Hall et al., 1998a) to as many as 8 animals (e.g. Juarez & Vazquez-Cortes, 2003) per SC condition. Moreover, the type of caging used to house the animals has varied widely between studies for both the IC and SC. The IC has included rearing/housing animals in hanging metal cages (Ellison et al., 1979) to the standard shoebox cage (Ehlers et al., 2007) with the SC rearing/housing animals in standard shoebox cages (Deehan et al., 2007) to large guinea pig cages (McCool & Chappell, 2009). The current experiments, taken together with past rearing experiments in this laboratory represent the only extended investigation of the effects of differential rearing environments on both the consumption of and responding for ethanol in 2 different lines of rats. Due to the fact that earlier consumption and responding studies utilized a 90-day rearing period, future studies will need to be conducted using the same paradigm to more fully classify the effects of differential rearing conditions on ethanol intake.

It has been suggested that the operant paradigm that the current research used to assess the effect of differential rearing environments on the appetitive (motivational) aspects of ethanol self-administration is confounded (Samson et al., 1999). It has been proposed that small amounts of ethanol earned in an operant situation, in this case 0.1 ml per reinforcement several times over the course of the session, may affect responding later in the session (Samson et al., 1998). Samson et al. (1998) outlined a procedure in which sessions are 20 minutes in length and rats are required first to lever press a certain number of times (usually a FR 30) to gain access to a sipper tube. The rats then have free access to the sipper tube and may consume ethanol for the remainder of the 20 min session. Samson et al. (1999) conclude that, by first having the rat respond without ethanol present, and then allowing access to ethanol from a sipper tube, one is able to separate the appetitive (lever responses) from the consummatory

(drinking) aspects of ethanol self-administration. While this "sipper" paradigm provides somewhat of a separation between appetitive and consummatory behaviors, the "dipper" paradigm used in the current project could still be considered an effective measure of motivation.

An early study investigating the differences between the "sipper" and "dipper" paradigm found that Long-Evans rats consumed, over the course of the 20 minute session, the same amount of ethanol in both paradigms (Samson et al., 1999). Due to the response requirement between each ethanol reinforcer in the "dipper" paradigm, rats responding for the dipper took longer to consume the same amount of ethanol as the rats in the "sipper" paradigm. However, the key point is that both paradigms produced comparable amounts of ethanol consumption with identical session lengths (Samson et al., 1999). Another issue with the "sipper" paradigm is that all animals are trained to respond up to a certain point (i.e., FR 30) once per session to gain access to the sipper tube. By having all animals respond to a single FR value once per session it becomes difficult to assess the variability between animals as to the motivational component of ethanol as a reinforcer (i.e., how many lever presses an animal will make to consume ethanol). The "sipper" paradigm attempts to evaluate appetitive behaviors via assessment of lever presses per time elapsed but it fails to account for individual differences in motivation (different levels of responding) between rats.

A viable alternative to the "sipper" paradigm, which successfully measures the appetitive behavior of responding for ethanol, is the progressive ratio (PR) schedule. For example, responding on a continuous reinforcement (CR) or low FR schedule results in a high ratio of reinforcers being received per number of responses made. By having animals respond on a PR, where the animals respond at incrementally

increasing values early in the session, the ratio of reinforcers earned to responses made is quite small (Richardson & Roberts, 1996). Thus a PR schedule is able to assess the highest number of responses that an animal is willing to perform to obtain ethanol while providing very little ethanol to consume.

For Experiment 2, the IC P group had the highest average break point across all of the sessions (both shallow PR and logarithmic PR) and as such received the highest number of dipper presentations. If the IC P rats consumed all of the 10% ethanol from every dipper cup that was presented, they would have consumed an average of 2.5 ml of 10% ethanol during the shallow PR schedule and an average of 1.5 ml of 10% ethanol during the logarithmic PR schedule. Therefore, due to the distribution of reinforcements in the PR schedule as well as the high functional and metabolic tolerance of the P rat (Gatto et al., 1987a; Gatto et al., 1987b; Lumeng & Li, 1986; Waller et al., 1983), the animals likely were not receiving high enough ethanol concentrations to affect their operant responding (motor activity) during the PR.

To summarize the present series of studies, P rats reared in an EC consumed significantly less ethanol and responded significantly less for ethanol compared to IC P rats. While IC P rats exhibited a significantly higher breakpoint for 10% sucrose at the conclusion of Experiment 2, it is believed that this occurred due to learning history and not due to the effect of differential rearing conditions. Additionally, in Experiment 3 there were no significant differences between P rats in the three rearing conditions when they responded for 10% sucrose on both levers. This suggests that the effects of differential rearing conditions are specific to ethanol and not other reinforcing solutions, which lack a pharmacological profile such as ethanol.

Another interesting finding was that by rearing P rats in an EC, it effectively decreased ethanol consumption and ethanol responding to the levels observed in the NP groups. That is, the consummatory and appetitive behavior exhibited by EC P rats toward 10% ethanol was not significantly different than all three NP groups throughout testing. Past research has shown an attenuation of the consumption of and/or responding for ethanol in the P line by using various agonists or antagonists. The current data represent a non-pharmacological suppression of both the consumption of and the motivation to respond for ethanol. This in itself provides evidence that rearing in an EC acts to protect rats against increased ethanol intake when they are genetically predisposed to consume high levels ethanol.

The current series of experiments add to the body of literature on the effect of differential rearing conditions on the intake of drugs of abuse. Furthermore, these experiments represent the first thorough documentation of the effects of differential rearing environments on the most established animal model of alcoholism and the interaction between nature and nurture. Given the consistent effects of differential rearing on ethanol intake in P rats, further research designed to delineate the underlying neurological changes that are affecting the consumption of and responding for ethanol is warranted. Certainly there are many candidate variables that are available that may underlie the effects observed. Therefore, the interactions between such neurological factors will need to be explored due to the great deal of overlap between the systems implicated in the increased intake of ethanol in the P line and those affected by differential rearing conditions.

General Summary

Alcoholism is a significant problem in the United States which has been further characterized through animal research. Currently, there are several animal models and experimental paradigms that allow researchers to ethically examine and develop novel treatments and interventions for those suffering from alcoholism. Perhaps the most established animal model of alcoholism is the alcohol-preferring (P) and alcohol non-preferring (NP) rat lines from Indiana University. The current research sought to examine the effects of differential rearing conditions on the consumption of, responding for, and preference for ethanol in the P and NP lines. This research represents the first thorough investigation of the interaction between genetic predisposition (nature) and environmental influences (nurture) on the proclivity of P and NP rats to consume, respond, and prefer ethanol.

Data from the current experiments show that differential rearing conditions significantly affected the consumption of, responding for, and preference for ethanol in the P rat line. Rearing P rats in an environmental enrichment condition (EC) reduced ethanol consumption, ethanol responding, and ethanol preference to levels that were significantly below those of P rats reared in the impoverished condition (IC). Furthermore, EC rearing decreased ethanol consumption, ethanol responding, and ethanol preference in the P line to levels that were not significantly different from those of NP rats. These represent important findings as it speaks to the importance of early rearing environment on adulthood behaviors toward drugs of abuse, specifically alcohol. Future research will need to focus on the underlying neurological mechanisms that are changing during differential rearing conditions and are affecting ethanol consumption, ethanol responding, and ethanol preference. Once such mechanisms are identified

steps can be taken to develop additional novel treatments and interventions that could help individuals suffering from alcoholism.

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Figures

Figure 1. Panel A represents the mean (± SEM) amount of sucrose consumed during limited-access testing prior to and following sucrose/ethanol fading and ethanol testing. Panel B shows the average (± SEM) grams of ethanol (EtOH) per kilogram body weight (g EtOH/kg BW) consumed during the fading in of EtOH. Panel C displays the mean (± SEM) consumption of ethanol (g EtOH/kg BW) during the fading out of EtOH after EtOH testing had occurred.

Figure 2. Panel A illustrates the mean (± SEM) g EtOH/kg BW consumed during limited-access EtOH testing in differentially reared P rats. Rats in the IC P group consumed significantly more EtOH than rats in the EC P group ($p<.05$). Panel B shows the mean (± SEM) g EtOH/kg BW consumed by differentially reared NP rats during limited-access testing.

Figure 3. Panel A shows the average (± SEM) g EtOH/kg BW consumed during free-access testing. IC P rats consumed significantly more EtOH than the EC P rats ($p<.05$). Panel B represents the mean (± SEM) preference scores for EtOH during free-access testing (total amount of 10% ethanol/ total amount of water consumed + total amount of ethanol consumed).

Figure 4. Mean (± SEM) number of sessions for each group to acquire operant responding for 6% ethanol. There were no significant differences between the groups for acquisition of operant responding.

Figure 5. Average (± SEM) number of responses for 6%, 8%, and 10% ethanol during the ethanol fading procedure. Differential rearing conditions did not have a significant effect on responding for any of the fading solutions presented.

Figure 6. Panel A shows the mean (± SEM) number of operant responses on the ethanol lever for 10% ethanol. Rats in the IC P groups responded significantly more than rats in the SC P and EC P groups ($p<.05$). Animals in the SC P group responded significantly more than the EC P group ($p<.05$). Panel B represents the mean (± SEM) number of responses made on the water lever for each group. Differential rearing conditions did not significantly affect responding on the water lever.

Figure 7. Mean (± SEM) number of responses on the ethanol and water levers for the IC P (Panel A), SC P (Panel B), and EC P (Panel C) groups. Rats in the IC P and SC P groups responded significantly more on the ethanol lever than the water lever ($p<.05$). Animals in the EC P group did not significantly differ in the number of responses made on the ethanol and water levers.

Figure 8. Average (± SEM) number of responses on the ethanol and water levers for the IC NP (Panel A), SC NP (Panel B), and EC NP (Panel C) groups. There were no significant differences between ethanol lever and water lever responding for any of the groups.

Figure 9. Mean (± SEM) number of responses on the active (Panel A) and inactive (Panel B) levers during the FR schedule increase. IC P rats responded significantly

more on the active lever compared to EC P rats during all three FR schedules (p<.05). IC P rats responded significantly more than the SC P group during the FR 2 and FR 5 schedules (p<.05) and the SC P group responded significantly more than the EC P group during the FR 2 and FR 5 schedules (p<.05) as well.

Figure 10. Mean (± SEM) break point on the active lever during progressive ratio (PR) testing with ethanol. IC P rats exhibited a significantly higher break point than EC P rats for the shallow PR (10% EtOH) and each logarithmic PR (10% and 15% EtOH) (p<.05). SC P rats had a higher break point than EC P rats for 10% ethanol during both the shallow PR and the logarithmic PR for 10% ethanol (p<.05).

Figure 11. Mean (± SEM) break point on the active lever during progressive ratio (PR) testing with 10% sucrose. IC P rats exhibited a significantly higher break point for 10% sucrose than EC P rats (p<.05). There were no other group differences.

Figure 12. Mean (± SEM) number of sessions for each group to acquire operant responding for 10% sucrose. There were no significant differences between the groups for acquisition of operant responding.

Figure 13. Average (± SEM) number of responses for 10% sucrose during experiment 3. Differential rearing conditions did not have a significant effect on the operant responding for 10% sucrose.

Figure 1.

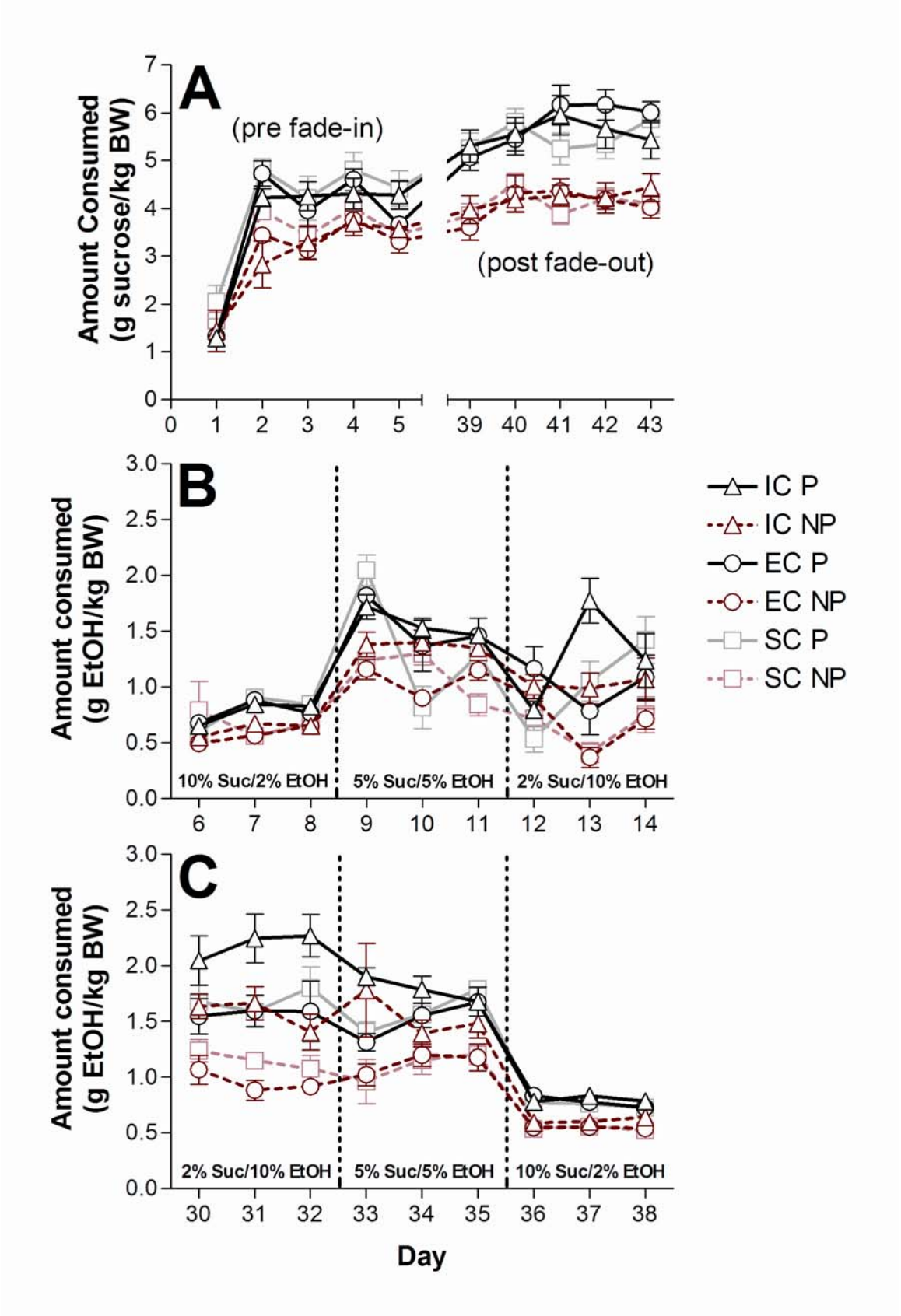

A
(pre fade-in)
(post fade-out)
Amount Consumed
(g sucrose/kg BW)
B
Amount consumed
(g EtOH/kg BW)
10% Suc/2% EtOH
5% Suc/5% EtOH
2% Suc/10% EtOH
C
Amount consumed
(g EtOH/kg BW)
2% Suc/10% EtOH
5% Suc/5% EtOH
10% Suc/2% EtOH
Day
IC P
IC NP
EC P
EC NP
SC P
SC NP

Figure 2.

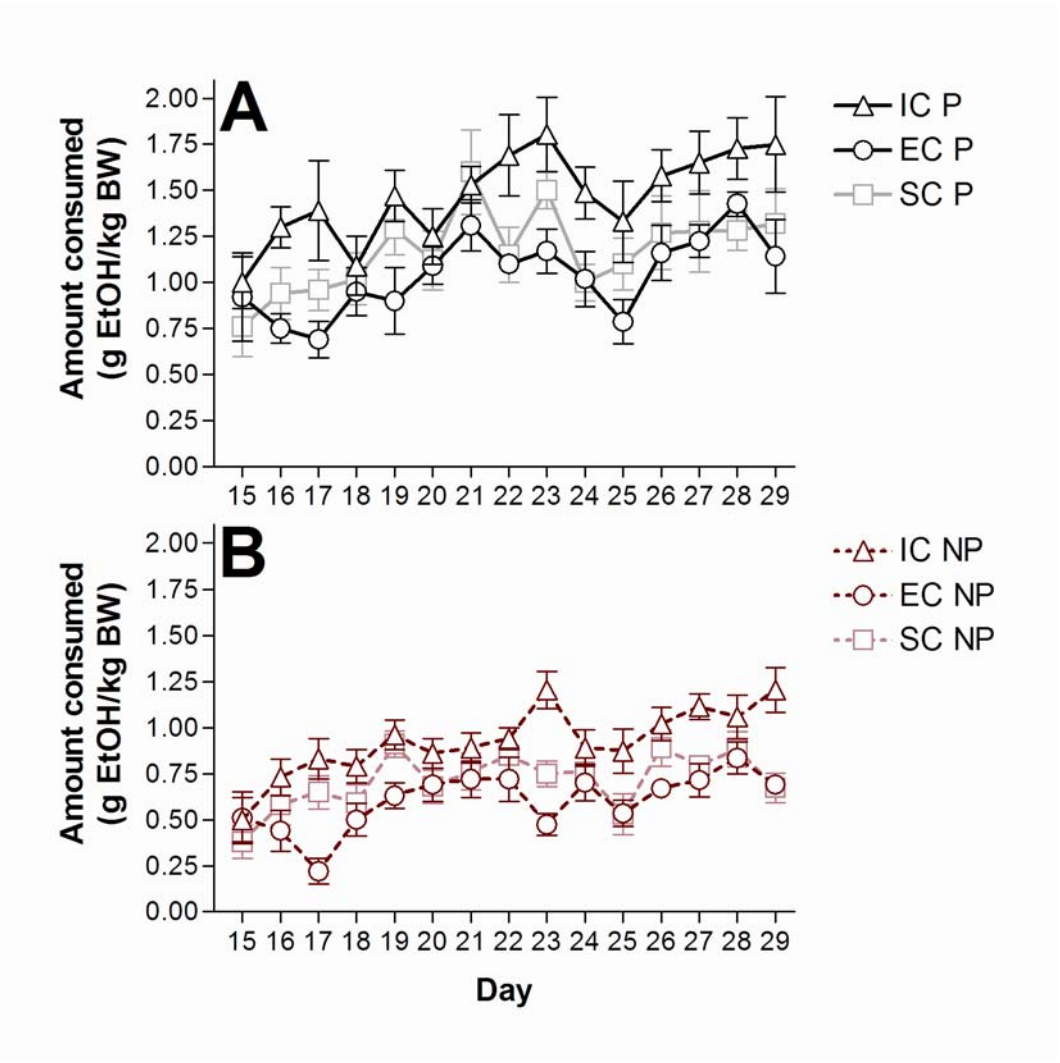

A
B
Amount consumed (g EtOH/kg BW)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Day
IC P
EC P
SC P
IC NP
EC NP
SC NP

Figure 3.

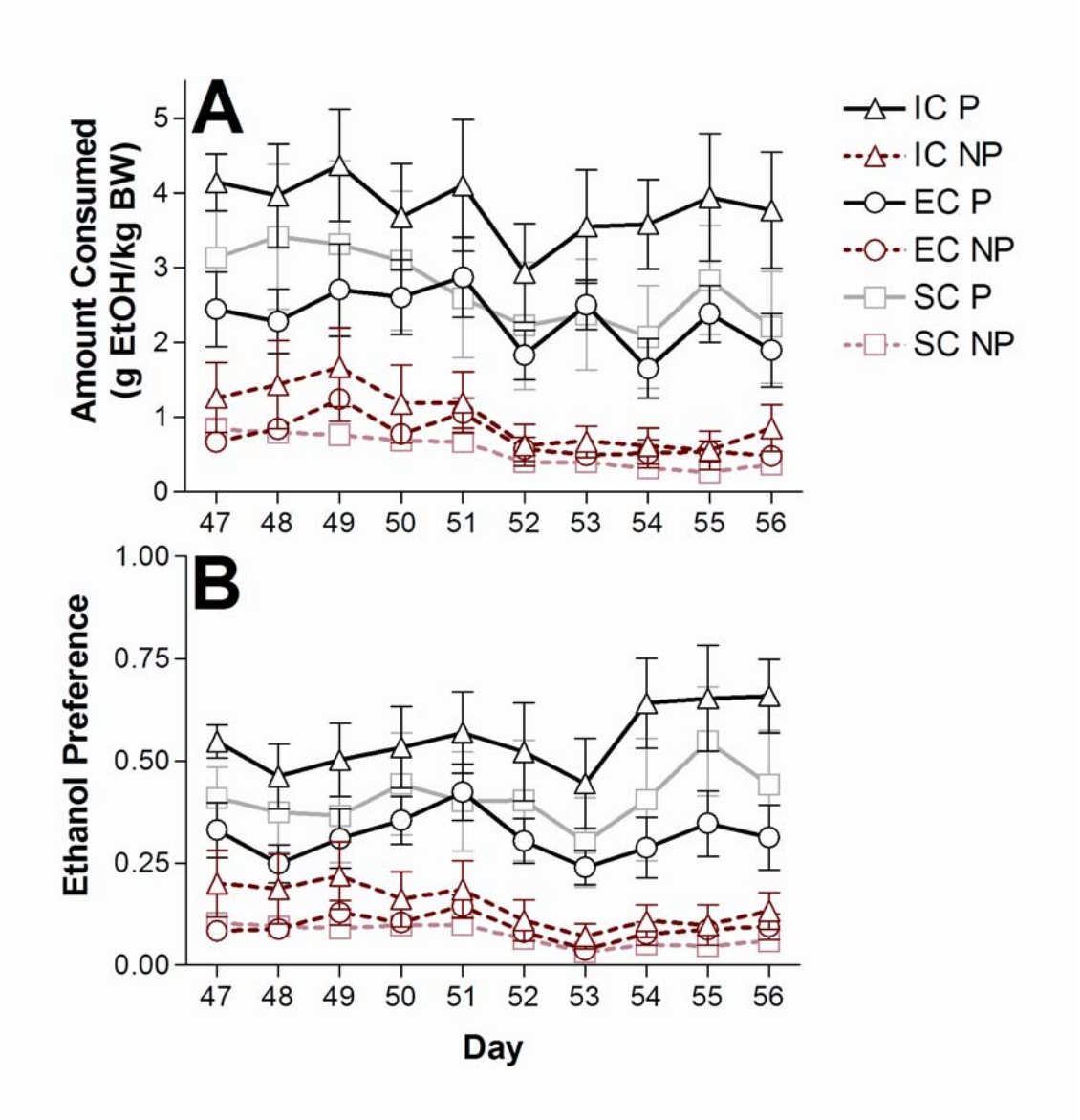

A
B
Amount Consumed
(g EtOH/kg BW)
Ethanol Preference
Day
IC P
IC NP
EC P
EC NP
SC P
SC NP
0
1
2
3
4
5
0.00
0.25
0.50
0.75
1.00
47
48
49
50
51
52
53
54
55
56

Figure 4.

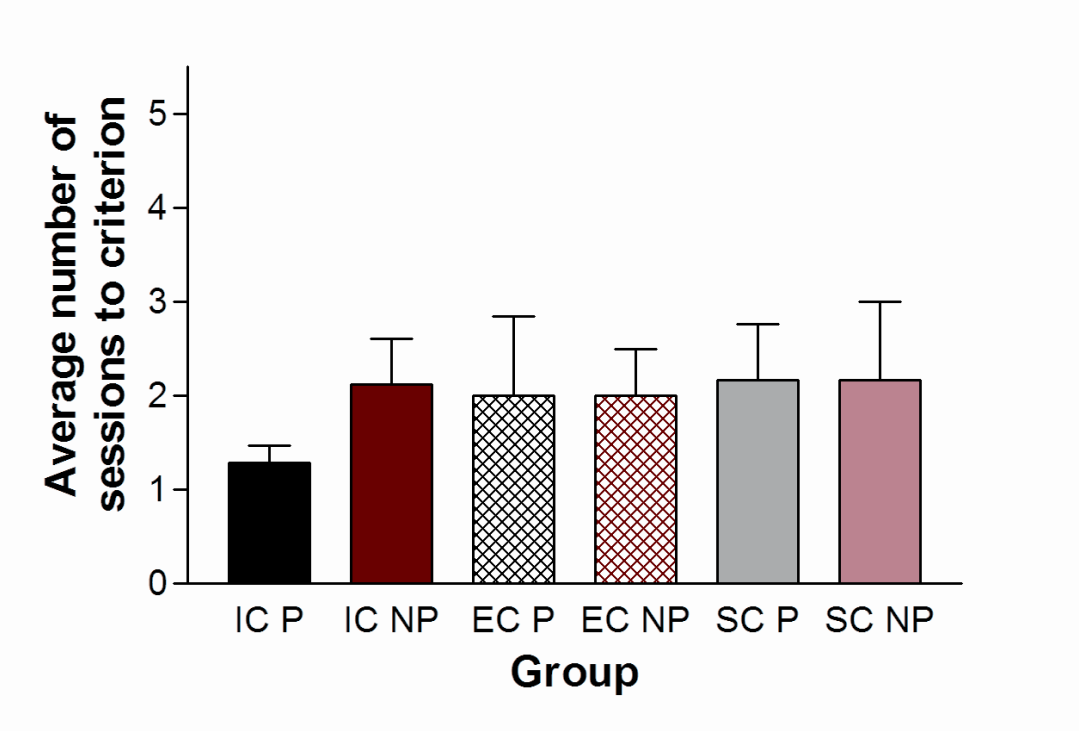

Average number of sessions to criterion
0
1
2
3
4
5
IC P
IC NP
EC P
EC NP
SC P
SC NP
Group

Figure 5.

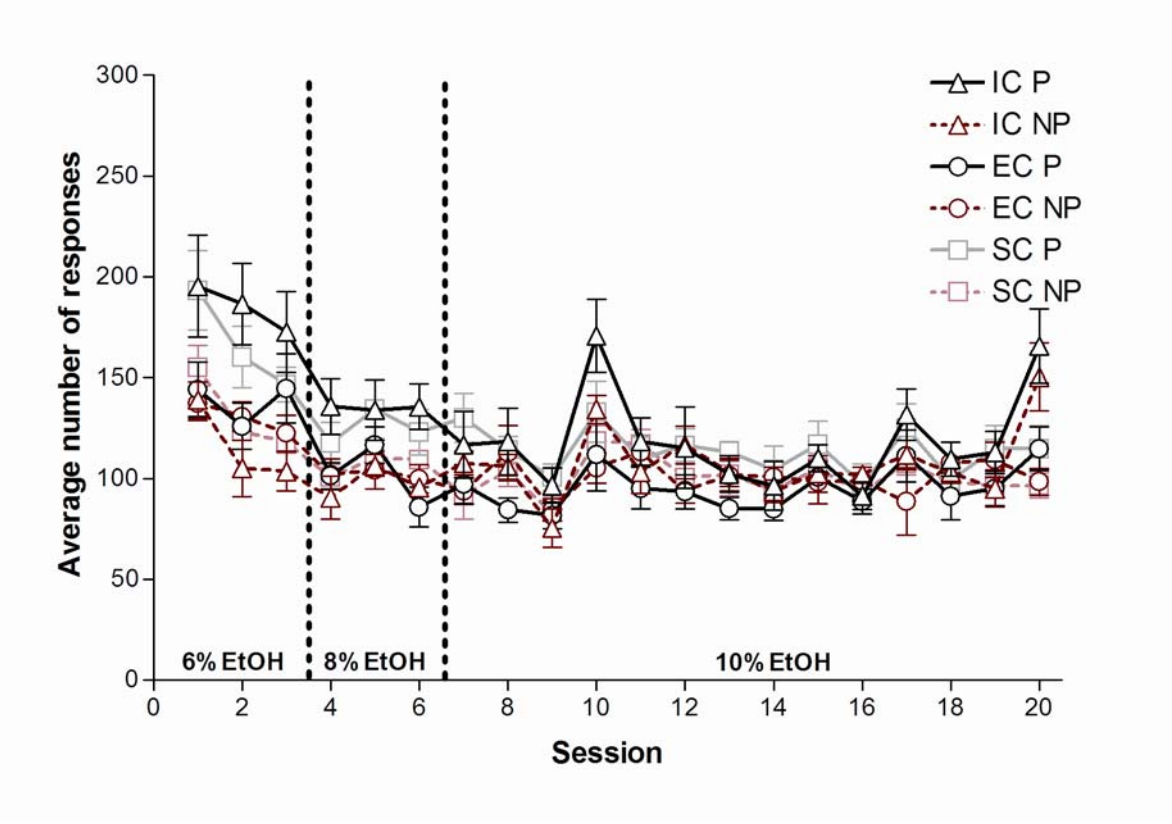

IC P
IC NP
EC P
EC NP
SC P
SC NP
Average number of responses
300
250
200
150
100
50
0
6% EtOH
8% EtOH
10% EtOH
0
2
4
6
8
10
12
14
16
18
20
Session

Figure 6.

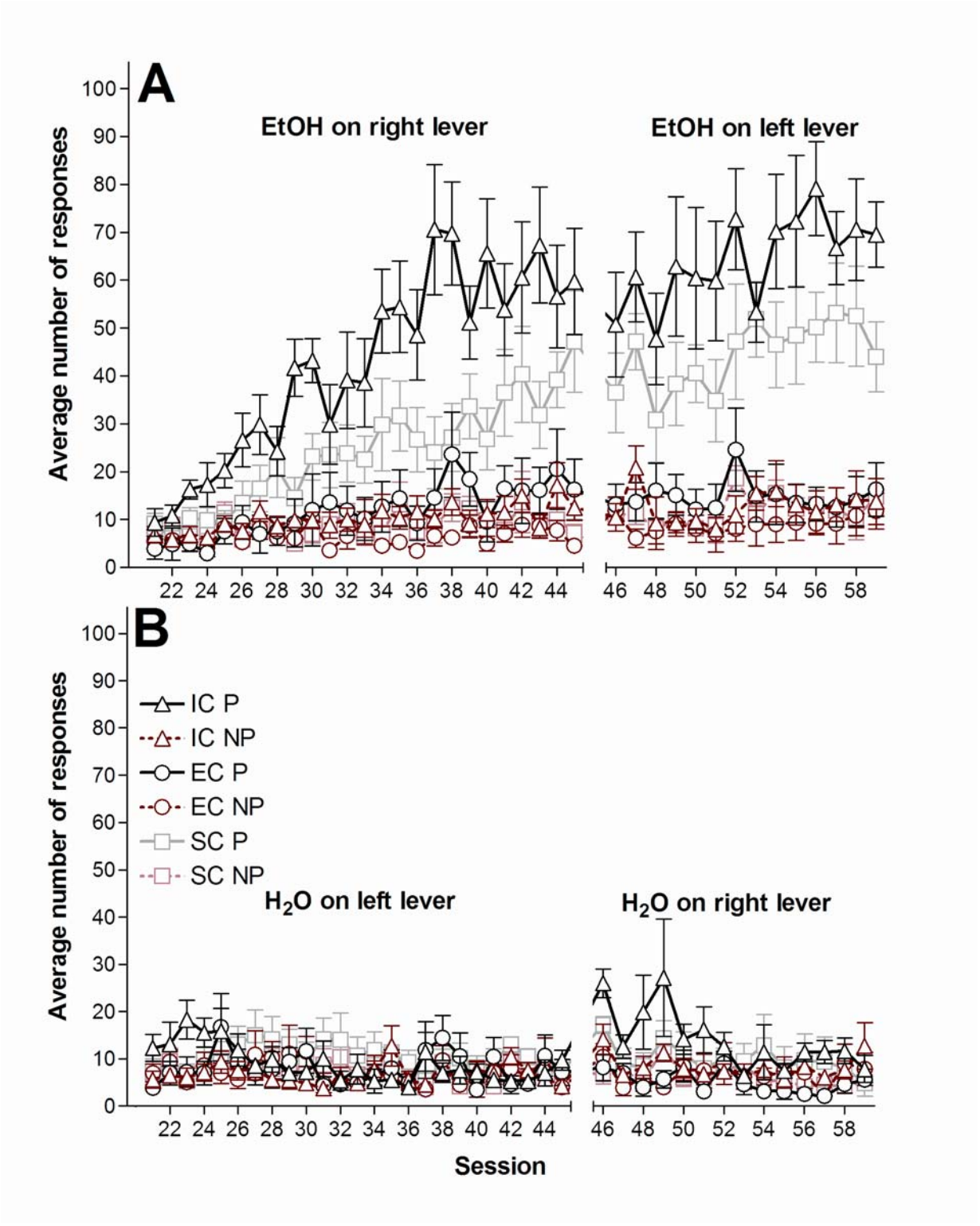

A
EtOH on right lever
EtOH on left lever
Average number of responses
B
IC P
IC NP
EC P
EC NP
SC P
SC NP
H2O on left lever
H2O on right lever
Session

Figure 7.

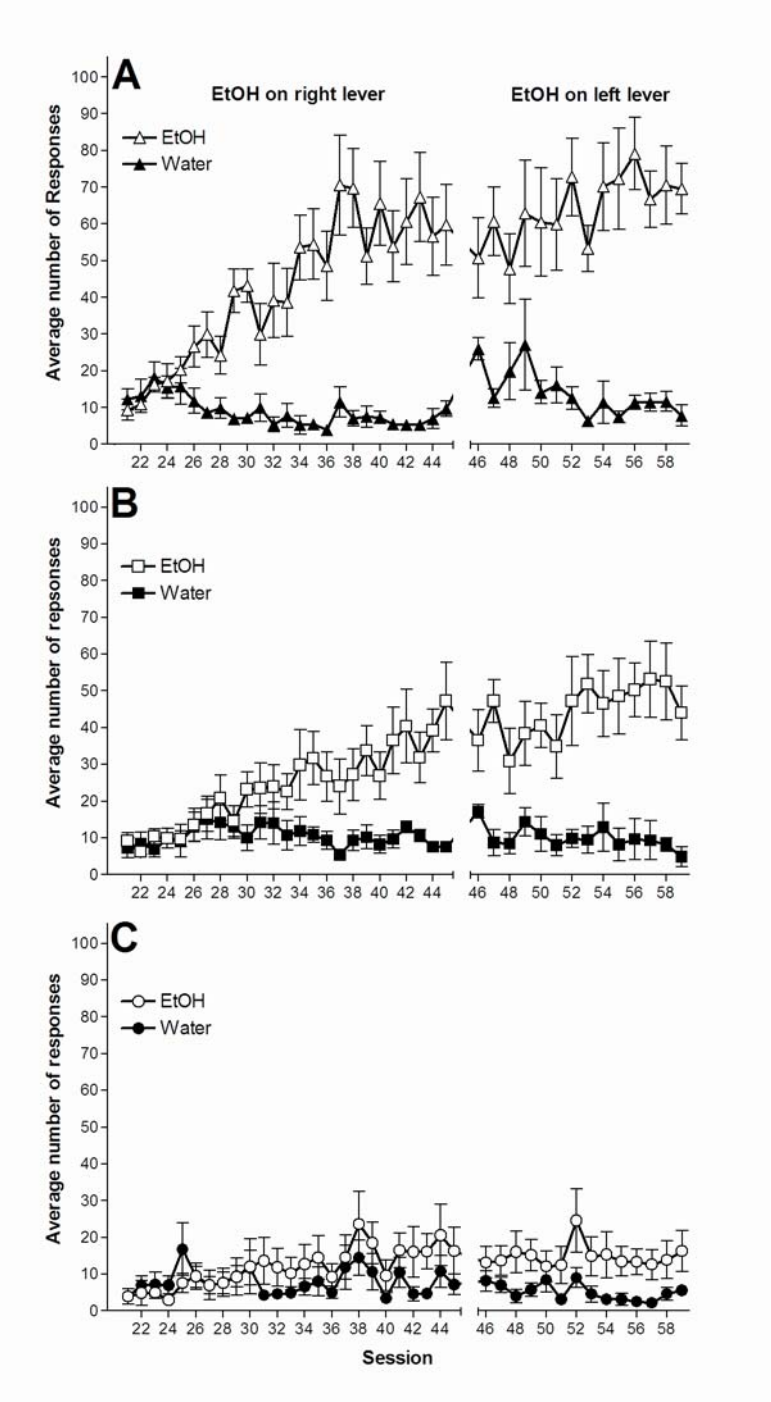

A
EtOH on right lever
EtOH on left lever
EtOH
Water
Average number of Responses
B
EtOH
Water
Average number of repsonses
C
EtOH
Water
Average number of responses
Session

Figure 8.

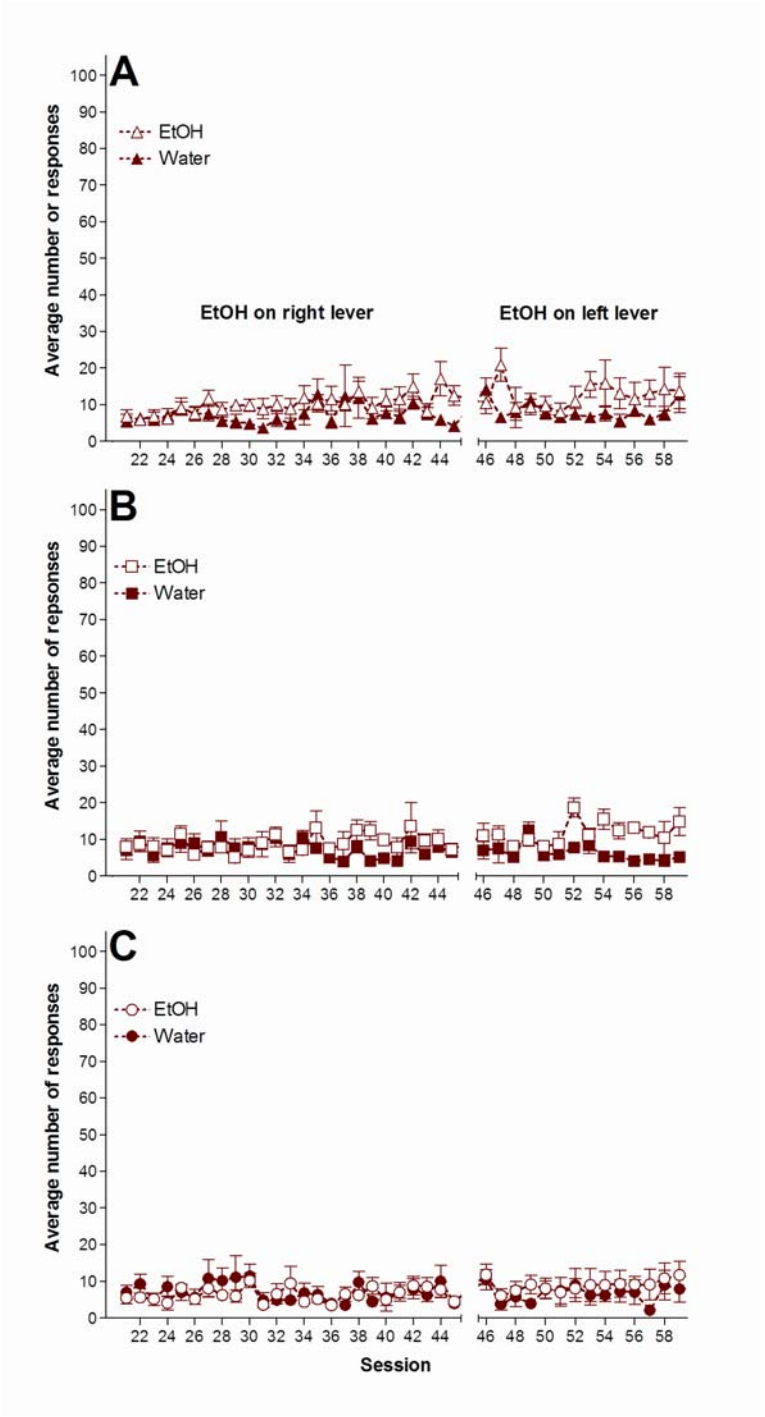

A
EtOH
Water
EtOH on right lever
EtOH on left lever
Average number or responses
B
EtOH
Water
Average number of repsonses
C
EtOH
Water
Average number of responses
Session

Figure 9.

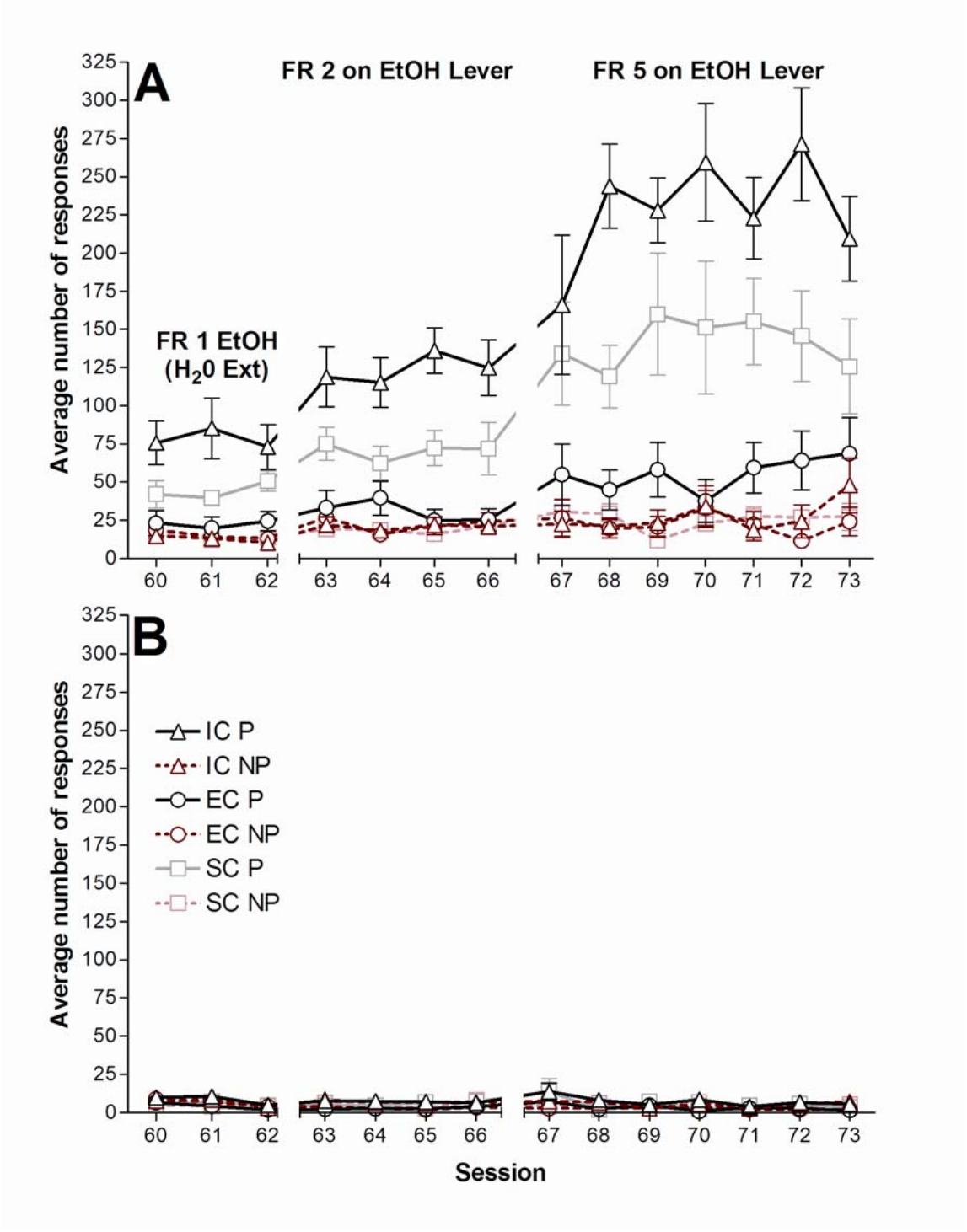

A
FR 2 on EtOH Lever
FR 5 on EtOH Lever
FR 1 EtOH
(H20 Ext)
Average number of responses
325
300
275
250
225
200
175
150
125
100
75
50
25
0
60
61
62
63
64
65
66
67
68
69
70
71
72
73
B
IC P
IC NP
EC P
EC NP
SC P
SC NP
Session

Figure 10.

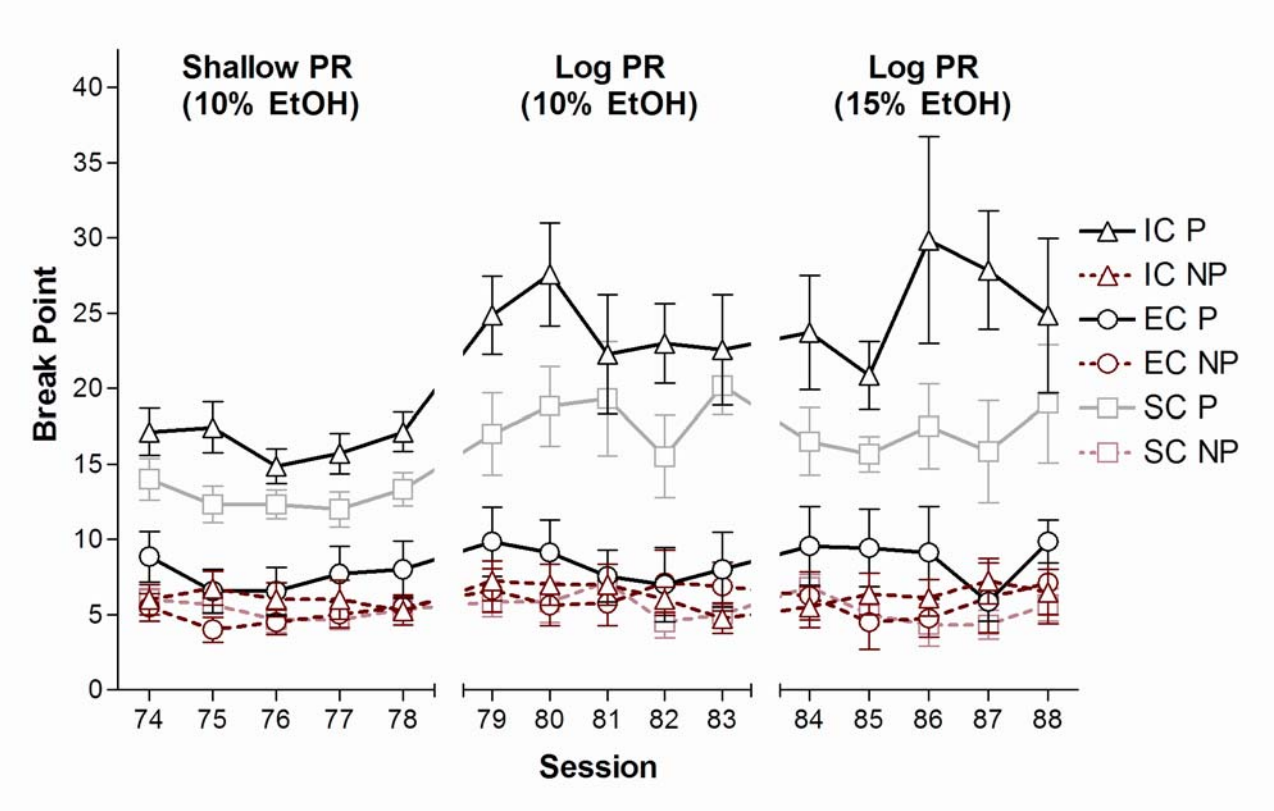

Shallow PR
(10% EtOH)
Log PR
(10% EtOH)
Log PR
(15% EtOH)
Break Point
0
5
10
15
20
25
30
35
40
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
Session
IC P
IC NP
EC P
EC NP
SC P
SC NP

Figure 11.

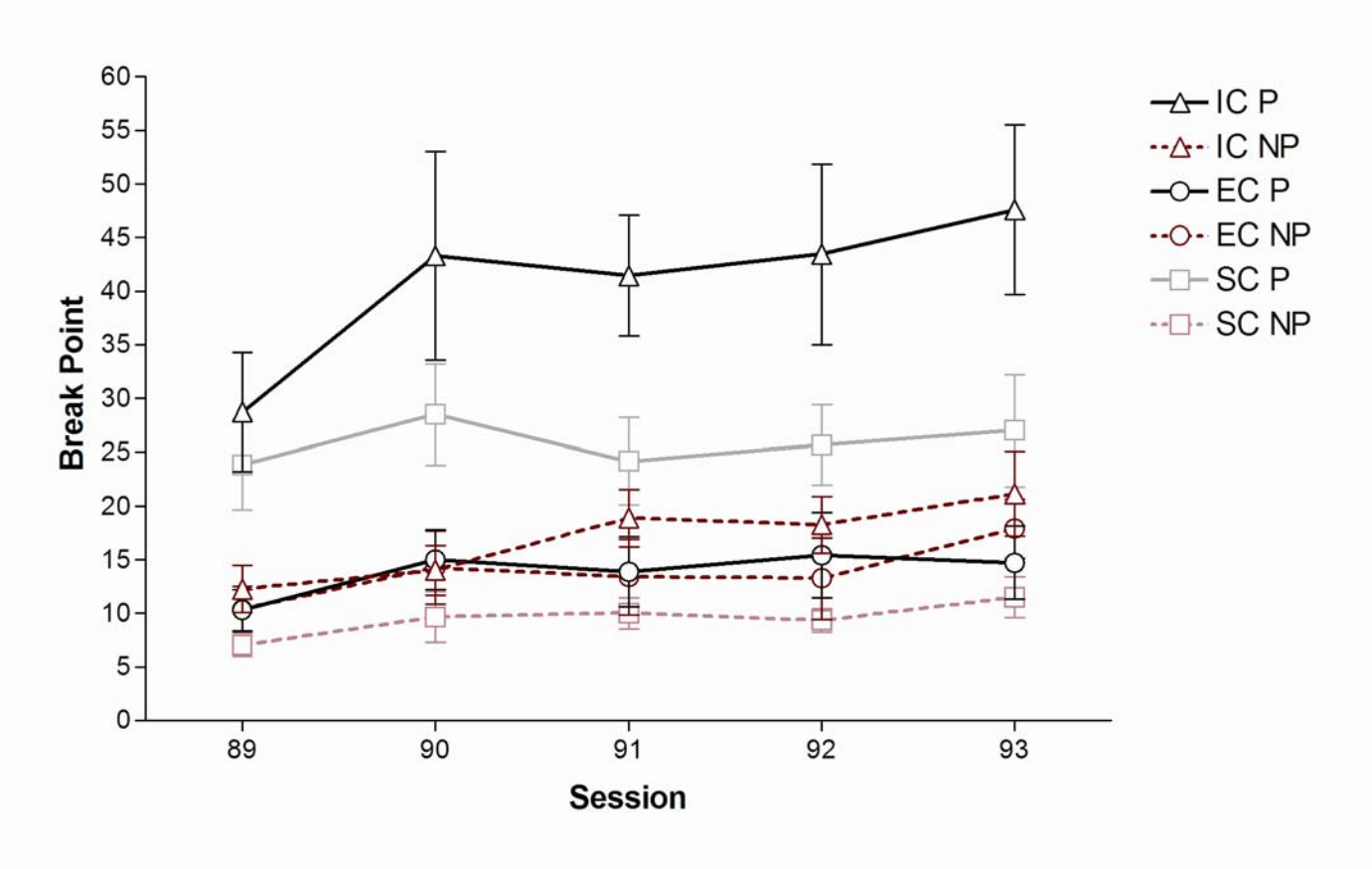

Break Point
60
55
50
45
40
35
30
25
20
15
10
5
0
89
90
91
92
93
Session
IC P
IC NP
EC P
EC NP
SC P
SC NP

Figure 12.

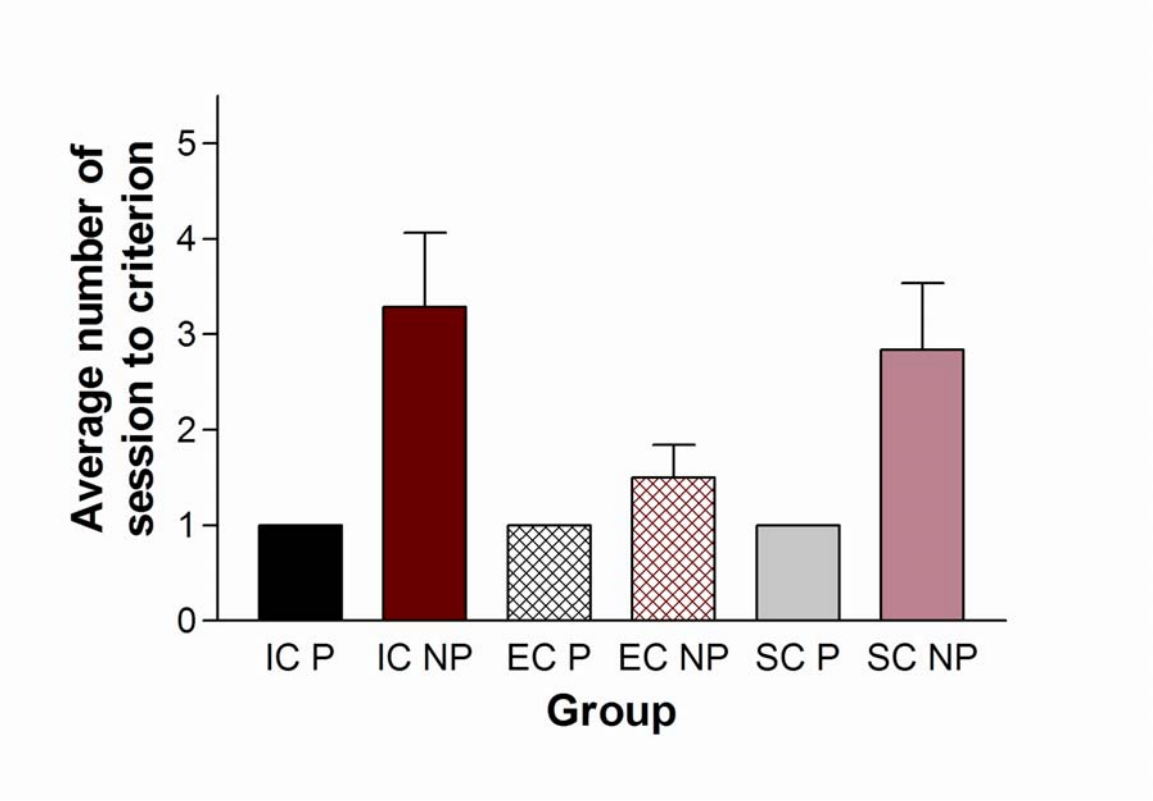
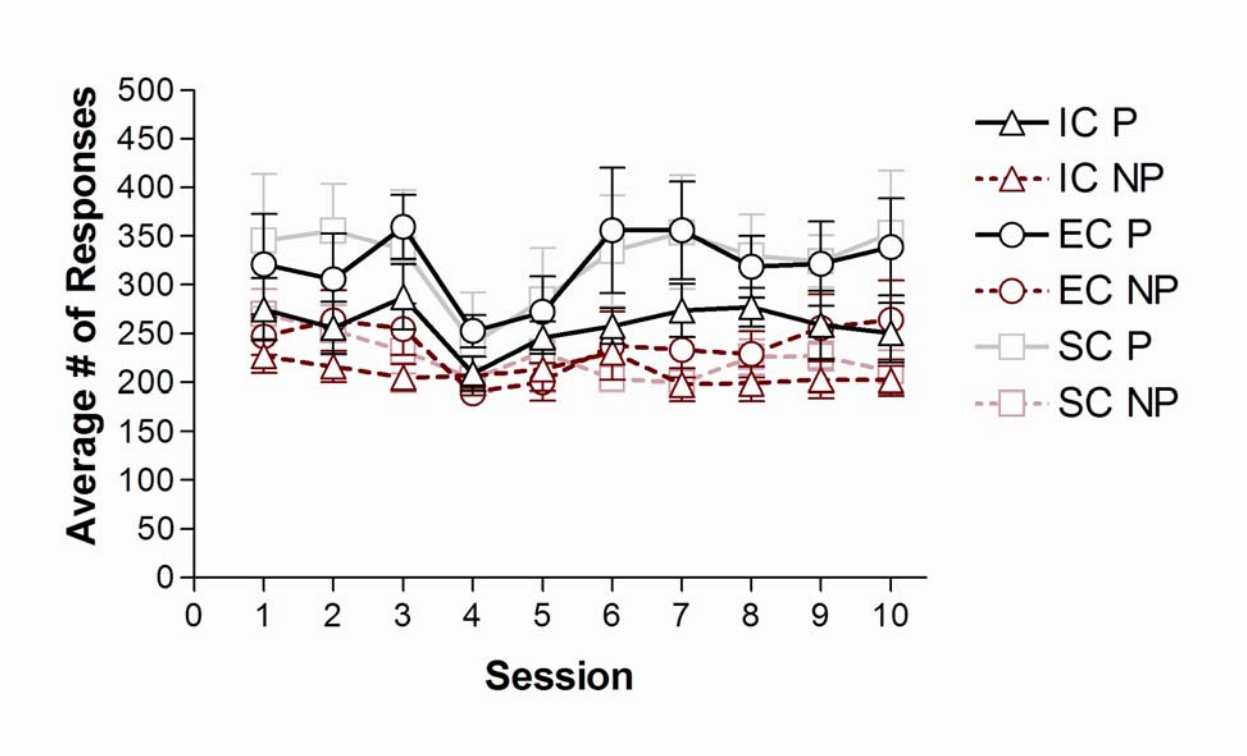

Average number of session to criterion
0
1
2
3
4
5
IC P
IC NP
EC P
EC NP
SC P
SC NP
Group

Figure 13.


Average # of Responses
500
450
400
350
300
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10
Session
IC P
IC NP
EC P
EC NP
SC P
SC NP